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RESEARCH ARTICLE

PROTEIN ANALYSIS FROM DIFFERENT BODY TISSUES OF THREE IMPORTANT COMMERCIAL RACES OF MULBERRY SILKWORM *Bombyxmori*(L)BY USING BRADFORD METHOD

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Abstract

The mulberry silkworm *Bombyxmori* L. is one of the economically beneficial insect. Natural silk is produced by silkworm which converts mulberry leaf protein in to silk. Three mulberry silkworm races viz.,two single hybrid $FC_1(CSR_6 \times CSR_{26})$, $FC_2(CSR_2 \times CSR_{27})$and one bivoltinedouble hybrid $FC_1 \times FC_2[(CSR_6 \times CSR_{26}) \times (CSR_2 \times CSR_{27})]$ were selected for the present study. The main aim of present study was made an attempt to analyse and compare the protein content in Haemolymph, Midgut, Silkgland and Fatbodyselected silkworm races of fifth instar larvae. Data obtained were statistically analysed with two way ANOVA method at ($p<0.001$), ($p<0.005$) and ($p<0.05$) level of significance. High level of significance ($p<0.001$)were recorded in silkgland compared to haemolymph, midgut and fatbody in all selected silkworm races whichcomparing the protein content in haemolymph and midgut of all selected silkworm $FC_1 \times FC_2$races shows ($p<0.001$) significance compared to FC_1 ($p<0.005$) and FC_2 ($p<0.05$), protein content in silkgland and fat body shows ($p<0.001$) in $FC_1 \times FC_2$ compared to FC_1 and FC_2respectively. The results therefore, showed bivoltinedouble hybrid silkworm is superior in protein content in all examined different tissues and the study would be of great help to farmers to choose the better silkworm races for rearing purpose.

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Introduction:-

Silk production is an art of sericulture, Mulberry silkworm *B.mori* (L.) is a lepidopteron which feeds only on mulberry leaves. Sericulture industry plays an important role in the development of national income and economic development of agriculture (Dandin, 2014; Mahmoudet al., 2019). Raw silk production is depends on the nutritive quality of silkworm breed. Production of raw silk has got a very good demand in the market and hybrid varities play an important role in sericulture. Bivoltine silk production in India could not reach to the most of the farmers despite efforts made by sericulture (KhasruAlam, 2020). Silkworm rearing depends on the breed of silkworm ie, whether Univoltine, Bivoltine or Multivoltine in nature (Savithriet al., 2013; Rohithet al., 2015). The different breeds of silkworm are of differ in their nutritional value, metabolic and physiological process, hence this studies are of great importance to understand the difference in the protein content of different tissues such as Haemolymph, midgut, silk gland and fat body of single hybrid ($FC_1$and FC_2) and Bivoltine double hybrid (FC_1X FC_2). Biomolecules such as carbohydrates, proteins, lipids, vitamins and hormones play an important role in biological activities such as growth and development of silkworm. Many important proteins in silkworm play a significant role such as metamorphosis,

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physiological functions, and biological activities in different tissues of silkworm. Midgut protein helps in larval development, silk gland protein for production silk filaments, Haemolymph protein for immune response and fat body for storage of proteins. Keeping this in view the aim of present study is comparative analysis of protein content in different tissues of three commercial important silkworm breed viz., FC_1, $FC_2$and FC_1X FC_2.

Materials and Methods:-

Silkworm Rearing

Before starting the experiments the laboratory was disinfected with limestone powder at DOSR in Zoology, Tumkur University, and Tumakuru. FC_1single hybrid(CSR_6× CSR_{26}) and FC_2single hybrid(CSR_2×CSR_{27}) DFL of silkworm (1^{st} instar larvae) were collected from farmers Ajjapanahalli, Yallapura, Tumakuru and FC_1×FC_2[(CSR_6× CSR_{26}) × (CSR_2×CSR_{27})]Bivoltine double hybrid breed of silkworm were obtained from chawki rearing centre Hollanahalli , Tumkur. These silkworms were reared under laboratory conditions of 25±3℃, 72±2% relative humidity. The larvae were divided into 3 groups; each group has 30 larvae which were supplied with sufficient amount of V_1 mulberry leaves. The larvae were reared under standard rearing conditions (Krishnaswami, 1983). The healthy larvae in the rearing trays were enumerated and the larvae which showed delayed moulting were removed periodically.

