



Research Article

Impact of Probiotic-Fortified Mulberry Leaves on the Enzymatic Activity and Economic Traits of *Bombyx mori* L.

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Keywords

Bombyx mori, Probiotics, Mulberry leaves, Fortification, *Saccharomyces cerevisiae*, *Lactobacillus rhamnosus*



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Abstract | Nutritional supplements play a crucial role in enhancing the growth and development of silkworms (*Bombyx mori* L.). This study evaluated the effects of probiotic-fortified mulberry leaves on biological and economic parameters as well as the digestive enzyme activity in silkworms. Fresh mulberry leaves coated with *Saccharomyces cerevisiae* and *Lactobacillus rhamnosus* at concentrations of (0.5, 1, and 2%) were fed to 5th instar silkworm larvae. Larval weight was measured for 7 days. A portion of the larvae were dissected, and their silk glands and guts were separated. The gut homogenate supernatant was used for enzyme activity analysis. The remaining larvae were allowed to complete the cocoon formation. The obtained cocoons were then used to estimate the effects of yeast treatments on economic and biological parameters. The results showed that the average weight of silkworm larvae on the 7th day was significantly higher ($P < 0.05$) in larvae fed with 0.5% *L. rhamnosus* + *S. cerevisiae* compared to the control group. This group also exhibited the highest cocoon weight, length, width, shell ratio, and fibroin content. Moreover, the enzymatic activity was significantly higher in larvae fed with 0.5% *L. rhamnosus* + *S. cerevisiae*. It was concluded that the 0.5% *L. rhamnosus* + *S. cerevisiae* supplement promotes the enzymatic activity of silkworms, leading to positive effects on their economic and biological parameters.

Novelty Statement | Pakistan, an agrarian economy, has a significant potential in sericulture. This study aims to enhance the economic viability of sericulture by supplementing silkworms' diet with probiotics-enriched mulberry leaves. The resulting economic benefits associated with improved silkworm growth and silk quality could encourage wider adoption of sericulture, contributing to rural development and sustainable agriculture in the country.

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Introduction

The silkworm, *Bombyx mori* L. (Lepidoptera: Bombycidae) is an economically important and well-known monophagous lepidopteran insect that produces

industrial silk (Zamani *et al.*, 2019). Silkworms exclusively feed on mulberry leaves (*Morus* spp.), the foundation of the sericulture industry (Sajgotra *et al.*, 2018). The nutritional quality of these leaves significantly impacts the development and growth of silkworm larvae (Riaz *et al.*, 2020; Thakur *et al.*, 2022). Silkworms convert 72-80% of the proteins found in mulberry leaves into silk proteins (Qadir *et al.*, 2022). Consequently, the quality and economic value of cocoons are highly dependent on the silkworm's diet

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(Sajgotra *et al.*, 2018; Sudan and Bukhari, 2021). Although various arthropods, such as honeybees, beetles, silkworms and spiders produce silk, a long natural fibrous filament of protein (Saha *et al.*, 2019). However, silkworm silk fiber is highly valued due to its superior qualities like luster, softness, tensile strength, dyeing capacity, biocompatibility, and biodegradability (Lee *et al.*, 2020). Moreover, *B. mori* silk is one of the strongest natural fibers and is considered an ideal replacement for synthetic fibers (polyester and viscose) (Ude *et al.*, 2019).

In developing countries, silk production by local farmers is relatively lower than in China and India (Pandiarajan *et al.*, 2018; Rahim *et al.*, 2021). However, sericulture is a prospective and promising industry with significant economic potential (de Barcelos *et al.*, 2020). The higher selling prices of silk products and by-products can help rural people find employment and improve their socioeconomic situation (Agustarini *et al.*, 2020). Moreover, the sericulture industry is crucial in preventing rural-urban migration for job opportunities (Vishaka *et al.*, 2020). In recent years, initiatives have been implemented to improve the sericulture industry by increasing cocoon yield or silk production through the use of various nutrients, including proteins, carbohydrates, amino acids, vitamins, hormones, probiotics, plant extracts, and antibiotics (Gunasekhar and Somayaji, 2020; Qadir *et al.*, 2022).

