CATRINA (2024), 30 (1):31-39

Potential of Egg Albumen as a Source of Amino Acids and Protein Supplement on the Production of Silkworm *Bombyx mori* L.

Marwa N. Moustafa*
Sericultural Department, Plant Protection Institute, Agriculture Research Center, Dokki, Giza, Egypt

ABSTRACT

The mulberry silkworm is a monophagous insect that depends solely on mulberry leaves as its primary feeding source. Therefore, the quality of mulberry leaves and nutritive supplements are significant factors affecting silk production. This investigation was carried out to evaluate the potential of hen egg albumen as a protein and amino acid source impacting various biological and biochemical characters of mulberry silkworm. Larvae were reared on mulberry leaves enriched with different concentrations of hen egg albumen (10%, 20%, 30%, and 40%) from 4th larval instar until the spinning stage (odd days). While hen egg albumen contains amino acids vital for silk production, excessive doses led to reduce the production due to an abundance of non-silk-producing amino acids or those not involved in silk structure, resulting in silk metabolism disorders. Larvae reared on leaves fortified with (10 %, 20%) concentrations showed improvements in all biological and biochemical parameters, with no significant differences observed between the two concentrations in cocoon criteria. However, 20% concentration treatment exhibited a significantly higher shell ratio percentage, 5th larval weight, growth index, cocooning and pupation ratios. The results also showed that higher concentrations of hen egg albumen were significantly inversely related to cocoon production.
Keywords: Biological parameters; Biochemical analysis; Cocoon production; Hen egg albumen; Mulberry silkworm.

INTRODUCTION

Sericulture is one of the most important industries that have attention in Egypt, recently. Silk is used to produce a wide range of products, including clothing, accessories, and home furnishings, also, in the (bio) medicine sector, silk is being used to develop new and innovative medical treatments Holland, *et al.* (2019). The economic significance of silk is primarily determined by the enhancement of silk gland growth and silk protein production Chang, *et al.* (2015) and Peng *et al.* (2019).

Mulberry silkworm is an herbivorous insect that feeds on mulberry leaves only to produce silk worldwide so it has attention in the countries that interested silk production, Aswartha *et al.* (2017) and Chauhan and Tayal (2017). Although mulberry leaves variety is very important to produce good silk but some additives may be required to produce silk with high quality. All scientific reviews that are interested with food supplementation of silkworm larvae referred to the improvement of commercial and biological aspects of silkworms Ahsan *et al.*, (2013) Kanafi *et al.*, (2007).

Nutritional supplements that can help to improve the efficiency of food consumption and utilization by silkworms, lead to higher silk quality Pawar, *et al.*, (2017). All nutritional additives that have vitamins, amino acids and proteins play a vital role in increasing the economic traits of silkworm Amalarani *et al.* (2011). Also, the progression of silk gland growth depends on the healthy nutrition of silkworm and its status (Kumar and Gangwar 2010). Enhancing mulberry leaves with 1% of glycine have beneficial effects on the silk production and economic traits saad *et al.* (2014). The green and sustainable way to modify silk fibers by directly feeding silkworms with various additives such as amino acids Teramoto and Kojima (2014). These additives are mainly distributed in silk fibroin and can improve the thermal and mechanical properties of silk fibers. It's a fascinating approach to in-sit functionalization.

Bombyx mori, utilizes all amino acids in Egg albumen especially the essentials during the growth and development to synthesis silk fiber Islam *et al.* (2020). Due to its technological properties anti-microbial and antiviral activity egg white protein used as a raw material for the food and pharmaceutical industries Kovacs-Nolan *et al.* (2005).

Silk production involves a series of connected steps. Initially, silkworms metabolize their food into protein, which ultimately forms the silk fiber. Proteins are distributed throughout various parts of insects, including the cytoplasm, organelles fat bodies and hemolymph, which serving as a metabolic reservoir that stores nutrients and energy (Zhou, *et al.*, 2015; Hou, *et al.*, 2010). There is no alternative resource available for producing fibroin and sericin in the fifth instar of silkworms aside from haemolymph protein (Shivkumar and Subramanya, 2015). Approximately 72-86% of the necessary amino acids for silkworms are sourced from mulberry leaves, with 60% being utilized for silk production, while the remaining amino acids are derived from the tissues and blood of the silkworm (Bhattacharyya *et al.*, 2016; Lu and Ziang, 1988). The synthesis of silk proteins necessitates the presence of free amino acids in the hemolymph (Mahmoud *et al.*, 2013). Egg yolk, known for being a crucial source of proteins, essential fatty acids, vitamins, and minerals, is distinct from egg albumen (Gray and Griffin, 2009). Egg white protein serves as a valuable source of dietary protein (Yin *et al.*, 2014).

Proteins found in egg albumen are glycoproteins

* *Corresponding author e-mail: marwamoustafa931@gmail.com*

(Harvey *et al.*, 2000; Raikos *et al.*, 2006). Egg white constitutes 58% of an egg and contains approximately 10-12% protein content, which is rich in ovalbumin, ovotransferrin, ovomucoid, globulins, and lysozyme (Mine, 2002). Egg albumen contains 18 amino acids, including all the essential amino acids required for silk production (Powrie, 1985). Egg whites are nutrient-rich, supporting healthy embryo growth and providing protection against harmful microorganisms (Cook *et al.*, 2005; Alabdeh *et al.*, 2011). In less than half a cup, which represents hundreds of grams, there are 5.5 gm of alanine, 2.9 gm of glycine, 6 gm of serine, and 1.9 gm of cysteine, along with about 4 grams of protein and 55 mg of sodium (Tajamul *et al.*, 2020).

This study aimed to investigate the impact of hen egg albumen, a natural and cost-effective additive rich in proteins and amino acids, on silk production, particularly during the summer season when the quality of mulberry leaves is typically low. The objective was to explore the potential of using egg albumen as a supplement to enhance the rearing of silkworms fed on mulberry varieties with lower quality.

MATERIALS AND METHODS

The experiment was carried out on Sericultural Research Department, Plants Protection Institute, Agricultural Research Centre in Giza.

