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Research Article

EFFECT OF SPIRULINA FORTIFICATION ON THE DIGESTIVE PROTEASE, CARBOHYDRASES AND COCOONS ECONOMIC TRAITS OF MULBERRY SILKWORM *BOMBYX MORI*

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ABSTRACT

Bombyx mori, the silkworm, is a voracious feeder that relies on digestive enzymes for mulberry digestion and its conversion in to silk. In the current study, fifth-instar larvae were given 2% spirulina supplementation with commercially graded spirulina samples, and a few digestive enzymes and cocoon traits were examined. The treatment groups of silkworms T1-control, T2-supplemented with spirulina powder graded for animal dietary consumption, T3- graded for human dietary consumption and T4-spirulina graded for medicinal applications. Spirulina supplementation was tested in silkworms for digestive protease, carbohydrases and cocoon traits. The results shows that the total protein content percentage difference (PD) in the gut wall was increased by 2.79% in T3, 1.96% in T4, and 0.78% in T2 compared to T1 (control). A similar trend was noticed in the gut content, with the PD of 15.81% in T3, 7.51% in T4, and 3.95% in T2. In total carbohydrate content, observed PD of 16.35% in T2, 11.27% in T3, and 9.38% in T4, while the gut content was 13% both in T2 and T3, and 1.22% in T4. The protease activity PD of gut wall tissue has 21.99% in T3, 5.13% in T2, and 4.30% in T4; similarly, the gut content has a PD of 112.38% in T3, 106.12% in T4, and 86.41% in T2. The amylase activity have shown highest PD in T4 (33.43%), T3 (23.37%), and moderate in T2 (19.23%) in the gut wall, and 93.10% in T3, 66.44% in T2, and 14.92% in T4 of gut content. Enzymes such as sucrase, cellulase, and pectinase have also improved with spirulina fortification. The sucrase activity in the gut wall and gut content were, respectively, 7.99% and 80.73% in T3 treatment. Noteworthy, the T2 showed 37.69% in the gut wall and 24.54% in the gut content for the cellulase activity. Unlike cellulase, pectinase activity has been observed with a PD of 93.15% in the gut wall and 64.63% in gut content. The study further confirms that the additional fortification of spirulina will have a beneficial effect on the digestive cells of the silkworm, *Bombyx mori,* resulting in the highest rate of enzyme activity and in turn, increasing survivability.

Keywords: Alpha amylase, *Bombyx mori*, Carbohydrases, Protease, Silkworm, Spirulina.

INTRODUCTION

Sericulture is an evolving industry based on the silkworm breeds, mulberry varieties, environmental and rearing factors that bring out the quality of cocoon production. Mulberry leaves, which are high in vitamins, minerals, and amino acids, are the sole source of nutrition for silkworms. The mulberry leaves has a significant impact on the worm's growth and development as well as the weight and quality of the cocoon (Andadari *et al*., 2021). The mulberry leaves contains 80% of water, 9.8% carbohydrates and some starch and fiber. The fibre (12%) is both soluble (25%) in the form of pectin and insoluble (75%) in the form of lignin, protein (12%) and fat (3%), and rich in many vitamins and minerals. Many researchers have made an effort to improve the quality and yield of cocoon by introducing new mulberry varieties that have good nutritional aspects and improving breeds with more silk synthesising and digestive efficiency. The variation in the input of fertilizer, irrigation, and poor maintenance made the promising quality mulberry yield unattainable. Thus, an

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effective fortification of nutrition implemented as a strategy for increasing the economic worth of cocoon is to fortify mulberry leaves with added nutrients and feed the silkworm.

Spirulina is the thread form of cyanophyceae, a type of blue-green algae used for the health benefits of humans, animals and growth and development of insects. It is a rod, disc, or spiral-shaped gram-negative bacteria that consist of complex sugars, protein, minerals, and beta-carotene. Mostly available in dried form, it is used as food additive or dietary supplements for animals and insects. Many researchers has been identified spirulina supplementation has improvement in animal growth, fertility, aesthetics, and the quality of nutritional products. It is good for many living beings due to the richness of antioxidants to boost immunity. Many animal studies have confirmed that spirulina ingestion has also been found to improved animal health and welfare. In cattle, it helps to increase the lactation. The spirulina acts as an effective agent in the silkworms' rearing to increase the growth, length, weight of the cocoon and pupa and digestive enzymes (Kumar *et al.,* 2018).

