Estimation of available protein content in different tissues of mulberry silkworm larva(*Bombyxmori*L.)

Abstract:

Mulberrysilkworm*Bombyxmori*L.istheonlysilkwormhavingmonophagousfeedinghabit with mulberry as the sole food material. Silk gland in silkworm body accounts for 60%ofthebodyweightduring5th instarandactsasabioreactorthatconvertsmulberryleafproteininto silk protein composed of sericin and fibroin. The biochemical analysis for detecting totalavailable protein content in different body tissues of the silkworm larvae like silk gland,haemolymphandwholelarvalbodyof5th instarlarvaewasanalysedandvaluesrecordedweref ound to vary considerably from one another as 69.2 and 76.5% in silk gland, 51.9 and 47.6%in haemolymph and 50% in whole larval body. In case of silkworm hybrid FC1xFC2, thestudied larval body sample revealed the optical density (OD) value in the range of 260nm,270nm and 180nm for larval body, haemolymph and silk gland sample respectively depictingthe presence of fairly pure proteins in sufficient amount in the studied sample. In case ofsilkwormhybridFC2xFC1,thestudiedlarvalbodysamplerevealedthe ODvalueintherangeof 197nm, 124nm and 199nm for larval body, haemolymph and silk gland depicting thepresence of fairly pure proteins. For estimation of total protein content in the form of cocoon,theweightswererecordedforthesinglecocoonandshellweight.Themaximumsinglecocoo nweightwasrecordedas1.46gforFC1xFC2and1.30gforFC2xFC1,cocoonweightwithflosswas recorded as 1.19g and 1.38g, deflossed cocoon as 1.40g and 1.08g, floss weight as 0.32gand0.46g,pupalweightas1.55gand1.27g,cocoonshellweightas0.78gand2.46g,100 %pupalpercentage,drycocoonweightas0.55gand0.44g,cocoonshellratioas53.42%and 35.38 %for FC1xFC2 and FC2 xFC1 respectively. The current observations on biochemicalprofiling could be utilized for future breeding prospects for development of region and seasonspecifichybrids with desirable metrictraits.

Comment [a1]: Disconnected flow of sentence from earlier sentence.

Comment [a2]: The whole abstract needs a redraft and should be precise and compact depicting the outcome of the study rather than the analytical results.

Keywords:silkworm,silk,biochemical,haemolymph,protein,cocoon,hybrid.

Introduction:

Sericulture is one of the most important economic sectors of the agriculture field.It involvesa continuous series of integral activities including moriculture (cultivation of host plant i.e.Mulberry), rearing of lepidopteron larvae i.e. silkworm *Bombyx mori L*. and various industrialaspects(manufacturingoffabricfromfibres).Silkwormbeingmonophagousinsectfeedsonlyon mulberry leaf because of the presence of morin pigment in it (Babu *et al*., 2009). Underdifferent environment, feeding and nutritional conditions and with ingestion of the samenumber of mulberry leaves, the silkworm shows significant difference in its ability to digest,absorbandconvertfoodtobodymatter.Hence,influenceofseason,temperature,humidityonfood intake, assimilation and conversion efficiency of the *Bombyx mori* is significant (Etebari&Matindoost2004).Ontheotherhand,environmentalfactorsarealsoresponsibleforregulationofphysiologyandmetabolismofsilkworm(Kerenhap*etal*.,2005andGanie2022).This in turn triggers the synthesis and accumulation of two invariably important proteinsnamely sericin and fibroin making a single unit of silk fibrils known as silk protein (Chen *etal*.,2022).

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Silk protein is the ultimate product forming the protective covering around the larvae,known as cocoon which provides shelter to the metamorphosizing pupa and is reeled inindustrial processes to extract silk. The quantity and quality of silk content present in thecocoon shell decides the market value of silk. Silk gland which is predominantly divided intofore, mid and posterior silk gland actually hosts the site of silk synthesis, which is the posteriorpart of the silk gland. Whereas, middle region is the region of storage and accumulation andanteriorpartisforexclusionthroughwhichlarvaeexpelsoutsilkfromspinneretandformsilkfilament that solidified after coming in contact with air. Therefore, in order to analyses thecontent of silk protein present in different tissues of silkworm body an attempt has been madetoestimatetheavailableproteincontentinsilkwormsilkgland,haemolymphandwholelarvalbody.

