



Associative Effects of *Rhodotorula glutinis* Yeast x Mulberry Leaves on the Morpho-Productive Parameters of Two Breeds of Silkworms *Bombyx mori* L.

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RESEARCH ARTICLE

Abstract

The nutritional value of mulberry leaves is correlated with the morpho-productive performances of silkworms *B. mori*. *Rhodotorula glutinis* is an oleaginous microorganism that may produce a wide range of metabolites. There is no evidence on using this yeast to silkworm. We aimed to assess the morpho-productive performance of RG90 and Maritza III silkworm breeds fed *Rhodotorula glutinis*. A bifactorial trial (3x2) was running on 600 larvae belonging to RG90 and Maritza III breads. The measurements were done on first, 5th, 7th, and 9th days (D) during 5th instar and after cocoon formation. Larvae were randomly distributed in 3 groups (2 replicates): 1) C group fed mulberry leaves; 2) E1 fed C diet and yeast 1x10⁷; 3) E2 fed C diet and yeast 1x10⁹. The larva characteristics tend to be impacted by the breed (>1.03 times higher on Maritza III. Compared to first D, a high significantly increase of larva was noticed in D9 (>1.74 times). The silk gland was positive correlated with larva length (r=0.76, P<0.0001) and larva weight (r=0.61, P<0.0001), and depend significantly of breed. The breed influence significantly certain cocoon parameters. We demonstrated that the mulberry leaves with yeast allow to silkworm to express their morpho-productive potential.

Keywords: Cocoon; larvae; *Rhodotorula glutinis*; silkworm, yeast.

INTRODUCTION

Mulberry leaves, known as a single source of feed for silkworm *Bombyx mori*, due to morin, a flavonoid compound with attractive characteristics, provide to silkworm's larvae all the nutrients need for growth and development. The quality of mulberry leaves has a major impact on the morpho-productive characteristics of silkworm larvae (Taha et al, 2017). Mulberry leaves are digested and assimilated by the silkworm to meet its nutritional requirement, which include proteins, water, vitamins, carbohydrates, lipids, and ascorbic acid (Muzamil et al, 2023). The modern approaches have been evaluated to increase the biological and economic properties of the silkworms, including larval growth, cocoon size and shell weight, and the quality of silk produced. The effects of nutritional supplements on economic indices, illness resistance, and the molecular structure of a protein in silkworms have all been studied. Masthan et al, (2011) explored enriching mulberry leaves with amino acids and antibiotics. Supplements including vitamins such as folic acid, ascorbic acid, thiamin, niacin, and multivitamins are also utilized for improving silkworm performance (Joyce and

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Sabura, 2021). In several studies, have been shown that probiotics such as *Saccharomyces cerevisiae* (*S. cerevisiae*) in combination with mulberry leaves can impact the economic parameters of *B. mori* larvae in their fifth instar (Esaivani et al. 2014, Yadav and Bagdi, 2016, Taha et al. 2017, Hăbeanu et al. 2024). According to Masthan et al. (2011) a treatment containing 300 ppm yeast greatly improve the weight of the pupal, the length of the silk thread, and the cocoon features. *S. cerevisiae* has several functions in silkworms, including immunomodulation properties (Esaivani et al. 2014). Thus, *S. cerevisiae* enhance the weight of the cocoon, pupae and shell weight, as well as the shell ratio, filament length and reeling of the silk (Esaivani et al. 2014 cited by Hăbeanu et al. 2024). In turn, Yadav and Bagdi (2016) investigated the effects of *S. cerevisiae* yeast on the growth performance of Eri silkworms. They observed an important impact on the larval characteristics, with noticeable differences between the 300 ppm and 100 or 200 ppm concentrations.

