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Yield performance of Bulgarian race of Silkworm fed on local Mulberry supplemented with Vitamins and Amino Acids

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ABSTRACT

Silkworm rearing is being practiced for commercial purpose in different parts of Pakistan by rural masses as a cottage industry. Silk seed improvement through importation is one of the key initiative towards popularising and ameliorating silk production in Pakistan. The performance of Bulgarian race of silkworm reared on local mulberry supplemented with vitamins(Band C) in addition to amino acids (Alanine and Glycine) was evaluated at24 - 28 °C and 70± 5 % RH during early larval instars ($1^{st}$and 2^{nd})and 24-28°C and 85 ± 5 % RH during last three (3^{rd} -5^{th}) larval instars. Early in stars were fed on chopped mulberry leaves whereas later instars were reared on mulberry leaves dipped with 10% solutions of vitamins (B and C) and 2%amino acid (Alanine and Glycine) in different combinations. Data collected at the end of 3^{rd},4^{th} and 5^{th} instars showed significant variations in larval body length (2.81, 4.27 and 6.02 cm),body weight (0.35, 1.03 and2.74 g) and food consumption (2.60, 3.26 and 4.12 g) when larvae were fed on mulberry leaves dipped in 10 % Vitamin C Solution + 2 % Glycine + Alanine (T_9).Significant differences were also observed in all growth parameters. Fecundity (439.67 #), Fertility (86.0%) and Hatchability (84.67%) were highest in T_9which were significantly different from control (T_0), 422.67, 79.33 and 76.33, respectively. Cocoon Weight (1.36 g), Shell Weight (0.33 g) and Cocoon shell percentage (24.02%) in T_9were significantly different from control, 1.26 g, 0.25 g and 19.89%, respectively. Bulgarian race has potential to thrive under prevailing environmental conditions if reared on mulberry leaves rich in vitamins (B and C) in addition to amino acids (Alanine and Glycine) contents or supplemented.

Key words: Sericulture, Silkworm, Rearing, Mulberry, Supplementation

INTRODUCTION

Sericulture is an eco-friendly cottage industry comprised of mulberry cultivation, rearing of silkworms, spinning of cocoon and processing of silk fibres (Mahmoud, 2013; Singh & Bandey, 2012). Key component of sericulture is silkworm *Bombyx mori,* an important commercial insect that transforms the mulberry proteins into natural silk spun by larvae prior to pupation in the form of cocoons (Tazima, 2001). History of this agro-based industry dates back in 4500 BC when Chinese queen Xi Ling Shi accidently discovered it (Yilmaz *et al*., 2015; Liu, 2010).Despite its great commercial significance, sericultureis expensive in nature due to procurement of disease free silk seeds, silkworm domestication, availability of specific climatic conditions and rearing equipment (Ramesha *et al.,* 2012; Hussain *et al.,* 2011).Silkworm domestication leads to inbreeding depression, thus, biological and commercial aspects of sericulture are prone to natural degradation. This has led researchers to work on the improvement in silkworm traits through importation, hybridization, development of hybrid mulberry varieties, alternate food sources, artificial diets, food supplementation and improvement in rearing procedures (Shah *et al.,*2007). Quality and quantity of mulberry leaves define the standard of silk produced (Nicodemo *et al.,* 2014).Various scientists reported improved performance of silkworm for commercial and biological traits by vitamin B and C supplementation (Balasundaram *et al.,* 2013; Singh & Bandey, 2012; Zannon *et al.,* 2008; Etebari & Mtindoost, 2005). Increment in larval weight, cocoon shell percentage and length of silk filament was observed by supplementation of mulberry leaves with vitamin B (Ahsan *et al.,* 2015; Suprakash & Pal, 2002). Enhancement in commercial traits of silkworm cocoons was observed by supplementation with Vitamin C (Zannon *et al.,* 2008). Nitrogen