Collection of Haemolymph from the silkworm

The fifth instar larvae were collected regularly till the end of fifth instar. The haemolymph was collected in pre-chilled micro centrifuge tube containing small amount of thiourea by puncturing caudal horn of silkworm and centrifuged at 3000rpm for 5 min and supernatant was used for estimation of total protein in haemolymph.

Collection of Tissues

The midgut, silkgland and fat body tissue were collected from fifth instar larvae by dissecting in ice cold water. The tissue was washed in distilled water.

Estimation of total protein

The total protein present in the haemolymph, midgut, silk gland and fat body were estimated by the Bradford method (Bradford, 1976) using Bovine serum albumin as standard protein.

Biostatical Analysis

For data analysis, statistical computer application Graph pad prism 8.30 was used. Two way analysis of variance ANOVA was used to know significance differences between silkworm breeds.

Results:-

The present study is comparative analysis of protein content in different tissue of three economically important silkworm*B.mori* (L.). The two potential single hybrid FC_1 and FC_2 and one Bivoltine double hybrid FC_1XFC_2were chosen for the experiment, in which they were subjected with V1 mulberry leaves, Fifth instar larvae were isolated from 1^{st} day to 7^{th} day for evaluation of protein content in haemolymph, midgut, silk gland and fat body

The estimated protein content in FC_1 silkworm *B.mori* were summarized and presented in the Table-1 and Fig-1, the protein content was found to be increased from 1^{st} day to 7^{th} day in all three races. With reference to protein content in haemolymph, midgut, silk gland and fat body. There was a significant difference and more protein content was found to be in silkgland,(28.0±0.12mg/gm tissue) followed by Haemolymph (24.23 µg of protein/µl), midgut (20.15 mg/gm tissue) and fatbody(17.02 mg/gm tissue) respectively.

Table 1:- Concentration of protein in different tissues of FC_1 Silkworm during fifth instar.

FC_1	1st Day	2nd Day	3rd Day	4th Day	5th Day	6th Day	7th Day
Heamolymph (µg of protein/µl)	15.12±0.21	15.93±0.65	16.20±0.66	18.17±0.32	20.19±0.95	24.01±0.88	24.23±0.13
Midgut (mg/gm tissue)	12.14±0.14	13.01±0.45	14.19±0.52	15.23±0.41	16.11±0.39	17.86±0.42	20.15±0.19
Silkgland (mg/gm tissue)	18.04±0.18	19.43±0.87	20.12±0.32	21.43±0.12	25.99±0.73	26.53±0.33	28.0±0.12
Fatbody (mg/gm tissue)	11.12±0.95	12.53±0.69	13.91±0.55	14.1±0.03	15.60±0.39	16.90±0.28	17.02±0.31

