International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 23-10-2024, Accepted: 22-11-2024, Published: 07-12-2024
Volume 9, Issue 12, 2024, Page No. 34-39

Comparative study on protein content and protease activity in (Pure Mysore & FC_1 x FC_2) races of silkworm *Bombyx mori* L. treated with chia seeds

Ameena Misba BA[1], Asiya Nuzhat F B[2*]
[1] Research Scholar, Department of Studies and Research in Zoology, Tumkur University, Tumakuru, Karnataka, India
[2] Associate Professor, Department of Studies and Research in Zoology, Tumkur University, Tumakuru, Karnataka, India

Abstract
Silk is called the queen of fibres due to its economic importance. Silk production involves rearing of silkworm by feeding mulberry leaves *Morus alba* which converts leaf protein into silk. The present study aims on the supplementary effect of plant protein chia seeds fortified with mulberry leaves of different concentrations (1, 1.5 and 2%w/v) on protein content and protease enzyme activity in midgut of two races of silkworm *Bombyx mori* L. multivoltine Pure Mysore (PM) and bivoltine double hybrid FC_1 X FC_2 race along with control. The chia seeds supplement were fed to silkworm from hatching to pupation at 25±2ºC with relative humidity of 75±5%. The protein content and protease enzyme activity in midgut was estimated in fifth instar from day 1st to till pupation in both the races. Results showed that the total protein content and protease activity in the larva of midgut was significantly high in silkworm reared with mulberry leaves fortified with the protein supplement chia seeds. As well, silkworm larvae of the bivoltine double hybrid race exhibited more positive response to supplementary protein than compared to multivoltine race.

Keywords: *Bombyx mori* L., protein, protease, midgut, chia seeds

Introduction
Sericulture has originated from two French words, serios meaning silk and culture means rearing. Sericulture is rearing of silkworms to obtain raw silk. Globally India is the second largest producer of silk. The leaf of mulberry plant is the sole source of food for Silkworm *Bombyx mori* L. Silkworm larva obtains different amino acids from the mulberry leaves which are highly essential to synthesize silk proteins secreted during spinning. Proteins and proteases in the midgut of larva exhibit an important physiological role in growth and development of silkworm and synthesis of silk proteins (Murthy, 2015) [15]. Chia seeds (*Salvia hispanica* L.) have become one of the world's most recognizable foods based on their nutritional properties and medicinal values (Ullah *et alf*., 2016; Das, A. 2018; Silva *et al*., 2016) [3, 24, 26]. It is a plant-based food source rich in protein and essential amino acids used by humans as a healthy diet. It has higher proportion of α-linolenic acid and a good source of Omega-3 fatty (about 65 % of the oil content). The chemical composition of chia seeds contains about (15–25%) of proteins, vitamins, minerals, and antioxidants (wet basis).

The insect gut is the principle site for secretion of digestive enzymes, digestion of food and absorption of nutrients (Pauchet *et al*., 2008) [21]. Protein is necessary for various biological activities during development, metamorphosis and maintenance of various physiological functions in different tissues (Kumar *et al.*, 2011; Murthy *et al*., 2014) [11, 16]. Almost 70% of the silk protein is found to be obtained from the leaves of mulberry, which is rich protein content. The digestive enzymes of silkworm midgut have been studied in detail by various scientists (Kanekatsu, 1972; Eguchi *et al.*, 1976; Sumida *et al.*, 1990; Nijagal & Kumara, 2017) [4, 9, 19, 25]. Among many digestive enzymes, protease enzyme plays an important role in converting the mulberry leaf protein to silk protein. The activity of protease enzyme varies from breed to breed and also between hybrids of silkworm races (Narayanan *et al*., 1967 & Kumar and Kalpana, 2009 and Hassan, 2020) [5, 12, 18]. The enzyme protease was found to be high during larval stage of silkworm (Kumari *et al.*, 1997; Nijagal, & Kumara, 2017) [13, 19]. Keeping this in view, the present experiment was carried to evaluate the effect of chia seeds supplement on protein concentration and protease enzyme activity during silkworm larval development in midgut of silkworm Pure Mysore and FC_1 X FC_2 race at 25±2ºC with relative humidity of 75±5%.