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supplementation of mulberry leaves improved quality and quantity of cocoon characters (Javed & Gondal, 2002). In silkworms, silk fibroin is composed of four amino acids: alanine, glycine, tyrosine and serine which are naturally present in mulberry leaves i.e. 1.23% alanine and 5.94% glycine (Yao *et al*., 2002). Alanine and Glycine having important role in glucose metabolism and biosynthetic pathways of silkworm may contribute to enhance its biological and economic performance (Radjabi, 2010). The studies has shown impact of food supplementation with amino acids and vitamins on silkworm biology and cocoon production, however, the response of different races reared under diverse environmental conditions and feeding on different varieties of mulberry result in variable response in terms of cocoon production. Bulgarian race was imported to strengthen local silk seed, improve yield and to boost the confidence of the farming community engaged in the sericulture. Thus, the study was designed to assess the impact of rearing silkworm larvae on local mulberry in addition to evaluate the impact of Vitamin (B and C)and amino acids (Alanine and Glycine) on biological and commercial traits of Bulgarian race silkworm.

MATERIALS AND METHODS

Sericulture as an allied sector of agriculture, greatly depends on the availability of suitable climate conditions, quality of mulberry leaves and rearing process. Rearing equipment and laboratory floors were disinfected with formalin solution (4%) followed by maintenance of airtight conditions for 24 hrs and aeration of laboratory for 12 hrs (Hussain *et al*., 2011).

Egg Incubation, Hatching, brushing and early instar rearing:

Eggs were placed on a single sheet and incubated at 24 - 26˚C and 70 – 80% RH with 16light:08 dark hrs of photoperiod at Sericulture Research Laboratory Lahore, Punjab. Uniform hatching of the larvae was accomplished by black boxing of incubated eggs on the 10th day of the incubation. Newly hatched larvae were transferred from hatching trays to petri dishes and rearing trays by placing mulberry leaves in the hatching trays, allowing them to crawl on it followed by shifting them to rearing trays. Rearing of 1stand 2ndinstar larvae was carried out at24-28°C and 80-90 % RH with Photoperiod (12 light:12 dark). Finely chopped mulberry leaves were fedsix times per day at an interval of four hrs. Bleaching powder and calcium oxide was taken in 1:1 ratio, mixed with sawdust and used to disinfect the larvae after each moult (Hussain *et al*., 2011).

Larval rearing (3rd - 5th instar) and mounting

Larval rearing (3rd - 5th instar) was carried out at 24-28°Ctemperature and 65 - 75%RH. These variations were kept to assess the performance of larvae in conditions usually prevail in the field rearing. Eucalyptus twigs / branches were provided at the end of 5th instar for pupation (Hussain *et al.,* 2011).

Experimental Design

In present work, larvae were provided with mulberry leaves dipped in different solutions of vitamins and amino acids to determine their impact on silkworm biology and cocoon parameters (Table I). These concentrations were prepared by diluting distilled water with specific quantity of amino acids and vitamins (Nicodemo *et al*.,2014). The study was conducted in Completely Randomized Design (CRD) with 04 replications and 50 larvae in each replicate.

Table I: Silkworm larval rearing on local mulberry variety supplemented with different treatments

Treatments	Description
T_0	Mulberry leaves (Control)
T_1	Mulberry leaves dipped in 10 % Vitamin B Solution
T_2	Mulberry leaves dipped in 10 % Vitamin C Solution
T_3	Mulberry leaves dipped in 10 % Vitamin B Solution + 10 % Vitamin C Solution
T_4	Mulberry leaves dipped in 10 % Vitamin B Solution + 2%Glycine
T_5	Mulberry leaves dipped in 10 % Vitamin B Solution + 2%Alanine
T_6	Mulberry leaves dipped in 10 % Vitamin B Solution + 2%Glycine + Alanine
T_7	Mulberry leaves dipped in 10 % Vitamin C Solution + 2%Glycine
T_8	Mulberry leaves dipped in 10 % Vitamin C Solution + 2%Alanine
T_9	Mulberry leaves dipped in 10 % Vitamin C Solution + 2%Glycine + Alanine

Cocoon harvesting, Moth emergence and Egg laying

Larvae spun cocoons at 24 ± 26 ^{0} C temperature and 70-80 % RH and harvested after 8 days from beginning of cocoon spinning. Moths were allowed to emerge from mature cocoons and were picked for pairing, mating and egg laying (Hussain *et al*., 2011).