Preparation of stock culture

Silkworm seeds were imported from India (PM X CSR2) then incubated at 27 ± 1°C and 80 ± 5% RH till hatching. Rearing was carried out under hygienic conditions according to Uma (2003). Newly hatched silkworm reared in the summer season at 29-32 °C and relative humidity 80-90% RH . Silkworm fed on clean mulberry leaves from hatching till the end of the 3rd larval instar.

Egg White Sampling

Fresh eggs of hen were collected from the farm of Poultry Department, Faculty of Agriculture, Cairo University, Egypt.

Experimental Design

At the beginning of 4th larval instar, the larvae divided to 4 groups each group consists of three replicates each one consists of 50 larvae. All groups fed on sprayed mulberry leaves with aqueous solution of freshly hen egg albumen at different concentrations (10%, 20%, 30%, and 40%). The sprayed mulberry leaves were kept in the shade for half an hour to remove excess moisture. Subsequently, the leaves were fed to the larvae from the first day of the fourth instar. The silkworms were fed hen egg albumen-treated leaves once and normal mulberry leaves three times on alternate days until the spinning stage of cocoons.

Biological analysis

The study involved the measurement of larval weight at the end of the 4th and 5th instars, overall growth index for the 4th and 5th instars, pupation ratio, cocooning percentage, pupal weight, as well as the weights of single cocoon and single shell. Additionally, the single cocoon shell ratio percentage was calculated.

Biochemical Analysis

Hemolymph was collected from treated larvae at 6th day of 5th larval instar by removal of thoracic leg in 1.5 ml Eppendorf tubes with small amount of phenyl thiourea crystal (PTU) as an anti-coagulant substance, Mahmoud (1988). The tubes were kept at -20°c, the blood samples were centrifuged at 10000 rpm for 10 minutes at 5°C. The supernatant was collected and assayed to determined different parameters as follow:

Total protein

Total protein was determined using the method of Bradford (I976).

Total carbohydrate

The total carbohydrate content was determined in the acid extract of the sample using the phenol-sulfuric acid reaction method established by Dubois *et al.* (1956). The extraction and preparation of total carbohydrates for the assay were conducted following the protocol defined by Crompton and Birt (1967). The total carbohydrate content is expressed as μg of glucose/g of fresh weight.

Total free amino acids determination

Total amino acids were colorimetrically assayed by ninhydrin reagent according to the method described by Lee and Takabashi (1966).

Data analysis

The values of the measured parameters were presented as Mean ± SE. One-way analysis of variance (ANOVA) was conducted using SAS software (SAS/STAT® 9.1, 2004). The Least Significant Difference (LSD) test was performed at a significance level of $p \leq 0.05$ within the same program.

RESULTS

Biological Analysis

Feeding silkworms on mulberry leaves enriched with different concentrations of egg albumen, showed adverse effect on 4th larval mean weight as, control larvae was the highest significantly mean weights (0.509 g) while the minimum mean weight was (0.406 g) for 40% treatment as recorded in Table (1). Whereas, the data elevated in 5th larval instar, the weight of larvae was affected significantly with supplementations. Since, treatment (20%) showed the maximum mean weight with (3.582g.) that represents about 47% over control. The minimum weight was recorded for treatments (30% and 40%) that were recorded (2.218 g. and 2.166 g.) respectively. There was a decline in larval mean weights with high concentrations of egg albumen.

The overall growth rate showed a similar pattern in the 4th larval weight, with the control treatment recording the maximum rate at 424.5%, significantly higher than the minimum result of 318% for the 40% treatment. In contrast, the data varied in the 5th larval stage, showing an increase as a result of feeding on mulberry leaves enriched with different concentrations of egg albumen. The highest results were recorded for the 20% and 10% treatments at 671.6% and 681.4%, respectively, with no significant variance between them but significant differences from the other treatments. The control and 30% treatments recorded the lowest values at 379.2% and 377.1%, respectively. The highest significant cocooning and pupation ratios were recorded for the 20% treatment, at 100% and 100% respectively, while the least results were recorded for the control at 93.60% and 98.166%, respectively.

Cocoon Traits

The data concerning the cocoons produced by larvae fed on various concentrations of hen egg albumen are documented in Table (2). The highest mean cocoon weights were 1.786g and 1.735g, for 10% and 20% concentration, respectively. While the cocoon shell weights were 0.312g and 0.324g, and pupal weights were 1.472g and 1.414g for larvae fed on mulberry leaves with 10% and 20% hen egg albumen treatments, respectively. There was no significant variance between these treatments, but significant differences were observed between them and the other treatments. In contrast, the control group exhibited the lowest weights for cocoon, cocoon shell, and pupa at 1.290g, 0.208g, and 1.084g, respectively. Regarding the cocoon shell ratio percentage values, the maximum value was 18.7% for the 20% treatment, which significantly differed from the other treatments in females.

In male the data recorded as follow: 1.462, 1.290 g for cocoon mean weight and 1.146, 1.015g. for pupal mean weight with significant variance between 10% and 20% treatments respectively, but cocoon shell mean weight results were (0.297,0.310g.) without any variance between them, respectively. Whereas, cocoon shell ratio values were 24.10, 20.30, 18.20, 17.81, 17.10 % for 20%, 10%, 30%, 40% and Control, respectively with significant difference among them.

Biochemical Analysis

Total protein and free amino acids

The results regarding the total protein and total free amino acids in 5^{th} instar silkworms reared on mulberry leaves enriched with various concentrations of hen egg albumen are presented in Table (3). The mean total protein values in the larvae hemolymph were 71.56, 66.76, 62.23, 57.13, and 54.56 mg/ml for larvae fed on mulberry leaves treated with 30%, 40%, 20%, 10% hen egg albumen, and the control, respectively. There was a significant difference between the maximum and minimum values. In meantime, the differences in total protein content among treatments were statistically significant at level of $p \leq 0.0005$.

For the mean value of free amino acid, the treatment with 40% hen egg albumen exhibited the highest total free amino acid levels (3603.33 µg alanine/ml), while the 10% treatment had the lowest level (3007.33 µg alanine/ml). There was a significant difference in total free amino acid levels among the treatments ($p \leq 0.0001$).

