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Nutrient supplementation using amino acid for growth and yield of silkworm, *Bombyx mori* L.

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Abstract

In sericulture, mulberry leaves are the main food source for silkworms. Over the years, researches were conducted on supplementation of essential nutrients to improve mulberry leaf quality in order to meet the nutrient requirements for silkworm growth and development. Hence, this study determined the effect of amino acid (AA) supplementation on the growth and yield of silkworm (*Bombyx mori* L) and on the occurrence of diseases. A Philippine silkworm hybrid, DMMMSU 346 was used as testing organism and fed with mulberry leaves sprayed with 0.5% AA (given 4x a day); 0.5% AA (given 2x a day); 1.0% AA (given 4x a day); and 1.0% AA (given 2x a day), then compared to the control. The treatments were laid out in Completely Randomized Design with three replications. Data gathered were computed and analyzed using Analysis of Variance and treatment means were compared using Duncan's Multiple Range Test. Results revealed that amino acid supplementation of mulberry leaves at 0.5% concentration and fed to silkworms 4x a day and reared during cold-dry season is ideal for cocoon yield per box, filament length and effective rearing rate. Supplementation of 1% concentration fed 2x a day positively affected larval weight at maturity. Grasserie disease was observed but did not significantly affect the survival rate of silkworm during rearing.

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Introduction

Sericulture involves mulberry cultivation, silkworm rearing and silk production. Although considered as a labor-intensive enterprise, it also contributes to rural development and economic growth in sericulture-engaged countries like the Philippines. Mulberry varieties have been evolved and introduced and traditionally cultivated. These varieties are continuously improved to ensure production of high quality leaf to meet essential nutrient requirement of silkworm (*Bombyx mori* L.), as silk productivity is related to the quantity and quality of mulberry leaves (Akuli *et al.*, 2012; Ramesh, Pushparaj, Prabu, & Rajasekar, 2018 and Ruth, Ghatak, Subbarayan, Choudhury, Gurusubramanian, Kumar & Bin, 2019). Further, nutrition plays a vital role in influencing the performances of different stages of silkworm (Kumar & Balasubramaniyan, 2013). Development of silkworm is greatly influenced by the nutrient composition of the mulberry leaves, which is also the determining factor of the quality of silk (Jyothi *et al.*, 2014 and Ruth *et al.*, 2019).

Amino acids as precursors of proteins are essential to all organisms including the silkworms. Literature on nutritional significance of amino acids in silkworm *Bombyx mori* reveals the quantitative requirements of amino acids (Wang *et al.*, 2017). Amino acids which when bind together as long chains in protein will be broken down to amino acids in the process of digestion mediated by the digestive enzymes in the midgut. The amino acids when separated are released into the blood stream and will be utilized by individual cells to assemble new and different protein required for specific functions. Silkworms do not have enzymes required to synthesize all the amino acids. The six amino acids which silkworm can produce are proline, alanine, glycine, serine, tyrosine and cystine. Essential amino acids, required for synthesis of silk (arginine, histidine, tryptophan, isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine) are obtained only through food. In addition, the silkworm larvae also require either aspartic or glutamic acid for normal growth and development (Ito & Arai, 2003).

In relation with silk productivity, cocoon shell percentage hardly exceeds 20% using artificial diet and to some extent, affects good growth and better silk quality. However, the introduction of hormone administration to silkworm larvae is essential to increase cocoon production as it ensures an improvement of 10-20% on cocoon and silk yield (Offord *et al.,* 2016).

The silkworm, *B. mori* is also susceptible to various diseases caused by viruses, bacteria, fungi and microsporidia. This susceptibility is further aggravated by environmental factors such as temperature, humidity, bad ventilation and nutritional deficiency, which consequently cause considerable damage to silkworm crop (Babu, Ramakrishna, Reddy, Lakshmi, Naidu, Basha & Bhaskar, 2009). Thus, measures are also made to prevent and control diseases which include nutrient supplementation. Aside from evaluating the effects of amino acid supplementation on the growth and yield of silkworm, the study also investigated its impact on disease occurrence in silkworm.

Materials and methods

The study was conducted at DMMMSU-SRDI silkworm rearing station during cold- dry (November-December) and warm-dry (April-May) seasons from 2012-2015 using silkworm hybrid, DMMMSU 346.