The digestive system, which hosts millions of microorganisms, is the primary site of bacterial colonization (Cristofori *et al.*, 2021). Probiotics are beneficial microorganisms ingested through food or water to improve gut microbial balance and promote good health (El-Saadony *et al.*, 2021). Gut probiotics facilitate food digestion, stimulate the innate immune system, and detoxify metabolites (Soliman, 2021). These beneficial microorganisms are purposely introduced into the intestinal microflora to compete with and eliminate harmful bacteria (Pachiappan *et al.*, 2021).

Probiotic-enriched food has been introduced in the various farming industries to enhance economic output (Saranya *et al.*, 2019; Pachiappan *et al.*, 2021). Commercial probiotics contain microorganisms ranging from 10^8 to 10^{11} CFU/mL (Nasri *et al.*, 2023). While widely used in aquaculture and veterinary medicine, there are currently no commercial probiotic formulations particularly manufactured for the sericulture industry. This study aimed to determine the effects of *S. cerevisiae* and *L. rhamnosus*, and a combination of both probiotics on basic digestive enzymes (amylase and invertase) in the digestive tract of silkworms.

Saccharomyces cerevisiae and *Lactobacillus rhamnosus* were selected as probiotic supplements due to their synergistic effects and potential health benefits (Suharja

et al., 2014). These probiotics exhibit synergistic anti-pathogenic activity (Moens *et al.*, 2019) and inhibit aflatoxin production both *in vitro* and *in vivo* (Nada *et al.*, 2010). Moreover, probiotics also play a significant role in enzyme regulation, particularly for amylase and invertase activities. Certain *Lactobacillus* strains exhibit probiotic characteristics and modulate the production of amylase enzymes (Guimarães *et al.*, 2021). Invertase, a key enzyme in carbohydrate metabolism, is subject to complex regulatory mechanisms. In *Saccharomyces cerevisiae*, the URE2 gene influences invertase activity (Silveira *et al.*, 2000).

This study was aimed at enhancing the economic and biological growth parameters, as well as the amylase and invertase activity of silkworms, by providing them with probiotic-fortified mulberry leaves. Previously, the effects of probiotics supplemented feed on silkworm were not explored. Although probiotics were reported to improve enzymatic profile but that was not confirmed in silkworms. With this context in mind, the current research was conducted to evaluate the effect of probiotics on the enzymatic profile and economic aspects of the silkworm *B. mori* L. Improved silk production would not only fulfill local silk demands but also increase overall silk exports, benefiting developing agricultural economies through the advancement of the sericulture sector.

Materials and Methods

Silkworm eggs hatching and rearing

Chinese race silkworm (*Bombyx mori* L.) eggs were obtained from the Sericulture Wing of the Forestry, Wildlife, and Fisheries Department, Ravi Road, Lahore (31° 36' 28" N, 74° 18' 11" E). The eggs were incubated in sterile trays under optimal conditions: humidity (75±5%), temperature (25±1°C), and photoperiod (12 light: 12 dark). Before rearing mulberry silkworms, the laboratory and all rearing equipment were disinfected with a 4% formaldehyde solution (Gupta and Dubey, 2021). The laboratory was closed for 24 hours for proper disinfection and then kept open for 12 hours to release the formalin vapors. After hatching, newly emerged larvae were carefully removed from the hatching eggs using a fine hairbrush. The larvae were fed with freshly chopped mulberry leaves until the end of the 4th instar. The 5th instar larvae were selected as the experimental model for this study (Zulfiqar *et al.*, 2022; Muzamil *et al.*, 2023).

Preparation of probiotic suspensions

Probiotics (*Saccharomyces cerevisiae* and *Lactobacillus rhamnosus*) were added to 100 ml of deionized water in specific quantities to prepare various concentrations (0.5, 1, and 2%) of probiotic suspensions. A total of 400 larvae were used in each of the three replicates, divided into 10 groups: nine experimental groups (0.5% yeast, 1% yeast, 2% yeast,

Table 1: The details of experimental groups.