Total carbohydrate content

Total carbohydrate content of silkworm, fed on enriched mulberry leaves with different concentrations of egg albumen, was expressed in Table (3). The data showed that the 40% treatment had the highest total carbohydrate content (22.300 mg/ml), followed by the 30% treatment (20.966 mg/ml). The 10% treatment showed the lowest total carbohydrate content at 16.100 mg/ml. However, there was no significant difference between the 40% and 30% treatments in terms of total carbohydrate content, but both were significantly higher than the 10% treatment ($p \leq 0.0001$). The L.S.D values for total protein, total free amino acid, and total

Table (1) Impact of fortified mulberry leaves with different concentrations of hen egg albumen on some biological parameters of silkworm (*Bombyx mori* L).

Egg albumen concentration (%)	Measured Parameters					
	4^{th} larval weight (g)	5^{th} larval weight (g)	Overall 4^{th} growth index (%)	Overall 5^{th} growth index (%)	Cocoon percentage (%)	Pupation ratio (%)
Control	0.509±0.01^{a}	2.427±0.04^{c}	4.245±0.25^{a}	3.792 ±0.10^{c}	0.936±0.44^{c}	0.981±0.16^{c}
10.0	0.430±0.01^{c}	3.286±0.05^{b}	3.345±0.22^{bc}	6.716 ±0.24^{a}	0.990±0.00^{b}	0.993±0.15^{b}
20.0	0.463±0.01^{b}	3.582±0.06^{a}	3.770±0.24^{abc}	6.814±0.25^{a}	1.000±0.00^{a}	1.000±0.00^{a}
30.0	0.470±0.01^{b}	2.218±0.05^{d}	3.838±0.23^{c}	3.771±0.15^{c}	0.982±0.14^{c}	0.980±0.00^{c}
40.0	0.406±0.01^{d}	2.166±0.04^{d}	3.180±0.19^{c}	4.355±0.14^{b}	0.981±0.14^{c}	0.990±0.00^{b}
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
L.S.D	0.0188	0.104	0.649	0.523	0.68	0.318

Table (2): Effect of egg albumen concentration on measured parameters in female and male silkworm *Bombyx mori* L, including Cocoon weight (C.w); Cocoon shell weight (C.S.W); Pupa weight (P.W) and Cocoon shell ratio (C.S.R%).

Egg albumen concentration (%)	Measured Parameters							
	Female (♀)				Male (♂)			
	C.W	C.S.W	P.W	C.S.R%	C.W	C.S.W	P.W	C.S.R%
Control	1.290±0.03^{c}	0.208±0.01^{c}	1.084±0.02^{bc}	0.161±0.32^{c}	1.178±0.04^{c}	0.200±0.01^{b}	1.005±0.04^{b}	0. 171±1.05^{c}
10.0	1.786±0.01^{a}	0.312±0.0^{a}	1.472±0.01^{a}	0.175±0.35^{b}	1.462±0.03^{a}	0.297±0.01^{a}	1.146±0.03^{a}	0.203±0.04^{b}
20.0	1.735±0.03^{a}	0.324±0.0^{a}	1.414±0.03^{a}	0. 187±0.33^{a}	1.290±0.03^{b}	0.310±0.01^{a}	1.015±0.02^{b}	0.241±0.19^{a}
30.0	1.291±0.04^{c}	0.194±0.0^{c}	1.044±0.03^{c}	0.149±0.13^{d}	1.145±0.02^{cd}	0.208±0.01^{b}	0.909±0.02^{c}	0.181±0.38^{c}
40.0	1.394±0.04^{b}	0.232±0.01^{b}	1.132±0.03^{b}	0.166±0.30^{cb}	1.093±0.02^{d}	0.195±0.01^{c}	0.864±0.02^{c}	0.178±0.29^{c}
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
L.S.D	0.099	0.021	0.085	0.009	0.091	0.022	0.084	0.015

Table (3): Impact of egg albumen concentration on protein, amino acid, and carbohydrate levels on some biochemical parameters of silkworm (*Bombyx mori* L).

Egg albumen concentration (%)	Measured Parameters		
	Total protein (mg/ml)	Total free amino acid (µg alanine/ml)	Total Carbohydrate (mg/ml)
Control	54.567±0.78[d]	2382.33±34.59[c]	16.733±0.12[cb]
10%	57.133±0.76[dc]	3007.33±51.15[b]	16.100±0.43[c]
20%	62.233±0.37[cb]	3145.67±72.77[b]	18.013±0.54[b]
30%	71.560±3.97[a]	2527.67±44.73[c]	20.966±0.54[a]
40%	66.760±0.78[ba]	3603.33±42.80[a]	22.300±0.36[a]
p	≤0.0005	≤0.0001	≤0.0001
L.S.D	5.94	160.56	1.363

carbohydrate were 5.94, 160.56, and 1.363, respectively. These values indicate the minimum difference required to establish significant variance between the means of different treatments.

DISCUSSION

The utilization of egg albumen as a potential source of amino acids and protein supplement in the production of Silkworm *Bombyx mori* L. presents an intriguing avenue for enhancing the nutritional content of silkworm feed. By incorporating egg albumen into their diet, silkworms may benefit from a richer amino acid profile, potentially leading to improved growth rates and silk production. Further studies could explore the optimal ratio of egg albumen to other feed components to maximize these benefits while ensuring the overall nutritional balance of the silkworm diet. Additionally, investigating the long-term effects of egg albumen supplementation on the health and productivity of silkworms could provide valuable insights into its viability as a sustainable protein source in sericulture practices.

Biological characters

The results of fortification were reflected in biological aspects such as 5th larval weight, overall growth rate, cocooning percentage, pupation ratio, and cocoon traits. Although, the supplements did not affect 4th larval weight and overall larval growth, it differed in the 5th instar and all criteria under studied. Despite the use of high doses of hen egg albumen, their effect was negative in all criteria under studied. These results were in line with Radjabi *et al.* (2009) who referred to a high level of amino acid supplementation not having a positive effect on silkworm growth and development whereas the fortification of silkworm diet with certain levels may be effective for improving the growth. Furthermore, Muzamil *et al.* (2023) confirmed the addition high concentrations of amino acids in feeding of mulberry silkworm leads to the toxicity so, it is important to use specific concentration of amino acids for normal growth. Helan and Zahra (2023) reported mulberry leaves smeared with 4% honey increasing 5th larval weight. The overall growth rate increased (8.3%) at honey (2%) Sharma *et al.,* (2023).

