International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 24, No. 1, p. 19-27, 2024

INNSPUB

RESEARCH PAPER OPEN ACCESS

Productive Performance of Philippine Silkworm (*Bombyx Mori* L.) Purelines Fed with Folic Acid-Enriched Mulberry Leaves

Janet D. Manuel*, **Roel D. Supsup**

Don Mariano Marcos Memorial State University, Bacnotan, 2515 La Union, Philippines

Key words: Nutrient enrichment, Silkworm, Larval growth, Mulberry, Folic acid

http://dx.doi.org/10.12692/ijb/24.1.19-27 Article published on January 03, 2024

Abstract

Low silk production has been attributed to the low quality of mulberry leaves fed to silkworms during rearing. Thus, quality of mulberry leaves should be enhanced. This study determined the efficacy of folic acid-enriched mulberry leaves in the performance of silkworms and silkmoth egg production. Fifth instar larvae of the six (6) silkworm purelines were used in the study. Mulberry leaves were supplemented with 0.001% Folic Acid (T_1) and 0 .002% Folic Acid (T_2) and compared to the Control (No Supplementation). Results showed that the larvae, cocoons and eggs produced by the silkworm purelines fed with folic acid supplemented mulberry leaves were significantly affected based on the weight of 10 matured larvae, single shell weight, single cocoon weight, effective rearing rate (ERR), cocoon yield per box (CYPB), and fecundity. An interaction effect on the effective rearing rate (ERR) and cocoon yield per box (CYPB) from silkworm fed with folic acid supplemented mulberry leaves revealed significant differences among all treatments and breeds. It can be noted that folic acid-enriched mulberry leaves fed to silkworms tend to have higher results as compared to the control treatment (CT) on all the parameters of the study.

* **Corresponding Author:** Janet D. Manuel ✉ jdmanuel@dmmmsu.edu.ph

Introduction

Silk, the finest and the most elegant natural fiber produced by silkworms is a result of a long chain, labor intensive, agro-based industry. Sericulture is an old developed industry in Japan, Korea, China, India and other countries in the world. In the Philippines, this is a potentially viable industry to increase rural employment and family income.

Success of sericulture as a cash crop depends on the vitality and robustness of silkworm seed (egg) supplied to the farmers (Narasimhanna, 1988; Singh, 2010; Pathan & Harale, 2016). Quality silkworm seed or egg is the backbone of the sericulture industry. A country's silk production and its quality are measured by the quality of the silkworm egg it produces. Eggs or seeds of superior silkworm breeds ensure high silk recovery. A well-organized healthy seed production ensures high cocoon yield. The quality of leaves fed to worms is considered to be the most important factor for good cocoon crop production. Since the silkworms attain short period of time to synthesize silk, the nutrient content of the mulberry leaf meals greatly affects and determine the growth and development of the larvae and cocoon production (Seidavi *et al.*, 2005; Kanafi *et al.*, 2007; Rahmathulla *et al.*, 2007). Leaves of superior quality enhance the chances of good cocoon crop and quality (Ravikumar, 1988; Ravi Kumara, 2016; Hosamani *et al.*, 2019).

Different researches have been conducted on supplementation to mulberry leaves and different results have been reported. Etebari *et al.* (2004) stated that the nutritional status of mulberry leaves can be improved by enriching them with vitamins and other nutrients and supplementations in order to increase larval growth and post cocoon characteristics. Vitamin B-complex groups and certain essential sugars, proteins, amino acids, minerals, and others are responsible for the proper growth and development of silkworms *Bombyx mori* L. (Faruki, 1998; Madrid *et al.*, 2021).

The Don Mariano Marcos Memorial State University-Sericulture Research and Development (DMMMSU-SRDI) hasstudied on the nutritional supplementation on mulberry leaves to improve certain economic characteristics of silkworms and eventually cocoons. These efforts included the use of honey (Almojuela *et al.*, 2022); amino acid (Madrid *et al.*, 2020); and turmeric tea (Garcia *et al.*, 2023), wherein these additives have proven to help enhance the growth and yield parameters of both silkworms and cocoons. Thus, with the above findings, it is also necessary to explore other supplements available to further improve the productivity of silkworms.