Data collection and Statistical analysis

Data on larval body length, larval body weight, cocoon weight, shell weight, cocoon shell percentage, food consumption, fecundity, fertility and hatchability were recorded. ANOVA was applied on means against each parameter to determine the significance at P< 0.05. Means were compared by applying Tukey's Test.

Larval Weight and Larval Length:

Larval weight (g) was recorded by using electronic weight balance whereas larval length (cm) was recorded with the help of measuring tape (Hussain *et al*., 2011).

Food Consumption:

The data relating to larval food consumption from 3rd-5th instar were calculated by following formula.

Cocoon Characteristics:

After the completion of cocoon spinning, electronic weight balance was used to calculate the weight of cocoons with pupa and weight of shell after removal of pupa by following formula (Hussain *et al*., 2011).

$$\mathbf{FoodConsumption} = \mathbf{Dry\ weight\ of\ offered\ leaves(g) - Dry\ weight\ of\ residual\ leaves(g)}$$

$$\text{Coccon Weight (g)} = \frac{\text{Weight of 5 female cocoons (g)} + \text{Weight 5 male cocoons (g)}}{10}$$

$$\text{Shell Weight (g)} = \text{Cocoon weight with pupa(g)} - \text{Cocoon weight without pupa(g)}$$

$$\text{Cocoon Shell Percentage} = \frac{\text{Shell weight (g)}}{\text{Cocoon weight (g)}} \times 100$$

Fig. 1: Rearing of silkworm larvae: a) 1st instar larvae, b) 2nd instar larvae, c) 3rd instar larvae, d) 4th instar larvae, e) 5th instar larvae, f) Cocoons,g) Egg laying, h) Eggs

RESULTS

The present study was conducted to evaluate the effect of vitamins(B and C) and amino acids (alanine and glycine) supplementation on biological parameters i.e. length, weight, food consumption, during 3rd, 4th and 5thlarval instars, cocoon weight, shell weight, cocoon shell percentage, fertility, fecundity and hatchability of Bulgarian silkworm race.

Larval body length in 3rd,4th and 5th instars:

Mean larval length (cm) at the end of the 3rdinstar showed maximum length in T_9 (2.81 ± 0.014) followed by T_7(2.80 ± 0.015) and T_8(2.80 ± 0.015) which were significantly different ($F_{(9, 29)}$ = 32.27, P = 0.0000) as compared to control T_0(2.63 ± 0.005).Mean larval length (cm) at the end of the 4thinstar showed maximum length in T_9 (4.27 ± 0.009) followed by T_7 (4.24 ± 0.014) which were significantly different ($F_{(9, 29)}$ = 127.04, P = 0.0000) as compared to control T_0 (3.80 ± 0.005). Mean larval length (cm) at the end of the 5thinstar showed maximum length in T_9 (6.02 ± 0.033) followed by T_7 (6.01 ± 0.024) were significantly different ($F_{(9, 29)}$ = 64.16, P = 0.0000) as compared to control (5.60 ± 0.015) as represented in Table II.

Larval body weight in 3rd, 4th and 5thinstars:

Mean larval weight (g) at the end of the 3rd instar showed maximum weight in T_9 (0.35 ± 0.014) followed by T_8 (0.35 ± 0.010) were significantly different as compared to control T_0(0.28 ± 0.008). Mean larval weight (g) at the end of the 4th instar showed maximum weight in T_9 (1.03 ± 0.017) followed by T_8 (1.00 ± 0.012) were significantly different as compared to control T_0(0.79 ± 0.006).Mean larval weight (g) at the end of the 5th instar showed maximum weight in T_9 (2.74 ± 0.017) followed by T_8 (2.73 ± 0.017) were significantly different as compared to control T_0(2.57 ± 0.014) as presented in Table II.

Larval food consumption (g) in 3rd, 4th and 5th instars:

At the end of the 3rd instar, maximum food consumption recorded by T_9 (2.60± 0.017) followed by T_8 (2.59 ± 0.05) were significantly different as compared to control T_0(2.28± 0.009).At the end of the 4th instar, maximum food consumption recorded by T_9 (3.26± 0.020) followed by T_8 (3.25± 0.08) were significantly different as compared to control T_0(2.63± 0.011).At the end of the 5th instar, maximum food consumption recorded by T_9 (4.12 ±0.037) followed by T_8 (4.10± 0.012) were significantly different as compared to control T_0(3.75± 0.014) as summarized in Table III.

