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Nutritional Efficiency and Economic Traits of Silkworm *Bombyx mori,* L. Reared on Mulberry Leaves Fortified with Synbiotics

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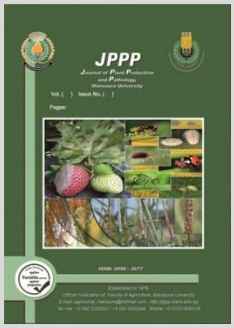

ABSTRACT

Mulberry leaves is the main food of silkworm. Silk production is dependent on nutritive value of mulberry leaves. The purpose of this investigation is studying the impact of prebiotic agents on nutrition and silk production of silkworm. So to explore the effect of *Helianthus tuberosus (Jerusalem artichoke)* as a source of inulin on the activity of some probiotic bacteria (*Lactobacillus rhamnosus* and *Bifidobacterium bifidum*) that added to mulberry leaves which offered to silkworm *Bombyx mori* L.. Mulberry leaves was fortified with prebiotic agent with two different concentrations (3%&6%) and offered 4 times day after day from 4th instar till cocooning. Results revealed that there is no a significant variance between 3% and 6% in all treatments under studied while using mixture of the two probiotic improved larval performance which recorded height value in consumed food, Assimilation, Tissue growth, nutritional parameters in 4th and 5th instars and recorded, the highest values in economic parameters, silk (length, weight and size in the two used concentrations compared with control.

Keywords: silkworm, prebiotic, *Bifidobacterium, lactobacillus,* food consumption.

INTRODUCTION

Silkworm, *Bombyx mori* L. is a monophagus that eats mulberry only (Triubhuvan, 1989). The quality of leaves provided to the worms for feeding has been considered as the main factor that effect on good cocoon production (Ravi Kumar, 1998). Although the mulberry leaves are complete diet for silkworm, the supplementation of extra nutrients results higher yield (Rahmathulla *et al*., 2007). Biologically active additives with living microbial cultures have favorable effects on living organisms which improve the intestinal microbial balance and immune processes. Salminen *et al* (1998) defined probiotics as "foods which contain live bacteria that are beneficial to health. The International Dairy Federation has recommended that the bacteria must be active and abundant in the product, and be present at level not less than 10^7cfu/g Ouwehand and Salminen 1998. The gut probiotics are involved in digestion, metabolic detoxification, stimulation of non-specific immune system also promote the vitamins production and increase host resistance (Singh *et al*., 2005). Improvement in growth and economic characters were noticed when probiotics *Lactobacillus plantarum* (Singh *et al*., 2005) and *Bifido bacterium bifidum* (Amala rani *et al*., 2011b) supplemented with *B.mori* larvae. Intestinal microbiocoenosis is a highly organized dynamic system that respond to changes in the function of an organism by certain qualitative and quantitative shifts (Grinevich *et al*., 2008). To enhance the growth of probiotics must add a prebiotic agent during application that promotes the component of the normal intestinal microflora and evince a health benefit to the host (Isalauri *et al*., 2011). Prebiotics are selectively fermented ingredients that lead to specific changes in the composition and/or activity of the gastrointestinal microbiota, with resulting benefits for the host's well-being and health (Roberfroid, 2007). Some studies, though, highlight the contribution of inulin or inulin-storing plants (Jerusalem artichoke) to human health enhancement (Williams and Jackson, 2002)**.**Inulin is considered as an important prebiotic substrate and has been well-studied due to its effects on intestinal *Bifido* bacteria (Watzl *et al*., 2005). Jerusalem artichokes, which are nearly 20% (w/w) carbohydrate that is composed of 70-90% (w/w) inulin (Ge, Xiang-Yang *et al.*2010).

The aim of this study was to evaluate the in vivo effects of the synbiotics by using prebiotics as the dietary administration of *Jerusalem artichoke* (a source of inulin) and probiotics as *Lactobacillus* and *Bifido bacterium* (a probiotic) combined on growth and cocoon production of silkworm *Bombyx mori* L.

MATERIALS AND METHODS

The present study was carried out during spring season of 2018 in Sericultural Research Department of Plant Protection Research Institute, Agricultural Research Center in Giza

Materials:

Mulberry silkworm, *Bombyx mori* eggs (Bulgarian hybride) was used.

Lactobacillus rhamnosus: **(**Dairy Dept., Fac. of Agric., Al-Azhar Univ., Cairo, Egypt).

Bifidobacterium bifidum: (Cairo MIRCEN), Fac. of Agric., Ain Shams Univ.).