Incubation of eggs and brushing of larvae

The silkworm eggs were incubated for 12 days. After hatching, the larvae were brushed at 10 o'clock in the morning and fed with plain mulberry leaves up to 4th instar- 1st day.

Treatment preparation and statistical design

Amino acid tablets were pulverized and mixed with distilled water to make a stock solution from which the different AA concentrations were formulated.

A_0 = no AA supplementation, given 4x a day

A_1 = 0.5% AA supplementation + distilled water, given 4x a day

A_2 = 1.0% AA supplementation + distilled water given 4x a day

A_3 = 0.5% AA supplementation + distilled water, given 2x a day
A_4 = 1.0% AA supplementation + distilled water, given 2x a day

The treatments were laid out using the Complete Randomized Design (CRD) with three replications, with 100 larvae per replication.

Treatment administration
Amino acid supplements were sprayed to mulberry leaves (*Batac* variety) at a volume of 100ml to 250 grams of mulberry leaves then fed to the larvae from 4th instar-2nd day until maturity. The AA supplements were administered at two feeding schedules: 4x a day (normal-5AM, 10AM, 3PM, 7PM) and 2x a day (5:00AM & 7:00PM).

The experiment was carried out at room temperature ranging from 27°C to 35°C and RH at 57% to 83% in warm-dry season; 24°C to 29°C and RH at 64% to 74.80% during cold-dry season (Figure 1).

Data collection and analysis
Data gathered include weight of 10 matured larvae, cocoon yield box^{-1}, single cocoon weight, cocoon shell percentage, filament length, effective rearing rate/survival rate, and cost and return analysis. These were analyzed using Analysis of Variance (ANOVA) and significant differences between treatments were further tested using Duncan's Multiple Range Test (DMRT).

Results and discussion
The growth and yield performance of silkworm across season as affected by different concentrations of AA supplementation was evaluated based on the following parameters.

Weight of 10 matured larvae (g)
There was no significant difference observed on the weight of 10 matured larvae on Year 1 of testing (Table 1). Larvae fed with mulberry leaves treated with AA had statistically similar weights with the untreated larvae at maturity. This implies that the nutrient quality of mulberry leaves with or without AA supplementation is sufficient for larval growth regardless of season.

Table 1. Mean weight of 10 matured larvae (g) as affected by treatment and season.

Treatment	Season	Y1 (2012) Mean	Y2 (2013) Mean	Y3 (2014) Mean
A_0 – no AA	S_1	33.20	23.43 c	21.27 f
(4x a day)	S_2	29.12	28.98 a	28.73 ab
A_1 – 0.5% AA	S_1	34.18	26.57 b	24.57 c
(4x a day)	S_2	31.03	28.05 ab	27.40 bcd
A_2 –1.0% AA	S_1	34.46	26.27 b	26.27 cde
(4x a day)	S_2	31.76	28.05 a	27.87 abc
A_3 –0.5% AA	S_1	33.98	-	25.10 e
(2x a day)	S_2	29.27	-	27.50 bc
A_4 –1.0% AA	S_1	-	-	25.53 de
(2x a day)	S_2	-	-	29.64 a
Level of significance		ns	*	*
C.V. (%)		4.63	4.48	3.92

*All means followed by same letter are not significantly different (DMRT).

Legend:
S_1 – (warm-dry season) April – May 2x a day: given 5AM & 7PM
S_2 – (cold-dry season) Nov – Dec 4x a day: given 5AM, 10AM, 3PM & 7PM

In Year 2 however, A_1 and A_2 supplementation gave the same weight of ten matured larvae (28.05g) when reared during S_2, which is comparable with the control (28.98g). On the other hand, the lowest weight was seen in larvae fed with non-AA-supplemented leaves (23.43g), reared in S_1 (warm-dry season, on the months of April-May). In Year 3, larvae fed with A_4 supplementation, gave the highest weight (29.54g) but comparable to those fed with A_2 (27.87g) and control (28.73g), all reared in S_2, while larvae fed with plain mulberry leaves registered the lowest weight (21.27g), reared in S_1.