On the other hand, Taha et al. (2017) conducted an experiment in which two silkworm hybrids received mulberry leaves supplemented with probiotics consisting of bacteria *Bifidobacterium bifidum* and *S. cerevisiae*. Furthermore, a technique of larval rearing they implemented in order to protect *B. mori* from microbial disease attacks and to boost the production of cocoons. *Bifidum* and *S. cerevisiae* were found to improved cocoon characteristics and silk filament, especially *Bifidum*. Taha et al. (2017) demonstrated hybrid influence on the productive parameters of silkworms. According to Shruti et al. (2019) mulberry leaf-based silkworm diets supplemented with spirulina, Azolla, yeast and soy milk have potential to alter larval growth and development parameters. In 2020, Abdelmegeed used mulberry leaves enriched with soybean meal and *S. cerevisiae* yeast at different concentrations to increase silkworm cocoon productivity and investigate their impact on fecundity and fertility. A year later, Soliman demonstrated that mulberry leaves mixed with two yeast extract and the blue-green algae *S. platensis* have beneficial influence on productive parameters of the larvae and cocoon of *B. mori*.

On the other hand, *Rhodotorula glutinis* (*R. glutinis*) yeast is a microorganism distinguished by a high protein, fat, and vitamin content and may produce a wide range of metabolites, including carotenoids, and enzymes (Kot et al. 2016).

Barnett (2004) emphases that Harrison, a Canadian microbiologist, introduced the term *Rhodotorula* when researching a variety of yeasts discovered in local cheeses in 1930s. The terms "*rhodos*" (red) and "*torula*" (feminine diminutive form of Neo-Latin torus, bulge) are the sources of the genus name (Kot et al. 2016). *R. glutinis* is included in the order Sporidiobolales, class Microbotryomycetes and phylum Basidiomycota.

Rhodotorula species, which were once considered to be nonpathogenic, have turned shown to be opportunistic pathogens that can colonize and infect vulnerable people, mainly immunoconmpromised patients (Wirth and Goldan, 2012, Jochum and Stecher's, 2020). However, Hof (2019) mentioned previously that doubts exist, regarding whether the gut is the primary source of all of the opportunistic infections. A significant presence in the gut suggests that the microbial flora's equilibrium has been compromised for a variety of reasons. Nonetheless, the colonization of the host organism with these yeasts may have health-promoting effects. *R. glutinis* can synthesize varying amounts of nutrients, β -carotene, torulene, and torularhodin (Latha et al., 2005). Thus, according to Jochum and Stecher's (2020) opinion in specific situations, certain microorganisms that are categorized as pathobionts can also benefit their host.

As far as we know there are no evidence on using this yeast to silkworm *B. mori* nor any information regarding its possible pathogenicity on insects. Beyond the fact that *R glutinis* was introduced in the category of pathobionts for certain species and under certain conditions, the physiological and structural differences exist between insects and other species along with the fact that they undergo rapid metamorphosis and molt. The hypothesis of the present study starts from the premise, *R. glutinis* will enable silkworms to manifest their productive potential.

The objective of this study was to assess the potential of *R. glutinis* yeast to fortify the nutritional qualities of mulberry leaves used for feeding two breeds of silkworm *B. mori* (RG-90 which belong to monovoltin type, and Maritza III , bivoltin type). The morpho-productive performances such as larvae weight and length, silk gland weight, and consequently row cocoon, pupa and shell weight, longitudinal and transverse axes and their ratio, shell proportion and the productivity were determined as well.

MATERIALS AND METHODS

Biological material

Two breeds, Maritza III and RG-90, were evaluated. The RG-90 breed belong to monovoltine type and was obtained in Romania in 2002. This breed is characterized by gray-green eggs, a zebra larvae and yellow belted cocoons. The Maritza III which belong to bivoltine breeds, is originated from Bulgaria, and is characterized by metallic gray embryonic eggs, vigorous larvae exhibiting larval signs, a pronounced appetite, average values for biological and technological characteristics, and a white belted cocoon.

R. glutinis yeast was provided from Culture Collection of Yeast (CCY) from Bratislava and was conserved in the Intern Collection of IBNA Balotesti-Romania, within the Laboratory of Animal Nutrition and Biotechnology.

In order to explore the influence of the yeast, two phases were required: (1) *in vitro* testing at the Animal Nutrition and Biotechnology Laboratory, IBNA - Balotesti; (2) *in vivo* testing at RSS Baneasa- Bucharest.