Many elements have a direct impact on the effective growth of silkworms and take part in the production of high-quality silk. Silkworms' nutrition is such that it plays a pivotal role in creating quality cocoons and silk productivity. The nutritive physiology of silkworms relies on the active ingestion, digestion, and absorption of mulberry and the consequent conversion of silk protein in the silk gland. The digestive system, i.e., the gut wall and the gut content, contains the digestive enzymes that act on the conversion of complex dietary molecules to simpler units. The major digestive system functions as a reactor for biochemical activity during digestion. The enzymatic study gives its digestive efficiency. The major biochemical constituents of the mulberry nutritive molecules like proteins and carbohydrates. The effective synthesis of wide range of digestive enzymes serves as an inducer for active digestion, absorption and regulates the silkworm's growth, development (Esaivani *et al*. 2014). Hence, the present study aims to study the quantitative enzymatic differences of different spirulina supplemented groups and its economic traits.

MATERIALS AND METHODS

The present study was conducted at the Silkworm Physiology Laboratory of the Central Sericultural Research and Training Institute, Mysore, India. The bivoltine silkworm double hybrid FC1 x FC2 was chosen for this study. Silkworm rearing was carried out with disease-free layings (DFLs) obtained from the Silkworm Seed Production Centre, Mysore, India. The DFLs were reared on V1 mulberry leaves until cocooning as per the standard rearing package (Rajan *et al.,* 2001). Up to IV instar, the larvae were reared in a single pool and divided into four experimental treatment groups. T1-control, T2-supplemented with spirulina powder graded for animal dietary consumption, T3-supplemented with spirulina powder graded as human dietary consumption and T4-spirulina graded for medical applications. The control batch, T1 was given 2 normal feedings as scheduled, at 10 a.m. and 4 p.m. For all the treatment batches, T2, T3 and T4 the 2nd feeding at 4 PM was replaced by mulberry leaves dipped in 2% spirulina solution as mentioned, air dried for 15 minutes, and fed to silkworms. The fortification was given on day-1, day-2, day-3, day-4 and day-5 of the V instar. During the fifth instar fifth day, the silkworm groups were dissected in the silkworm ringer solution. Gut wall (the alimentary canal wall without digestive fluid) and gut content (digestive fluid in the alimentary canal) were collected and preserved at -4ºC for biochemical analysis. Total proteins (Lowry *et al*., 1951), total carbohydrates (Carrol *et al*., 1956), and enzyme activities including protease (Davis and Smith, 1955), alpha amylase (Bernfeld, 1955), pectinase (Meneghel *et al*., 2014), sucrase (Ishaaya Swiski, 1970) and cellulase (Miller, 1959) were investigated. The mature larvae were mounted in plastic collapsible montages separately, treatment- and replication wise, and cocoon harvesting was done on the 6th day, and subsequently, cocoon assessment was carried out (Bohidar *et al.,* 2007; Rahmathulla *et al.,* 2007). The economic traits, such as single cocoon weight, shell weight, and pupation percentage, were also assessed. The data were analysed using one-way ANOVA.

RESULTS AND DISCUSSION

In insects like silkworms, growth and development were associated with digestive efficiency, i.e., the active synthesis of digestive enzymes in the gut and their efficient conversion in the respective organs. Many supplementation studies on silkworms will aim to improving cocoon yield via mulberry. In the silkworm *Bombyx mori,* digestive enzymes like proteases and carbohydrases have a prodigious role in digestive function. Since the host plant of silkworms, mulberry contains a rich source of proteins and different carbohydrate moieties, the enzymes; protease and carbohydrases are required to digest them. The present study aims to study the effect of spirulina supplementation on a few digestive enzymes activity level. In silkworms, the biochemical constituents undergo dynamic changes during their developmental stages. The quantitative variation in the major biochemical constituents, proteins and carbohydrates, was assessed in the gut wall and the gut content, and the results are as follows: Significant differences were not seen among the test groups in the protein content of the gut wall (Table 1). The highest protein content of 19.21 mg/g wet weight of tissue was noted in T3, followed by 18.82 mg in T2, 18.68 mg in T1, and 18.317 mg in T4. Unlike the gut wall, the digestive juice, i.e., the gut content has shown a significant difference among the test groups (Table 1). A protein content of 2.26 mg/ml was recorded in T3, followed by 2.08 mg in T4, 1.933 mg in T1, and 1.858 mg in T2. Dietary protein uptake and storage in the gut wall have been observed for functional protein synthesis to perform cell growth, metabolism, and antimicrobial activity. Furthermore, it contributes to the active synthesis of digestive enzymes by the goblet cells (Anand *et al*., 2010). The maximum percentage difference (PD) value of 2.79% has been observed in T3, followed by 1.96% in T4, and 0.783% in T2. But in the gut content, the variations are evidently shown with respect to treatments. The treatment T3 has shown the highest percentage difference (PD) value of 15.81% with respect to T1 (control), followed by PD values of 7.51% by T4 and 3.95% by T2 (Figure 3). Since the gut content is the biochemical reactor for the digestive biochemical reaction and the site for digestion, the higher possibilities of protein release from food have been pinpointed (Bhuvaneswari *et al*., 2012a). In addition, spirulina (for human consumption) supplementation geared up the protein level slightly in the gut wall and at the highest rate in the gut content.