These results imply that rearing season affects larval growth (Hussain *et al.*, 2011) and that S_2 (cold-dry season, on the months of November to December) was found more favorable for comparably high weight of ten matured larvae using both A_1 and A_2, fed 4x a day; and A_4, fed 2x a day. The same finding was reported by Radjabi (2010) where larval growth was positively affected with enrichment of two amino acids, alanine and aspargine.

Single Cocoon Weight (SCW, g)

Feeding of mulberry leaves treated with different concentrations of AA during warm and cold dry season did not affect the weight of single cocoon across years (Table 2). This implies that supplementation of AA in the diet of silkworm did not increase the cocoon size in both rearing seasons.

Table 2. Mean weight of single cocoon (g) as affected by treatment and season.

Treatment	Season	Y1 (2012) Mean	Y2 (2013) Mean	Y3 (2014) Mean
A_0 – no AA (4x a day)	S_1	1.41	1.12	1.13
	S_2	1.36	1.38	1.44
A_1 – 0.5% AA (4x a day)	S_1	1.69	1.19	1.20
	S_2	1.52	1.45	1.45
A_2 –1.0% AA (4x a day)	S_1	1.71	1.14	1.21
	S_2	1.53	1.58	1.45
A_3 –0.5% AA (2x a day)	S_1	1.65	-	1.22
	S_2	1.52	-	1.42
A_4 –1.0% AA (2x a day)	S_1	-	-	1.23
	S_2	-	-	1.49
Level of significance		ns	ns	ns
C.V.		7.69	6.72	7.30

Legend:

S_1 – (warm dry season) April – May 2x a day: given 5AM & 7PM

S_2 – (cold dry season) Nov – Dec 4x a day: given 5AM, 10AM, 3PM & 7PM

Table 3. Mean cocoon shell percentage (%) as affected by treatment and season.

Treatment	Season	Y1 (2012) Mean	Y2 (2013) Mean	Y3 (2014) Mean
A_0 – no AA (4x a day)	S_1	18.71	17.01	18.67
	S_2	17.44	19.32	19.51
A_1 – 0.5% AA (4x a day)	S_1	18.60	17.69	19.19
	S_2	19.34	20.98	19.21
A_2 –1.0% AA (4x a day)	S_1	20.08	19.30	19.94
	S_2	10.11	20.66	19.03
A_3 –0.5% AA (2x a day)	S_1	18.65	-	18.58
	S_2	18.94	-	19.56
A_4 –1.0% AA (2x a day)	S_1	-	-	19.74
	S_2	-	-	19.23
Level of significance		ns	ns	ns
C.V.		5.74	6.43	6.69

Legend:

S_1 – (warm dry season) April – May 2x a day: given 5AM & 7PM

S_2 – (cold dry season) Nov – Dec 4x a day: given 5AM, 10AM, 3PM & 7PM

Cocoon shell percentage (CSP, %)

Amino acid supplementation did not affect CSP of larvae reared during warm and cold dry season. Recorded CSPs ranged from 10.11% to 20.98%. This could be attributed to the nutritive content of the leaves which is enough for the growth and yield of silkworm and the rearing environment is favorable for their growth and development which ranged from 27°C to 29°C and relative humidity of 70% to 75%.

Cocoon Yield Box^{-1} (CYB, kg)

Amino acid supplementation significantly affected CYB only in Year 3 where the highest CYB (23.26kg) was recorded in silkworms fed with plain mulberry leaves reared in S_2 (Table 4). Moreover, comparatively lower CYB were observed using A_1 and A_3 during S_1 and S_2; and A_4 during S_1. On the other hand, A_4 resulted to the lowest CYB (14.15kg), reared during S_2. Based on these results, more cocoons can be produced, thereby higher cocoon yield can be obtained even without amino acid supplementation.

Table 4. Mean cocoon yield box^{-1} (kg) as affected by treatments and rearing season.