***In vitro* testing**

To maintain the culture conditions, the yeast was activated for 24-48 h at 28°C, 150 rpm (in a shaker incubator) under aerobic conditions and refreshing at least three times in yeast-peptone-dextrose (YPD) broth medium (Himedia M1365). The YPD broth contained in a 1:10 ratio (w/v) the following components: 2% peptic digest of animal tissue, 1% yeast extract, and 2% dextrose dissolved in distilled water. Prior to sterilization at 121°C for 15 min., the pH was adjusted to 6.5±0.2. Following incubation, the purity of the yeast culture was verified using YPD agar plates. Physiological traits of the yeast were assayed based on morphology of the colony. The optical density (OD) at 600 nm colony-forming units (CFU) per mL were determined in triplicate. Under the microscope analyses, *R. glutinis* cells are gram-positive, so they will stain purple on Gram stain, small, oval, mostly in different stages of budding.

***In vivo* testing**

A bifactorial experiment (2 breeds x 3 diets) was carry out in 2024 at RSS located in Baneasa in the north side of Bucharest, 44°29'33"N 26°04'45"E, during 5th instar (9 D). A number of 600 larvae of silkworm *B. mori* belonging to RG-90 and Maritza III breeds, diseases free, rearing under hygienic conditions, were kept at 24–26°C and 70%–80% relative humidity in order to evaluate biological and productive traits.

Experimental design

Larvae were randomly assigned in 3 groups with 2 replicates each one: 1) Control (C group) fed mulberry leaves; 2) Experimental 1 (E1 group) fed C diet plus *R. glutinis* yeast concentration 1×10^7 ; 3) Experimental 2 (E2 group) fed *R. glutinis*, concentration 1×10^9 added to the mulberry leaves.

Every morning, a specific quantity of leaves (100 g per group) was subjected to the yeast treatment by spraying with 20 mL solution with different concentration. On the following meal shots x leaves were administered based on *ad libitum* basis throughout the subsequent feeding. The larvae health status was monitored every day.

Treatment and Measurements

The solution was prepared (1 L with the known concentration of *R. glutinis*).

Every morning, before the first feeding of the day, 8:00 and 9:30h, throughout 9D, 20 mL of known concentration solution was used, depending on the silkworm fed group. 100 g of mulberry leaves (50 g/ replicate) were sprayed at room temperature, and left for 30 min., after which it was dried. During the 5th instar, twenty larvae (10 per replicate) were randomly selected for the measurements of the biological characteristics (larvae weight and length) on 1stD, 5thD, 7thD and 9thD.

In order to determine whether the addition of *R. glutinis* yeast to the diet affect the weight of the silk glands, on 5, 7 and 9 days, prior to spinning, 4 silkworms per group (2 within each replicate) were dissected and the silk glands were removed for weighting it.

From each experimental group, twenty cocoons (10 / replicate) were randomly selected, and the raw cocoon, pupa and shell weight, longitudinal (L) and transverse axis (l), and their ratio were assessed after the cocoons were gently opened with a cutter for remove pupae. An electronic scale and digital calliper were utilized for this.

For shell ratio, the Sekar et al. (2016) formula was used: S.R.% = weight of cocoon shell/weight of cocoon x 100, while for silk productivity (cg/day) we adopting the formula of Saranya et al. (2019): shell weight / duration of 5th instar (9D).

Chemical analyses

The analyses were performed in duplicate at the Chemistry Laboratory of IBNA Balotesti. The gross chemical composition was determined using standardised methods according to Commission Regulation (EC) no. 152 (2009). Briefly, for crude protein, a semiautomatic classical Kjeldahl method was used (Auto 1030 Analyzer, Tecator Kjeltex, SR EN ISO 5983-2, 2009). The fats (ether extractives) were performed according to SR ISO 6492, (2001), using an adapted version of the classical procedure, which involved continuous solvent extraction followed by fat measurement using Soxhlet after the solvent was removed. The cellulose was extracted using an intermediate filtering method according to European Commission (EC) Regulation no. 152 (2009), standard SR EN ISO 6865:2002. Dry matter (ISO 6496/2001) and ash (ISO 2171/2010) were determined by gravimetric method.

Statistical analysis

The recorded data were statistically analyzed by using the Statistical Package SPSS software, version 20 (2011), General Linear Model (GLM) multivariate test. The Least Significant Difference (LSD) test was used to determine

the differences between the means. Response data, expressed as the mean and standard deviation (SD), were displayed. The applied treatments and breeds were considered the fixed influencing factors. Every larva and cocoon were regarded as experimental units.