Carbohydrates in the mulberry leaves are the prime source for the silkworms. Ingestion, digestion, and conversion are governed by a group of enzymatic reactions in the digestive compartments. The total carbohydrate content in the gut wall is highest in T3 (11.24 mg/g of tissue), followed by T4 (10.480 mg), T1 (9.541 mg), and T2 (8.523 mg). Similarly, the gut fluid contains the highest quantity of T3 (18.650 mg/ml), followed by T4 (16.450 mg), T1 (16.250 mg), and T2 (14.250 mg) (Table 1). Both tissues have shown a similar trend in response to the spirulina supplementation, with significant variation. The amount of carbohydrates varies with the mulberry leaf age and its quality (Benchamin and Jolly, 1986) and the digestive frequency of silkworms, i.e., the active release of carbohydrases and glucose moieties in the gut. According to the current research, all spirulina treatments have a positive effect on carbohydrate metabolism, with a finely tuned regulation between the gut wall and gut content. Improvements in carbohydrate release and conversion in the gut wall were observed in all treatment groups. In T3, the gut wall cells have shown the highest reserves of carbohydrates have a strong correlation with the highest release of carbohydrates in the gut content, with PD values of 16.35% and 13.75%, respectively (Figure 3). Similarly, the PD was 11.27% and 13.11% in T2; 9.38% and 1.22% of PD in T3 were noted in the gut wall and gut content, respectively. The late-age silkworms instantly reserve carbohydrates in the form of trehalose to maintain homeostatic equilibrium and later convert glycogen for metamorphic events.

Table 1.Changes in the level of total proteins and total carbohydrates of gut wall and gut content of silkworm (FC1xFC2) *Bombyx mori* fortified with spirulina.

Name of the treatment	Total proteins		Total carbohydrates	
	Gut Wall (mg/g)	Gut Content (mg/ml)	Gut Wall (mg/g)	Gut Content (mg/ml)
T-1	18.681 (±0.641) NS	1.933 (±0.141) *	9.541 (±0.850) *	16.250 (±0.214) *
T-2	18.828 (±1.256) NS	1.858 (±0.051) *	8.523 (±0.840) *	14.250 (±0.150) *
T-3	19.210 (±0.389) NS	2.265 (±0.184) *	11.240 (±0.350) *	18.650 (±0.231) *
T-4	18.317 (±0.867) NS	2.084 (±0.081) *	10.480 (±0.250) *	16.450 (±0.080) *

*Statistically significant (P<0.005), NS- Statistically not significant.

Values represent the mean ± standard deviation (SD±) of three separate observations. For each observation tissue samples from 10 to 15 larvae were pooled. The percentage difference (PD) for spirulina fortified (T2, T3 and T4) larvae were calculated over control (T1).

Table 2.Changes in the level of few digestive enzyme activity (µ moles) levels in the gut wall and gut content of silkworm (FC1xFC2) *Bombyx mori* fortified with spirulina.