Furthermore, Helaly (2018) and Saad *et al*. (2014) have noted that mulberry leaves enriched with supplements such as hen egg and glycine have a significant impact on full-grown larvae, silk gland weights, and cocooning percentage. Larvae that were fed on mulberry leaves fortified with raw whey protein, royal jelly, and egg white showed increased weight, as mentioned by Islam et al. (2020). Similarly, Horie and Watanabe (1983) suggested that the highest silkworm growth rate was observed when hemolymph protein and amino acids increased due to feeding on mulberry leaves treated with egg albumen. Additionally, feeding on SERIFEED as a food supplement containing protein and additives increased cocoon yield and 5th body weight, as reported by Narayanaswamy and Ananthanarayana (2006). Zhen *et al*. (2021) demonstrated that larval growth increased with certain concentrations of lactic acid in their diet. Recent findings by Murugesh *et al*. (2022) indicated that larvae fed on mulberry leaves enriched with Glycine, Alanine, and Serine showed improvements in biological traits and cocoon parameters. On the other hand, the increase in larval growth and weight observed with 20% hen egg albumen may be attributed to its ability to accelerate the protease enzyme responsible for growth. These findings align with Nirmala *et al*. (2001), who highlighted the importance of increasing protease enzyme levels to enhance silkworm weight by feeding on mulberry leaves treated with soy protein.

Cocoon traits

Fresh cocoon weight and cocoon shell ratio are the more important criteria for treatments evaluation. The significant improvement of cocoon traits was due to the suitable concentration of egg albumen that contains adequate protein, amino acids. The present findings are in agreement with the findings of Nalini *et al.* (1994), cocoon weight increased by addition of egg albumen source of protein. Silkworm needs a high-quality food to produce good cocoons (Das *et al.*, 2001, Kumar and Vadamalai 2010). The highest shell ratio resulted from glycine additives (Babu, 1994). Also, Mala *et al.* (2017) add Aloe Vera as a source of protein on mulberry leaves to increase cocoon traits. Others findings confirmed that mulberry leaves supplemented with cow milk increased cocoon shell weight by 23.33% over control Amit *et al.* (2015).

Similarly, Radjabi *et al.* (2009) supported our results by demonstrating that a specific alanine concentration (1%) enhanced cocoon commercial parameters compared to other concentrations. Tajamul *et al.* (2020) illustrated a positive relationship between the increase in shell ratio and shell weight, attributed to the influence of egg albumen on protein synthesis in the larval silk gland. These findings are consistent with Hossain *et al.* (2015) and Amit *et al.* (2015), who observed an 11% and 9.01% increase in shell percentage, respectively, over the control group by adding milk and waste pupal protein. Horie and Watanabe (1983) documented the highest shell ratio resulting from protein supplementation of mulberry leaves with amino acids. These results align with the perspectives of Pallavi and Muthuswami (2012) and Rekha (2004), who concluded that an increase in cocoon weight and shell weight occurred when larvae were fed on soy flour and soybean.

Biochemical analysis

Biochemical analysis suggests that the quality of silk production is closely linked to the nutritive content of mulberry leaves and supplements, particularly those rich in pure protein and essential amino acids. While the 20% treatment emerged as the most effective dosage in terms of biological and cocoon characteristics, it did not demonstrate elevated levels of hemolymph components (total protein, amino acids, and carbohydrates) as presented in Table (3). Nonetheless, this concentration likely provides a sufficient amount of protein essential for synthesis and utilization processes. These results align with the observations of Horie (1961), emphasizing the significant impact of food quality and utilization on the biochemical profile, including protein, carbohydrates, and amino acids, in silkworms. Moreover, Nagata and Kobayashi (1990) showed that the protein content in the silkworm diet has a significant impact on hemolymph storage protein. The notable increase in protein levels observed may suggest a positive nitrogen balance accumulation, a hallmark of the growth phase. Similarly, a study conducted by El-Gendy (2021) found that protein content plays a crucial role in worm growth. Studies by Venkataramana *et al.* (2003) and Krishnan *et al.* (1995) have suggested that hemolymph proteins are enhanced when larvae are fed with soy protein (2-2.5%).

Mulberry leaves sprayed with (2.5%) of Aloe Vera increased the level of hemolymph protein that affect positively on silk production marwa, (2020). On the other side, it was noticed the excess dose of hen egg albumen (30% and 40%) increased hemolymph protein, amino acid and carbohydrate level but affect negatively on the silk production. Due to its containing a high amount of non-essential amino acid that not required for silk production which leads to imbalanced amino acid. Also, may hyper-proteinemia and extra amino acids affect protein gene expression, silk gland growth progression, which led to the toxicity. These results are in conformity with Wani *et al.* (2021) who referred to the variance concentration of hemolymph protein may be as a result of the variance of protein metabolism and synthesis on seventh day of fifth instar.

High dose of ascorbic acid cause elevation in hemolymph total protein that reduce cocoon production Thulasi and Sivaprasad (2013). Zhou, *et al.* (2008) was in line with our suggestions formula contains extra amino acids affect negatively the hemolymph protein expression that responsible for silk synthesis in silk glands. Younus, *et al.* (2021) confirmed that total protein concentration differ as a result of different rates of metabolism and synthesis of proteins. Xuedong, *et al.* (2022) reported, larvae fed on formula enriched with high protein levels have imbalanced amino acids in the hemo-lymph protein that led to an increase of uric acid and amino acid levels which affect negatively on silk production. Dong, *et al.* (2017) and Qin, *et al.* (2020) referred to the disturbance of amino acid metabolism increased nitrogen metabolism that produced urea and uric acid that led to silk reduction. Chen *et al.*, *(*2018) and Wang *et al.* (2022) concluded that excessive high protein or amino acid levels in hemolymph caused developmental disorders. Balanced hemolymph amino acids in protein are easily digested, absorbed, and used by insects (Watford and Wu, 2018, Weekes *et al.* 2006).