In GLM (SPSS software), the repeated measurements test was employed for statistical testing of the variations resulting from the measurement in different time points. The Pearson correlation was applied to assess the relationship between various characteristics. Variations were considered significantly or highly significantly when P values < 0.01; 0.001, or 0.0001, significantly if $P \leq 0.05$, and tendency was considered at $P < 0.10$. Regression analysis was used to determine the statistical measure of the proportion of variance in the dependent variable that can be explain by an independent variable.

RESULTS AND DISCUSSIONS

The present investigation explored the biological and productive characteristics of *B. mori* following their feeding with mulberry leaves enriched or not with yeast *R. glutinis* two different concentrations.

In vitro test

The yeast morphology involved a rapid growth with red-colored colonies on YPD agar plate (Figure 1).

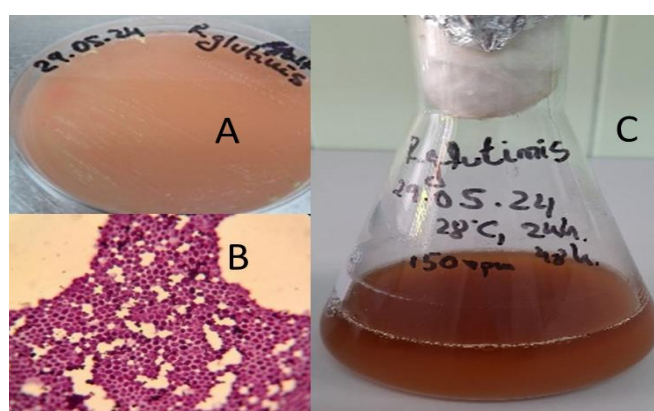


Figure 1. (A) Pure culture of the yeast strains *Rhodotorula glutinis* CCY 020-002-033 on YPD agar. (B), Micro-morphology of RY1801 observed under 100× with Gram staining. (C) YBD broth liquid culture of RG.

The initial viable cells count was 11.45 Log₁₀ which was adjusted to about 1×10^9 CFU /mL and 1×10^7 CFU /mL for subsequent experiments.

In vivo test

The chemical composition of mulberry leaves is shown in Table 1.

Table 1. Chemical composition of the mulberry leaves (% DM)

Group*	DM	CP	EE	CEL	Ash
C	25.44	25.79	1.79	13.67	12.34
E1	25.29	24.59	2.51	15.24	11.24
E2	28.33	25.10	2.49	15.41	13.61

Note: *abbreviation: DM, dry matter, CP, crude protein, ether (crude fat), Cel, cellulose.

The leaves in the E2 fed group contained a higher amount of dry matter (DM), which could be explained by the way in which the treatment with yeast was applied and then dried.

Morpho-productive parameters of the larvae and cocoons

The main factors influencing the silkworm *B. mori* performance are diet and nutritive value of the diet. Although mulberry leaves have a great nutritional potential for *B. mori*, recently many studies were directed to find new solutions to fortify its value (Yadav and Bagdi, 2016; Sekar et al., 2016; Saranya et al. 2019; Abdelmegeed, 2020). Nguku et al. (2007), obtained favorable effect on the production parameters by fortifying mulberry leaves with royal jelly. Saranya et al. (2019) improved economic factors and larval growth of *Bombyx mori* L. by using probiotic

strains, specifically *Staphylococcus arlettae* BMGB 17 and *Staphylococcus gallinarum* SWGB 6 extracted from the silkworm gut.

Our findings showed that by fortifying the mulberry leaves with microorganism *R. glutinis* yeast the performances of the silkworms were favourable modified, regardless the breed (Table 2). In both experimental fed groups final weight and average daily gain (ADG) of the larvae recorded a higher values compared to C diet ($P < 0.0001$). Such as, *R. glutinis* addition determined an increase of the final body weight with 14.78% in E1 group compared to C fed group and 12.89% higher in E2 group compared to C on RG-90 breed, as well as 10.72% in E1 vs. C diet, respective 10.64% in E2 vs. C on Maritza III breed. Regarding the ADG we noticed a similar trend: i) in RG-90 breed, the ADG was 21.16% higher in E1 than C group, 16.06% higher in E2 vs. C; ii) in Maritza III breed the ADG was 14.16% higher in E1 vs. C and 12.97% E2 vs. C group. A strong coefficient correlation was between body size regardless the breed ($R > 0.88$).