This study determined the effects of enriching mulberry leaves with vitamin supplement, Folic acid on the performance of silkworm larvae and eggs; and to evaluate the interaction effect of the different levels of folic acid supplemented to mulberry when fed to six (6) bivoltine silkworm purelines in terms of larvae, cocoon, and egg production parameters of parental hybrids.

Methodology

Materials and Procedures

Prior to the conduct of the study, the rearing house and grainage building were disinfected. All the other materials used in the study were sterilized to avoid any contamination or infection of the worms.Mulberry leaves were gathered and preserved with wet clothes when not yet in use for feeding within twenty-four hours.

Formulation of Treatments

The solutions for folic acid were prepared based on the treatments. For Factor A, the Treatments included FA1 (0.001% Folic acid), FA2 (0.002% Folic acid, and the Contron (No Treatment). On the other hand, Factor B included the different silkworm breeds, DMMMSU 100, DMMMSU 101, DMMMSU 102, DMMMSU 103, DMMMSU 107, and DMMMSU 115. Five (5) mg Folic acid capsules were used in the preparation of the Folic acid solution. FA1 (0.001% FA) solution was prepared by dissolving one capsule in 500 ml distilled water while for FA2 (0.002% FA) solution consisted of one capsule dissolved in 250 ml distilled water.

Administration of the different treatments

Freshly-gathered mulberry leaves were sprayed with the Folic acid solutions based on treatments before feeding the silkworms. To ensure uniform volume of solutions used, 20 ml of each treatment was sprayed to every 100 grams of mulberry leaves. This was done before each feeding schedule, for 5 days.

Rearing of silkworms

Three hundred (300) 5th instar larvae from each of the six (6) DMMMSU-SRDI silkworm purelines (DMMMSU 100, DMMMSU 101, DMMMSU 102, DMMMSU 103, DMMMSU 107, and DMMMSU 115) were used as testing organisms.

The recommended rearing management practices of the silkworm in terms of sanitation, feeding schedule (4 AM, 10 AM, 3 PM, and 8 PM) and bed cleaning were strictly observed and followed during the conduct of the study.

When silkworms showed signs of maturity, 10 matured larvae were randomly picked from each treatment and were weighed then placed in cocooning frames for spinning. All mature worms were mounted then covered with plastic nets and left undisturbed for five days.

Data gathering and analysis

Five days after spinning, the cocoons were harvested from each treatment. Cocoons were assessed based on the economic parameters such as the weight of 10 matured larvae, single cocoon weight (SCW), single shell weight (SSW), cocoon shell percentage (CSP), cocoon yield box (CYB), and effective rearing rate (ERR). After the egg production activities, counting the fecundity (number of silkworm eggs laid) and hatching percentage were done.

The study used the Factorial Randomized Complete Block Design (RCBD) with three (3) replications per treatment. Analysis of Variance (ANOVA) was used in determining the significant difference among the treatments. Significant treatment means were compared using Duncan Multiple Range Test (DMRT)to determine the best formulation of folic acid that could be sprayed to mulberry leaves that could affect and improve the performance of silkworm purelines.

Results and discussion

Growth and Yield Performance of Silkworms

Table 1 presents the performance of silkworm larvae fed with mulberry leaves enriched with folic acid. Results show significant differences among treatment means on the weight of 10 matured larvae, single shell weight, single cocoon weight, effective rearing rate, cocoon yield per box, and the fecundity of silkworm eggs produced. On the other hand, no significant difference was observed among the means on cocoon shell percentage and hatching percentage.

These parameters revealed that silkworms fed with mulberry leaves with 0.002% Folic Acid (FA2) obtained the highest mean but werecomparable to silkworms fed with .001% Folic Acid (FA1) mulberry leaves. The lowest means were from the silkworms in the Control Treatment (CT).

Table 1. Performance of silkworms fed with mulberry leaves enriched with Folic Acid.