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Jerusalem artichokes: *Jerusalem artichoke* tubers (*Helianthus tuberosus*) that harvested by Dairy Dept., Fac. of Agric., Al–Azhar Univ., Cairo, Egypt, in Dec. 2018 from the Experimental Station, Agricultural Research Centre, El-Kanater El-Khayria, Egypt.

Method:

Silkworm Rearing Technique:

Silkworm rearing was carried out under laboratory conditions 25±1°c and 75±5%R.H, According to Krishnaswami (1978). The diseased free egg cards were incubated in an incubator at 25°c and 75%R.H till hatching, newly hatched larvae were transferred to rearing trays, using cleaning nets for cleaning the rearing bed. At the beginning of 4th larval instar, larvae were divided into three groups, each group refer to type of probiotic bacteria under studied, each group divided to two sub groups for two concentrations(3% and 6%), each concentration with 3 replicates , each replicate contain 50 larvae. Mulberry leaves were sprayed with *(Jerusalem artichoke)* at concentration 2% in 4th instar and 3% in 5th instar as a recommended doses then let to dry about 10 minute after that spray the treated leaves with one of the two concentrations of prebiotic bacteria day after day then left to dry again and offered to disinfected larvae 4 times till cocooning. The control group was fed with mulberry leaves sprayed with distilled water.

Bioassay:

Microbial isolation and growth media:

***Lactobacillus rhamnosus* AZ1 KY123807 :**was cultured at 37ºC for 24 h. under an aerobic condition in 54 g modified MRS broth.

Bifidobacterium bifidum ATCC 15696: was cultured at 37°C for 48hr in 54g modified MRS broth.

MRS medium: which containing of Peptone 10 g; meat extract 10 g; yeast extract 5 g; potassium mono-hydrogen phosphate 2 g; ammonium citrate 2 g; glucose 20 g; tween (80) 1 ml; sodium acetate (3H2O) 5h; salt solution 5 ml; distilled water 1000 ml; pH 6.2-6.6. Salt solution: MgSO4.7H2O, 11.5 g; MnSO4. 2H2O, 2.4 g; distilled water, 100 mol. supplemented with 0.05% L–Cysteine HCL and 0.3% lithium chloride. Ingredients were dissolved in distilled water; pH was adjusted to 6.5 before sterilization at 121°C for 20 minutes.

Probiotic properties of strains:

1. **Acid tolerance**: Strains were evaluated for their ability to grow in low pH values according to Pereria and Gibson (2002)
2. **Bile tolerance**: Bile tolerance was estimated as described by Pereira and Gibson (2002), strains were evaluated for rapidity of growth in a broth medium with bile salts.
3. **Tolerance of artificial gastric juice (AGJ) and artificial intestinal juice (AIJ):** Gastric and pancreatic juices were prepared fresh by dissolving pepsin (Sigma- 3050 Spruce street Saint Louis, Missouri 6310- USA) from porcin stomach mucosa (3g/ L) and pancreatin (Sigma) from porcin pancreas (1g/ L) in sterile saline (5g/ L) according to Charteris *et al.* (1998).

Prebiotic agent: *Jerusalem artichoke* was used as a prebiotic prepared as a powder according to (Modler *et al*., 1993).

Determination of nutritional indices:

The healthy larvae were counted daily in each treatment of three replications and the unequal weak / unhealthy larvae if any, were replaced by healthy ones of the same age from the reserve stock. The left over leaves and the excreta were dried in open air, and the values were recorded also, initial and final weight of larvae was recorded to determine the larval growth. Food consumption, utilization and other nutritional indices such as approximate digestibility(AD), efficiency of converting leaf ingested (ECI) and leaf digested (ECD), consumption rate (RCR), and coefficient of metabolism (COM) were calculated as suggested by Waldbauer (1968) and Kaushal *et al*. (1988). The nutritional efficiency parameters were calculated by the following formula:

- **Food consumption = Initial weight of leaves — weight of unconsumed leaves (in g dry weight / day / larva).**
- **Assimilation = Food consumed - weight of litter (in g dry weight / day / animal).**
- **Tissue growth = Final body weight - Initial body weight of larvae (in g dry weight / day / animal).**

$$\text{AD (in \%)} = \frac{\text{Assimilation}}{\text{Food consumed}} \text{ X100} \qquad \text{RCR (in \%)} = \frac{\text{Food consumed}}{\text{Fresh leaves supply}} \text{ X100}$$

$$\text{ECI to larva (in \%)} = \frac{\text{Tissue growth}}{\text{Consumption}} \text{ X100} \qquad \text{ECD to larva (in \%)} = \frac{\text{Tissue growth}}{\text{Assimilation}} \text{ X 100}$$

$$\text{COM (in \%)} = \frac{\text{Assimilation - Tissue growth}}{\text{Assimilation}} \text{ X100}$$

Economic Analysis:

Filament length:

Twenty cocoons produced from *B. mori* larvae of different treatments were separately reeled and length of filament was recorded. Cocoons were softened by being soaked in boiling water. The special apparatus for reeling individual cocoon silk was used to measure the total length of silk filament and average length was calculated.