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Materialsandmethods:

Thecurrentexperimentwascarriedoutbyusingrulinghybridsof JammuandKashmirnamely ; FC1 x FC2 and its reciprocal cross i.e. FC2 x FC1. For the study different tissues ofsilkwormlarvalbodywereutilizedincluding~~silkgland~~silk gland,haemolymphandwholelarvalbodyof5thinstarlarvaefoeestimationofavailableproteincontent~~Proteincontent~~(%)byusingslightlymodifiedLowery*et al*., (1951)method.

PROTOCOL:

1. 0.2mlofBSAworkingstandardin5testtubesandmakeupto1mlusingdistilledwater.
2. Thetest tubewith 1mldistilled waterservesasblank.
3. Add4.5ml ofreagentIandincubate for10minutes.
4. Afterincubationadd0.5mlof reagentIIandincubatefor30minutes.
5. Measuresthe absorbance at660nmandplotthestandardgraph.
6. Estimatetheamountofproteinpresentinthegivensamplefromthestandardgraph.Inaddition toproteincontent,followingcocooncharacterswerealsostudiedduringtheexperiment;
    a) Green cocoonweight.
    b) Cocoonwithfloss.
    c) Deflossedcocoon.
    d) Drycocoon weight.
    e) Shellweight.
    f) Pupalweight.
    g) Cocoonshellratio.
    h) Cocoongrains.
    i) Cocooncompactness.
    j) Cocoonsize.
    k) Pupalpercentage.

SATISTICALANALYSIS

TherawgeneratedfromthecurrentexperimentbyCRDdesignhavebeenpooledandsubjectedtoAnalysisofVariance(ANOVA)onSPSSsoftwareVersion;2021,todeterminethesignificantvalues fortheselected parameters.

Results:

Proteincontent(%):

ForFC1 x FC2 silkworm hybrid:

a) Wholelarvalbodysample:

Totalproteincontent (%)insilkwormbodyof5thinstarlarvaewasdetectedonphoto-calorimeter at an optical density (OD) value of 660nm. The studied larval body samplerevealedtheODvalueintherangeof180nmwhichdepictedthepresenceoffairlypure

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proteinsinsufficientamountinthestudiedsample(i.e.,50%sericinand50%fibroin),withthenoticeable increaseinthetotal protein contentwith every subsequent day.

b) Haemolymph:

Totalproteincontentpercentageinsilkwormhaemolymph(5th instarlarvae)wasdetectedon photo-calorimeter at an optical density (OD) value of 660 nm. The studied haemolymphsamplerevealedtheODvalueintherangeof260nmwhichdepictedthepresenceoffairlypureproteins in sufficient amount in the studied sample i.e., 51.9 %of protein content in silkwormhaemolymph,with considerable increaseafter each day.

c) ~~Silkgland~~Silk gland:

Total protein content percentage in Silk gland of the silkworm (5th instar larvae) wasdetermined on photo-calorimeter at an optical density (OD) value of 660nm. The studied silkgland sample revealed the OD value in the range of 270nm which depicted the presence offairly pure proteins in sufficient amount in the studied sample i.e.89.2 %of protein content insilk gland of silkworm. Similar to whole larval body and haemolymph a remarkable increasehadbeen noticed with each pairing during in silk glandalso.

II. ForFC2xFC1 silkworm hybrid

a) Wholelarvalbodysample:

The studied larval body sample revealed the OD value in the range of 197nm whichdepictedthepresenceoffairlypureproteinsinsufficientamountinthestudiedsample(i.e., 75.7 %protein content in silkworm larvae). Maximum OD value was recorded in silkwormlarvaeof 5th day.

a)Haemolymph:

Total protein content percentage in silkworm haemolymph (5th instar larvae) wasdetectedonphoto-calorimeteratanopticaldensity(OD)valueof660nm.Thestudiedhaemolymphsamplerevealedthe ODvalueintherangeof154nmwhichdepictedthepresenceof fairly pure proteins in sufficient amount in the studied sample i.e., 57.6 ~~percent~~ %of proteincontentin silkworm haemolymph.

a)Silkgland:

Total protein content percentage in Silk gland of the silkworm (5th instar larvae) wasdetermined on photo-calorimeter at an optical density (OD) value of 660nm.The studied silkgland sample revealed the OD value of 199nm which depicted the presence of fairly pureproteinsinsufficientamountinthestudiedsamplei.e.76.5%proteincontentinsilkglandof

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silkworm. Maximum and minimum values are recorded in case of 5th and 1st day of larvalsamplerespectively (Table-2).

