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Host plants induced changes in the digestive enzymes activities and growth parameters of Eri silkworm (*Samia ricini)* Larvae

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Abstract

In leaf feeding insects, proper nourishment of larvae is dependent on the balance between nutritional content of food plants and the action of digestive enzymes in the digestive tract. In present investigation, gut digestive enzyme activities (α-amylase, proteinase and lipase) and their influence on the larval growth parameters of Eri silkworm (*Samia ricini*) larvae reared on different food plants viz. castor (*Ricinus communis),* tapioca (*Manihot esculenta*) and papaya (*Carica papaya*) were assessed. The larvae on castor showed highest α-amylase (1.50 ± 0.045 U/ml) and lipase (0.61 ± 0.012 U/ml) activities followed by larvae on tapioca (1.15 ±0.007 U/ml and 0.53 ± 0.059 U/ml respectively) and the lowest in papaya fed larvae (1.04 ± 0.009 U/ml and 0.39 ± 0.011 U/ml respectively). The highest proteinase activity was recorded in papaya fed larvae (0.045 ± 0.004 U/ml) and the lowest in castor (0.033 ± 0.001 U/ml). Our results showed highest larval survivability in castor (94.56 ± 1.84%) followed by tapioca (85.56 ± 1.93%) and lowest in papaya (62.22 ± 1.92%). Furthermore, the larvae fed on castor had the highest larval weight (6.92 ± 0.59 g) and silk gland weight (1.42 ± 0.07 g) whereas the lowest values of larval weight and silk gland weight were recorded in larvae reared on papaya leaves (4.33 ± 0.61 g and 0.65 ± 0.14 g respectively). Above results indicated that enzyme activities are functionally related with the type of food plants which subsequently determines the nutritional responses of the silkworm fed on different food plants.

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Introduction

Eri silkworm (*Samia ricini*), a member of leaf feeding insects, is a vital component of ericulture. In India, eri silkworm is extensively reared in north eastern states which provide income for livelihood of the rearers. Assam has the highest output among the north eastern states accounting for almost 85% of total eri silk production in India (Chakrabarty *et al.*, 2012). Eri silkworm being polyphagous in nature is reported to feed on about 29 different host plants (Reddy *et al.,* 2000), besides their primarily food plants Castor (*Ricinus communis* L.) and Kesseru (*Heteropanax fragrans* Seem.) leaves (Das *et al.*, 2020). Individual food plants contains different proportions of nutritive value and exert their effect on the insect's survivability, intake rate of food, digestion and absorption process of nutrients, which ultimately influences the overall economic parameters of the silkworm (Kumar and Elangovan, 2010).

The lepidopteran insect gut is a hollow tube where the secretion of digestive enzymes, digestion, nutrient absorption and detoxification takes place (Pauchet *et al.,* 2008). Silkworms require specific nutrients for normal growth and development which comes through digestion and absorption of food plants. In polyphagus insects, breakdown of complex food materials into simpler forms in the gut requires a complex of digestive enzymes. The adjustments in the digestive enzyme activity levels help to obtain better nourishment from the diet. A balanced action of digestive enzymes is vital for availing the nutrients from the feed (Kotkar *et al.*, 2009) which influences the growth and development of insect.