No	Group	Treatment
1	Control group	Fresh mulberry leaves
2	Yeast 0.5%	Leaves fortified with 0.5% yeast suspension
3	Yeast 1%	Leaves fortified with 1% yeast suspension
4	Yeast 2%	Leaves fortified with 2% yeast suspension
5	LR 0.5%	Leaves fortified with 0.5% <i>L. rhamnosus</i> suspension
6	LR 1%	Leaves fortified with 1% <i>L. rhamnosus</i> suspension
7	LR 2%	Leaves fortified with 2% <i>L. rhamnosus</i> suspension
8	L+Y 0.5%	Leaves fortified with 0.5% <i>L. rhamnosus</i> + yeast suspension
9	L+Y 1%	Leaves fortified with 1% <i>L. rhamnosus</i> + yeast suspension
10	L+Y 2%	Leaves fortified with 2% <i>L. rhamnosus</i> + yeast suspension

0.5% *L. rhamnosus*, 1% *L. rhamnosus*, 2% *L. rhamnosus*, 0.5% *L. rhamnosus* + yeast, 1% *L. rhamnosus* + yeast, 2% *L. rhamnosus* + yeast) and one control group (Table 1). Each group consisted of 40 larvae reared in separate cardboard boxes (30 × 30 × 5 cm). The control group received freshly picked mulberry leaves after washing and cleaning. For the experimental group, fresh, dirt-free, and healthy mulberry leaves were dipped in prepared probiotic solutions (1g of leaves/ml of probiotic suspension) for 5 minutes to prepare probiotic-fortified mulberry leaves. These fortified leaves were then shade-dried for 5 minutes to ensure proper and uniform probiotic absorption. The probiotic-fortified leaves were then fed to silkworms in the treatment groups to assess the individual and synergistic effects of the probiotics (Shakl and Essa, 2022).

Assessment of biological traits

The weight of all 5th instar silkworm larvae was observed daily using an electric weight balance until they began spinning or cocoon formation. Ten silkworms from each group were dissected to remove their silk glands and measure any potential change in silk gland weight caused by the addition of probiotics to their diet. The gut of larvae was also removed for the investigation of enzymatic activity.

Preparation of gut homogenate

The exposed guts of the silkworm was longitudinally dissected and thoroughly washed with ice-cold PBS (pH 7.4). The gut contents were removed, washed with PBS, and homogenized with chilled PBS using a mortar and pestle. The samples were centrifuged at 7000 rpm for 10 minutes at 25°C. The supernatant was collected to assess the enzyme activity of amylase and invertase (Esaivani *et al.*, 2014).

Quantitative assay of amylase

A standard calibration curve of known concentrations of starch was prepared through spectrophotometric absorptions at 540 nm using the method described by Kadam *et al.* (2021). DNS reagent (500 mL) was prepared

by using the method described by Zin *et al.* (2022) by using Dinitrosalicylic acid, distilled water, sodium hydroxide and potassium sodium tartarate-4-hydrate.

The activity of the amylase was measured at pH 7.4 and 27°C. Ten test tubes (one for each group) were used in each replicate, with each test tube containing 20 µL of gut/enzyme extract and 250 µL of 1% starch substrate solution. DNS reagent (250 µL) was added to each test tube, incubated for 60 minutes at 27°C, and then heated in a water bath for 10 minutes. When the samples cooled, optical density at 550 nm was measured using a spectrophotometer (Esaivani *et al.*, 2014). The OD values were compared to the standard curve of glucose to determine amylase activity.

Quantitative assay of invertase

The activity of invertase was measured at pH 7.4 and 27°C using a similar experimental setup. Each test tube received 20 µL of the homogenate supernatant sample (invertase solution) and 250 µL of the 4% sucrose solution before being incubated at 27 °C for 10 minutes. DNS reagent (250 µL) was added to each test tube and the mixture was heated in a water bath for 10 minutes. The cooled samples were analyzed for optical density at 550 nm using a spectrophotometer (Esaivani *et al.*, 2014; Taha *et al.*, 2017; Pachiappan *et al.*, 2021). The OD values were compared to the standard curve of glucose to determine invertase activity.