Treatment	Protease		Alpha amylase		Sucrase		cellulase		Pectinase	
	Gut Wall	Gut Content	Gut Wall	Gut Content	Gut Wall	Gut Content	Gut Wall	Gut Content	Gut Wall	Gut Content
T-1	1.234 (±0.057)*	0.023 (±0.001) NS	2.984 (±0.096) *	0.031 (±0.001) *	3.411 (±0.127) *	0.130 (±0.007) *	2.239 (±0.083) *	0.082 (±0.001) NS	2.251 (±0.204) *	0.111 (±0.016) *
T-2	1.299 (±0.057) *	0.058 (±0.030) NS	3.619 (±0.086) *	0.062 (±0.003) *	3.434 (±0.014) *	0.241 (±0.001) *	3.279 (±0.143) *	0.105 (±0.001) NS	3.169 (±0.517) *	0.140 (±0.004) *
T-3	1.539 (±0.118) *	0.082 (±0.002) NS	3.774 (±0.248) *	0.085 (±0.008) *	3.7209 (±0.012) *	0.306 (±0.002) *	3.272 (±0.358) *	0.089 (±0.120) NS	6.1763 (±0.049) *	0.217 (±0.003) *
T-4	1.182 (±0.047) *	0.075 (±0.700) NS	4.182 (±0.110) *	0.036 (±0.002) *	3.4934 (±0.001) *	0.270 (±0.001) *	1.961 (±0.027) *	0.078 (±0.014) NS	5.5046 (±0.148) *	0.109 (±0.001) *

Values represent the mean ± standard deviation (SD±) of three separate observations. For each observation tissue samples from 10 to 15 larvae were pooled. The percentage difference (PD) for spirulina fortified (T2, T3 and T4) larvae were calculated over control (T1).Statistically significant (P<0.005), NS- Statistically not significant

Figure 1. Fifth instar larvae of silkworm (FC1xFC2) *Bombyx mori* T1 (control), fortified with spirulina T2 (animal consumption), T3 (human consumption) and T4 (medicinal use).

Proteases are the only enzymes that contribute to total proteolytic activity in the silkworm digestive system (Holtof *et al*., 2019), by the sole synthesis of gut epithelial cells and not by the microbial community (Sharma *et al*., 1984). The highest protease activity level of 1.539 µ moles hass noticed in the gut wall of T3, followed by 1.29 µ moles in T2, 1.18 µ moles in T4, and 1.23 µ moles in T1 (control) (Table 2). But in gut content, the activity levels are found to be highest in T3 (0.082 µ moles), followed by T4 (0.075 µ moles), T2 (0.058 µ moles), and least in T1 (0.023 µ moles). Significant variations are noticed in the gut cells except for the gut juice. The fifth instar of the silkworms consumes a bulk volume of mulberry in an insatiable manner and is fine-tuned by the synthesis and release of proteases. In the gut wall, T3 has been shown the highest rate of synthesis with a PD of 21.997%, followed by T2 and T4 at 5.132% and 4.304%, respectively. Similar findings are made in gut content. The T3 has the highest protease activity (PD of 112.38%), followed by the T4 (106.12%), and the T2 (86.41%) (Figure 4). The quantitative difference in the rate of enzyme activity has been noted in spirulina fortified, which functions as an inducer for the protease enzyme's release and activation. Spirulina has an endogenous proteases are playing protective role directly or indirectly to the host tissues (Del Rosso, 2012) and the enzyme efficiency also varied on the food chemical constituents (Kumari *et al*., 1997). Our findings are consistent with the earlier work of Selin *et al*. (2018) that spirulina aids protein digestion by increasing protease activity and hence many food industries are widely using this spirulina.

Figure 2. Cocoons of silkworm (FC1xFC2) *Bombyx mori* T1 (control), fortified with spirulina T2 (animal consumption), T3 (human consumption) and T4 (medicinal use).

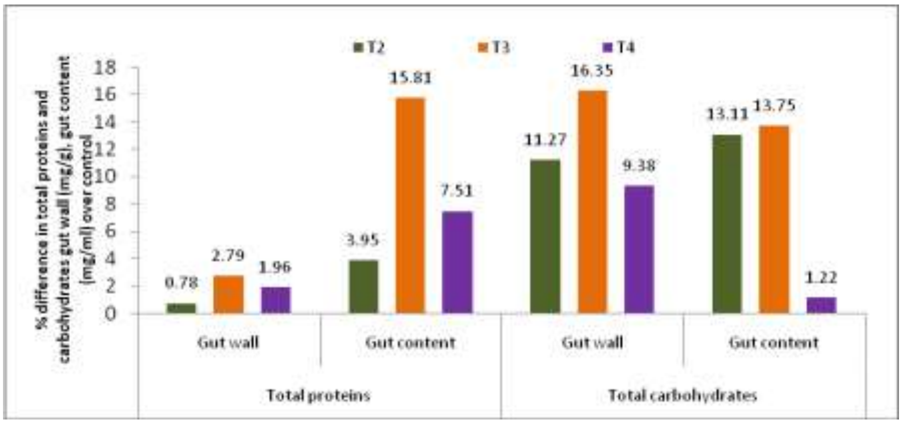

Figure 3. Percentage difference in the total proteins and carbohydrates in the gut wall and gut content of the spirulina fortified silkworm (FC1xFC2) *Bombyx mori* over the control.