Materials and methods
Silkworm *Bombyx mori* L.
Mulberry silkworm *Bombyx mori* L. eggs of two breeds; Pure Mysore (local race) and FC_1 X FC_2 (hybrid race) were procured from chawki rearing center Maralur, Department of Sericulture, Tumakuru, Karnataka.

Protein source
Chia seeds (*Salvia hispanica* L.) were obtained from the market by Neuherbs brand (GLOBAL HEALTHFIT RETAIL (INDIA) LLP). Chia seeds were ground using an electric grinder to obtain the grain powder and dissolved in 100ml of distilled water to prepare the different concentrations (1, 1.5 and 2% w/v) as a feed to silkworm along with mulberry leaves from I to V instar.

Silkworm rearing technique
The disease free laying of Pure Mysore and double hybrid FC_1 X FC_2 race of silkworm, *Bombyx mori* L. eggs were obtained from Sericulture model grainage, Maralur Dinne, Tumakuru. After a period of 10 days freshly hatched larvae were transferred to sterilized enamel trays (36 × 26 × 4 cm) covered with paraffin paper to prevent loss of water from the leaf bed. The experiment was conducted with two

different silkworm races larvae from I to V instar were reared under standard rearing conditions on V_1 mulberry variety leaves with the different concentrations of chia at room temperature of 25±2ºC with a relative humidity of 75±5%, continued till pupation, arranged in triplicate. Appropriate rearing technique and proper disinfection was adopted according to the rearing technique of Krishnaswami (1978) [10]. Necessary precautions were taken during rearing to avoid diseases and contamination. The healthy larvae in the rearing trays were enumerated and the larvae which showed delayed moulting were removed periodically.

Collection of midgut tissue

Midgut tissue was excised by cutting larval skin dorsally in a dissection tray containing ice cold solution of PBS buffer Solution (pH 7.4). The tissue sample was collected during each day in 5th instar at an interval of 24hrs till spinning. Midgut was collected by separating anterior and posterior part of the gut and transferred to a pre-cooled plastic vial and used as sample for the assay.

Estimation of Protease enzyme activity in midgut of silkworm *B. mori* L.

The activity of protease enzyme was determined by adopting the method of (Ishaaya and Swiriski, 1970) [7]. The larvae from each treated group along with control were selected on each day of V instar till pupation. The midgut was dissected out and homogenized with 0.7% NaCl. The supernatant was taken as enzyme extract obtained by centrifugation of homogenate. 1ml of Casein was used as substrate to determine activity of protease enzyme along with 0.5ml of enzyme extract followed by 1ml of buffer and the reaction mixture was heated for 30 min in water bath. The reaction was incubated for 30min and 2.5ml of DNSA was added. The DNSA reagent was prepared for the estimation as described by (Noelting and Benfeld, 1948) [20]. Further the reaction mixture was heated for 5 min and cooled immediately. After cooling 2.5ml of distilled water was added and mixed in the test tube and reading was measured in Spectrophotometer at 660nm. The protease enzyme activity in midgut tissue of silkworm was expressed in terms of µg tyrosine/ml/g tissue.

Estimation of total protein in midgut of silkworm *B. mori* L.

The total protein content present in the midgut of silkworm treated with different concentrations of fortified mulberry with chia seeds was estimated according to the method of (Lowry *et al*., 1951) [14] using Bovine serum albumin (BSA) as standard for protein estimation.

Biostatical analysis

The values are expressed as mean ± standard deviation mean of each stage. The statistical data was analyzed using one way ANOVA to know significance differences between silkworm breeds in MS Excel.

Results

Total protein concentration in midgut of 5th instar silkworm

In multivoltine silkworms PM race, protein concentration was minimum on 1st day (21.31 µg/ml/g) and gradually increased on each day of 5th instar till pupation and reached peak on 6th day (65.24 µg/ml/g) and decreased to (59.08 µg/ml/g) on 7th day at control. In Bivoltine silkworm FC_1 X FC_2 race larvae the protein concentration was minimum on 1st day (24.20 µg/ml/g) and maximum on 6th day (86.92 µg/ml/g) and decreased on 7th day (80.11 µg/ml/g). In treated batch the protein concentration was found to be increased with increase in chia seed concentrations (1, 1.5 and 2%). Among that the maximum protein concentration in the midgut was found in the 2% concentration of chia seeds treated larvae in both multivoltine and bivoltine races respectively.