Assessment of economic trait

The remaining silkworms (n=30) from each group began spinning cocoons at the end of the fifth instar stage. After harvesting the cocoons, they were placed in sunshine to kill the moths inside and remove any moisture. The silk production of the treatment groups was measured and compared to the control group. An electronic weighing balance and a Vernier Caliper were used to calculate cocoon weight (g) and width (mm). The dried cocoons were sliced using a cutter. Dead pupae were removed, and the shell ratio and weight were calculated using the following methods:

$$\text{Shell weight} = \text{Cocoon weight} - \text{Pupal weight}$$

$$\text{Shell ratio \%} = \frac{\text{Shell Weight (gm)}}{\text{Cocoon Weight (gm)}} \times 100$$

(Saranya *et al.*, 2019)

Fibroin/sericin content

To determine the fibroin and sericin content, the cocoons were degummed by autoclaving them in a specific volume of water at 121°C for two hours (Tahir *et al.*, 2020). After degumming, the fibroin content was equivalent to the dry weight of the shell/filtrate. However, the sericin content was calculated using the following formula:

$$\text{Sericin content (mg)} = \text{initial weight of cocoons} - \text{dry weight of fibroin}$$

Statistical analysis

The data was analyzed using Statistical Package for the Social Sciences (SPSS) version 21.0. One-way ANOVA followed by Tukey's test was used to compare the increase in larval weight among various groups on a single day. Moreover, One-way ANOVA was used to compare the silk gland weight among experimental groups. The economic traits of silkworms as well as the enzymatic activities across various groups were also analyzed using One-way ANOVA.

Results

Evaluation of biological traits

The 5th instar larval weight of silkworms increased gradually from day 1 to 7 in both the control and experimental groups. On day 2, there were no significant differences in larval weight between the experimental and control groups ($F_{9, 390} = 2.973$; $P > 0.05$). However, a statistically significant increase in larval weight of all silkworm groups was observed from days 3 to 7. The most significant increase in larval weight occurred in the groups of silkworm fed on 1% and 0.5 % *L. rhamnosus*

+*S. cerevisiae* (L+Y) fortified mulberry leaves. On day 6, the larvae in these two groups showed significantly higher weight gain than the control group and the majority of treatment groups (Table 2).

All experimental groups showed a higher percentage ratio of silk gland to body weight ($F_{9, 90} = 23.882$; $p < 0.05$) than the control group. Silkworms fed with probiotic-coated mulberry leaves of 0.5% *L. rhamnosus* +*S. cerevisiae* exhibited the highest percentage ratio of silk gland to body weight (37.58 ± 1.421), followed by the 1% *L. rhamnosus* +*S. cerevisiae* treatment group (35.44 ± 1.249) (Figure 1).

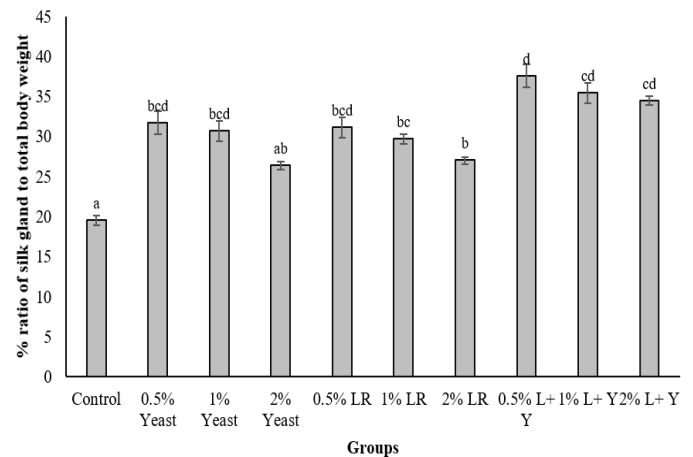


Figure 1: Percentage ratio of silk gland weight to total body weight of silkworm larvae in experimental and control groups.

Note: The bars with different superscripts are statistically significant ($P < 0.05$).

Evaluation of economic parameters

Significant differences ($F_{9, 190} = 8.985$; $P < 0.001$) were observed between the cocoon length of the treatment groups and the control group. Similar trends were observed for other economic parameters, including cocoon width, length, and weight. Furthermore, the silkworms fed with

Table 2: Larval weight (g) of silkworm in control and treatment groups.