Treatments	Wt. of 10 matured larvae (g)	Single shell weight (g)	Single cocoon weight (g)	Cocoon shell percentage (%)	Effective rearing rate (%)	Cocoon yield per box (kg)	Fecundity	Hatching percentage (%)
CT – control (no spray)	30.09b	.26b	1.37b	19.38	90.00b	24.73b	499.94b	97.69
FA1 .	30.80ab	.28a	1.43a	19.79	93.19a	26.64a	523.56a	97.73
FA2	31.56a	.28a	1.46a	19.06	94.58a	27.49a	508.06b	97.54
Significance	**	**	**	ns	**	**	**	ns

*- All means followed by the same letter are not significantly different in .05 level (DMRT).

Silkworm Egg Production

On the production of silkworm eggs, the fecundity or the number of silkworm eggs laid by the moths was significantly higher when fed with mulberry leaves sprayed with0.001% Folic Acid (FA1), as compared to the silkworms fed with FA2 formulation (0 .002%

Folic Acid) and the Control Treatment (CT).

These results imply that the silkworm purelineshave positively responded to folic acid supplemental feeding at the rate of 0.001%.

Further, in terms of silkworm larvae growth and yield parameters, supplementation of mulberry leaves with folic acid in both concentrations, tends to increase the weight of 10 matured larvae, single shell weight, single cocoon weight, effective rearing rate, cocoon yield per box, and fecundity of silkworm eggs produced as compared to the control treatment (CT). Rahmathulla *et al.* (2007) reported that administration of folic acid significantly increased the larval weight, shell weight, cocoon weight, and the yield per weight of cocoon (ERR weight). Similar results were obtained by Nirwani & Kaliwal (1996); Rai *et al.*(2002) and Etebari (2002) on the administration of folic acid.

Table 2. Performance of silkworm purelines fed with mulberry leaves enriched with Folic Acid.

Treatments	Wt. of 10 matured larvae (g)	Single shell weight (g)	Single cocoon weight (g)	Cocoon shell percentage (%)	Effective rearing rate (%)	Cocoon yield per box (kg)	Fecundity	Hatching Percentage (%)
DMMMSU 100	31.97a	0.28bc	1.51a	18.94bc	96.67a	29.03a	514.78b	97.46
DMMMSU 101	32.08a	0.27c	1.42b	19.60b	93.61b	27.89ab	537.7a	97.95
DMMMSU 102	31.45a	0.29ab	1.42b	21.06a	92.78bc	26.85bc	511.6b	97.86
DMMMSU 103	31.08a	0.30a	1.55a	19.75b	91.39bc	26.00c	504.4bc	97.42
DMMMSU 107	29.27b	0.25d	1.43b	18.01c	90.83cd	25.77c	498.44c	97.53
DMMMSU 115	29.20b	0.22e	1.19c	19.11bc	90.28d	22.16d	496.0c	97.72
Significance	*	**	**	**	**	**	**	ns

*- All means followed by the same letter are not significantly different at .05 level (DMRT).

The present result showed that folic acid acts as a growth promoter and can significantly increase the economic characteristics of silkworms such as the single cocoon weight, single shell weight (Devi & Yellama, 2013), effective rearing rate, and cocoon yield per box. On the other hand, results indicated no significant differences in the cocoon shell percentage and hatching percentage of the silkworm eggs produced by feeding silkworms with unsprayed mulberry leaves (CT) or mulberry leaves supplemented with folic acid. This result implies that supplementation of mulberry leaves with folic acid solution at 0.001 or 0.002% can be done and still yield similar effects on 5th instar silkworms.

Table 3. Egg production (hybrid silkworm eggs) performance of silkworms fed with mulberry leaves enriched with Folic Acid.

Treatments	Fecundity	Hatching percentage (%)
CT – Control (no spray)	533.33 c	98.22
FA1– 0.001% folic acid (FA)	557.73 a	98.46
FA2 – .002% folic acid (FA)	548.27 b	98.25
Significance	**	ns

- All means followed by the same letter are not significantly different at .05 level (DMRT).

Growth and Yield Performance of Silkworm Purelines

Table 2 shows the performance of silkworm larvae, cocoon production, and egg production of silkworm purelines fed with mulberry leaves supplemented with folic acid. The weight of 10 matured larvae of the silkworm purelines was significantly affected by folic acid supplementation. Similarly, the single shell weight, single cocoon weight, cocoon shell percentage, effective rearing rate, cocoon yield per box, and fecundity have shown significant differences among treatment means in all the silkworm purelines when fed with mulberry leaves enriched with folic acid.