Filament weight: The previous filaments were afterwards oven dried at 40˚C to constant weight and then average weight of filament was calculated.

Filament size: The size of silk reeled per cocoon was calculated according to the following equation (Tanaka, 1964).

$$\text{Size of silk filament (denier)} = \frac{\text{Wt. of silk filament (gm)}}{\text{Length of filament (m)}} \text{ x 9000}$$

Where, Denier = Weight of reeled thread with Length 9000 meters by Gram.

Statistical analysis: The obtained data were analyzed using one way ANOVA using SAS soft wear (Snedecor, 1990). Mean separation followed using LSD in the same program.

RESULTS AND DISSCUSSION

Results in Table (1), indicated that, there is a significant variance between treatments in larval weight, since the addition two types of bacteria registered the highest value followed by *Bifido* after that *Lactobacillus* in both instars 4th and 5th as follow (0.829 ,0.816 ,0.802 ,0.769,0.750 and 0.731) and (4.029, 3.925, 3.775, 3.688,

3.509 and 3.403), respectively. But control recorded the least value in both instars as follow (0.664 and 2.325).

Also, we notice there is no significant variance of the two concentrations in the same treatment.

Data obtained in Tables (2, 3), cleared that, the maximum amount of consumed food and assimilation was recorded for treatment $_3$ in both concentrations without a significant variance between them in each larval instar, which registered in 4th and 5th larval instar (0.581, 0.574\gm) and (1.805, 1.783\gm) respectively, for consumed food and assimilation (0.517, 0.508), (1.331, 1.309), respectively while the curve declined in treatment 2 especially in 6% concentration until it reaches to the least value in control. Also, treatment$_3$ recorded the maximum results in T.G, AD%,RCR%,ECI% and ECD% with a significant variances between treatments in 4th and 5th larval instar that enrolled (0.128, 0.354),(88.98, 73.72%), (77.87, 78.79%), (22.02, 19.41%) and (20.89, 26.56%) ,respectively. But the results differed in COM% since, control give the maximum value in both instars which indicate there is a reverse relationship between EDI% and COM%.

Table 1. Influence of synbiotic on grown larval weight.

Treatments	Sub treatments	4th Larval weight	5th Larval weight
Bifido	3%	0.769b ±0.079c	3.688±0.338b
	6%	0.802b±0.075b	3.775±0.317b
Lactobacillus	3%	0.731c±0.075d	3.403±0.313c
	6%	0.750d±0.075cd	3.509±0.310d
Bifido+	3%	0.816a±0.092ab	3.925±0.315a
Lactobacillus	6%	0.829a±0.085a	4.029±0.259a
Control	0%	0.664e±0.069e	2.325±0.198e
F value		47.31	334.82
P value		0.0001	0.0001
LSD		0.02307	0.0884

Means with the same letter are not significantly different.

Table 2. Influence of synbiotic on assimilation and food consumption of 4th larval instar silkworm *Bombyx mori.* L. in per larva.

Treatments	Sub treatment	C.F \gm	Assimilation	T.G \gm	A.D %	R.C.R %	E.C.I %	E.C.D %	COM %
Bifido	3%	0.552±0.002b	0.479±0.391bc	0.116±0.001d	86.60±0.391bc	74.08±0.203b	20.92±0.167b	24.16±0.239bc	75.930±0.155cbd
	6%	0.56±0.006b	0.489±0.144b	0.122±0.002c	87.37±0.144bac	75.06±0.805b	21.84±0.240a	24.99±0.284a	74.967±0.333
Lactobacillus	3%	0.543±0.007c	0.469±0.771cd	0.110±0.002f	86.79±0.771bc	72.78±0.942c	20.25±0.070c	23.45±0.078c	76.537±0.078b
	6%	0.540±0.002c	0.460±0.511d	0.108±0.001e	85.75±0.511c	71.84±0.231c	20.14±0.133c	23.49±0.161c	76.500±0.161bc
Bifido +	3%	0.574±0.001a	0.508±0.985a	0.125±0.001b	88.33±0.985ba	77.03±0.156a	21.86±0.225a	24.98±0.696a	75.587±0.982ced
Lactobacillus	6%	0.581±0.003a	0.517±0.060a	0.128±0.000a	88.98±.060a	77.87±0.402a	22.02±0.115a	24.75±0.145ab	75.237±0.145ed
C	0%	0.524±0.007d	0.451 ±2.402 e	0.094±0.001g	85.95±2.402c	70.32±0.892d	17.97±0.275d	20.89±0.771d	79.093±0.771a
F value		60.20	28.76	237.80	3.88	60.00	175.02	34.51	22.94
P value		0.0001	0.0001	0.0001	0.017	0.0001	0.0001	0.0001	0.0001
L.S.D		0.007	0.013	0.002	1.845	1.070	0.329	0.745	0.874

Means with the same letter are not significantly different.