Amylase and protease are the digestive enzymes involved in the processes of starch and protein digestion respectively, and are important for proper nutrient acquisition and growth of insect (Hemati *et al.*, 2012; Mansouri *et al.*, 2013; Namin *et al.*, 2014). Lipids also constitute an important component of insect diet. Lipases are the enzymes which play a key role in lipid acquisition, storage, and mobilization (Santana *et al.*, 2017). These enzymes play an important role in the digestion and effective transformation of organic food molecules of the leaves into simpler assimilable nutrients ultimately determining the overall growth and development of the silkworm larvae (Mala and Vijila, 2017). Any changes in the type of food and its nutritional contents results in the alteration of the activities of enzymes (Mardani-Talaee *et al.,* 2014). Nutrition is the most crucial factor for silkworm growth and the ability of silkworm to secrete digestive enzymes is largely influenced by the nutrient components present in the food (Manjula *et al.*, 2010). The activity of digestive enzymes is affected by various factors including dietary state, steroid hormone ecdysone (Suzuki *et al.*, 2011) and developmental stage of the insects (Suzuki and Iwami, 2021). The relationship between insect host digestive enzymes and food plants had been studied by earlier researchers (Kotkar *et al.*, 2009; Mansouri *et al.*, 2013; Mardani-Talaee *et al.,* 2014; Oftadeh *et al.*, 2014; Naseri *et al.*, 2014). However, there has not been such report on the gut digestive enzyme responses of *S. ricini* reared on different food plants.

Therefore, the present study was conducted to investigate the effect of different food plants on the activity of digestive enzymes of *S. ricini*. This study will also provide a generalized idea about the relationship between digestive enzyme activities and larval development responses with respect to feeding on different food plants.

Materials and method

Collection of silkworm

Disease free layings (DFLs) of *S. ricini*, Kokrajhar eco race were collected from the Directorate of Sericulture, Kokrajhar, India with their prior permission. The eggs were incubated at room temperature for hatching.

Selection of food plants

Three food plants, one each from primary, secondary and tertiary namely Castor (*Ricinus communis* L.), Tapioca (*Manihot esculenta* Crantz) and Papaya (*Carica papaya* L.) respectively were selected for the experiment. The selection of food plants was solely done based on the availability of plants in the area and their easy accessibility.

Rearing of silkworm

Kokrajhar eco race of eri silkworm was selected for the experiment. Rearing was done under standard rearing conditions at the animal house, Department of Zoology, Bodoland University, Kokrajhar. The newly hatched larvae were first fed on castor leaves till 2nd instar stage. Then the larvae were separated into three groups and each group (n=50) was fed with different food plants viz. Castor, Tapioca and Papaya till the mature 5th instar stage.

Assessment of Larval parameters

The growth parameters such as larval duration, length, weight, percentage survivability, silk gland weight were recorded. The data were presented as means ± SD.

Digestive Enzyme Assay

Preparation of Enzyme Extract

The 3 days old 5th instar larvae were selected for the experiment. 10 individuals were picked up randomly from each experimental group and kept on starvation for 24 hours prior to gut dissection. The larvae were surface sterilized with 70% ethanol for 5 seconds (Kannan *et al*., 2015) followed by rinsing with distilled water to remove surface impurities. The guts were then dissected in ice-cold phosphate-buffered saline (PBS, pH 7.4) using forceps and dissection scissors under a simple microscope. The whole gut along with lumen contents was transferred to a cold homogenizer with 1X PBS buffer. Guts from 10 individuals were pooled and used as one sample and homogenized. The homogenate was then transferred to a 1.5mL centrifuge tube and centrifuged at 13,000 rpm for 15 min at 4^0C. The supernatant was collected and used as an enzyme extract. Each sample was prepared thrice and each experiment consisted of three replicates.

α-amylase assay

The α-amylase activity of gut extract was assayed using dinitrosalicylic acid (DNS) method (Bernfeld, 1955). 1% starch was used as a substrate for the activity assay. The reaction was stopped by using 3, 5-dinitrosalicylic acid reagent and the absorbance of the reaction mixture was measured using uv-vis spectrophotometer at 540 nm. One unit of enzyme activity corresponds to the enzyme amount required for producing 1 µmol of maltose per min under the assay conditions.

Proteinase assay

Proteolytic activity of gut homogenate was assayed according to the protocol described by (Oyebanji *et al.,* 2014), using 1% casein as substrate. Trichloroacetic acid (TCA) reagent was used as stopper of enzyme activity. The sample absorbance was measured at 660 nm. Proteinase enzyme activity of samples were calculated from the tyrosine standard curve and one unit of protease activity was defined as the quantity of enzyme that is required to produce 1 µmol of tyrosine equivalents per mL of TCA filtrate under the assay conditions.