In 2% chia seeds treated group of multivoltine race the total protein content in midgut was found to be minimum (28.03 µg/ml/g) on 1st day and gradually increased till 6th day (35.81 µg/ml/g, 43.63 µg/ml/g, 62.52 µg/ml/g, 79.12 µg/ml/g and 84.78 µg/ml/g on 2nd day, 3rd day, 4th day, 5th day and 6th day respectively). Then decreased gradually on 7th day (78.12 µg/ml/g) as the larvae proceeds for pupation (Table 1 and Fig. 1).

Similar findings were found in 2% chia seeds treated group of bivoltine race of silkworm, the protein concentration was found to be minimum (33.86µg/ml/g) on 1st day and gradually increased till 6th day (53.77 µg/ml/g, 59.12 µg/ml/g, 79.14 µg/ml/g, 116.85 µg/ml/g and 125.13µg/ml/g on 2nd day, 3rd day, 4th day, 5th day and 6th day respectively). Then decreased gradually on 7th day (115.85 µg/ml/g) as the larvae proceeds for pupation (Table 2 and Fig. 2). Bivoltine silkworms recorded maximum protein concentration compared to multivoltine silkworms during 5th instar.

Protease enzyme activity in midgut of 5th instar silkworm

According to the data attained in Table (3 and 4), a highly significant increase in protease enzyme activity of silkworm larvae was found in Bivoltine silkworm race than compared to multivoltine race. All the concentration of chia seeds (1, 1.5 and 2% w/v) treated larvae increased the enzyme activity significantly but maximum was found in larvae reared on 2% of chia seeds in both multivoltine and bivoltine races of silkworms.

In multivoltine PM race the protease enzyme activity at 2% chia seeds treated group was found to be minimum (94.13 µg/ml/g) on 1st day and gradually increased till 6th day (132.21 µg/ml/g, 182.56 µg/ml/g, 275.41µg/ml/g, 315.13 µg/ml/g and 343.2µg/ml/g on 2nd day, 3rd day, 4th day, 5th day and 6th day respectively). Then decreased gradually on 7th day (301.4 µg/ml/g) as the larvae proceeds for pupation (Table 3 and Fig. 3).

In bivoltine FC_1 X FC_2 race similar results were found at 2% chia seeds treated larvae the protease enzyme activity was found to be minimum (355.12 µg/ml/g) on 1st day and gradually increased till 6th day (457.25 µg/ml/g, 488.77 µg/ml/g, 567.85 µg/ml/g, 624.12 µg/ml/g and 643.2 µg/ml/g on 2nd day, 3rd day, 4th day, 5th day and 6th day respectively). Then decreased gradually on 7th day (588.20 µg/ml/g) as the larvae proceeds for pupation (Table 4 and Fig. 4). According to the results obtained compared to multivoltine race the highest protease enzyme activity was recorded in bivoltine race.

Table 1: Effect of prebiotic supplement chia seeds on total protein content in midgut of 5th instar silkworm *B. mori* in multivoltine Pure Mysore (PM) race fed *ad libitum Morus alba* at 25±2ºC and 75±5% R.H. The values are expressed in µg BSA/ml/g tissue

Total Protein content in midgut (µg BSA/ml/g tissue)	Days	Control	1% conc. of Chia seeds	1.50% conc. of Chia seeds	2% conc. of Chia seeds
PM race	1st day	21.31 ± 0.14	24.33 ± 0.25	26.45 ± 0.20	28.03 ± 0.18
	2nd day	29.94 ± 0.52	31.25 ± 0.31	33.20 ± 0.45	35.81 ± 0.54
	3rd day	35.32 ± 0.31	38.12 ± 0.38	43.02 ± 0.57	48.63 ± 0.41
	4th day	46.76 ± 0.12	49.63 ± 0.65	61.84 ± 0.59	62.52 ± 0.55
	5th day	61.38 ± 0.31	66.28 ± 0.11	71.80 ± 0.25	79.12 ± 0.27
	6th day	65.24 ± 0.17	74.23 ± 0.11	76.14 ± 0.14	84.78 ± 0.12
	7th day	59.08 ± 0.02	71.44 ± 0.19	74.21 ± 0.24	78.12 ± 0.28