Table 2. Effects of supplementing mulberry leaves with *R. glutinis* (1×10^7 or 1×10^9) on productive parameters of RG-90 and Maritza III breeds

Parameters*	RG-90			MARITZA III			SEM	P-value**	
	C	E1	E2	C	E1	E2		Diet effect	Breeds effect
Initial weight (mg)	868	833	903	776	756	795	10.64	NS	<0.0001
Final weight (mg)	3334	3827	3764	3831	4242	4239	48.48	<0.0001	<0.0001
ADG (mg)	274	332	318	339	387	383	5.70	<0.0001	<0.0001
Initial length (mm)	39.54	39.30	40.69	39.38	37.57	37.49	0.24	NS	<0.0001
Final length (mm)	63.85	65.38	67.08	69.57	71.66	69.58	0.30	0.034	<0.0001

Note: * ADG, average daily gain.

** Significant differences; (LSD test, $p < 0.05$); Highly significant difference (LSD test, $P < 0.0001$); non-significant differences (NS, $P > 0.05$).

While on RG-90 breed, the larvae length before cocoon spinning recorded a higher value in group fed mulberry leaves supplemented with *R. glutinis* 1×10^9 , on Maritza III larvae the more pronounced effect was observed in group fed mulberry leaves fortified with *R. glutinis* 1×10^7 ($P = 0.034$). Our findings on the body weight of the larvae in the Maritza III breed were higher than those reported by Sekar et al. (2016), however the results in the RG-90 breed were comparable. In comparison to the results of supplementing mulberry leaves with *Lactobacillus casei* (*L. casei*) by Sekar et al. (2016), significantly greater values were observed for both of our breeds.

In comparison to the RG-90 breed, following the 1stD of the 5th instar up to 9D, the Maritza III breed showed a more noticeable linear increase in larval weight and length (Figure 1A and Figure 1B). Before cocoon spinning phase, in RG-90, the weight and length of the larvae increased 4.18 times respectively 1.64 times, whereas in Maritza III breed a 5.25-fold rise in weight and a 1.84-fold increase in length was noticed ($P < 0.0001$). The average values are closed in D7 vs. D9, possible as a result of the continuation of the metamorphosis process, namely the transformation of the larva into a pupa and subsequently molting.

The repeated measure test highlighted a higher significance interaction between breed x time and breed x diet.

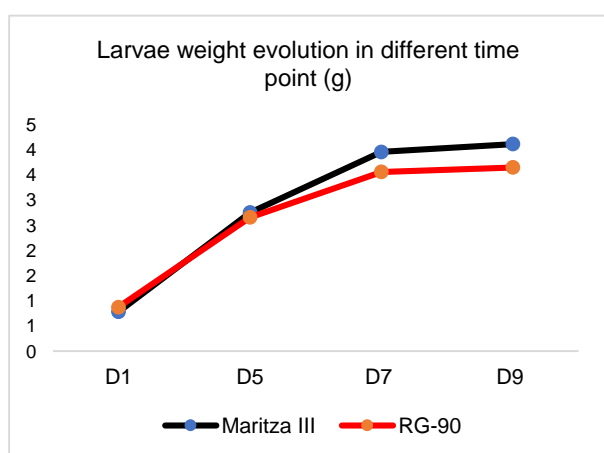


Figure 1 A) Larvae weight changes in time.

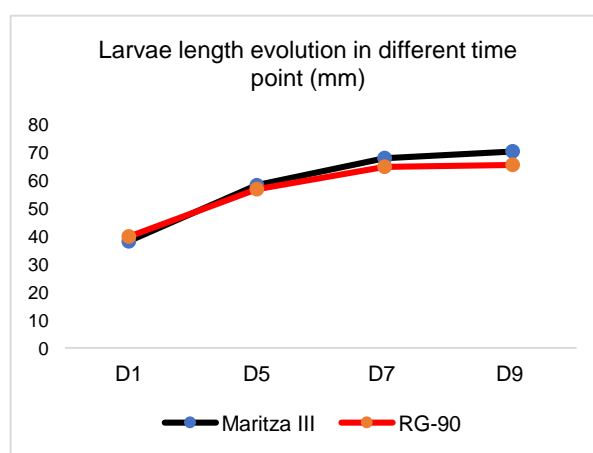


Figure 1 B) Larvae length changes in time.