Lipase assay

p-nitrophenylpalmitate (pNPP; Sigma Aldrich) assay (Winkler and Stuckmann, 1979) was used to determine lipolytic activity of gut homogenate following the protocol described by (Sarate *et al*., 2012). Absorbance of the samples was measured spectrophotometrically at 410 nm against a substrate free blank. One unit of enzyme activity was expressed as 1 µmol of p-nitrophenol released per minute under the assay conditions (Massadeh and Sabra, 2011).

Statistical analysis

The data obtained were subjected to one way analysis of variance (ANOVA) followed by comparison of the means with Fisher's least significant difference (LSD) test at $\alpha = 0.05$. The results were presented as means ± SD. All the experiments were carried out in triplicates.

Results

Larval parameters

The data of the larval growth parameters assessed in the experiment showed significant differences ($p<0.05$) among the larval groups fed on different food plants (Table 1). Larval duration was found to be shortest in castor (19.87 ± 0.73 d) followed by tapioca (20.63 ± 0.96 d) and the longest in papaya fed larvae (24.67 ± 1.03 d). Similarly, the castor fed larvae showed highest larval length (7.57 ± 0.53cm) and weight (6.92 ± 0.59 g) followed by the larvae fed on

tapioca (6.97 ± 0.51cm) and (5.71 ± 0.74 g) respectively. Least larval length and weight were observed in papaya fed larvae (6.02 ± 0.42cm) and (4.33 ± 0.61 g) respectively. Significant differences in percent of larval survivability were observed in different treatment groups. The maximum survivability percent of larvae was recorded in larvae reared on castor (94.56 ± 1.84%) and lowest in papaya fed larvae (62.22 ± 1.92%) whereas larvae reared on tapioca showed moderate survivability percent (85.56 ± 1.93%). The silk gland weight of larvae was recorded highest in castor (1.42 ± 0.07 g) followed by larvae fed on tapioca (1.01 ± 0.19 g) and lowest in papaya fed larvae (0.65 ± 0.14 g).

Table 1. Effect of different food plants on the larval growth parameters of *Samia ricini*.

Growth parameters	Food plants		
	Castor	Tapioca	*Papaya*
Larval duration (days)	19.87 ± 0.73a	20.63 ± 0.96b	24.67 ± 1.03c
Larval length (cm)	7.57 ± 0.53a	6.97 ± 0.51b	6.02 ± 0.42c
Larval weight (g)	6.92 ± 0.59a	5.71 ± 0.74b	4.33 ± 0.61c
% survivavility	94.56 ± 1.84a	85.56 ± 1.93b	62.22 ± 1.92c
Silk gland weight (g)	1.42 ± 0.07a	1.01 ± 0.19b	0.65 ± 0.14c

Data are represented as mean ± SD. Mean with different letters in the same rows are significantly different (LSD, P < 0.05).

Digestive Enzyme Assay

α-amylase assay

Significant differences in α-amylase activity ($p<0.05$) were observed among the three treatment groups (Table 2). The gut α-amylase showed highest activity in larvae fed on castor (1.50 ± 0.045 U/ml) followed by the larvae reared on tapioca (1.15 ± 0.007 U/ml) and papaya fed larvae with the lowest gut α-amylase activity (1.04 ± 0.009 U/ml).

Proteinase assay

The gut proteinase activity assay revealed variations in the results in larvae fed with three types of food plants (Table 2). The larvae reared on papaya exhibited significantly higher gut proteinase activity (0.045 ± 0.004 U/ml) than other two food plants. The tapioca fed larvae showed moderate proteinase activity (0.035 ± 0.003 U/ml) whereas castor fed larvae was recorded with lowest gut proteinase activity (0.033 ± 0.001 U/ml).