Mulberry carbohydrates, which are an important source of energy and a primary component of food, such as sucrose, cellulose, pectin, xylan, and others, require cascade enzymatic hydrolysis for active absorption and conversion. In mulberry leaves, these carbohydrates are associated with the physiological and biochemical pathways and maintain the integrity of plants. The enzymes that react upon these complex molecules were also simultaneously identified in the silkworm's gut. A group of carbohyrases and their activations are controlled by different factors; the silkworm gut does not have the potential for all carbohydrases, and the microbial community existing in it will synthesize, release, and act on the complex carbohydrates. Bhuvaneswari *et al*. (2015), Thirupathaiah *et al*. (2018) and Anand *et al*. (2010) are well documented the functional role of gut microbes and their impact on carbohydrate digestion. The mutualism of gut microbes further enhances the digestive function of silkworms. Kumar and Balasubramanian (2015) investigated the synergistic effect of spirulina supplementation on consumption and conversion traits. Hence the present study focused on the impact of spirulina on carbohydrases. Alpha amylase aids starch digestion by cleaving the 1, 4 glycosidic linkages of repeated units of D-glucose in the silkworms alimentary canal (Jenner, 1982; Strobl *et al*., 1998; Bhuvaneswari *et al.,* 2012b). The results show that the amylase activity has been modulated by the supplementation of spirulina.

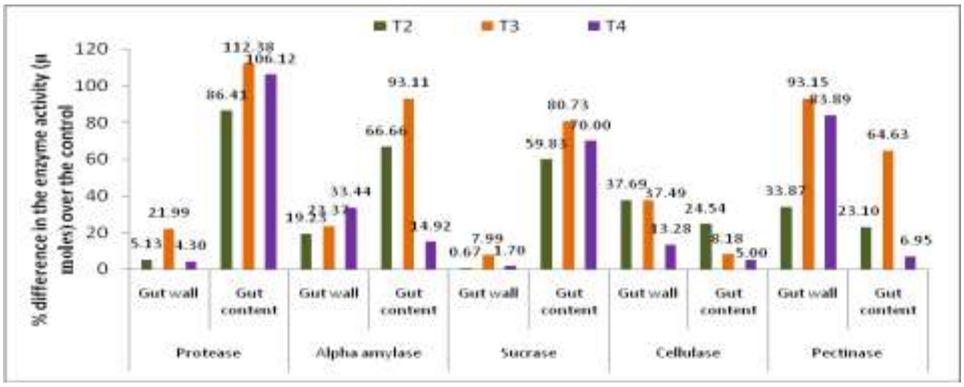

Figure 4. Percentage difference in the protease, alpha amylase, sucrase and pectinase activities in the gut wall and gut content of the spirulina fortified silkworm (FC1xFC2) *Bombyx mori* over the control.