Our results also coincide with Smitha *et al*, (2006) who exhibit that larvae exposed to a certain amount of selenium activate the synthesis of protein which effects on physiological state of larvae and leads increase in the silk production. Therefore, a specific dosage of protein and amino acids that enhance silk production is essential. Carbohydrates serve as the primary energy source, and therefore, when silkworms feed on mulberry leaves enriched with egg albumen, noticeable fluctuations in carbohydrate levels are observed, as depicted in the data presented in Table (3). The highest value was recorded for the 40% treatment, which exhibited a decline in all biological parameters. Excessive total carbohydrate accumulation can lead to metabolic disorders that hinder production.

Our findings align with those of Wu *et al.* (2004), who noted a conflict in carbohydrate metabolism stemming from its increased levels. Furthermore, Sundara *et al.* (2018) confirmed that an excessive dose of vitamin C reduces hemolymph total carbohydrate levels, negatively impacting silk production. Notably, feeding on pollen at a 5% concentration was found to have a positive effect on various physiological aspects (total protein, carbohydrates, and amino acids) as well as biological characteristics of the silkworm, as reported by Mohanny (2022).

CONCLUSION

The study demonstrates that hen egg albumen can serve as a valuable protein and amino acid supplement for mulberry silkworms, impacting their biological and biochemical attributes. While the larvae showed improvements in various parameters when fed with 10% and 20% egg albumen concentrations, excessive dosages led to reduced silk production due to an imbalance of amino acids. The optimal concentration of 20% exhibited superior outcomes, including a higher shell ratio percentage, larval weight, growth index, cocooning, and pupation ratios. However, a direct relationship was observed between higher egg albumen concentrations and decreased cocoon production, underscoring the importance of maintaining a delicate balance for optimal silk production in *Bombyx mori* L.

REFERENCES

AHSAN, K., KHAN, A.R. AND FERDOUS, T. 2013. Growth and development of the mulberry silkworm, *Bombyx mori* L. on vitamin B and C supplemented diet. Bangl. J. of Zoology, 41(2):199-206.

ALABDEH M, LECHEVALIER V., NAU F., GAUTIER M., COCHET M.F., GONNET F., JAN S., BARON F. 2011 Role of incubation conditions and protein fraction on the antimicrobial activity of egg white against *Salm-onella enteritidis* and *Escherichia coli*. J Food Prot. 74(1):24-31. doi: 10.4315/-0362-028X.JFP-10-157.

AMALA RANI, G., PADMALATHA, C. AND RANJITH SINGH, AJ. 2011. Probiotic supplementations to improve commercial characteristics, disease resistance and protein in the silkworm *Bombyx mori* L. World Journal of Biological Research 4(2): 12-26.

AMIT, K., GOVIND, M. AND HUGUR, I.I. 2015. Biopotentiality of Waste pupae bio-protein as nutritional supplement to improve the economic traits in B. mori L. International Journal of Science Research, 4(11):75-76. Research Foundation

ASWARTHA, H. R., NAGESWARI, G. AND VENKATAPPA, B. 2017. Silkworm (*Bombyx mori*) and its constituents: A fascinating insect in science and research. Journal of Entomology and Zoology Studies, 5(5): 1701-1705.

BABU, V. P. 1994. Influence of supplementation of L-glycine on parent silkworm, *Bombyx mori* L. and grainage, rearing and cocoon parameters using successive generation. M.Sc. (Seric.)Thesis, University Agriculture Science Bangalore (India).

BERNARDINI, D., GERARDI, G., CONTIERO, B. AND CALDIN, M. 2009. Interference of haemolysis and hyperproteinemia on sodium, potassium, and chloride measurements in canine serum samples. Veterinary Research Commu-nications, 1: 173–176. doi: 10.1007/s112-59-009-9280-x.

BHATTACHARYYA, P., JHA, S., MANDAL P. AND GHOSH, A. 2016. Artificial diet-based silkworm rearing system-A Review. International J. of Pure and Applied Biosience, 4(6): 114-122.

BRADFORD, M. I976. A rapid and sensitive method for the quantitation of microgram quantities of proteins utilizing the principle of protein-dye binding. Anal Biochimistry, 72: 248-254.

CHANG, H., CHENG, T.,WU,Y., HU,W., LONG, R., LIU, C., ZHAO, P. AND XIA, Q. 2015. Transcriptomic analysis of the anterior silk gland in the domestic silkworm (*Bombyx mori*) - insight into the mechanism of silk formation and spinning. doi:10.1371/journal.pone.0139424.

CHAUHAN, S. AND TAYAL, M. K. 2017. Mulberry Sericulture. International Indian, Entomology, Springer, Singapore, 197- 263.

CHEN, X. D.,YONG-FENG, W., YU-LONG , W., QIU-YING, L., HUAN-YU, M., LU, W., YANG-HU, S. AND SHI-QING, X. 2018. Induced hyperproteinemia and its effects on the re-modelling of fat bodies in silkworm, *Bombyx mori*. Front. Physiology, 9: 302.

COOK, M. I., BEISSINGER, S. R., TORANZOS, G. A., AND ARENDT, W. J. 2005. Incubation reduces microbial growth on egg shells and the opportunity for trans-shell infection. Ecology Letters, 8: 532-537.

CROMPTON, M. AND BIRT, L. M. 1967. Changes in the amounts of carbohydrates, phosphagen, and related compounds during the metamorphosis of the blowfly, *Lucilia cuprina*. Journal of Insect physiology,13:1575-1595.

DAS, B. C., SAHU, P. K. SENGUPTA, T., MISRA, A. K., SARATCHANDRA, B. AND SEN, S. K. 2001. Genetic variability in some physiological traits in mulberry. Indian Journal of Plant Physiology, 6:162–165. doi: 10.3390/plants-8050118.