Table 3. Influence of synbiotic on assimilation and food consumption per larva of in 5th larval instar silkworm *Bombyx mori* L.

Treatments	Sub treatments	C.F \gm	Assimilation /gm.	T.G \gm	A.D %	R.C.R %	E.C.I %	E.C.D %	COM %
Bifido	3%	1.745±0.014b	1.245±1.398b	0.324±0.001d	70.937±1.398bc	76.317±0.704b	18.460±0.199cb	26.030±0.266a	73.960±0.266b
	6%	1.762±0.009b	1.264±0.601b	0.330±0.001c	71.687±0.601bac	76.767±0.393b	18.697±0.125b	26.083±0.183a	73.673±0.391b
Lactobacillus	3%	1.685±0.014c	1.175±1.175c	0.306±0.002e	69.047±1.175c	73.397±0.595c	18.170±0.121cd	26.063±0.107a	73.927±0.107b
	6%	1.643±0.007d	1.058±2.387d	0.296±0.003f	64.363±2.387d	71.553±0.305d	18.007±0.081d	25.897±4.521a	71.987±0.886c
Bifido +	3%	1.783±0.005ba	1.309±0.611a	0.344±0.001b	73.367±0.611ba	77.680±0.218ba	19.303±0.084a	26.310±0.104a	73.680±0.104b
Lactobacillus	6%	1.805±0.006a	1.331±0.287a	0.354±0.001a	73.727±0.287a	78.797±0.127a	19.413±0.219a	26.560±0.131a	73.430±0.131b
C	0%	1.55±0.041e	0.746±2.278e	0.184±0.002g	49.917±2.278e	67.530±1.785e	11.890±0.41e	23.903±1.267a	76.087±1.267a
F value		77.42	308.90	4362.33	98.66	76.83	479.69	0.73	11.51
P value		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.632	0.0001
L.S.D		0.314	0.035	0.002	2.567	1.380	0.362	3.118	1.078

Results in Table (4), revealed that, significant variance between mixture of probiotic and all treatments for silk length, and size (1063.80, 1019.80m) and (2.51, 2,38d), respectively with no variance between two concentrations in the same treatment. On the other hand statistically analysis showed a significant variance between the high concentration (6%) of *Bifido* + *Lactobacillus* and other treatments for silk weight that recorded (0.30g).While control recoded the least value in all economic.

T**able 4. Influence of synbiotic on Economic traits of silkworm *Bombyx mori* L..**

Treatment	Sub treatment	Silk length (m)	Silk weight (g)	Silk size (d)
Bifido	3%	920.40±114.34bdc	0.24±0.014c	2.36±0.147ba
	6%	1012.60±79.02ba	0.26±0.006bc	2.36±0.181ba
Lactobacillus	3%	951.60±93.79bac	0.24±0.010c	2.31±0.053ba
	6%	851.40±60.16dc	0.21±0.009d	2.22±0.156c
Bifido+Lactobacillus	3%	1019.80±46.85ba	0.27±0.007b	2.38±0.053ba
	6%	1063.80±105.49a	0.30±0.017a	2.51±0.156a
C	0%	827±94.14c	0.21±0.034e	2.28±0.512b
F value		5.14	170.86	146.56
P value		0.001	0.0001	0.0001
L.S.D		113.7	0.024	0.195

Means with the same letter are not significantly different.

Nutrition plays a vital role in silk industry, addition prebiotic agent enhance larva, cocoon weight and commercial characters of cocoon. The previous results showed that larvae fed on mulberry leaves fortified with

Jerusalem artichoke as a source of prebiotic agent with probiotic bacteria under studied increased in food efficiency and larval weight of 4^{th} and 5^{th} instars with a significant variance between concentration 6% and all other treatments while there was no significant variance between the two concentrations in every treatment of most characters under studied. This may be due to the nutritional supplementation as reported earlier (Amala Rani *et al*., 2011a and b; Balasundaram *et al*., 2013). There is a direct relation between Food consumption and weight of larvae, cocoon, pupae and shell (Shiva kumar, 1995). Variance in consumption and productivity depending on the type of nutrition and silkworm breeds as suggested by (Rema devi *et al*., 1992). In general, the present results are in agreement with the observations of earlier workers (Balasundaram *et al*., 2008; Rath, 2010; Lakshmi Bai and Ramani Bai, 2011).