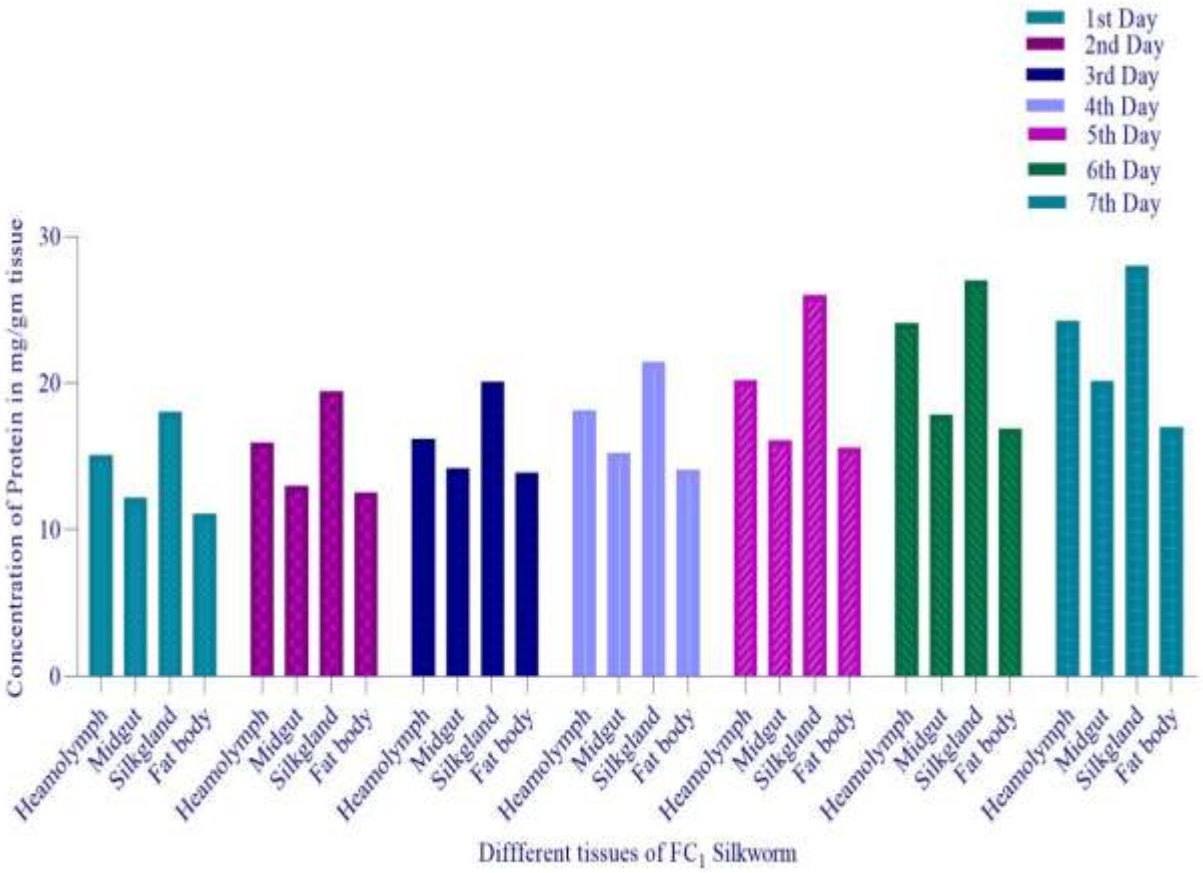

Fig 1:- Concentration of protein in different tissues of FC_1 Silkworm during fifth instar.

The result clearly reflect that the silkgland have highest protein content as compare tohaemolymph, midgut andfatbody, and maximum protein content was observed in last day fifth instar in all the three silkworm breeds and the results are summarised in Table-2 and Fig-2 represented that protein content in FC_2 silkworm *B.mori* .The results show that thereis significant difference among the different tissue. Such as haemolymph, midgut, silk gland and fatbody.In FC_2 also maximum protein content was found to be observed in silkgland(19.23 mg/gm tissue), followed by haemolymph(13.56 µg of protein/µl), midgut (13.14 mg/gm tissue) and fatbody(12.91 mg/gm tissue). The protein content in Haemolymph, Midgut, Silk gland, and Fat body of silkworm FC_1XFC_2bivoltine double hybrid is presented in Table-3 and Fig-3, The concentration of protein was observed in haemolymph is (27.90 µg of protein/µl) followed by silkgland(31.06 mg/gm tissue), Midgut(20.11 mg/gm tissue) and Fatbody (15.12

mg/gmtissue). Significant difference was observed among the tissues in which silkgland shows maximum protein concentration compared to midgut, silk gland, and fatbody.

Table 2:-Concentration of protein in different tissues of FC_2 Silkworm during fifth instar.

FC_2	1st Day	2nd Day	3rd Day	4th Day	5th Day	6th Day	7th Day
Haemolymph (µg of protein/µl)	11.91±0.10	12.10±0.11	12.31±0.45	12.49±0.77	12.93±0.65	13.11±0.47	13.56±0.84
Midgut (mg/gm tissue)	10.14±0.37	10.93±0.59	11.13±0.93	11.46±0.24	11.99±0.19	12.23±0.35	13.14±0.73
Silkgland (mg/gm tissue)	14.13±0.95	15.23±0.64	16.30±0.55	17.16±0.11	18.88±0.10	19.01±0.24	19.23±0.39
Fatbody (mg/gm tissue)	8.34±0.44	9.12±0.66	10.16±0.13	11.23±0.22	12.14±0.81	12.23±0.26	12.91±0.46