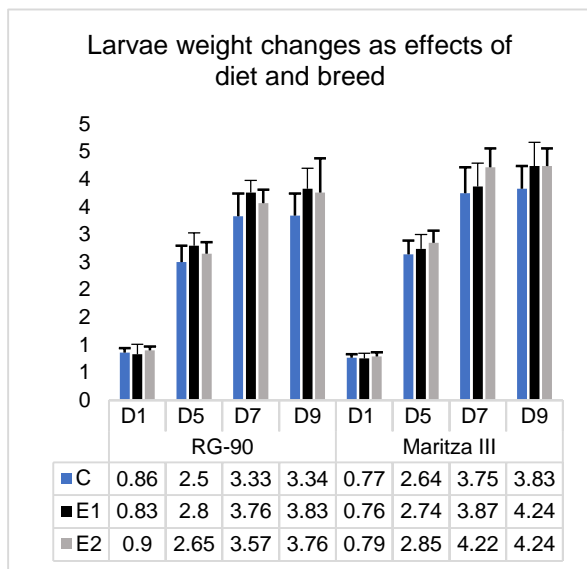


Figure 2 A) Larvae weight depending of diet in the RG-90 and Maritza III breeds at different times points.

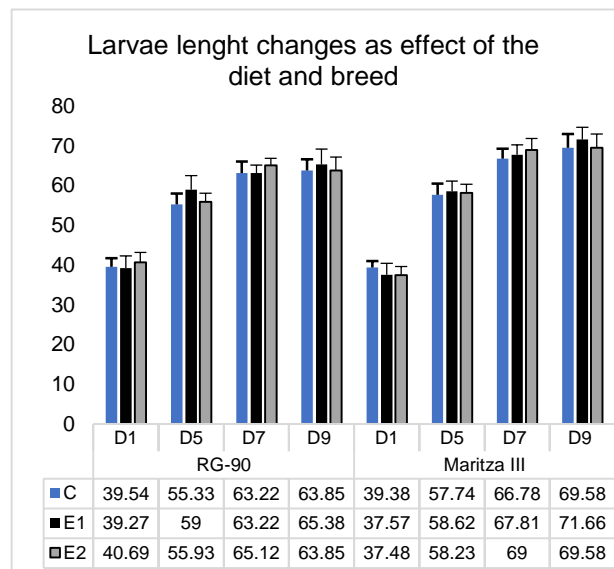


Figure 2B) Larvae length depending of diet in the RG-90 and Maritza III breeds at different times points.

In RG-90 breed in group C on D5 the larvae weight was 2.87 times higher, and in D7 3.83 times higher compared to D1. A more pronounced effects had E1 diet in which weight was 3.73 times higher in D5, respectively 4.53 times in D7. Unexpectedly the effect in group E2 was less intense than in E1, although it was still more noticeable than in C, the weight being 2.94 times higher in D5, respectively 3.96 times higher in D7 vs. D1. The length evolution in RG-90 was similar to weight, respective: i) in group C length was 1.40 times higher in D5, 1.59 times higher in D7, respective 1.61 times higher vs. D1; ii) in E1 group in D5 1.50 times higher, D7 1.61 times higher and in D9 1.66 times higher compared to D1; iii) in E2 group D5 the increase was 1.37 times, in D7 1.60 times and in D9 1.57 times vs. D1 (Figure 2A and Figure 2B). On breed Maritza III, on D5 compared to D1 weight gain was 3.40 times higher in group C, 3.62 times higher in E1 and 3.58 times higher in E2 group. In D7, the weight value recorded was 4.82 times higher in C group, 5.12 times higher in E1 group and 5.30 times higher in E2 group. In group C, the weight at D9 was 4.91 times higher than D1, in group E1 it was 5.58 times higher, and in group E2 5.3 times higher ($P < 0.0001$). Regarding the average length of larvae (Figure 2B) during 5th instar, in group C in D9 increased 1.77 times compared to D1, in group E1 by 1.91 times, and in group E2 by 1.86 times.