Fecundity (no.):

Number of eggs laid by a single female moth was highest in T_9(439.67± 3.527) followed by T_3(437.67 ± 1.666) which were significantly different as compared to control T_0(422.67± 2.333) as summarized in Fig.2.

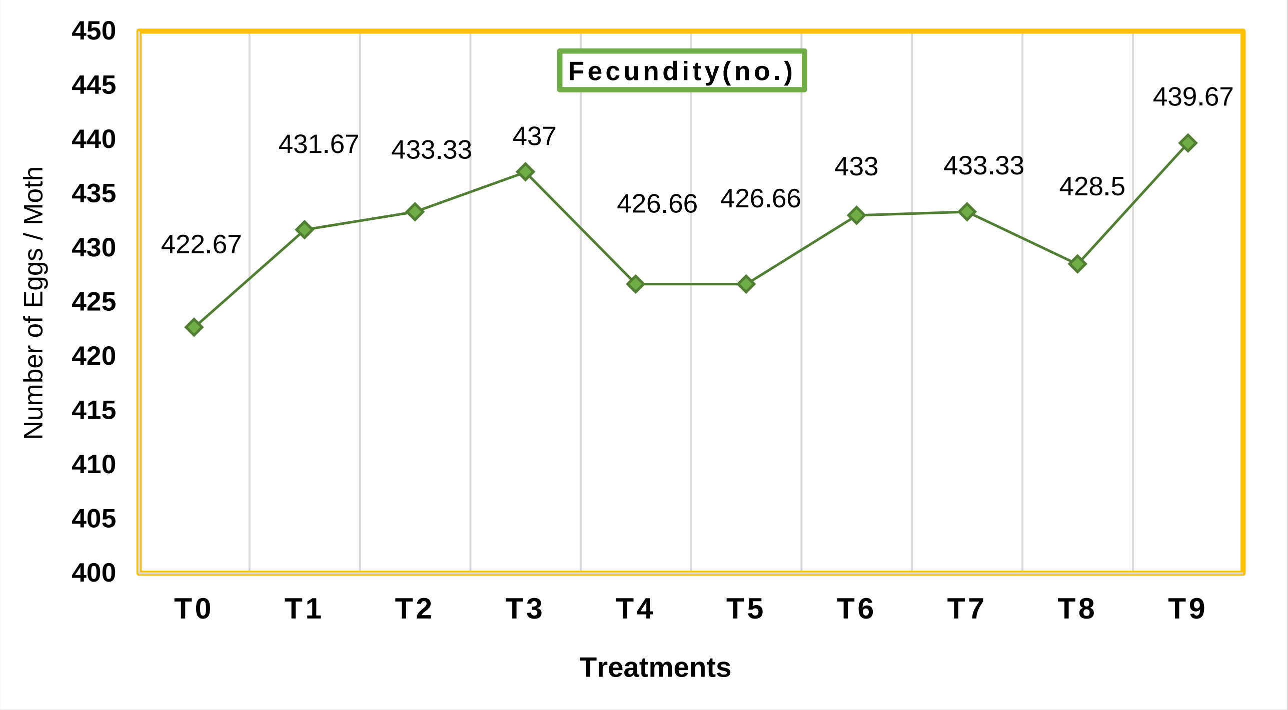

Fig. 2: Fecundity of Bulgarian race reared on local mulberry under different treatments of vitamins (B and C) and amino acids (alanine and glycine)

Fertility (%):

Fertility rate was highest in T_9(86.00± 1.154) followed by T_3(84.33± 4.333) which were significantly different as compared to control T_0(79.33± 2.603) whereas fertility in other treatments showed significant variations (Fig.3).

Hatchability (%):

Hatchability (%) was highest in T_9 (84.67± 1.201) followed by T_3(83.33± 5.004) which were significantly different from control T_0(76.33± 2.403) whereas hatchability in other treatments showed significant differences (Fig.3).