Lipase assay

Among the three different food plants used for rearing significant differences ($p<0.05$) in gut lipase activity was observed (Table 2). The highest lipase activity was found in larvae fed with castor leaves (0.61 ± 0.012 U/ml). In tapioca fed larvae, the lipase activity was recorded as moderate (0.53 ± 0.059 U/ml) and the lowest gut lipase activity was observed in larvae fed with papaya plant leaves with activity (0.39 ± 0.011 U/ml).

Table 2. Digestive enzyme activities of eri silkworm larval gut extract reared on different food plants.

Food plants	amylase activity (U/ml)	Proteinase activity (U/ml)	Lipase activity (U/ml)
Castor	1.50±0.045a	0.033±0.001a	0.61±0.012a
Tapioca	1.15±0.007c	0.035±0.003a	0.53±0.059b
Papaya	1.04±0.009b	0.045±0.004b	0.39±0.011c

Data are represented as mean ± SD. Mean with different letters in the same column are significantly different (LSD, P < 0.05).

Discussion

The economic parameters of silkworm such as cocoon yield, shell weight and quality of silk, etc. are influenced by the type of food plant used for rearing silkworm larvae (Gangwar, 2010). In present study, significant variations in the results have been observed in three larval groups each fed on different food plants. The castor fed larvae showed better larval parameters such as shortest larval duration, better larval length, larval weight and silk gland weight as compared to larvae reared on tapioca and papaya food plants. The better larval parameters on castor fed larvae had also been reported by earlier workers (Kumar and Elangovan, 2010; Kumar and Gangwar, 2010; Rajadurai *et al.*, 2010; Borah *et al.*, 2020). This indicated that castor served as the primary food for

the proper growth and development of silkworm larvae. Kumar and Elangovan, (2010) reported that the larval duration of castor leaves fed larvae was shortest (19.25 d) compared to larvae fed with the leaves of tapioca (20.50 d) and papaya (22.00 d). They also observed maximum larval weight in castor fed larvae (7.38 g) compared to tapioca (6.45 g) and papaya (5.55 g) food plants. A similar trend of observations in larval duration and larval weight was also reported by Kumar and Gangwar (2010).

The type of food plants and their nutritional quality also affect the survivability of insects feeding on them. Sarate *et al.* (2012) observed that larval growths are affected significantly by the quality of food plants. Similarly the food plants taken under this study differ in terms of nutritional contents in their leaves (Deuri *et al.,* 2017; Ugo *et al.*, 2019). It is evident form these studies that the leaves of castor, tapioca and papaya show variations in the content of total carbohydrates, protein, fats, crude fibres and mineral elements. Such variations have subsequently affected the growth and development of silkworm larvae. Significant differences in the larval survivability were observed in present investigation. The castor fed larvae showed the maximum survival percent as compared to tapioca and papaya fed larvae.

The above findings are comparable with the reports of Birari *et al.* (2019) where significantly higher larval survivability, shorter larval duration and heavier mature larval weight were found on castor fed larvae (94.00%, 22.57 d and 7.65 g respectively) than tapioca (90.00%, 24.65 d and 6.57 g respectively). Similar observations indicating superior larval performances in castor fed larvae than larvae reared on tapioca was also reported by Rajadurai *et al.* (2010). Papaya plant leaves being tertiary food plant showed poorest growth parameters and lowest survivability rate in present investigation, suggesting that the papaya plant is not suitable for larvae in terms of growth and development. Similarly, the result of Konno *et al.* (2004) also reported high mortality of *S. ricini* larvae reared on papaya leaves. Their result clearly indicated that papaya leaves contain papain in latex which is responsible for strong toxicity against lepidopteran insects.