Table 2: Effect of prebiotic supplement chia seeds on total protein content in midgut of 5th instar silkworm *B. mori* in bivoltine double hybrid (FC_1 x FC_2) race fed *ad libitum Morus alba* at 25±2ºC and 75±5% R.H. The values are expressed in µg BSA/ml/g tissue

Total Protein content in midgut (µg BSA/ml/g tissue)	Days	Control	1% conc. of Chia seeds	1.50% conc. of Chia seeds	2% conc. of Chia seeds
FC_1 x FC_2 race	1st day	24.20 ± 0.28	28.81 ± 0.25	30.23 ± 0.33	33.86 ± 0.18
	2nd day	36.23 ± 0.41	44.23 ± 0.28	49.21 ± 0.19	53.77 ± 0.15
	3rd day	42.52 ± 0.31	53.21 ± 0.29	55.23 ± 0.14	59.12 ± 0.22
	4th day	58.26 ± 0.16	71.74 ± 0.54	74.24 ± 0.45	79.14 ± 0.35
	5th day	82.11 ± 0.41	98.21 ± 0.17	105.45 ± 0.37	116.85 ± 0.12
	6th day	86.92 ± 0.19	106.12 ± 0.55	117.02 ± 0.41	125.13 ± 0.19
	7th day	80.11 ± 0.44	102.21 ± 0.75	113.45 ± 0.64	115.85 ± 0.23

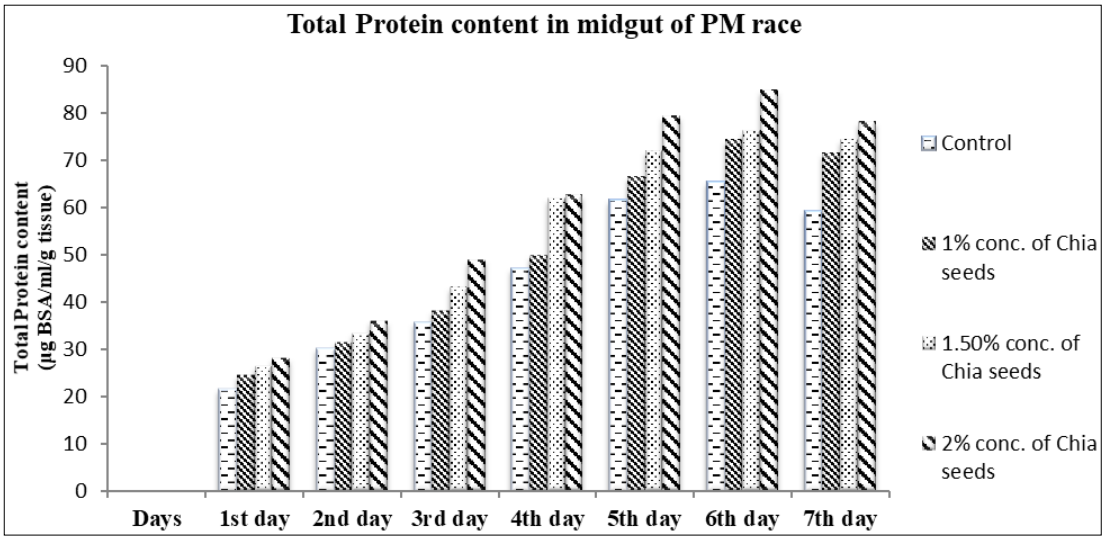

Fig 1: Effect of prebiotic supplement chia seeds on total protein content in midgut of 5th instar silkworm *B. mori* in multivoltine Pure Mysore (PM) race