Further, the synthesis was noted to be highest in T4 (4.18 µ moles) followed by T3 (3.774 µ moles), T2 (3.619 µ moles), and T1 (2.984 µ moles) (Table 2). The changes in the enzymatic activity clearly showed PD values of 33.43%, 23.379%, and 19.23% in T4, T3, and T2, respectively. Unlike the gut wall, the digestive fluid has shown the highest activity in T3 (0.085 µ moles), followed by T2 (0.062 µ moles), T4 (0.036 µ moles), and T1 (0.031 µ moles), with PD values of 93.10%, 66.66%, and 14.92%, respectively (figure 4). Alpha amylase plays a pivotal role in the digestive functions of complex carbohydrates and improves the quality of silk. The current study found that the treatment T3 is beneficial for increasing the enzyme activity level in the digestive fluid.Silkworms require stimulation to feed on mulberry leaves because their physiological responses to leaf phytochemicals determine acceptance and rejection, as well as the rate of ingestion and digestion. The silkworms identify plants using their keen sense of taste, which enables quality assessment (Tsuneto *et al.,* 2020). The mulberry contains a stimulative compound, a carbohydrate moiety called sucrose, which performs its core duty as a stimulating agent for silkworm feeding (Ito, 1960; Bhuvaneswari *et al.*, 2013). Sucrose often functions as a chemical inducer for feeding behaviour and drives energy cycles in many insect groups. A sucrose molecule of this type is digested in the digestive compartments by columnar epithelial and goblet cells, with the synthesis and release of sucrase into glucose and fructose monomer (Kanekatsu *et al.,* 1992). The current study found a significant difference in sucrase activity between different treatments groups (types of spirulina derived for different purposes). In the gut wall, the sucrase activity is not showing the greatest difference among the treatments; in T3 (3.72 µ moles), the highest activity has been observed, followed by T4 (3.49 µ moles), T2 (3.43 µ moles), and T1 (3.41 µ moles), with the PD of 7.79%, 1.70%, and 0.67%, respectively, in T3, T4, and T2. Contrary to the gut wall, prominent results were observed in the gut content. T3 (0.30 µ moles), T4 (0.37 µ moles), T2 (0.24 µ moles), and T1 (0.13 µ moles) have the highest activity, with PD values of 80.73%, 70%, and 59.83%, respectively, when compared to the T1 (control) (Table 2 and Figure 4). The gut lumen of the silkworm acts as a metabolic reactor, and its fluid is a reaction mixture known for its active digestion and the formation of end products. The enzymatic reaction in this site contains a rich source of sucrose from mulberry; hence, the highest activity has been noticed in the gut content. Gut cells will synthesise and release digestive enzymes into the gut content in a time-dependent manner. In the posterior part of the digestive system, sucrase (molecular weight: 66000 kDa at pH 6.6) in association with maltase, lactase, and glucoamylase hydrolyzes different sugars (Kanekatsu *et al*., 1992). Contrastingly, the dietary sucrose concentration affects the activity levels of sucrase in the gut lumen, as noted by Sumida and Ueda (2003). The sucrase enzyme levels in the inherent physiological gut cells are especially intense during the fifth instar. Interestingly, in the present investigation, the fortification of spirulina triggers the enzyme activity level in T3, which has a percentage difference of 80.73% compared to control (T1). Because of the high ingestion of mulberry and sucrose accumulation, this clearly shows that spirulina has an effective role in the elevation of sucrase activity.

Table 3.Silkworm (FC1xFC2) *Bombyx mori* cocoon economic traits fortified with spirulina**.**

Name of the treatment	Final larval weight (10 nos) (g)	Single cocoon weight (g)	Single shell weight (g)	Shell Ratio (%)	Pupation (%)
T-1	47.50 (±0.105)*	1.644 (±0.035) NS	0.348 (±0.104) NS	21.26 (±0.007) NS	92.00 (±0.005)*
T-2	50.26 (±0.120)*	1.740 (±0.102) NS	0.372 (±0.150) NS	21.41 (±0.063) NS	98.00 (±0.012)*
T-3	49.82 (±0.098)*	1.710 (±0.100) NS	0.365 (±0.047) NS	21.40 (±0.033) NS	97.00 (±0.010)*
T-4	48.00 (±0.088)*	1.658 (±0.024) NS	0.350 (±0.327) NS	21.20 (±0.029) NS	95.00 (±0.020)*

Values represent the mean ± standard deviation (SD±) of three separate observations. Statistically significant (P<0.005), NS- Statistically not significant.

Cellulose is a chief dietary carbohydrate, a linear polymer of D-glucose residues; the primary structural component of plant cells is digested by a series of enzymes known as cellulases in the digestive system of the silkworm *Bombyx mori* (Bhuvaneswari *et al.,* 2015). Interestingly, cellulase-synthesising genes were not found in the organism; its digestion is aided by the symbiotic microbial flora present in it (Anand *et al*., 2010; Shi *et al.,* 2011). The current study found that cellulase activity was modulated between tissues and was effective with the fortification of spirulina. T2 and T3 had the highest cellulase activity (3.27 mol/L), followed by T1 (2.23 mol/L) and T4 (1.96 mol/L) (Table 2 and Fig 4). The variations among treatments are quite statistically significant. Further, it indicates that the fortification of spirulina triggered the relative synthesis of cellulase enzymes in the microbial flora that resides in the gut cells. *Proteus, Klebsiella,* and *Citrobacter* are likely to have contributed to enzyme synthesis (Nakashima *et al*., 2002; Anand *et al*., 2010; Belda *et al*., 2011). A PD of about 37% was noted in T2 and T3, and 13.28% in T4 compared to T1. This distinction is due to the fact that spirulina strengthens the gut microbiome through its symbiotic function. Whereas in gut content, the variations are noted as non-significant among the treatment groups and found to be 0.08 µ moles in T1, 0.10 µ moles in T2, 0.08 µ moles in T3, and 0.07 µ moles in T4. It is necessitated by the silkworms or the symbiotic microbes to maintain an optimum level of cellulase in the gut for digesting the cellulose. Because cellulose is the major carbohydrate source, i.e., 19–25% present in the mulberry, it has to convert into a functional molecule in a continuous manner. This has been made possible by the maintenance of an optimised level of enzyme activity in gut content.