The highest mean on the weight of 10 matured larvae was seen in DMMMSU 101 (32.08 g) which was comparable to DMMMSU 100 (31.97 g), DMMMSU 102(31.45 g), and DMMMSU 103 (31.08 g).Lower means were observed in DMMMSU 107 (29.27 g) and DMMMSU 115 (29.20 g).

The highest single shell weight was obtained from DMMMSU 103 (0.30 g) but comparable to DMMMSU 102 (0.29 g).The single cocoon weight of DMMMSU 103 (1.55 g) and DMMMSU 100 (1.51 g) were significantly higher among the other breeds of purelines using both treatments. For the cocoon shell percentage, DMMMSU 102 (21.06%) gave a significantly higher result than the other purelines used. Significant differences among treatment means on theERR of silkworm purelines were also noted where DMMMSU 100 gave the highest ERR (96.67%).Consequently, DMMMSU 100(29.03 kg) obtained the highest cocoon yield per box but comparable to DMMMSU 101 (27.89 kg).

Table 4. Parental hybrid silkworm eggs produced from silkworm purelines fed with mulberry leaves enriched with Folic Acid.

Treatments	Fecundity	Hatching percentage (%)
DMMMSU 100x101	549.22 a	98.09
DMMMSU 100x102	550.89 a	98.18
DMMMSU 101x107	551.67 a	98.50
DMMMSU 102x107	553.33 a	98.59
DMMMSU 115x107	527.11 b	98.20
Significance	**	ns

*- All means followed by the same letter are not significantly different at .05 level (DMRT).

Egg Production Performance of the Silkworm Purelines

The egg production parameters of the different purelines were also influenced by folic acid supplementation (Rahmatulla *et a.l*, 2011; Rahmathulla *et al.*, 2007). Among all the treatment breeds for parental rearing used in the study, it could be noted that DMMMSU 101 (537.78) produced a significantly higher number of silkworm eggs, which coincides with the findings of Radjabi *et al.*(2007) and Nezhad *et al.* (2010). However, the hatchability of silkworm eggs produced from all the purelines fed with folicacid-supplemented mulberry leaves was comparable.

The above results show that feeding silkworms with folic acid-supplemented mulberry leaves affects the larvae and cocoon characters, and egg production performance. According to Chapman (1998) and the National Research Council (1987), folic acid plays a major role in cellular metabolism including the synthesis of some components of DNA and pigment precursor (Radjabi, 2010; Kanafi *et al.*, 2007).

A significant increase in female and male cocoon and shell weight were determined in folic acid supplementation (Nirwani & Kaliwal, 1996; Jayabal & Manjula, 2019). Para-amino benzoic acid (PABA) is a growth regulator and represents one of the forms of folic acid. PABA is one of the substances belonging to the vitamin B-complex group and supports vital function in insects and especially in silkworm (Pai *et al.*, 1988). The supplementation of Fe-PLUS® (ferrus fumarate+folic acid) significantly increased larval, pupal and adult weight in comparison with controls with lowest and highest growths obtained at the concentrations of 0.32 and 0.64 %, respectively (Khan & Saha, 1996).

Fecundity Silkworm egg production and quality, as influenced by folic acid supplementation on mulberry leaves is shown in Table 3. Fecundity or the number of parental hybrid silkworm eggs produced by silkworms fed with folic acid-supplemented mulberry leaves showed significant differences among the treatment means. Silkworms fed with 0.001% Folic Acid (FA1) supplemented mulberry leaves produced the highest number of silkworm eggs laid followed by those fed with mulberry leaves supplemented with0.002% Folic Acid (FA2) concentration. The silkworms in the Control treatment have the lowest number of hybrid (F_1) silkworm eggs produced. This implies that supplementation of folic acid to the mulberry leaves can increase fecundity. Khan and Saha (1996) reported that fertility increased significantly in all treatments of Fe-PLUS (ferrus fumarate+folic acid) supplementation when compared to control except for0.64 % concentration. However, there are no significant differences among the treatment means in terms of hatching percentage.