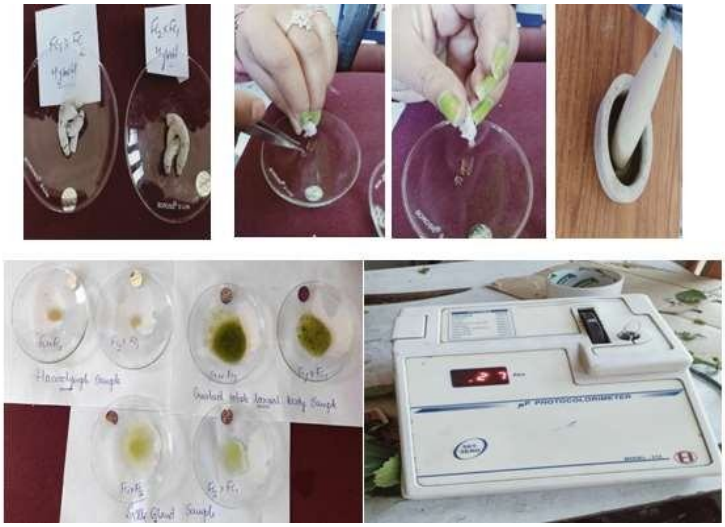

Plate-01:BiochemicalprofilingofSilkwormlarvae.

Table-01: Values depicting Percentage of Protein content on the basis of OD values instudied samples of whole larval body, haemolymph and silk gland of silkworm hybridsviz.,FC1 x FC2 andFC2 x FC1

Particulars	FC1xFC2		FC2xFC1	
	ODValue (nm)	%	ODValue (nm)	%
Wholelarvaebody	180nm	50%	197nm	75.7%
Haemolymph	260nm	51.9%	154nm	57.6%
Silkgland	270nm	89.2%	199nm	76.5%

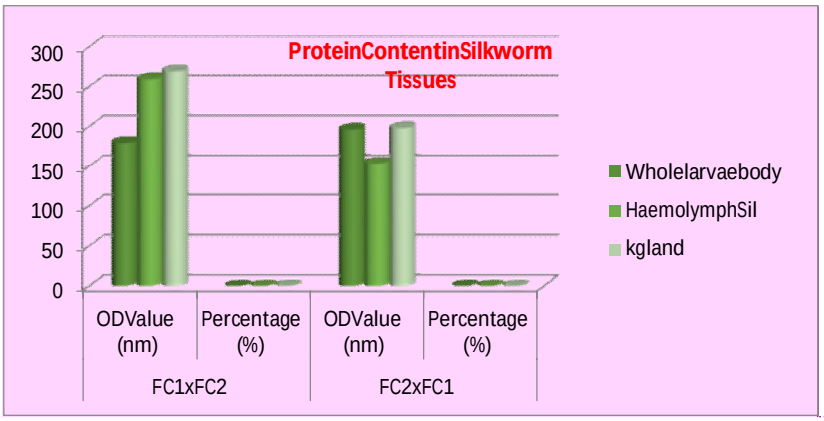

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Fig-01:Protein content insilkworm tissues.

Someoftheimportantmorphologicalcharactersofcocoonsrecordedincludes:

a) Greencocoonweight

Thecocoonharvestedon6th dayofspinning(FC1xFC2andFC2xFC1)wererecordedto possess (1.46gm,1.30gm) of single and (14.61gm ,13.36 gm) for ten cocoons forgreencocoon weight.

b) Cocoonwithfloss

The same cocoons harvested from FC1 x FC2 and FC2 x FC1 was determined toevaluate cocoon weight with floss and recorded to possess (1.38gm, 1.19gm) of singleand(15.54gm,13.32gm)fortencocoonsincaseofFC1xFC2andFC2xFC1respectivel y.

c) Deflossedcocoon

The cocoons observed on the 6th day of spinning (FC1 x FC2 and FC2 x FC1) weresubjectedtodeflossingbymanualdeflossingmethodwithhelpofwoollenstickandthest udied hybrids were recorded to possess (1.40gm, 1.08gm) of single cocoon and(14.98gm,10.81gm)of10cocoonsincaseofFC1xFC2andFC2xFC1,respectively.