As seen in the Figure 3, the silk gland weight, recorded a higher value in Maritza III breed regardless of the diet. While on D9 the silk gland had a greater weight in group E2, in D5 and D7 the highest value was observed in E1 on RG-90 breed. In Maritza III breed in D7 and D9 the mean values of silk gland were similar. While breed influenced higher significantly the silk gland weight, the diet no significantly impacted, although slight increase was noticed. Our data are in agreement with that presented by Radjabi (2010) by supplemented mulberry leaves with amino acids, but were not similar with that obtained by Muzamil et al. (2023). Thus, Muzamil et al. (2023) showed that at 1–7D of the 5th instar, the percentage ratio of silk gland weight to body weight of larvae fed mulberry leaves enriched with amino acids was considerably higher than that of larvae in the C group. Similar results were obtained by Radjabi (2010). When silkworm larvae were given mulberry leaves supplemented with raw whey protein, royal jelly, and egg white, there was also a similar rise in larval weight and silk gland weight (Abdel-Rahman, 2018; Islam et al. 2020).

The silk gland follows a similar linear evolution as the larvae gain weight and length. Table 3 shown the Pearson correlation values between larvae growth parameters and silk gland weight on both breeds.

The coefficient of correlation was interpreted according to Akoglu (2018). A very strong positive relationship between silk gland and length of the larvae of Maritza III breed was observed. The coefficient of determination ($R^2 = 0.69$) showed that 69% of the variability observed in the target variable can be explained by the regression model. The correlation coefficients in RG-90 breed were fair although a significant influence was observed and only 22% ($R^2 = 0.22$) of regression model can explains observed data.

In our investigation, we no recorded larval mortality and no found any signs of disease.

Despite the fact that in specific condition *R. glutinis* was considered pathobiont for immune-deficient patients, specific interaction between *B. mori* silkworm and yeast are not yet elucidated.

There are certain structural and physiological variations among insects and other species. Insect-yeast interactions have demonstrated the significance of yeasts for both attracting feed and influencing the behavior and

development of insects (Stefanini, 2018). The microbiotas of some insects can be dominated by yeasts, which mostly form commensal or symbiotic interactions with their host. Intense feeding activity in last instar of silkworms lead to a gradual increase of microorganisms population in digestive tract. Given the capacity for *R. glutinis* to produce enzymes and their significance for nutritive substances absorption and digestion, we presume that exogenous yeast possesses associative effects with the host microbiota.

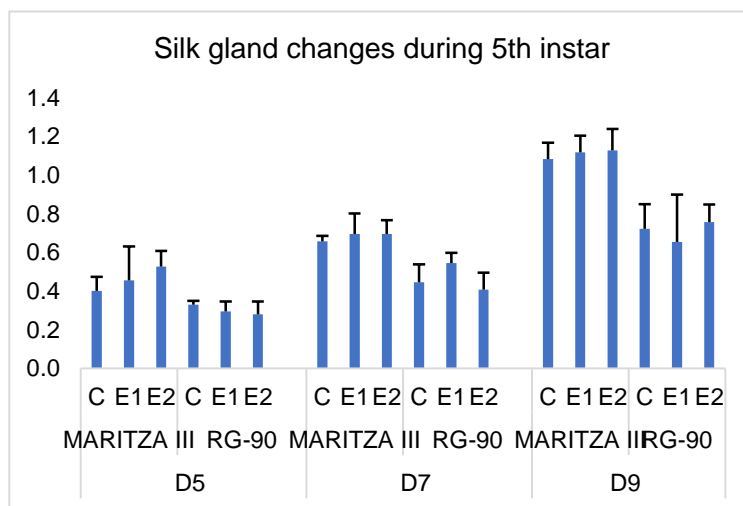


Figure 3. Changes of the silk gland weight in RG-90 and Maritza III breed differentiated by type of feed administered.