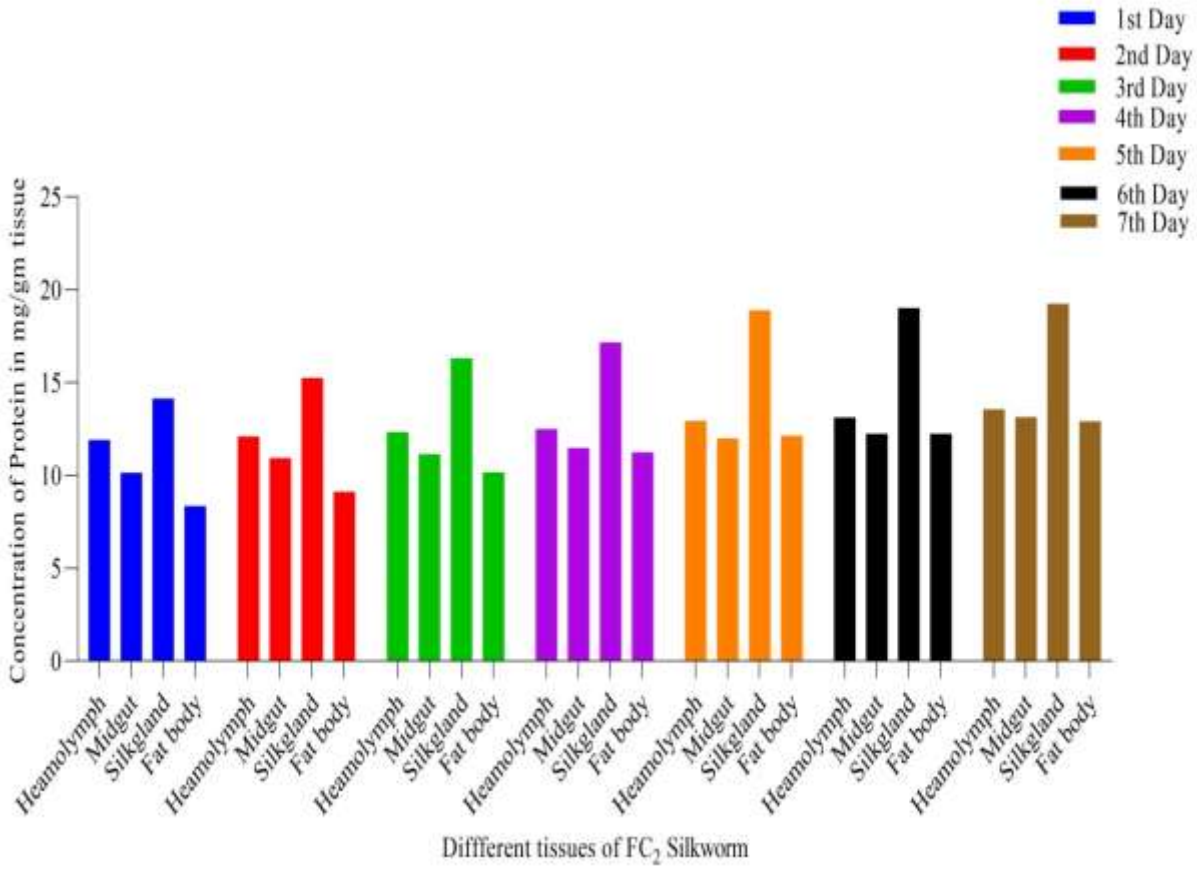

Fig 2:- Concentration of protein in different tissues of FC_2 Silkworm during fifth instar.

Table 3:- Concentration of protein in different tissues of $FC_1 \times FC_2$ Silkworm during fifth instar.