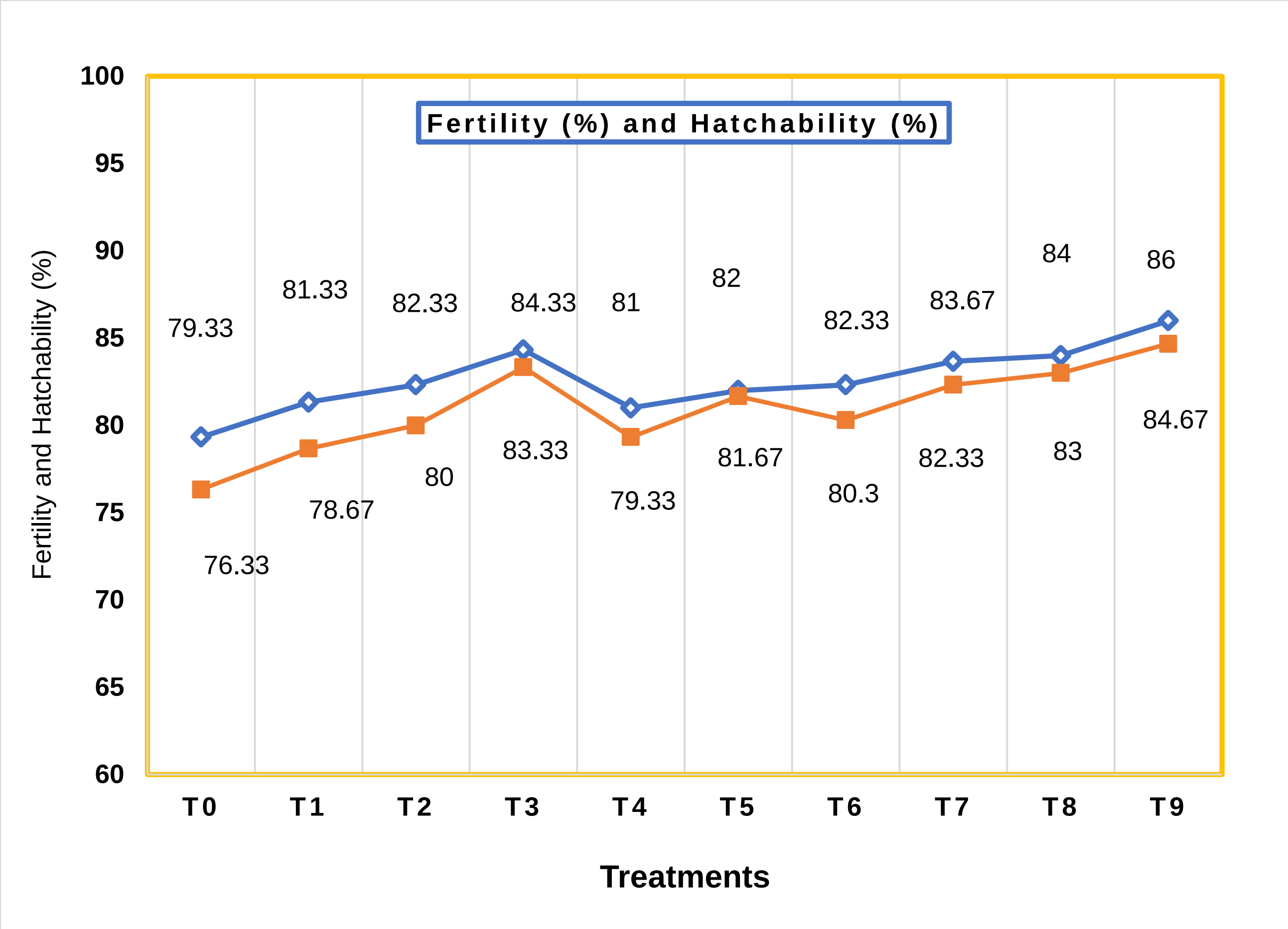

Fig. 3: Fertility and hatchability of Bulgarian race reared on local mulberry under different treatments of vitamins (B and C) and amino acids (alanine and glycine)

Cocoon weight (g):

At the end of spinning, maximum cocoon weight was recorded by T_9(1.36 ± 0.015) followed by T_8 (1.34 ± 0.014) that were significantly different ($F_{(9, 29)}$ = 11.346, P = 0.0000) from control T_0(1.26 ± 0.008) and cocoon weight means in other treatments also showed significant differences (Table III).