Groups	Increase in weight (g)					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Control	0.166 ^a ±0.005	0.286 ^a ±0.004	0.379 ^a ±0.008	0.526 ^a ±0.007	0.669 ^a ±0.014	0.787 ^a ±0.018
Yeast 0.5%	0.190 ^{bc} ±0.003	0.303 ^a ±0.004	0.466 ^{cde} ±0.006	0.674 ^d ±0.007	0.869 ^{bcd} ±0.007	0.992 ^{cd} ±0.007
Yeast 1%	0.190 ^{abc} ±0.003	0.302 ^a ±0.004	0.463 ^{bcd} ±0.005	0.641 ^{bcd} ±0.008	0.854 ^{bc} ±0.007	0.965 ^{bcd} ±0.008
Yeast 2%	0.182 ^{ab} ±0.005	0.290 ^a ±0.005	0.442 ^{bcd} ±0.006	0.626 ^b ±0.010	0.830 ^b ±0.008	0.959 ^{bcd} ±0.008
LR 0.5%	0.185 ^{ab} ±0.004	0.327 ^a ±0.004	0.445 ^{bcd} ±0.007	0.631 ^{bc} ±0.009	0.865 ^{bcd} ±0.008	0.973 ^{bcd} ±0.008
LR 1%	0.176 ^{ab} ±0.004	0.298 ^a ±0.002	0.439 ^{bc} ±0.008	0.622 ^b ±0.009	0.849 ^{bc} ±0.008	0.949 ^{bc} ±0.008
LR 2%	0.179 ^{ab} ±0.005	0.290 ^a ±0.008	0.425 ^b ±0.007	0.614 ^b ±0.008	0.841 ^{bc} ±0.008	0.929 ^b ±0.009
L+Y 0.5%	0.200 ^{bc} ±0.004	0.307 ^a ±0.002	0.491 ^{ef} ±0.005	0.689 ^{de} ±0.007	0.884 ^{cd} ±0.010	1.063 ^{ef} ±0.014
L+Y 1%	0.210 ^c ±0.003	0.309 ^a ±0.003	0.510 ^f ±0.006	0.717 ^e ±0.004	0.914 ^d ±0.006	1.108 ^f ±0.06
L+Y 2%	0.197 ^{bc} ±0.003	0.304 ^a ±0.002	0.482 ^{def} ±0.005	0.657 ^{bcd} ±0.005	0.873 ^{bcd} ±0.005	1.020 ^{de} ±0.007
$F_{(9,390)}$ P_{ANOVA}	10.845 $P < 0.001$	2.973 $P > 0.05$	31.325; $P < 0.001$	47.531 $P < 0.001$	56.680 $P < 0.001$	73.508 $P < 0.001$

Note: Means ± SE with different superscripts in the same columns are statistically significant ($P < 0.05$).

Table 3: Comparison of economic traits of silkworm in control and experimental groups.

Groups	Cocoon weight (g)	Cocoon length (mm)	Cocoon width (mm)	% Shell ratio	% Fibroin ratio	% Sericin ratio
Control	0.40 ^a ±0.010	27.57 ^a ±0.344	15.76 ^a ±0.176	41.51 ^a ±1.409	69 ^a ±0.490	31 ^f ±0.490
Yeast 0.5%	0.63 ^{bc} ±0.021	29.76 ^{abc} ±0.355	16.81 ^{ab} ±0.162	47.27 ^{ab} ±1.298	75 ^{cd} ±0.632	25 ^{bcd} ±0.632
Yeast 1%	0.60 ^b ±0.023	29.59 ^{abc} ±0.337	16.49 ^{ab} ±0.239	47.07 ^{ab} ±1.399	75 ^{cd} ±0.283	25 ^{bc} ±0.283
Yeast 2%	0.55 ^{bc} ±0.010	29.45 ^{abc} ±0.369	16.42 ^{ab} ±0.179	45.27 ^{bc} ±1.832	72 ^{abc} ±0.490	28 ^{def} ±0.490
LR 0.5%	0.57 ^{bcd} ±0.013	29.46 ^{abc} ±0.379	16.45 ^{ab} ±0.231	46.49 ^{abc} ±1.892	73 ^{bc} ±0.400	27 ^{cde} ±0.400
LR 1%	0.54 ^{bcd} ±0.022	29.44 ^{abc} ±0.293	16.14 ^{ab} ±0.242	43.58 ^{bc} ±2.498	72 ^{abc} ±0.632	28 ^{def} ±0.632
LR 2%	0.52 ^b ±0.022	28.35 ^{ab} ±0.237	15.99 ^a ±0.109	41.89 ^{bc} ±1.890	71 ^{ab} ±0.400	29 ^{ef} ±0.400
L+Y 0.5%	0.71 ^d ±0.019	31.20 ^c ±0.367	17.54 ^b ±0.278	50.61 ^c ±0.724	82 ^e ±0.400	18 ^a ±0.400
L+Y 1%	0.67 ^{cd} ±0.020	30.88 ^c ±0.333	16.98 ^{ab} ±0.237	49.16 ^{bc} ±1.770	81 ^e ±0.522	19 ^a ±0.522
L+Y 2%	0.65 ^{bcd} ±0.022	30.18 ^{bc} ±0.345	16.87 ^{ab} ±0.310	47.95 ^{bc} ±1.793	77 ^d ±0.632	23 ^b ±0.632
ANOVA	F _{9, 190} = 7.812; P<0.001	F _{9, 190} = 8.985; P<0.001	F _{9, 190} = 19.790; p<0.001	F _{9, 190} = 10.311; P<0.001	F _{9, 40} = 58.265; P<0.001	F _{9, 40} = 52.139; P<0.001