$FC_1 \times FC_2$	1st Day	2nd Day	3rd Day	4th Day	5th Day	6th Day	7th Day
Heamolymph (µg of protein/µl)	18.19±0.2	19.01±0.5	19.13±0.13	21.00±0.36	24.35±0.74	26.18±0.12	27.90±0.89
Midgut (mg/gm tissue)	14.91±0.01	15.23±0.25	16.14±0.19	17.23±0.59	18.19±0.78	19.10±0.98	20.11±0.69
Silkgland (mg/gm tissue)	22.9±0.36	24.3±0.74	26.9±0.68	28.72±0.95	29.32±0.19	30.12±0.36	31.06±0.34
Fatbody (mg/gm tissue)	10.12±0.43	10.23±0.85	11.43±0.33	12.73±0.13	13.16±0.66	14.86±0.11	15.12±0.55

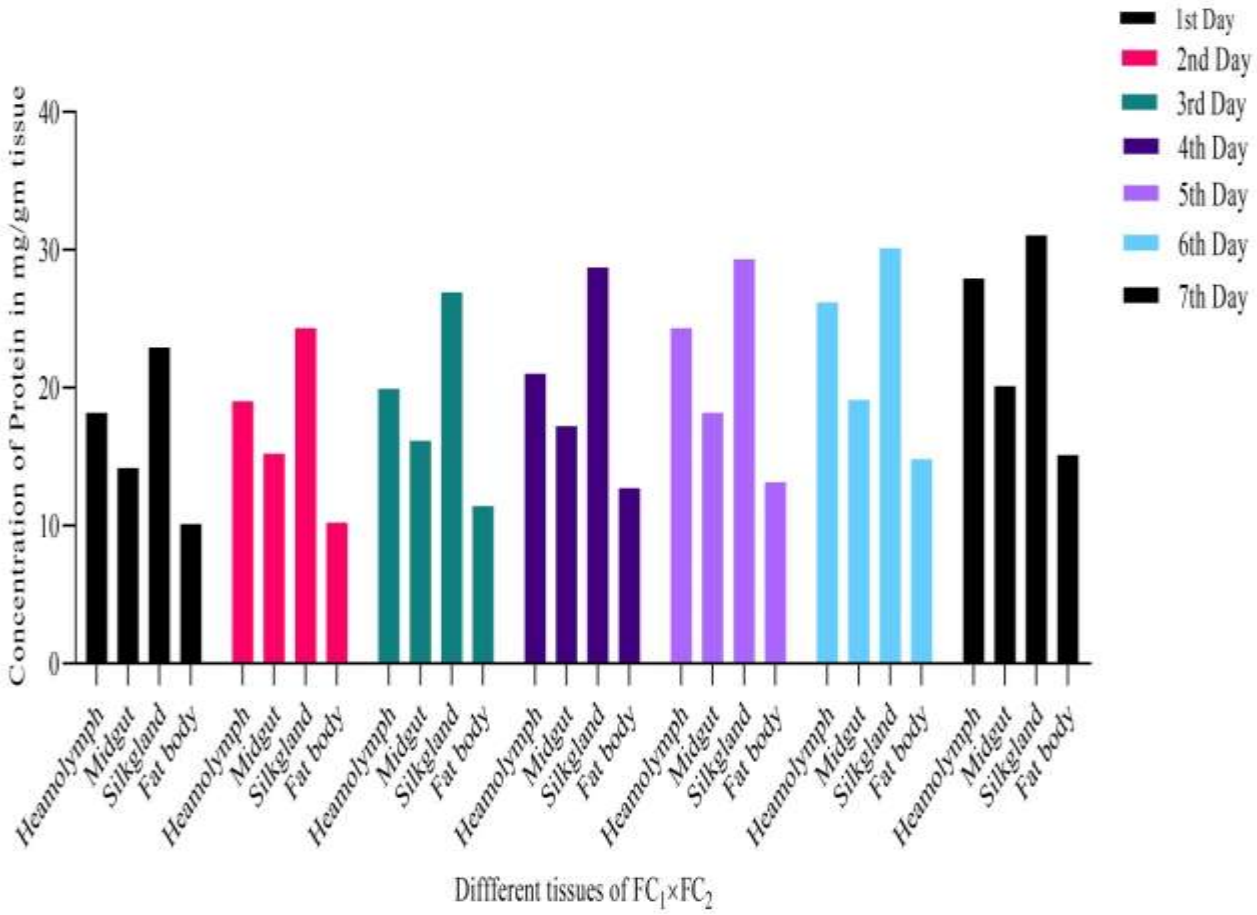

Fig 3:- Concentration of protein in different tissues of $FC_1 \times FC_2$ Silkworm during fifth instar.