DONG, H. L., ZHANG, S. X., TAO, H., CHEN, Z. H., LI , X. QIU, J. F., CUI, W. Z., SIMA, Y. H., CUI W. Z. AND XU, S. Q. 2017. Metabolomics differences between silkworms (*Bombyx mori*) reared on fresh mulberry (Morus) leaves or artificial diets. Scientific Reports, 7 :10972.

DUBOIS, M., GILLES, K. A., HAMILTON, J. K., REBERS, P. A. AND SMITH, F. 1956. Colorimetric method for determination of sugars and related substances. Analytical Chemistry, 28: 350-356.

EL-GENDY, R., M. 2021. Toxicological, Histological and Biochemical Effects of Lepidium sativum Seeds Extract on *Galleria mellonella* L. Lepidoptera: Pyralidae) Larvae Extract. CATRINA: The Egyptian Society for Environmental Sciences, 23(1): 1-10

GRAY, J. AND GRIFFIN, B. 2009. Eggs and dietary cholesterol–dispelling the myth. Nutrition Bulletin, 34: 66-70.

HARVEY, D.,WING, D., KUSTER, B. AND WILSON, I. 2000. Composition of N-linked carbohydrates from ovalbumin and co-purified glycoproteins. Journal of American Society Mass Spectrometry, 11 : 564-571.

HELALY, W.M. 2018. Evaluation of two food additives on *Bombyx mori* L. characters. Journal of Entomology and Zoology Studies, 6(2): 3119-3123.

HELAN, A. W. M. AND ZAHRA, N. A. 2023. Effect of supplemental feeding on the weight of silk glands and larval development of mulberry silkworm (*Bombyx mori*). Journal of University of Duhok, 26(1): 183-189.

HOLLAND, C. H., KEIJI, N., JELENA, R. K. AND PHILIPPI, S. F. 2019.The Biomedical Use of Silk: Past, Present, Future. Advanced Healthcare Mater, 8(1):1-26.

HORIE, Y. 1961. Physiological studies on the alimentary canal of the silkworm, *Bombyx mori*. III-Absorption and utilization of carbohydrates. Bulletin Sericulture Experimental Station Japan, 16: 287-309.

HORIE, Y. AND WATANABE, K. 1983. Effect of various levels of dietary protein and supplementation with limiting amino acids on growth, haemolymph components and uric acid excretion in silkworm, *Bombyx mori*. Journal of Insect Physiology, 29:187-199.

HOSSAIN, M. S., AFTAB, U.,SAIFUL, I. AND ABDUL, A. 2015. Effect of Cow Milk on the Growth and Economic Traits of Silkworm (*Bombyx mori*). Intenational Journal of Science and Engineering Research, 6(3): 517-520.

HOU, Y., ZOU, Y., WANG, F., GONG, J., ZHONG, X., XIA, Q. AND ZHAO, P. 2010. Comparative analysis of proteome maps of silkworm hemolymph during different developmental stages. Proteome Science, 8: 45–54.

ISLAM,T., KHAN, I. L., GANIE, N. A., AHMED, K., SAHAF, N. J., AND GORA, M. M., 2020 . Impact of egg albumen (egg white) fortified mulberry leaf on rearing and some cocoon parameters of silkworm, *Bombyx mori* L. (CSR6× CSR26) × (CSR2×CSR27) double hybrid. Intern-ational Journal of Chemistry Studies, 8(4): 276-271.

KANAFI, R. R., EBADI, R., MIRHOSSEINI, S. Z., SEIDAVI, A. R., ZOLFAGHARI, M. AND ETEBARI, K. 2007. A review on nutritive effect of mulberry leaves enrichment with vitamins on economic traits and biological parameters of silkworm *Bombyx mori* L. Invertebrate Survival Journal, 4(2): 86-91.

KOVACS-NOLAN, J., PHILLPIS, M. and MINE, Y. 2005. Advances in the value of eggs and egg components for human health. Journal of Agriculture and Food Chemistry, 53: 8421-8431.

KRISHNAN, M., SUBBURATHINAM, K. M. AND JANARTHAN, S. 1995. Effect of hydrolysed protein (P-Soyatose) on haemolymph protein profile, larval and pupal characters of the silkworm, *Bombyx mori* L. (Lepidoptera:Bombycidae). Sericologia, 35: 227- 235.

KUMAR, R.AND GANGWAR, S. K. 2010. Impact of varietal feeding on Samia ricini Donovan in spring and autumn season of Uttar Pradesh. Journal of Agriculture and Biological Science, 5(3): 46-51.

KUMAR, R., AND VADAMALAI, E. 2010. Rearing Performance of Eri Silkworm Philosamia ricini in Monsoon Season of Uttar Pradesh. Asian Journal of Expermintal Biology Science, 1:303–310.

LEE, Y. P. AND TAKABASHI, T. 1966 An improved colorimetric determination of amino acids with the use of ninhydrin. Anal Biochemistry, 14: 71-77.

LU, S. L. AND ZIANG, Z. D.1988. Absorption and utilization of amino acids in mulberry leave by *Bombyx mori* L. Acta Sericulture San.,14:198-204.

MAHMOUD, S. M. 1988. Activation of Silk Secretion by Silkworms, Philosamia ricini and Bombyx mori L. After Applying Antibiotics. Ph. D. Thesis, Faculty of Agriculture, Cairo University.

MAHMOUD S. M., AKILA, M., MOUSTAFA, A. A., El-BANNA A. A. AND MARWA, N. 2013. Enzymatic activity of the silkworm, *Bombyx mori* L. hemolymph reared on different mulberry varieties. Egyptian Journal of Agriculture Rese
arch, 91: 1407-1413.

MALA, N., SADATILLA, F. AND BABU, H. 2017. Strengthening of sericulture industry through fortification of mulberry leaves to enhance commercial cocoon characteristics of silkworm. Agriculture Updates, 12: 210-217.

MARWA, N. M. 2020.Improving the silk industry by studying the effect of several aqueous extracts to enhance the efficiency of silkworm *Bombyx mori* L. Production. Journal of Plant Protection and Pathology, Mansoura Univ., 11 (2): 121-126.

MINE, Y. 2002. Recent advances in egg protein functionality in the food system. World's Poultry Science Journal, 58: 31-39.