Note: Means ± SE with different superscripts in the same columns are statistically significant (P < 0.05).

0.5% *L. rhamnosus* + *S. cerevisiae* registered the maximum cocoon weight (0.71±0.019), length (31.20±0.367), and width (17.54±0.278) among all study groups. The shell ratio of the treatment groups was significantly higher (F_{9, 190} = 10.311; P < 0.001) compared to the control group. The shell ratio of the 0.5% *L. rhamnosus* + *S. cerevisiae* treatment group (50.61±0.724) was the highest compared to other groups (Table 3).

The percentage of sericin content was significantly higher in the control group (F_{9, 40} = 52.139; P < 0.001) compared to the treatment groups. The control group exhibited the highest sericin percentage (31±0.490), while the groups of silkworm cocoons fed with 0.5% and 1% *L. rhamnosus* + *S. cerevisiae* treated mulberry leaves showed the lowest percentage of sericin content (18±0.400 and 19±0.522, respectively) (Table 3). The percentage of fibroin in the control and experimental groups showed the opposite trend to the percentage of sericin. The fibroin content in the treatment groups was significantly higher (F_{9, 40} = 58.265; P < 0.001) compared to the control group. The 0.5% *L. rhamnosus* + *S. cerevisiae* treatment group revealed the highest percentage of fibroin content (82±0.400), followed by the 1% *L. rhamnosus* + *S. cerevisiae* treatment group (81±0.522) (Table 3).

Enzymatic activity

The enzymatic activities of both amylase and invertase in the probiotic treatment groups were significantly higher (p<0.001) than in the control group. It was highest in the silkworms fed with 0.5% *L. rhamnosus* + *S. cerevisiae* probiotic-fortified mulberry leaves (Figure 2).

Discussion

The mulberry leaves diet significantly affects the growth, development, and subsequent cocoon production

of silkworm (*B. mori* L.) (Sarkar *et al.*, 2023). The present study aimed to access the digestive enzymatic activity, as well as the commercial and biological traits of silkworms fed on probiotic-fortified mulberry leaves. Previous studies have reported similar effects of mulberry leaf fortification with various substances, such as whey proteins (Abdel-Rahman, 2018), egg albumin (Islam *et al.*, 2020), sericin (Qadir *et al.*, 2022), amino acids (Radjabi, 2010), multivitamins (Khedr *et al.*, 2013), and royal jelly (Gomaa, 2010), on the enrichment of biological and commercial traits of silkworms. The obtained result showed that enzymatic activity (amylase and invertase) was enhanced when the silkworms were fed on fortified mulberry leaves. Moreover, significant improvements were observed in the economic and biological parameters of silkworms fed with probiotic-fortified mulberry leaves.