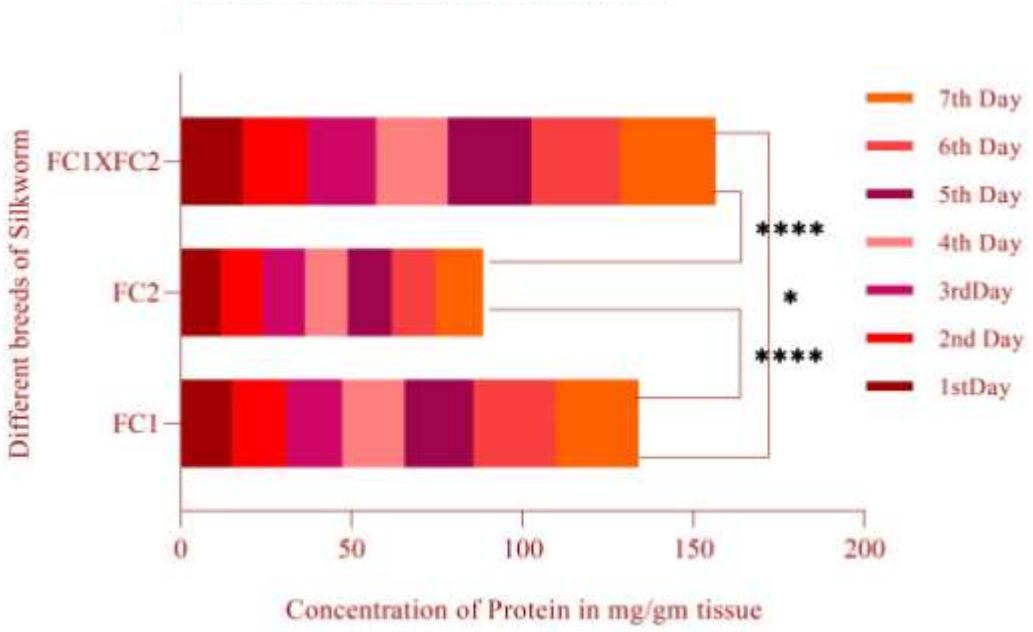

Fig 4:- Concentration of protein in Haemolymph of FC_1, FC_2 and $FC_1\times FC_2$ silkworm during fifth instar.

The Fig-4, 5, 6 and 7 represented that comparative analysis of protein content inhaemolymph, midgut, silk gland and fat body in three selected races there was significant difference between concentration of protein in (FC_1 and FC_2) and (FC_2 and $FC_1\times FC_2$) ie,($p<0.001$) but in (FC_1 and $FC_1\times FC_2$) show significant difference at ($p<0.05$) in Haemolymph and midgut of silkworm. But there is a significant difference between in (FC_1 and FC_2), (FC_1 and $FC_1\times FC_2$) and (FC_2 and $FC_1\times FC_2$) which shows significance at ($p<0.001$) in silk gland and fat body respectively.