Between two concentrations, 3% concentration reported asignificant increase compared to the control. The present results are in agreement with the observations of Amala Rani *et al*. (2011a) and Ganesh prabu *et al*. (2012). Magadam *et al*. (1996) as they reported that there is a direct relation between ingesta and digesta increases. The rate of digestion in silkworm increases with the advance of instar (Ueda, 1982). During larval advancement, the study revealed that, there was a significant increase in tissue growth, RGR, ECI, ECD that increases with instar.

Rath (2010) revealed that 95 - 96% of the total food intake of different larval instars was ingested during the last two instars, which confirms the result of the present findings. Probiotics produce vitamins and breakdown the digestible compounds, that lead to the nutritional improvement and stimulate appetite Irianto and Austin (2002). Gibson and Robert froid (1995) emphasized that prebiotics with probiotic promoting bacteria in the intestinal tract, that improving the host intestinal balance. Digestive enzymes such as amylase, protease and lipase are produced due to oral administration of dietary pre and probiotics in rabbit fish enrich the concentration of intestinal enzymes (Lee and Lee, 1990 and El - Dakar *et al*., 2007) and promote faster digestion.

The previous results revealed that 3% concentration of the last treatment recorded significantly the maximum value in silk length weight also size. This agreement in the earlier findings in the same insect, with supplementation of antibiotic Amoxicillin (Thilagavathi *et al*.,2013), *B.bifidum* and yeast (Amala Rani *et al*., 2011b) and commercial pre and probiotic (Lakshmi Bai and Ramani Bai, 2011). Narayanan *et al*. (1969) indicated that neatness is the important character by which the quality as well as excellence of silk fiber is judged. Mulberry leaves fortified with glycine (Babu, 1994).

Previous observations may be attributed due to increased efficiency of digestion and food assimilation that leading to increase protein synthesis and subsequent accumulation of storage protein in the body as a result of activity of probiotic microbial flora in the gut of host. Enrichment of mulberry leaves by nutrient supplementation is one of the strategies by which cocoon and silk productivity can be increased and quality can be enhanced and maintained.

CONCLUSSION

Silkworm survive only on a mulberry leaves. Nutritional quality of mulberry leaves increased by fortification with live micro-organisms but these micro-organisms require undigestible materials to maintain it's survive for a long time to perform its role completely. From the previous results it was concluded that, in all treatments there was no significant variance between 3% and 6%. As application of 3% that is a small dose give the same results as 6% concentration, we can use small amount of probiotic bacteria to give high result.

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الكفائة الغذائية والصفات الإقتصادية لدودة الحرير التوتية *Bombyx mori* L. التي تم تربيتها علي أوراق التوت المضاف إليه كائنات تكافلية

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أجريت هذه التجربة بمعامل قسم بحوث الحرير مركز البحوث الزراعية بالجيزة. من المعروف أن أوراق التوت هي الغذاء الرئيسي و الوحيد ليرقات دودة الحرير التوتية . و أن إنتاج الحرير يعتمد علي القيمة الغذائية لأوراق التوت . لذلك كان الغرض من إجراء هذا البحث هو دراسة تأثير بريبيوتيك علي كمية الغذاء المستهلكة و إنتاجية الحرير ليرقات دودة الحرير التوتية. وذلك لإظهار تأثير نبات *Helianthus tuberosus* كمصدر للإنولين علي نشاط بعض البروبيوتيك باكتيريا مثل*Lactobacillus Rahamnosus* and *Bifidubacterium* Bifidum المضافة لأوراق التوت. تم إضافة تركيزين من البريبيوتيك إلي أوراق التوت (3% & 6%) وتقديمها لليرقات أربع مرات يوم بعد يوم من بداية العمر الرابع إلي التعشيش. و قد أظهرت النتائج أنه لا يوجد فروق معنوية في النتائج بين التركيزين في كل المعاملات تحت الدراسة بينما استخدام خليط من البكتيريا بالتساوي حسنت أداء اليرقة حيث سجلت أعلي القيم في كل من (C.F,AD,AD%,T.G,ECI%,ECD%) في العمرين الرابع والخامس وأيضا سجلت أعلي النتائج في الخواص الإقتصادية للحرير (طول خيط الحرير, وزنه و حجمه) .