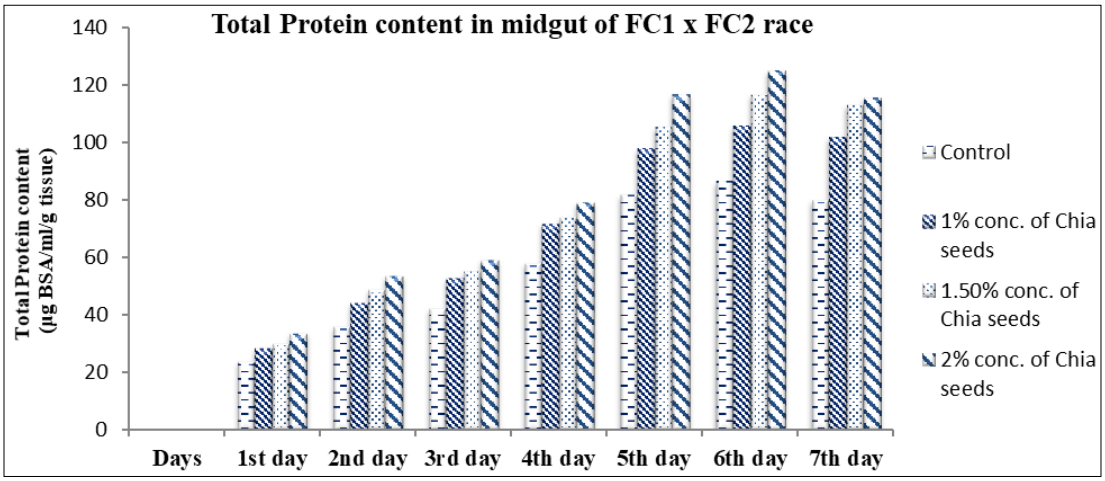

Fig 2: Effect of prebiotic supplement chia seeds on total protein content in midgut of 5th instar silkworm *B. mori* in bivoltine double hybrid (FC_1 x FC_2) race

Table 3: Effect of prebiotic supplement chia seeds on protease enzyme activity in midgut of 5th instar silkworm *B. mori* in multivoltine Pure Mysore (PM) race fed *ad libitum Morus alba* at 25±2ºC and 75±5% R.H. The values are expressed in µg tyrosine/ml/g tissue

Protease enzyme activity in midgut (µg tyrosine/ml/g tissue)	Days	Control	1% conc. of Chia seeds	1.50% conc. of Chia seeds	2% conc. of Chia seeds
PM race	1st day	81.14 ± 0.41	86.55 ± 0.65	88.02 ± 0.14	94.13 ± 0.56
	2nd day	95.23 ± 0.62	108.12 ± 0.23	115.2 ± 0.44	132.21 ± 0.71
	3rd day	106.01 ± 0.51	156.22 ± 0.16	165.4 ± 0.21	182.56 ± 0.48
	4th day	185.11 ± 0.32	205.18 ± 0.21	224.86 ± 0.28	275.41 ± 0.35
	5th day	235.12 ± 0.45	251.88 ± 0.24	284.14 ± 0.55	315.13 ± 0.49
	6th day	255.14 ± 0.69	285.6 ± 0.86	309.2 ± 0.75	343.2 ± 0.85
	7th day	215.03 ± 0.75	229.77 ± 0.69	278.58 ± 0.66	301.4 ± 0.92

Table 4: Effect of prebiotic supplement chia seeds on protease enzyme activity in midgut of 5th instar silkworm *B. mori* in bivoltine double hybrid (FC_1 x FC_2) race fed *ad libitum Morus alba* at 25±2ºC and 75±5% R.H. The values are expressed in µg tyrosine/ml/g tissue

Protease enzyme activity in midgut (µg tyrosine/ml/g tissue)	Days	Control	1% conc. of Chia seeds	1.50% conc. of Chia seeds	2% conc. of Chia seeds
FC_1 x FC_2 race	1st day	256.23 ± 0.61	316.21 ± 0.41	325.13 ± 0.52	355.12 ± 0.13
	2nd day	330.22 ± 0.49	401.14 ± 0.52	421.47 ± 0.46	457.25 ± 0.71
	3rd day	384.14 ± 0.33	463.10 ± 0.36	475.12 ± 0.57	488.77 ± 0.54
	4th day	475.85 ± 0.46	515.15 ± 0.12	544.28 ± 0.26	567.85 ± 0.31
	5th day	554.23 ± 0.15	585.45 ± 0.89	601.12 ± 0.96	624.12 ± 0.81
	6th day	587.2 ± 0.52	604.4 ± 0.85	621.2 ± 0.52	643.2 ± 0.74
	7th day	501.26 ± 0.85	531.23 ± 0.56	562.21 ± 0.66	588.20 ± 0.91