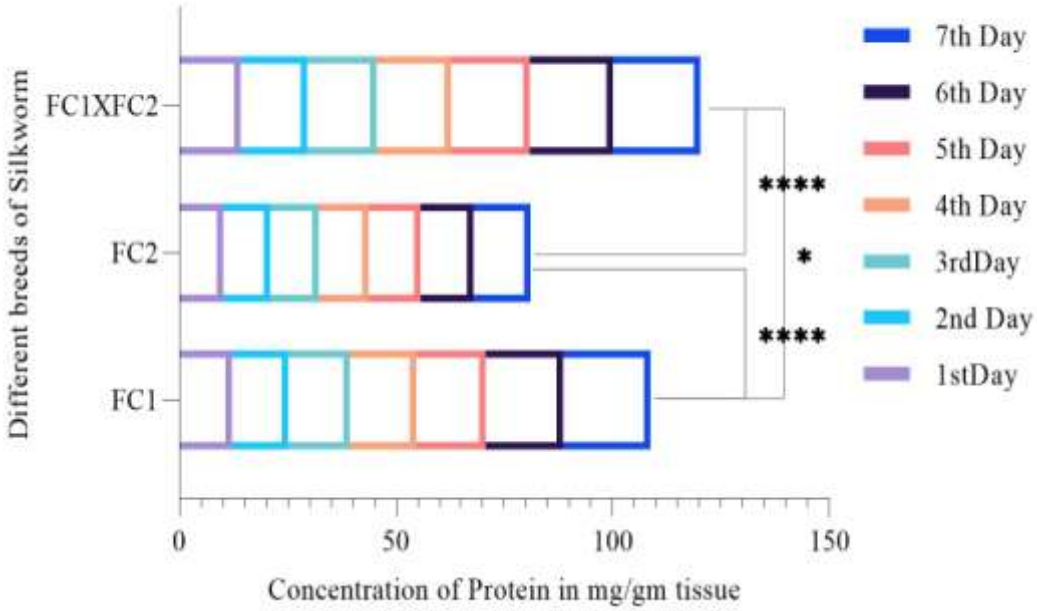

Fig 5:- Concentration of protein in Midgut of FC_1, FC_2 and $FC_1\times FC_2$ silkworm during fifth instar.

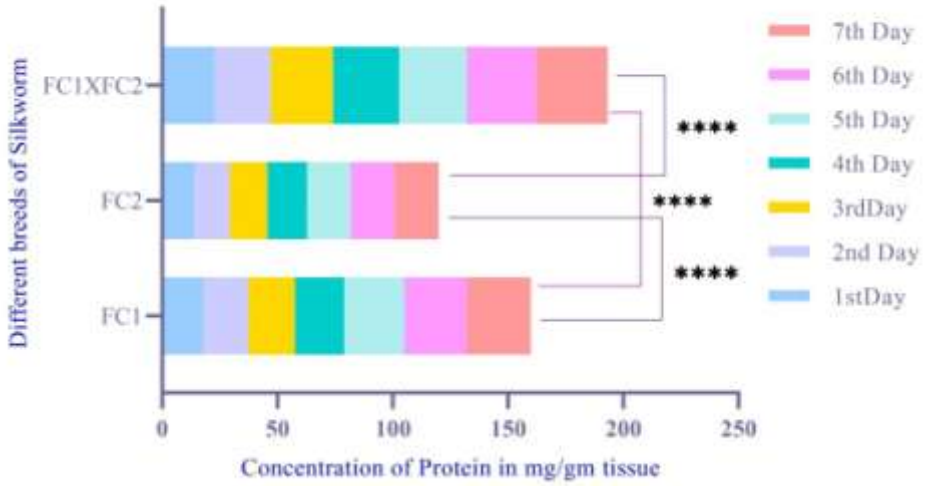

Fig 6:- Concentration of protein in Silkgland of FC_1, FC_2 and FC_1× FC_2 silkworm during fifth instar.