Pectin, a natural polymer, accounts for 4.6% of mulberry leaf (Ghosh *et al*., 2003). Its digestion in the silkworm digestive system, like cellulose digestion, it also been driven by pectinase synthesised by the microbial community (Taylor, 1982). The current investigation spirulina fortification on pectinase and found the highest activity in T3 (6.17 µ moles) followed by T4 (5.50 µ moles), T2 (3.169 µ moles) and T1 (2.25 µ moles) in the gut wall. The PD of 93.15% in T3, 83.89% in T4, and 38.87% in T2 were observed compared to T1. Similarly, in gut content, the highest activity was noted in the T3 (0.21 µ moles), followed by the T2 (0.14 µ moles), the T4 (0.109 µ moles), and the T1 (0.11 µ moles), and the difference could be 64.63% in T3, 23.10% in T2 and 6.95% in T4 (Table 2 and Fig 4). In insects, pectinase production has been identified as a microbial source not involving any specialised cells (Dillon and Dillon 2004). As shown in the earlier work of Anand *et al*. (2010), the pectin content in the digestive system of silkworms is greatly utilised by the bacterial colony of *B. circulans, P. fluorescens,* and *Erwinia sp.* Our findings confirm that T3 has the ability to stimulate pectinase and cellulase production, and a study on mice published by Hu *et al*., 2019, also found that a single dose of spirulina supplementation has an effect on the microbial community. The modulation has an effect on enzyme synthesis and digestive efficiency. Additionally, it maintains the physiological status of the silkworm.

There is a large volume of data available on the fortification studies with multivitamin mixture, botanical extracts, flour diets, medicinal plant powder, probiotic consortium, nutrilite products and homeo medicine to improve the silkworm, *Bombyx mori*, and its economic traits (Ganga and Gowri, 1990; Gobena and Bhaskar, 2015: Babu *et al*., 1992; Etebari and Matindoost, 2005; Rani *et al*., 2011; Thulasi *et al*., 2015a and 2015b; Bhuvaneswari *et al*., 2019). In the present investigation, a significant weight difference was noticed in the larval growth, with the highest weight in T2 (50.26 g), followed by T3 (49.82 g), T4 (48.00 g), and T1 (47.50 g) (Table 3 and Fig 1 and 2). Furthermore, no significant results were obtained for the single cocoon and shell weight. The highest cocoon and shell weights were obtained in T2 (1.74 g and 0.37 g), followed by T3 (1.71 g and 0.36 g), T4 (1.65 g and 0.35 g), and T1 (1.64 g and 0.34 g). Significant results were seen in the percentage of shell (21.20% to 21.41%) and in pupation. An improvement in the survival rate, *i.e.*, the pupation rate 98% in T2, 97% in T3 and 95% in T4 is an important determining factor of nutrient efficiency in their health development and production of quality cocoons, and our results are augmented by Kumar *et al*., 2018. A fact is pointed out from the study that spirulina has the ability to improve the health status of the silkworms, and it has been seen in the results with the PD of 6.31%, 5.29%, and 3.20% by T2, T3 and T4 over the control. Salmean *et al.* (2015) also suggested that spirulina has an essential level of protein, vitamins, minerals, and amino acids that enable the growth of a wide range of organisms when consumed in appropriate quantities.

CONCLUSION

It has been concluded that spirulina has a significant role in the enhancing digestive proteases and carbohydrases. The efficient digestion of ingested food and its ultimate conversions are aided by the production of digestive enzymes, which are well assisted by the spirulina fortification. Further, it has been proven that the spirulina graded for human as well as animal consumption purposes have beneficial impacts on silkworm *Bombyx mort's* digestive performance.

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