Treatment	Season	Y1 (2012) Mean	Y2 (2013) Mean	Y3 (2014) Mean
A_0 – no AA (4x a day)	S_1	11.06	16.24	16.80 bc
	S_2	22.30	24.58	23.26 a
A_1 – 0.5% AA (4x a day)	S_1	12.50	15.76	19.10 abc
	S_2	25.67	24.44	20.29 ab
A_2 –1.0% AA (4x a day)	S_1	15.05	15.35	17.39 bc
	S_2	27.62	25.91	16.86 bc
A_3 –0.5% AA (2x a day)	S_1	20.15	-	19.45 abc
	S_2	27.27	-	17.37 abc
A_4 –1.0% AA (2x a day)	S_1	-	-	16.37 abc
	S_2	-	-	14.15 c
Level of significance		ns	ns	*
C.V.		10.66	7.95	15.16

*All means followed by same letter are not significantly different (DMRT).

Legend:

S1 – (warm dry season) April – May

2x a day: given 5AM & 7PM

S2 – (cold dry season) Nov – Dec

4x a day: given 5AM, 10AM, 3PM & 7PM

Filament Length (FL)

As reflected in Table 5, the effects of AA treatment on FL was seen significant only in Year 2 where A_1 gave

longer filaments in both rearing seasons (S_1=786.20 m and S_2=792.40m). These figures were comparable with A_2 but only in silkworms reared in S_1 (747.90m). Silkworms can be fed with AA-supplemented leaves at 0.5% concentration and reared during cold-dry season to give comparably long filament.

Table 5. Mean filament length (cm) as affected by AA and season.

Treatment	Season	Y1 (2012) Mean	Y2 (2013) Mean	Y3 (2014) Mean
A_0 – no AA (4x a day)	S_1	781.37	784.60 a	820.27
	S_2	836.54	591.10 b	818.54
A_1 – 0.5% AA (4x a day)	S_1	728.63	786.20 a	872.27
	S_2	764.20	792.40 a	891.45
A_2 –1.0% AA (4x a day)	S_1	794.70	747.90 a	872.46
	S_2	781.00	503.70 c	850.22
A_3 –0.5% AA (2x a day)	S_1	821.19	-	721.28
	S_2	847.67	-	830.20
A_4 –1.0% AA (2x a day)	S_1	-	-	739.50
	S_2	-	-	800.10
Level of sig.		ns	*	ns
C.V.		5.22	6.57	5.88

*All means followed by same letter are not significantly different (DMRT).

Effective Rearing Rate (ERR, %)

The ERR of silkworms was significantly affected by AA supplementation only in Year 1 where highest ERR was recorded using A_1 with 94.56% but comparable with A_2 during cold dry season (Table 6). The lowest ERR was in silkworms fed with plain mulberry leaves with 34.66%, reared during warm-dry season. This implies that 0.5% AA supplement when fed to silkworm can increase the survival rate.

Table 6. Mean effective rearing rate as affected by treatment and season.

Treatment	Season	Y1 (2012) Mean	Y2 (2013) Mean	Y3 (2014) Mean
A_0 – no AA (4x a day)	S_1	34.66 b	72.67	84.00
	S_2	88.36 a	89.00	80.00
A_1 – 0.5% AA (4x a day)	S_1	38.33 b	64.67	86.67
	S_2	94.56 a	84.33	72.00
A_2 –1.0% AA (4x a day)	S_1	46.33 b	66.67	79.67
	S_2	93.95 a	88.00	60.00
A_3 –0.5% AA (2x a day)	S_1	71.00 a	-	84.00
	S_2	94.22 a	-	68.33
A_4 –1.0% AA (2x a day)	S_1	-	-	84.00
	S_2	-	-	51.33
Level of sig.		*	ns	ns
C.V.		10.10	5.95	15.05

*All means followed by same letter are not significantly different (DMRT).

Disease incidence

In the entire course of the study the occurrence of Grasserie disease was observed but minimal. This may be accounted to the finding that although grasserie is known to be one of the most serious diseases infecting silkworms in tropical countries that occurs throughout the year, its intensity varies with seasons (Joshi and Ahmad, 2017).

Conclusion

Based on the results, supplementing mulberry leaves with 1% amino acid concentration, administered 2x a day during cold-dry season positively affects larval weight, whereas 0.5% amino acid concentration, given 4x a day can improve economic parameters such as cocoon yield box^{-1}, filament length and effective rearing rate of silkworms when reared during cold-dry season. Moreover, colder temperature is seen more favorable for silkworm rearing when amino acid supplementation is used. Grasserie disease can be observed at a minimal level.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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