In polyphagus insects, digestion and absorption is correlated with the type and quality of food ingested which directly influence the overall physiology and biochemical condition. The type of nutrient source ingested effect the digestibility of the food ingested along with the marked variation in growth rate and efficiency of conversion of ingested food (Gururaj *et al.*, 2017). Significant differences were observed in digestive enzymes activities of eri silkworm larval gut. Castor fed larvae showed highest α-amylase and lipase activities than other two food plants whereas lowest activities of α-amylase and lipase were observed in larvae reared on papaya. However, the larvae reared on papaya also showed highest protease activity than the larvae fed on castor and tapioca. In present study, the higher activities of α-amylase and lipase in larvae fed on castor leaves was positively correlated with the better larval survivability whereas lowest activities of both the enzymes in papaya fed larvae was associated with poor larval growth parameters. Tapioca fed larvae showed moderate enzyme activities which was associated with moderate larval parameters. The higher activity of α-amylase may be associated with better digestion of carbohydrate rich leaves and better nutrient availability for the larval growth. This could have resulted in the better compatibility of eri silkworm with castor leave feeding habit which directly related to better growth and development of silkworm larvae fed on castor leaves followed by tapioca and papaya. This result is supported by the findings of (Namin *et al.*, 2014; Mendiola-Olaya *et al.,* 2000; Ahmadi *et al.*, 2012). They found that the effectiveness of amylases are important for the survival of the insect and thus influences establishment of compatible insect-host relationship.

Proteinase enzymes degrade the protein component of ingested diet and their activity depends on the protein content present in the leaves of host plants. In the present investigation, gut proteinase activity was found significantly higher in the larvae fed with papaya leaves as compared to tapioca and castor fed larvae.

Papaya leaf latex has been found to contain a significant amount of cystein protease, called papain (Konno *et al.*, 2004). The higher proteinase activity in the gut tissue of the larvae fed with papaya leaves could be due to the plant cysteine proteinase activity of papain present in the latex of papaya plant leaves.

The response of the insect to the ingested protease inhibitors (PIs), a defense strategy of plants may result in hyperproduction of proteases by the midgut cells (Namin *et al.*, 2014; Holtof *et al.*, 2019). Higher protease activity resulted in the higher larval mortality and the lowest values of larval growth index in papaya fed larvae. This finding was supported by the work of (Namin *et al.*, 2014; Pechan *et al.*, 2002). Such variations in the digestive enzyme activities in eri silkworm larvae may be due to differences in either dietary carbohydrate, protein, lipid and nitrogen contents in different food plants used for rearing larvae (Hemati *et al.*, 2012; Sarate *et al.,* 2012; Deuri *et al.,* 2017) or to the response of the insect to dietary enzyme-inhibitors.

The lipases plays a crucial role in digestion of lipid components of ingested food materials into simpler absorbable form. In the present investigation, higher lipase activity was recorded in castor fed larvae than other two host plants. This result might be due to the variation in the level of lipid content present in the food plants. Deuri *et al.* (2017) reported significantly higher level of lipid content in the leaves of castor leaves (10.95%) than the tapioca leaves (0.22%).

Lower lipid concentration in the ingested food resulted in lower digestive lipase activity in tapioca and papaya fed larvae. Thus, it is evident that dietary contents exert crucial role in activity levels of digestive enzymes in the insect host. This can be supported by the work of Namin *et al.* (2014) who reported that the digestive enzymes activities are correlated with presence of different concentration of nutritional components and plant metabolites which in turn correlated with developmental parameters of the insects.

Conclusion

This study revealed that different food plants i.e, castor, tapioca and papaya were found to exert different influences in overall physiology and development of *S. ricini* larvae. It was also prominent that the efficient digestive system was necessary for ensuring proper digestion and nutrient utilization by the host organism. From the above findings, we concluded that the castor was the most nutritive for eri silkworm rearing among the experimental food plants as supported by the higher activities of amylase and lipase which ensured better digestibility of the carbohydrate and lipid component. This finding will help in understanding the physiology of host nutrient acquisition from different food plants by regulating the digestive enzyme machinery. However, more detailed study may be carried out in terms of specific host-food plants relationship in order to properly understand the larval growth responses of silkworm to different food plants.

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