Shell weight (g):

Maximum shell weight was recorded by T_9(0.33 ± 0.003) followed by T_8 (0.31 ± 0.003) that were significantly different ($F_{(9, 29)}$ = 40.729, P = 0.0000) from control T_0(0.25 ± 0.005) shell weight means in other treatments also showed significant differences (Table III).

Cocoon shell percentage:

Maximum cocoon shell percentage was recorded by T_9(24.02 ± 0.056**)** followed by T_8 (23.45 ± 0.499) that were significantly different ($F_{(9, 29)}$ = 18.419, P = 0.0000) from control T_0(19.89 ± 0.324) and cocoon shell percentage in other treatments also showed significant differences (Table III).

Table II: Larval body length and body weight of Bulgarian race reared on local mulberry in response to different treatments

Treatments	Larval Body Length (Mean ± SE)			Larval Body Weight (Mean ± SE)		
	3rd instar	4th instar	5th instar	3rd instar	4th instar	5th instar
T_0	2.63 ± 0.005 a	3.80 ± 0.005 a	5.60 ± 0.015 a	0.28 ± 0.008 a	0.79 ± 0.006 a	2.57 ± 0.014 a
T_1	2.66 ± 0.005 b	3.91 ±0.014 b	5.67 ± 0.012 b	0.28 ± 0.006 a	0.84 ± 0.008 b	2.62 ± 0.014 b
T_2	2.72 ± 0.008 c	4.04 ± 0.023 c	5.81 ± 0.009 c	0.34 ± 0.008 b	0.94 ± 0.009 c	2.69 ± 0.014 c
T_3	2.79 ± 0.011 d	4.15 ± 0.015 d	5.88 ± 0.014 d	0.34 ± 0.006 b	0.96 ± 0.017 c	2.68 ± 0.014 c
T_4	2.79 ± 0.011 d	4.11 ± 0.006 d	5.94 ± 0.011 e	0.30 ± 0.008 a	0.85 ± 0.011 b	2.63 ± 0.012 b
T_5	2.78 ± 0.011 d	4.12 ± 0.003 d	5.97 ± 0.005 e	0.31 ± 0.005 b	0.84 ± 0.008 b	2.65 ± 0.015 c
T_6	2.79 ± 0.012 d	4.15 ± 0.009 d	5.97 ± 0.014 e	0.33 ± 0.006 b	0.89 ± 0.012 c	2.66 ± 0.018 c
T_7	2.80 ± 0.014 d	4.24 ± 0.014 e	6.01 ± 0.024 f	0.34 ± 0.014 b	0.96 ± 0.014 c	2.73 ± 0.012 d
T_8	2.80 ± 0.015 d	4.14 ± 0.014 d	6.00 ± 0.029 f	0.35 ± 0.010 c	1.00 ± 0.012 d	2.73 ± 0.017 d
T_9	2.81 ± 0.014 de	4.27 ± 0.009 e	6.02 ± 0.033 f	0.35 ± 0.014 c	1.03 ± 0.026 d	2.74 ± 0.017 d

Mean values with different letters in each column are significantly different from each other at P<0.05,Tukey's Test

Table III: Food consumption of Bulgarian race reared on local mulberry in response to different treatments