d) Drycocoonweight

Dryweightofsinglecocoonwasobservedas(0.55gm,0.44gm)and(5.94gm,4.44gm)for10 cocoons on the6thday of spinning (FC1x FC2 and FC2x FC1).

e) Shellweight

Shell weight of the single cocoon and 10 cocoons was recorded as (0.78gm, 046gm)and(7.89gm,4.63gm)onthe6th dayspinning(FC1xFC2andFC2xFC1),respectively.

f) Pupalweight

Thecocoonharvestedon6th dayofspinning(FC1xFC2andFC2xFC1)wereevaluatedfor determination of pupal weight and values depicting (1.55gm, 1.27 gm) of pupalweightforsinglecocoonand(15.92gm, 12.73gm) fortencocoons, respectively.

g) Cocoonshellratio

Thecocoonshell ratiowascalculated bytheformula

$$=\frac{\text{WeightofthecocoonshellW}}{\text{eightof cocoon}} \text{X100}$$

And the results showed higher cocoon shell ratio for the hybrid FC1xFC2 as (53.42%,35.38%)as to that ofFC2xFC1as (54.41%, 36.41%).

h) Cocoongrains

Thecocoonharvestedon6th dayofspinning(FC1xFC2andFC2xFC1)wereobservedto possess as deep and coarse grains, depicting the comparatively good quality ofcocoons.

i) Cocooncompactness

The same cocoon was observed as hard and compact in texture showing the superiorqualityof the cocoons onthe basis of visualexamination descriptor.

j) Cocoonsize

For both the hybrids the cocoons were recorded to possess oval shaped cocoon withslightconstriction at thecentre as shown in theplate.

k) Pupalpercentage

Pupal percentage were determined after stifling process and the studied hybrid wererecorded to possess high pupal percentage with values as (100%, 100%) and (100%,90%)forFC1xFC2 andFC2xFC1respectively.

Discussion:

Theproteincontentwasdeterminedbythepresenceoffairlypureproteinsinsufficientamountin the studied silkworm larval body sample in case of FC1xFC2 hybrid i.e**.,** 50% sericin and50% fibroin, with the noticeable increase in the total protein content with every subsequentday,51.9%ofproteincontentinsilkwormhaemolymph,withconsiderable increaseaftereachday and 69.2% of protein content in silk gland of silkworm. Similar to whole larval body andhaemolymph a remarkable increase had been noticed with each passing during in silk glandtoo.IncaseofFC2xFC1hybrid,75.7%proteincontentinsilkwormlarvaemaximumODvalue was recorded in silkworm larvae of 5th day, 47.6 ~~percent~~ % of protein content in silkwormhaemolymphand76.5%proteincontentinsilkglandofsilkworm.Maximumandminimum

values are recorded in case of 5th and 1st day of larval sample respectively which shows closeconformitywiththatoftheDeviandChapman(2013),Zhou*etal.*,(2015)andRamchandra*etal.*, (2020) observed total proteins in silk gland decreased in early stages and increased in laterstagesofsilkwormlarvaldevelopment.Monda*etal.*,(2011)revealedthatproteinconcentration wasfoundtobeinhighconcentrationsinfullymaturedsilkglandofthe5thinstarlarvaeandReddy*etal.*, (2015)whoreportedmaximumweightsandlengthsofsilkglandin7thday of 5th instar of larval period. Subramaniam *et al.*, (2011), Sunderraj *et al.*, (2015), Sharma*etal.*,(2020)andViswansath.S.(2018),suggestedincreaseinproteincontentinsilkglandand hemolymph of the 5th instar silkworm larvae at an exponential rate thus confirming the resultsofpresent investigation.