MOHANNY, K.M., SAFAA, I.A.H.A. AND GHADA, S. M. 2022. Effect of pollen a bee hive product on some physiological, biochemical and economical characteristics of silkworm, *Bombyx mori* L. SVU-International Journal of Agricultural Sciences, 4 (4): 79-89.

MURUGESH, K.A., ARUNA, R.AND CHOZHAN, K. 2022. Influence of amino acids on the economic characters of silkworm, *Bombyx mori* (L.). Madras Agriculture Journal, 108 (7–9): 376–382.

MUZAMIL, A., MUHAMMAD, H.T., ALI, A., FAROOQ, M. B., MUNIR, F., IJAZ, F., ADNAN, M., ASHRAF, H. K. AND ABDUL, K.Q. 2023. Effect of amino acid fortified mulberry leaves on economic and biological traits of *Bombyx mori* L.. Heliyon, 9 : 1-7 e21053 .

NAGATA, M. AND KOBAYASHI, M. 1990. Quantitative changes in storage proteins during larval development of silkworm, Bombyx mori. Journal Sericulture Science Japan, 59(6): 461-468.

NALINI, R., GUNATHILAGARAJ, K., RADHA, N.V. AND NATARAJAN, K. 1994. Supplementation of protein through soybean flour (Defatted) for mulberry silkworm. Second National Symposium on prospects and problems of Sericulture in India. 7th-9th march, Madras university, 54.

NARAYANASWAMY, M. AND ANANTHANARAYANA, S. R. 2006. Biological role of feed supplement ‘SERIFEED’ on nutritional parameters, cocoon characters and cocoon yield in silkworm, L. *Bombyx mori* L.. Ind. J. of Sericult., 45(2):110-11.

NIRMALA, X., VANISHREE, V. AND KRISHAN, M. 2001. Effect of P-soyatose on the developmental and economic characteristics of the silkworm *Bombyx mori* L. Entomology, 27: 347-35.

PALLAVI, M. AND MUTHUSWAMI. 2012. Application of feed additives on young age silkworm *Bombyx mori.* L. and its effect on cocoon parameters. International Journal of Science and Nature, 3(2): 380-383.

PAWAR, SH., KANCHANMALA, G., ARCHANA, K., SHILPA, G. AND ASHISH, S. 2017. An overview of malignancy cases reported on histopathology over a Span of 5 years (2011-2015) at Terna Medical College, Nerul, Navi Mumbai. Journal of Research in Dental and Medical Science, 5(1):19-22.

PENG, Z.,YANG, X., LIU, C., DONG, Z.,WANG, F.,WANG, X., HU,W., ZHANG, X., ZHAO, P. and XIA, Q. 2019. Structural and mechanical properties of silk from different instars of *Bombyx mori*. Bio macromolecules, 20 :1203–1216.

POWRIE, W. D. 1985. Characteristics of edible and fluids of animal origin egg. Inernational Food chemistry, 829-855.

QIN, D. Y., WANG, G., DONG, Z., XIA, Q. AND ZHAO, P. 2020. Comparative fecal metabolomes of silkworms being fed mulberry leaf and artificial diet. Insects, 11(12): 851.

RAD JABI, R., EBADI, R., MIRHOSEINI, S. Z. AND NAIR, S. 2009. Effects of feeding alanine-enriched mulberry leaves on the economic characters of silkworm *Bombyx mori* (Lepidoptera: Bombycidae). Form Entomology, 29: 73-78.

RAIKOS, V., HANSEN, R., CAMPBELL, L.AND EUSTON, S.R. 2006. Separation and identification of hen egg protein isoforms using SDS– PAGE and 2D gel electrophoresis with MALDI-TOF mass spectrometry. Food Chemistry, 99: 702-710.

REKHA, K. M. 2004. Influence of feed additives on the performance of the mulberry silkworm *Bombyx mori.* L.. M.Sc. (Seri.) Thesis, Univ. Agril. Sci., Bangalore (India).

SAS/STAT® 9.1 User’s Guide. 2004. SAS Institute Inc., SAS Campus Drive, Cary, North Carolina 27513, USA.

SAAD, I., TAHA, H. and SAAD, I. 2014. Effect of mulberry leaves enriched with the amino acid glycine on some biological aspects of the silkworm, *Bombyx mori* L. Minufiya Journal of Agriculture Research, 39(2): 759-769.

SHARMA, A., SHARMA, P., BANDRAL, RS., GUPTA, RK. AND BALI, K. 2032. Influence of protein fortification on larval growth parameters of silkworm, *Bombyx mori* L.The Pharma Innovation Journal, 2(3): 2978-2982.

SHIVKUMAR, G. AND SUBRAMANYA, G. G. 2015. Quantitative estimation of haemolymph protein during different days of fifth instar larvae in bivoltines, multivoltines and mutants of the silkworm, *Bombyx mori* L.. Global Journal of Bioscience and Biotechnology, 4(3): 239-241.

SMITHA, S., SHAJESH, P., ARAVIND, P. R., KUMAR, S. R., PILAI, P. k. AND WARRIER, K. G. K. 2006. Effect of aging time and concentration of aging solution on the porosity characteristic of sub-critically dried selica earogels. Microporus and Misoporous Materials, 91(1-3):286-292.

SUNDARA, R. J., KERENHAP, E. W. AND AKHIL, K. S. 2018. Effects of Vitamin C on Carbohydrate Profile of Silk Worm, *Bombyx mori* LXCSR2 and Pure Mysore. International Journal of Innovative Science and Research Technology, 3 (10).

TAJAMUL, I., IRFAN, L., NISAR, A. G., KHURSHEED, A. S., NAYEMMA, J. AND MEHREEN, M. G. 2020. Impact of egg albumen (egg white) fortified mulberry leaf on rearing and some cocoon parameters of silkworm, *Bombyx mori* L. (CSR6 × CSR26) × (CSR2 × CSR27) double hybrid. International Journal of Chemistry Studies, 8(4): 267-271.

TERAMOTO, H. AND KOJIMA, K. 2014.Production of *Bombyx mori* silk fibroin incorporated with unnatural amino acids. Biomacromolecules, 15: 2682–2690.