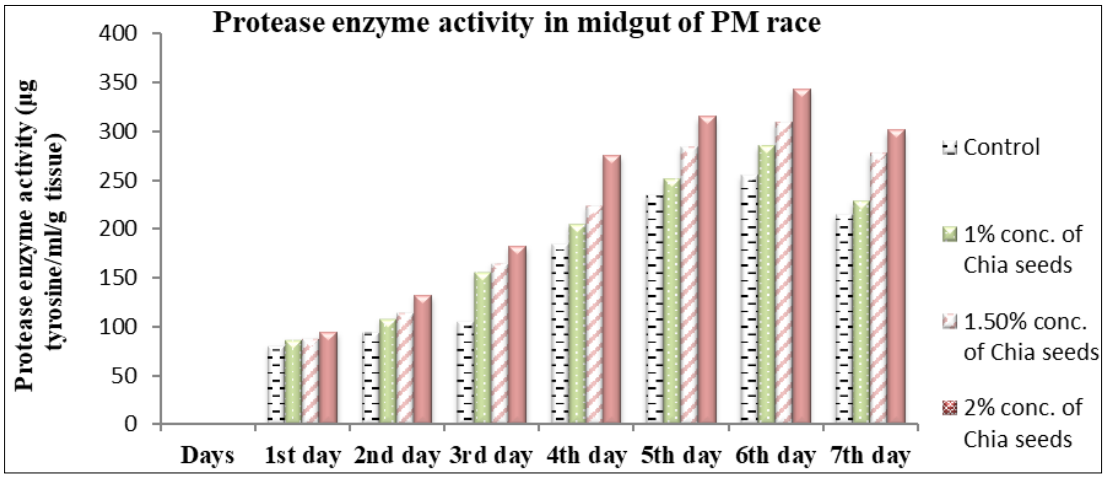

Fig 3: Effect of prebiotic supplement Chia seeds on protease enzyme activity in midgut of 5th instar silkworm *B. mori* in multivoltine Pure Mysore (PM) race

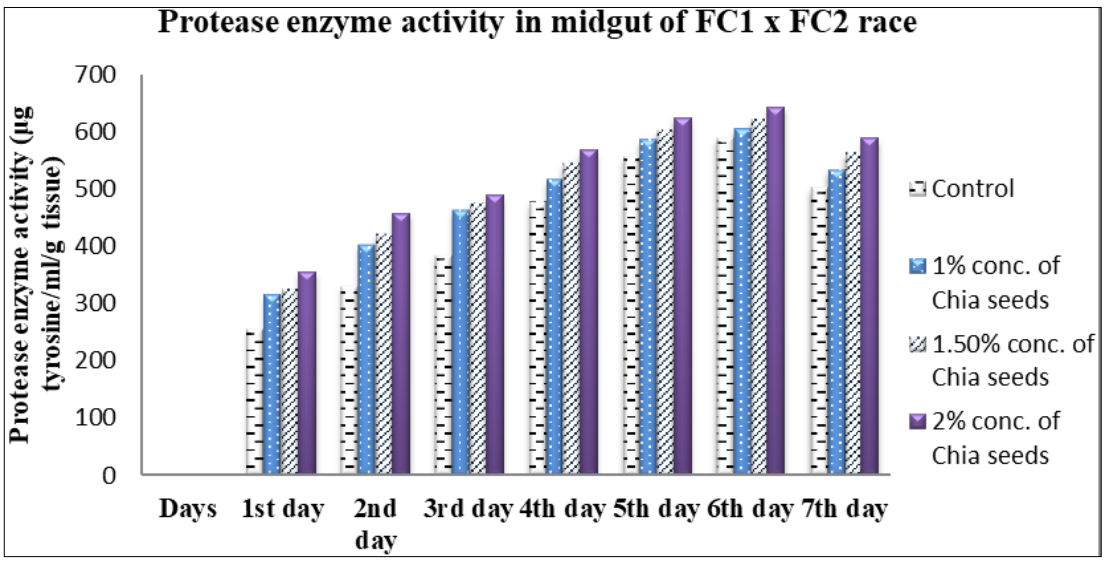

Fig 4: Effect of prebiotic supplement Chia seeds on protease enzyme activity in midgut of 5th instar silkworm *B. mori* in bivoltine double hybrid (FC_1 x FC_2) race

Discussion

Data revealed that, protein concentration in multivoltine PM race and bivoltine FC_1 x FC_2 race silkworms varied significantly. Midgut protein content increases from 1st day to 7th day of all 5th instar silkworms. Bivoltine race exhibits high midgut protein content in comparison to multivoltine race, similar findings were also observed by (Murthy, 2015**)** [15]. This is due to double hybrid variety of FC_1 x FC_2 bivoltine race and high efficiency rate for conversion and accumulation of proteins in the body of silkworm compared to multivoltine larvae. Proteins are very important during the developmental stages of insects. Quantitative and qualitative protein plays a critical role in influence the growth and development of *B. mori* (Hassan, 2020) [5].