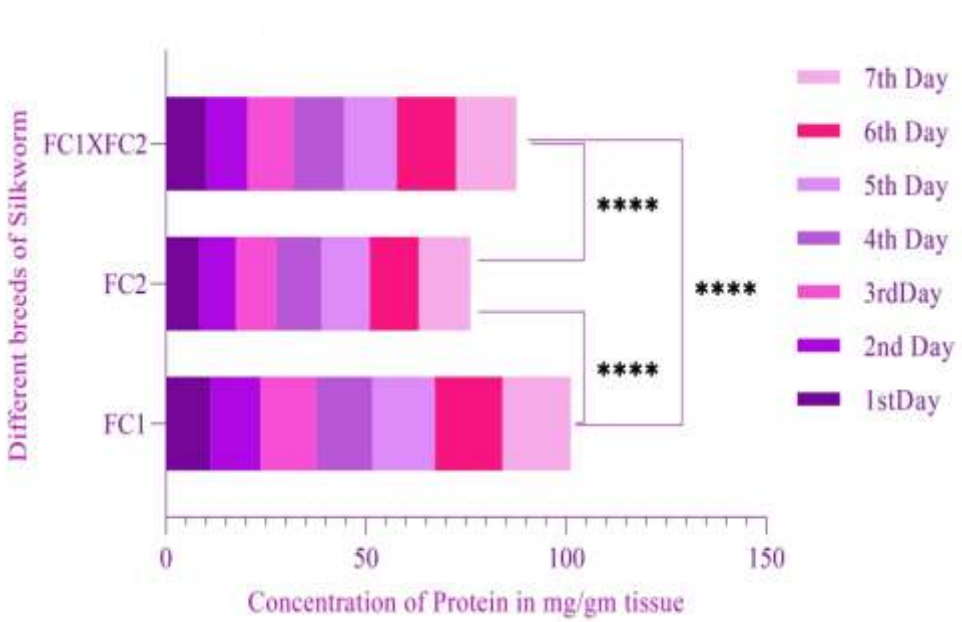

Fig 7:- Concentration of protein in fatbody of FC_1, FC_2 and FC_1× FC_2 silkworm during fifth instar.

Discussion:-

Sericulture depends on a successful raring of best quality cocoon crop throughout the year besides the breed of silkworm which are tolerate to temperature and diseases. Quantitative and qualitative protein plays a critical role in influence the growth and development of *B.mori*(Eman M. Hassan, 2020). So, in the present study we analysed the total protein content in haemolymph, midgut, silk gland, and fat body in three selected silkworm breeds, in order to find out better silkworm breed for silk production. The result showed that variation in protein content amonghaemolymph, midgut, silk gland and fat bodyand significant higher protein content was recorded in silkgland ($p<0.001$) followed by haemolymph ($p<0.005$) and in midgut and fatbody ($p<0.05$) in three selected silkworm races. Similar findings were observed by (Yoganandamurthyet al., 2015) in CSR_2, CB race and PM race, the quantitatively total protein of haemolymph and silkgland showed variations during larval development and maximum was on last day of 5^{th} instar and minimum on 1^{st} day after fourth moult. (Maheshaet al., 2015) also observed that protein content in the haemolymph,midgut and fatbody of different breeds of silkwormviz, PM × CSR_2, CSR_2, Nistari×NB_4D_2,

NB_4D_2, Nistari and PM races in which protein content was comparatively less with our obtained results. Haemolymph protein plays an important role in insects for transportation as well as for their enzyme action (SudhakarRaoet al., 2011). The increase in the protein concentration of Haemolymph in silkworm may be due to induced cellular and humoral response to undergo microbial immunization(AswarthaHarinathReddy and BadaVenkatappa, 2016).

Midgut protein involved in carbohydrates metabolism and also many other enzymes like phosphopyruvatehydrate which is responsible for catalysis of 2- phosphoglycerate (2-P) to phosphoenolpyruate (PEP) in the glycolytic pathway (Mitsuharaet al.,2014 and Parafaithet al.,2016). Silkworm larvae mainly in midgut protein required for growth, development and midgut digestive enzymes play vital role in digestion absorption of nutrients (HuiWang et al.,2019)Silkgland protein of silkworm plays an important role in formation of silkfilaments mainly two silk proteins (Fibroin and Sericin) and many other proteins are involved in cellular activity, diseases resistance, microfilament formation, protease activity and heat shock mechanism(Thulasi and Sivaprasad,2015). The increase in total proteincontent fromhaemolymph, midgut to convert protein in to silk protein for the formation of silk filaments. Fatbody acts as the storage organ for a variety of protein and high rate of protein synthesis was reported in fat body metabolism(Thangapandiyan andDharanipriya R,2019). This indicates that the Bivoltine single hybrid and double hybrid breed may be the highest protein in silkgland,haemolymph,midgut and fatbody. In the present study it helps us to understand the protein content in silkgland,haemolymph, midgut and fatbody of selected silkworm races.

Conclusion:-

The present study clearly concludes that protein content was more in the silkgland, followed by haemolymph, midgut and fatbodyof selected silkworm races respectively. In which increased protein content in silkgland, haemolymph, midgut and fatbody of bivoltine double hybrid FC_1X FC_2 have favourable influence on growth of silkworm and also increasing the quantity of silk production. So, bivoltine double hybrid could be advised to the farmers for quality cocoon production and silk production.

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