Treatments	Food Consumption (Mean ± SE)			Cocoon (Mean ± SE)		
	3rd instar	4th instar	5th instar	Weight(g)	Shell weight(g)	shell (%)
T_0	2.28 ± 0.009 a	2.63 ± 0.011 a	3.75 ± 0.014 a	1.26 ± 0.008 a	0.25 ± 0.005 a	19.89 ± 0.324 a
T_1	2.29 ± 0.008 a	2.65 ± 0.012 a	3.76 ± 0.008 a	1.27 ± 0.012 a	0.26 ± 0.003 a	20.16 ± 0.366 b
T_2	2.38 ± 0.010 b	2.89 ± 0.008 b	3.87 ± 0.012 b	1.30 ± 0.008 b	0.26 ± 0.006 a	20.17 ± 0.538 b
T_3	2.37 ± 0.003 b	2.98 ± 0.006 b	3.91 ± 0.014 b	1.33 ± 0.008 c	0.27 ± 0.006 a	20.25 ± 0.526 b
T_4	2.39 ± 0.015 b	2.67 ± 0.014 a	3.76 ± 0.014 a	1.26 ± 0.006 a	0.26 ± 0.003 a	20.90 ± 0.281 b
T_5	2.42 ± 0.014 c	2.74 ± 0.012 a	3.79 ± 0.014 a	1.27 ± 0.008 a	0.26 ± 0.003 a	20.78 ± 0.179 b
T_6	2.41 ± 0.008 c	2.74 ± 0.012 a	3.83 ± 0.012 b	1.27 ± 0.012 a	0.27 ± 0.003 a	21.58 ± 0.081 c
T_7	2.53 ± 0.012 d	3.21 ± 0.012 c	3.87 ± 0.014 b	1.32 ±0.014 c	0.31 ± 0.003 b	23.12 ± 0.381 d
T_8	2.59 ± 0.005 e	3.25 ± 0.008 d	4.10 ± 0.012 c	1.34 ± 0.014 c	0.31 ± 0.003 b	23.45 ± 0.499 d
T_9	2.60 ± 0.017 e	3.26 ± 0.020 d	4.12 ± 0.037 c	1.36 ± 0.015 d	0.33 ± 0.003 c	24.02 ± 0.056 d

Mean values with different letters in each column are significantly different from each other at P<0.05, Tukey's Test

DISCUSSION

To intensify the economic status of sericulture industry, nutritional supplementation plays major role. In this context, the study was conducted to evaluate the impact of vitamin B, C and Amino acids(alanine and glycine) on larval, cocoon and biological parameters of silkworm *Bombyxmori.* Supplementation with these additives provide significant results in larval parameters (larval length, larval weight, food consumption), cocoon traits (cocoon weight, shell weight and cocoon shell percentage) and biological parameters (fecundity, fertility and hatchability).Our findings are similar to previous researchers who investigated that provision of supplemented food and artificial diet with mulberry leaves yielded high quality cocoons. Myriad of work was done on supplementation of mulberry leaves with various additives like vitamins, minerals, proteins, sugars and amino acids (Thulsai &Sivaprasad, 2015; Ahsan *et al.,* 2015; Khyade & Shandage, 2012; Faruki, 2005). Some researchers gave positive results by supplementation of vitamin B and C (Ahsan *et al.,* 2015; Balasundaram *et al.,* 2013; Singh & Bandey, 2012) while in certain cases, vitamin supplementation showed no effect on larval and cocoon characteristics (Castillo *et al.,* 2016). In present work, cocoon traits enhanced by supplementation with vitamin C that are in collinear with previous findings in which treated cocoons were superior over control (Balasundaram*et al.,* 2008).It was investigated by Etabari (2002) that ascorbic acid with 2% concentration leads to an increase in cocoon shell weight that was similar to our results.During 5thinstar, 5% alanine and 5% Glycine made significant increase in larval weight (Sarkar *et al.,* 1995) that was similar to our findings. Some researchers reported that larval and cocoon parameters were increased by supplementation with vitamin B and C (Ahsan *et al.,* 2015) and our findings were collinear with them. Food ingestion and food consumption activity was enhanced upon multivitamin and mineral supplementation of mulberry leaves (Prassad, 2004).Augmentation of mulberry leaves with multivitamins leads to enhancement in female cocoon shell weight (Etabari&Mtindoost, 2005). Radjabi(2009) suggested that alanine has led to increase in larval weight but mean differences are not significant that is near to this recent work. Radjabi (2010) reported that alanine does not paly role to improve economic parameters of silkworm while Asparagine showed positive results at some extent. It was demonstrated from the present study that if Bulgarian race of silkworm would be provided with local mulberry leaves supplemented with vitamins (B and C) and amino acids (alanine and glycine) it would lead to an increase in output of sericulture industry. Provision of silk free seeds coupled with highly nutritive mulberry varieties would lead to successful harvesting of silk cocoons ultimately promoting sericulture industry in Pakistan.

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