Table 5. Interaction Effect of Silkworm Purelines and Folic Acid Supplemented Mulberry Leaves on the Effective Rearing Rate (ERR) and Cocoon Yield Per Box.

Breed x Treatment	Effective Rearing Rate (ERR)	Cocoon Yield/Box
DMMMSU 100 CT	95.83 ab	28.98 a
DMMMSU 100 FA1	97.50 a	29.31 a
DMMMSU 100 FA2	96.67 a	28.06 a
DMMMSU 101 CT	90.83 cde	25.17 bc
DMMMSU 101 FA1	95.83 ab	27.33 ab
DMMMSU 101 FA2	94.17 abcd	28.06 a
DMMMSU 102 CT	90.00 de	24.58 cd
DMMMSU 102 FA1	90.00 de	25.50 bc
DMMMSU 102 FA2	94.17 abcd	27.25 ab
DMMMSU 103 CT	88.33 e	24.36 cd
DMMMSU 103 FA1	95.00 abc	29.71 a
DMMMSU 103 FA2	95.00 abc	29.60 a
DMMMSU 107 CT	87.50 e	24.46 cd
DMMMSU 107 FA1	89.17 e	25.34 bc
DMMMSU 107 FA2	95.83 ab	28.20 a
DMMMSU 115 CT	87.50 e	20.84 e
DMMMSU 115 FA1	91.67 bcde	22.65 de
DMMMSU 115 FA2	91.67 bcde	23.00 cde
Significance	*	*

*- All means followed by the same letter are not significantly different at .05 level (DMRT).

*Egg Production Performance of Hybrid Eggs*Table 4 shows the egg production of hybrid silkworm eggs from the silkworm fed with mulberry leaves supplemented with folic acid. Results indicated that silkworm egg production of the breeds used in parental rearing obtained a highly significant difference among treatment means.

This implies that the supplementation of folic acid to mulberry leaves led to a significant increase in the eggs laid by the moths (Khan & Saha, 1996; Bentea *et al.*, 2011; Marghitas & Sara, 2011;). It could be noted that four hybrids (DMMMSU 102x107, 101x107, 100x102, 100x101) were significantly higher from DMMMSU 115x107. The F1 (hybrid) parents silkworm eggs were used to produced the 3-way and 4-way cross hybrids for the farmer clients of sericulture. These were also used by the seri-cooperator seed cocoon producers. However, the hatching of the eggs produced had a comparable result.

Interaction Effect of Folic Acid and the Silkworm Purelines

Table 5 shows the interaction effect on the ERR and cocoon yield per box of silkworm purelines as affected by the treatments. The results showed significant differences among treatment means on the two parameters. Silkworm pureline DMMMSU 100 fed with folic acid-supplemented mulberry leaves with both concentrations obtained the highest ERR and CYB (Jha *et al.*, 2015; Ferdous, 2016; Hasan, 2020). This was comparable to silkworm purelines, DMMMSU 101 FA1, DMMMSU 101 FA2, DMMMSU 107 FA2, DMMMSU 103 FA1 and FA2, and DMMMSU 102 FA2. It can be noted that most of the silkworm purelines responded positively to folic acid supplementation but in varying concentrations (Nirwani & Kaliwal, 1996; Etebari, 2002; Rai *et al.*, 2002; Ratmathulla *et al.*, 2007). This result showed that folic acid acts as a growth promoter that can significantly increase the yield of cocoons (Bhojne *et al.*, 2014; Meeramaideen *et al.*, 2017).

Conclusions

Based on theresults, folic acid supplementation on mulberry leaves in both concentrations (FA1 and FA2) significantly affected the larval growth and cocoon characteristics of silkworm purelines as indicated by an increase in weight of 10 matured larvae, single shell and cocoon weights, cocoon shell percentage, effective rearing rate and cocoon yield per box. Egg production increased using FA1 formulation. There is an interaction effect of using folic acid as supplement for mulberry leaves and silkworm breed on effective rearing rate and fecundity of the silkworm eggs produced.

Recommendations

Supplementation in mulberry leaves with 0.01 and 0.02% folic acid potentially improves silkworm larval growth, cocoon and egg production.Study trials using higher concentrations of folic acid as a mulberry leaf supplement can be conducted.

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