The enzyme found in the silkworm body plays a vital role for the effective transformation of organic food molecules of mulberry leaf into useful biomolecules to build the body of silkworm. One among the enzymes is protease enzyme which plays a direct role in the conversion of mulberry protein into silk protein. The final instar records for the high protease activity in the midgut due to high rate of mulberry consumption. In 2% concentration of chia seeds the protease activity was highest than compared to 1 and 1.5% chia seeds treated group in both the multivoltine PM race and bivoltine double hybrid FC_1 x FC_2 race of silkworms. It is due to increased concentration of supplementary protein in the diet of mulberry silkworms and similar findings were found with other high protein diet such as soy flour and Spirulina fed to silkworm races documented by (Venkataramana *et al*, 2003) [27]. These results are similar to the observations of (Anil Kumar, 2009) [1, 12], who has concluded that increase in protease activity in silkworm breeds and hybrids with advancement of age. Similar results was also reported by (Horie and Watanabe, 1983) [6], (Rathinam *et al*., 1994) [22] and (Nagata and Kobayashi, 1990) [17].

The total protein content and protease enzyme activity of 5th instar silkworm varies accordingly to age, race and feedings. Food consumed in the larval stage is effectively utilized for the production of silk proteins as well as to support its metabolism (Nijagal & Kumara, 2017) [19]. The protein and protease enzyme content was more in chia seeds treated group in both bivoltine and multivoltine race compared to control. It results in growth and development of silk gland for the formation of silk filaments as well as increase in conversion and assimilation rates during larval development and it is found that the supplement of protein rich diet affects the physiology and biochemical activities of silkworm.

Conclusion

From the data obtained in present study it is evident that the chia seeds with different concentration affects the biochemical characteristics of silkworm which has significantly increased protein and protease enzyme content in midgut at 2% concentration followed by 1.5% and 1% concentration of chia seeds than compared to control in both the races of silkworm. It is due to high plant based protein present in chia seeds which acted as efficient prebiotic supplement. This further confirms that by adding supplementation of food like chia seeds along with mulberry leaves resulted in the increase larval growth and silk production which can be implemented in Sericulture practices.