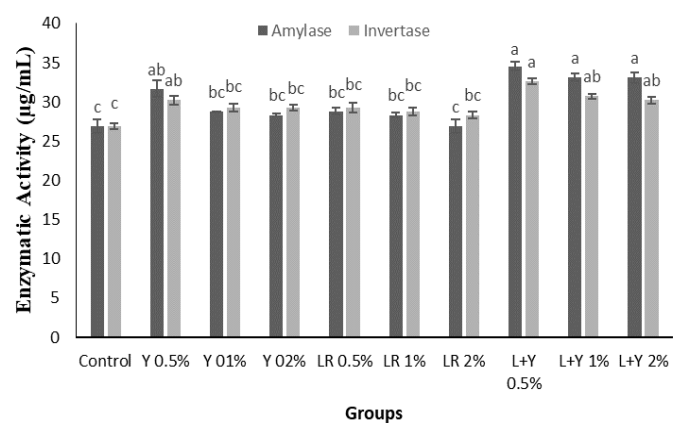


Figure 2: Enzymatic activity of amylase and invertase in control and experimental groups.

Note: The bars of the same enzyme with different superscripts show significant differences (P < 0.05).

The larval weight of 5th instar silkworms increased when treated with different concentrations of probiotics

(*S. cerevisiae* and *L. rhamnosus*). Similar results were observed when silkworms were fed with probiotics including *Saccharomyces cerevisiae* (Esaivani *et al.*, 2014), *Spirulina platensis* (Ashoka *et al.*, 2019), *Lactobacillus acidophilus*, *Lactobacillus sporogens* (Masthan *et al.*, 2017), *L. rhamnosus*, *Saccharomyces boulardii*, *Bifidobacterium longum* (Pachiappan *et al.*, 2021), and *Bifidobacterium bifidum* (Taha *et al.*, 2017). The highest increase in weight was recorded when silkworms were fed with 0.5% *S. cerevisiae* + *L. rhamnosus* (L+Y) fortified mulberry leaves. The reason behind this increased growth and body weight of silkworm is that the probiotics are well recognized growth promoters and immune modulators. Moreover, their growth promoting ability has been tested against various economically important animals (Angelakis, 2017).

The enzymatic activity (amylase and invertase) of silkworms fed with probiotic-fortified mulberry leaves varied significantly compared to the control group. Among the different concentrations used, 0.5% of the L+Y treatment had the most significant impact on the enzymatic activity of both amylase and invertase. Esaivani *et al.* (2014) also reported a similar increase in enzymatic activity when fed with *S. cerevisiae*. Pachiappan *et al.* (2021) have reported similar results with *Lactobacillus rhamnosus*. Furthermore, the probiotics supplements produce digestive enzymes and upregulate the enzymatic activities (Assan *et al.*, 2022).

The application of probiotics supplements (*S. cerevisiae*, *L. rhamnosus*, or a combination) on silkworms positively impacted their enzymatic profile, leading to improved commercial characteristics of the cocoon. The results showed that probiotics supplementation enhanced economic parameters, including cocoon weight, width and length, shell ratio, and sericin fibroin content). Silkworms, fed with 0.5% L+Y had the best economic parameters among all experimental and control groups. Ashoka *et al.* (2019) reported enhanced economic parameters with *S. cerevisiae*, and Pachiappan *et al.* (2021) reported similar results with *L. rhamnosus*.

The increased economic parameters of silkworms are attributed to the increased growth rates and activities of digestive enzymes (amylase and invertase), which are directly related to the gut microbiota influenced by the digestive process of the silkworm. Among commercially available probiotics, *S. cerevisiae* is the most widely accessible and cost-effective (Albrektsen *et al.* 2022). A single 30 g packet of commercial yeast is sufficient to feed 1000 silkworms. While *S. cerevisiae* and *L. rhamnosus* are readily available, other probiotics are often found in combinations and may not be easily available in rural areas where sericulture is prevalent.

Conclusions and Recommendations

Here it is concluded that the probiotic-fortified mulberry leaves can significantly enhance the biological and commercial traits of silkworms. The combination of *S. cerevisiae* and *L. rhamnosus* (at a concentration of 0.5%) appears to be particularly effective in improving growth, development, and economic parameters. These findings highlight the potential benefits of using probiotics in the sericulture industry.

Declarations

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Ethical statement

The study was conducted after Ethical Approval from Bioethical Committee Government College University Lahore, Pakistan.

Declaration of generative AI and AI-assisted technologies in the writing process

No generative AI and AI-assisted technologies were used in the writing process.

Conflict of interest

The authors have declared no conflict of interest.

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