During silkworm development, the removal of particular tissues due to consecutive molts has a profound effect on yeast populations, boosting the immune system. The immune system is stimulated by the presence of yeast species that are regarded as pathogens and are not found naturally in insect tissues (Malassigné et al 2021). As a result of these factors and given the relatively short administration duration, it is plausible that *R. glutinis* primarily exhibits advantageous rather than harmful effects on silkworm larvae traits.

The cocoons traits are described in Table 4. The higher values were recorded on Maritza III breed regardless the diets or characteristics evaluated. When compared to the results of Sekar et al. (2016), who added *L. casei* probiotic to the diets of local hybrids, and Samami et al. (2019), who evaluated the best-performing hybrid line in terms of feed conversion ratio to larva weight gain and to cocoon weight, shell to cocoon ratio, and wet manure per g of dry matter intake per silkworm, the bivoltine breed Maritza III demonstrated superior characteristics. Conversely, the cocoon characteristics of our native monovoltine breed, RG-90, were similar to those reported by Sekar et al. (2016); nevertheless, our findings were better when compared to the data obtained by Samami et al. (2019).

Table 3. Bivariate correlation coefficient Pearson' R between silk gland and larvae weight and length in RG-90 and Maritza III breeds

Item	Breeds	Pearson correlation	Larvae weight	Larvae length
		/ P value		
Silk gland	RG-90	R	0.43**	0.49**
		P	0.01	0.002
	Maritza III	R	0.67**	0.83**
		P	<0.0001	<0.0001

Note: ** Correlation is higher significant at the 0.01 level (2-tailed).

Such as, effect of supplement mulberry leaves with probiotic *L. casei* was obvious in the weight of the silkworm cocoon. The average weight in feed-supplemented silkworm cocoons was 1.399 g, while control cocoons weighed 1.187g. In a comparable manner, a 0.369 g increase in shell weight was noted in the probiotic-supplemented silkworm *B. mori*.

Although our cocoon weight data were superior to that reported by Muzamil et al. (2023), the SR% obtained by Musamil by adding amino acids to diets was much higher that resulted in our trial.

Overall data showed a higher mean value in experimental group, especially when the yeast concentration was 1×10^7 .

Table 4. Breeds and diets effects on cocoon traits

Specifications	RG-90			Maritza III			SEM	P-value*	
	C	E1	E2	C	E1	E2		Diet effect	Breeds effect
Cocoon weight (g)	1.70	1.85	1.87	2.00	2.05	1.93	0.24	NS	<0.0001
Shell weight (g)	0.28	0.29	0.30	0.38	0.39	0.38	0.05	NS	<0.0001
Pupae weight (g)	1.41	1.56	1.56	1.61	1.65	1.56	0.21	NS	<0.0001
Longitudinal axes (L, mm)	32.11	33.73	33.56	33.60	33.61	34.46	1.71	NS	<0.0001
Transversal axes (l, mm)	16.17	16.08	16.66	17.33	17.31	17.03	0.90	NS	<0.0001
L/l	1.99	2.10	2.02	1.94	1.94	2.03	0.13	NS	<0.0001
SR%	16.55	15.74	16.36	19.45	19.09	19.91	2.62	NS	<0.0001
Silk productivity (cg/day)	3.10	3.19	3.37	4.29	4.33	4.21	0.62	NS	<0.0001

Note: * Highly significant difference (LSD test, $P < 0.0001$); non-significant differences (NS, $P > 0.05$).

CONCLUSION

In this research we demonstrated that the mulberry leaves with *R. glutinis* yeast allow to silkworms to express and ameliorate their morpho-productive potential (larva, silk gland, and cocoon characteristics), regardless the breed. Our results indicated that, the morphological and productive features of the silkworms was positively altered by fortifying the mulberry leaves with the microorganism *R. glutinis* yeast, in both breeds, although bivoltine breed Maritza III showed superior characteristics.

Author Contributions: M.H. establish experimental protocol, the methodology; wrote the paper and make statistical analyses; A.G. performed the analysis, participated to the measurement; N.A.L. collected the data and participated to the measurement; T.M. participated to the measurement; M.D. *in vitro* test; M.F.A. investigation, A.M. review the manuscript, participated to the establish of the experimental design; D.S.D. visualization and validation.

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Conflicts of Interest

The authors do not have any conflict of interest.

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