References

1. Anil Kumar MN. "Studies on the seasonal variations in protease activity and their correlation with the economic characters in the popular silkworm breeds and their hybrids of *Bombyx mori* L." Ph.D. Thesis, University of Mysore, Mysuru, 2009, 146-210.
2. Banno Y, Tochihara S, Kawaguchi Y, Doira H. Protein of young larva of silkworm *Bombyx mori* L. Journal of Sericulture Science, Japan,1993:62(3):187-194.
3. Das A. Advances in Chia Seed Research. Adv. Biotechnol. Microbiol,2018:5:5–7.
4. Eguchi M, Iwamoto A. Alkaline protease in the midgut tissue and digestive fluid of silkworm, *Bombyx mori* L. Insect Biochem,1976:6:491-496.
5. Hassan EM. Comparative Study on Effects of some High Protein Content Nutritional Additives on some Hybrids of Mulberry Silkworm, *Bombyx mori* L. Journal of Plant Protection and Pathology,2020:11(8):403-410.
6. Horie Y, Watanabe K. "Effect of various levels of dietary protein and supplementation with limiting amino acids on growth, haemolymph components and uric acid excretion in silkworm, *Bombyx mori*." J Ins. Physiol,1983:29:187-199.
7. Ishaaya I, Swirski E. Invertase and amylase activity in the armoured scales *Chrysomphalus aonidum* and *Aonidiella aurantii*. J. Insect Physiol,1970:16:1599-1606.
8. Ito T, Arai N. Nutrition of silkworm *Bombyx mori* L. Amino acid requirements and nutritive effects of various proteins. Bulletin of Sericultural Experiment Station,1965:19:345-373.
9. Kanekatsu R. Amylase in the digestive juice of silkworm larvae, *Bombyx mori*. The Journal of Sericultural Science of Japan,1972:41:445-451.
10. Krishnaswami S. "New technology of silkworm rearing." Central Sericulture Researches and Training, Inst., Mysore Bull,1978:2:1-10.
11. Kumar D, Pandey JP, Jain J, Mishra PK, Prasad BC. Qualitative and quantitative changes in protein profile of various tissues of tropical tasar silkworm, Antheraea mylitta Drury. *Int. J. Zool.* Res,2011:7:147-155.
12. Kumar MNA, Kalpana GV. Studies on the seasonal variation in the protease activity in different developmental stages of productive and robust silkworm breeds and their hybrids of *Bombyx mori* L. National Journal of Life Sciences,2009:6(1):113-119 ref.12
13. Kumari L, Ananthanarayana SR, Jayaprakash B. Effect of radiation on the activity of digestive enzymes in the silkworm, *Bombyx mori* L. Sericologia,1997:37:221-228.
14. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. "Protein measurement with folin phenol reagent." J. Biol. Chem,1951:193:265-275.
15. Murthy VY. Estimation of protein concentration in different tissues of popular silkworm (*Bombyx mori* L.) races. International Journal,2015:3(1):254-261.
16. Murthy VY, Ramkumar B, Jayaram GN, Lokesh G. Critical biochemical analysis in different body tissues in three commercial silkworm (*Bombyx mori* L.) races. Asian Journal of Natural & Applied Sciences, 2014, 3(2).

17. Nagata M, Kobayashi M. Quantitative changes in the Storage Proteins during larval development of the silkworm *Bombyx mori* L. *Journal of Sericulture* Science Japan,1990:59(6):461-468.
18. Narayanan ES, Kasiviswanathan A, SitaramaIyengar MN. "Preliminary observations on the effect of feeding leaves of varying maturity on the larval development and cocoon characters of *Bombyx mori*. L." Indian J Seric,1967:1:109-113.
19. Nijagal BS, Kumara GP. Effect of Food Additives on the Protein Content and Midgut Enzyme Activities In the 5th Instar Larvae of *Bombyx mori* (L). International Journal of Recent Scientific Research,2017:8(10):21074-21078.
20. Noelting G, Bernfeld P. Sur les enymes amylolytiques. III. La β-amylase: dosage d'activite´ et contole de l'absence d'αamylase. Helv. Chim. Acta,1948:31:286-290.
21. Pauchet Y, Muck A, Svatos A, Heckel DG, Preiss S. Mapping the larval midgut lumen proteome of *Helicoverpa armigera*, a generalist herbivorous insect. Journal of Proteome Research,2008:7(4):1629-1639.
22. Rathinam KMS, Janarthan S, Krishnan M. "Improvement of multivoltine rearing through nutritional fortification of mulberry leaves." Global Silk Scenario 2001, Oxford and IBHpub. Co. pvt Ltd., New Delhi, 1994, 248-251.
23. Seo RW, Youn CY, Kang CS, Kim HR. A Study on Protein Pattern of Haemolymph during last larval and pupal stages of *Bombyx mori* L. Bulletin of Entomological Research,1985:11:153-164.
24. Silva C, Garcia VAS, Zanette Scriber JM, Slansky F Jr. The nutritional ecology of immature insect. Ann. Rev. Entomol,2016:26:183-211.
25. Sumida M, Yuan XL, Mah YI, Mori H, Matsubara F. Changes in kinetic parameters and total activity of midgut sucrase in the silkworm, *Bombyx mori* during larval-pupal-adult development. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry,1990:96(3):605-611.
26. Ullah R, Nadeem M, Khalique A, Imran M, Mehmood, S, Javid A, *et al*. Nutritional and therapeutic perspectives of Chia (*Salvia hispanica* L.): A review. J. Food Sci. Technol,2016:53:1750–1758.
27. Venkataramana P, Srinivasa Rao TVS, Srinivasula Reddy P, Suryanarayana N. "Effect of Spirulina on the larval and cocoon characters of silkworm, *Bombyx mori* L." Proc. Nat. Acad. Sci. India,2003:73:B(1):89-94.