THULASI, N. AND SIVPRASAD, S. 2013.Synergetic effect of ascorbic acid and lemon juice on the growth and protein synthesis in the silkworm, *Bombyx mori* and its influence on economic traits of Sericulture. J. of Bio Innovation, 2(4):168-183.

UMA, KRISHNASAMY. (2020). An analysis of mulberry and cocoon production in traditional areas of Tamil nadu. IX:466-469.

VENKATARAMANA, P., SRINIVASA, R. T. V. S., SRINIVASULA, R. P. AND SURYANARAYANA, N. 2003. Effect of Spirulina on the larval and cocoon characters of silkworm, *Bombyx mori* L. Processing of National Academy of Science India, 73(1): 89-94.

WANG, L., GAO, H., SUN, C. and HUANG, L. 2022. Protective application of Morus and its extracts in animal production. Animals, 12: 3541.

WANI, M.Y., GANIE, N.A., MIR, M.R., MIR, S. A., WANI, T. A., YAQOOB, M. AND MURTAZA, I. 2021. Total protein and total free amino acid estimation in peculiar bivoltine breed of mulberry silkworm (*Bombyx mori* L.). Journal Expermintal Zoology India, 24(2): 901-908.

WATFORD, M. AND WU, G.2018. Protein Advanced in Nutrition 9: 651–653.

WEEKES, T. L., LUIMES, P. H. AND CANT, J. P. 2006. Responses to amino acid imbalances and deficiencies in lactating dairy cows. Journal Dairy Science, 89: 2177–2187.

WU, Y., SUN, Z., CHE, S. AND CHANG, H. 2004. Effects of zinc and Selenium on the disorders of blood Glucose and lipid metabolism and its molecular mechanism in diabetic rats. 1: Wei Sheng Yan Jiv., 33(1): 70-3.

XUEDONG, C., AIHONG, Y., XUEHUI,W., ZHIGANG, Q., SHIQING, X.,YANGHU, S., YUJUN, W., RUIMIN, H., FEN, J.,PENGFEI, Z., JINRU, C., AND WENLIN, Z. 2022. Combined analysis of silk synthesis and hemolymph amino acid metabolism reveal key roles for glycine in increasing silkworm silk yields. International Journal of Biological Macromolecules, 209 : 1760–1770.

YIN, C.,YANG, L., ZHAO, H. AND LI, C. 2014. Improvement of antioxidant activity of egg white protein by phosphorylation and conjugation of epigallocatechin gallate. Food Research International, 64: 855-863.

YOUNUS, W. M., NISAR, A. G., MIR, M. R., MIR, S. A., TANVEER, A. W., MUNAZAH, Y. AND IMTIYANZ, M. 2021. Total protein and total free amino acid estimation in peculair bivoltine breeds of mulberry silkworm (*Bombyx mori* L.). Journal of Experimental Zoology India, 24(2): 901-908.

ZHEN, H., YANG, F., DE-CHEN, L., DENG-SONG, Ch. AND FAN, W. 2021. Effect of Lactic Acid Supplementation on the Growth and Reproduction of *Bombyx mori* . (Lepidopteria: Bombycidae). Journal of Insect Science, 21(2): 1–6.

ZHOU, Z., YANG, H., CHEN, M., LOU, C., ZHANG, Y., CHEN, K.,WANG,Y.,YU, M.,YU, F., LI, J. AND ZHONG, B. 2008. comparative proteomic analysis between the domesticated silkworm (*Bombyx mori*) reared on fresh mulberry leaves and on artificial diet. Journal of Proteome Research, 7 : 5103–5111.

ZHOU, L., LI, H., HAO, F., LI, N., LIU, X., WANG, G., YULAN, W. AND TANG, H. 2015. Developmental changes for the hemolymph metabolite of silkworm (*Bombyx mori* L.). Journal of Proteome Research, 14:2331–2347.

.

إمكانية استخدام زلال البيض كمصدر للأحماض الأمينية والبروتين في إنتاج دودة القز ***Bombyx mori* L.**

مـــــروي نبـيـــل مصـطـفى
باحث أول في بحوث الحرير، معهد وقاية النباتات، مركز البحوث الزراعية

الملخص العربـي

دودة الحرير التوتية هى حشرة وحيدة العائل حيث أنها تعتمد في تغذيتها علي أوراق التوت فقط كمصدر وحيد للغذاء . لذلك جودة أوراق التوت المقدمة و الإضافات الغذائية تعتبر من أهم العوامل المؤثرة علي إنتاج الحرير. كان الهدف من هذا البحث هو تقييم لقوة إضافة تركيزات مختلفة من زلال بيض الدجاج كمصدر للبروتين و الأحماض الامينية علي بعض الخواص البيولوجية و البيوكيميائية ليرقات دودة الحرير التوتية. و تمت تربية يرقات دودة القز علي (10%, 20%, 30 %,40%) من زلال بيض الدجاج بداية من العمر الرابع و حتي التعشيش وذلك في تتابع يوم بعد يوم. و كانت النتائج التي تم الحصول عليها أن بالرغم من إحتواء زلال البيض علي الأحماض الامينية المتعلقة بإنتاج الحرير إلا أن الكميات الزائدة تقلل من الإنتاجية نتيجة لوجود الأحماض الامينية لا تدخل في تركيب الحرير و تؤدي إلي إختلال في الايض الحيوي لليرقة. لكن اليرقات التي تمت تغذيتها علي أوراق التوت المضاف إليها (20%, 10%) من زلال البيض تحسنت لديها معظم الخواص البيولوجية والكيميائية ولكن دون فروق معنوية بينهم. و كان تركيز(20%) ألاعلي معنويا في متوسط أوزان العمر الخامس و أيضا في المحتوي الحريري للشرانق و نسبة التعشيش و نسبة التشرنق. وحيث أن أهم الصفات وهي المحتوي الحريري للشرانق والتي تأثرت إيجابيا بتركيز(20%) من زلال البيض، فكان هذا التركيزهو الانسب بدون منافسة لأوراق التوت و يرقات دودة القز محل الدراسة.