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Morphological characters of cocoon are predominant descriptors for assessment ofbreeds for distinctiveness, uniformity and stability; but are always influenced by prevailingenvironment. In present study, the morphological characters were recorded wide phenotypicvariation in cocoon colour, cocoon shape, cocoon shell weight, cocoon grains, cocoon shellratio, pupal percentage and pupal weight. The observations recorded are in line with earlierworkers, Rangeaswami and Govinda (2017) who reported the cocoon shape as an extremelyrelevant variable in commercialization, since automated wiring admits only elliptical cocoonsintothemachines.However,thebreedsevaluatedinthepresentstudywererecordedwithoval,elongated with fair constriction and coarser constricted presenting the superiority of thecocoons. Mallikarjunappa and Etebari (2019) observed that cocoon weight, shell ratio andfilament length are highly heritable traits determining the quality, quantity and efficiency ofthe reeling. Present findings are also in conformity with the report of Singh *et al.*, (2015) whoconcluded that environmental factors influence the physiology of the insect and also havedeleterious effect on the economic traits. Cocoon shell weight is an important character indeterminingthesilkcontent.Inpresentstudy,highlysignificantandpositivecorrelationswererecorded between single cocoon weight and 5thday larva weight (0.864g) followed by singleshell weight and 5thday larval weight (0.816g), single shell weight and single cocoon weight(0.775g), malformed cocoon and 5th instar duration (0.730g), single shell weight and pupationrate (0.624g), total larval duration and 5th instar duration (0.559g), single cocoon weight andhatching percentage (0.551g), larval weight 5th day and hatching percentage (0.545g), singlecocoon weight and pupation rate (0.536g), pupation rate and larval weight 5th day (0.524g),single shell weight (0.492g) and silk percent and single shell weight (0.470g). These findingsare in accordance with the results of Chakaraborty *et al.*, (2020), Murthy. Y.N.V. (2015),Narayanan*etal.*,(2020)andSaratchandra*etal.*,(2011).Whereas,Trivedy*etal.*,(2020)

reported that the cocoon weight differs significantly when silkworms were fed with leavesharvestedfromdifferenttreemulberrygenotypes.Sharma*etal*.,(2017)andSudoandChunking (2016) also reported highest values of single cocoon weight 1.28±0.04g whensilkworms were fed with mulberry leave harvested from tall trees. The present results areconformitywiththefindingsofWangandGilbert (2011),whorecordedthemaximumcocoonweight as 1.74±0.03g and shell weight 0.39 ± 0.01g when silkworms were grown on leavesharvestedfromtreetypeofmulberryplantation.Thepresentresultsareonparwiththeresultsof earlier workers, Radhakrishna *et al.,* (2017), ~~and~~ Rahmathulla and Suresh (2012), recordedthe maximum pupal weight 1.36 ± 0.02g when silkworms were reared on quality mulberryleaves possessing highest mean cocoon shell ratio (17.57%). The results of the present studyrevealed conformity with the reports cited by Chakraborty *et al.,* (2020), Fang *et al.,* (2020)andKaviraj*etal*.,(2021)withthecocoonshellratioandshellpercentageas(53.42%,35.38%)as to that of FC2xFC1 as (54.41%, 36.41%) and (0.78gm, 046gm) and (7.89gm, 4.63gm),FC1xFC2andFC2xFC1,respectively.Therefore,currentinvestigationrevealedsignificantandsatisfactoryresultswith greatvalidation onthebasisofearlierreportsofvariousworkers.

Conclusions:

Silk gland in silkworm body accounts for 60% of the body weight during 5^{th} instar and acts asa bioreactor that converts mulberry leaf protein into silk protein composed of sericin andfibroin.Thebiochemicalanalysisfordetectingtotalavailableproteincontentindifferentbodytissues of the silkworm larvae like silk gland, haemolymph and whole larval body of 5^{th} instarlarvaewasanalysedandvaluesrecordedwerefoundtovaryconsiderablyfromoneanotheras 69.2 and 76.5% in silk gland, 51.9 and 47.6% in haemolymph and 50% in whole larval body.In case of silkworm hybrid FC1xFC2, the studied larval body sample revealed the opticaldensity (OD) value in the range of 260nm, 270nm and 180nm for larval body, haemolymphand silk gland sample respectively depicting the presence of fairly pure proteins in sufficientamount in the studied sample. In case of silkworm hybrid FC2xFC1, the studied larval bodysample revealed the OD value in the range of 197nm, 124nm and 199nm for larval body,haemolymph and silk gland depicting the presence of fairly pure proteins. For estimation oftotal protein content in the form of cocoon, the weights were recorded for the single cocoonand shell weight. The maximum values of all the studied parameters were recorded as high incase of hybrid FC2xFC1 as compared to its reciprocal one. This short experiment could beutilized to extend further biochemical profiling of different breeds for quantification of all thebiochemicaland metrictraits of silkworm.

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