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Nutritional Evaluation of Eri Silkworm Pupa Strains: Addressing Entomophobia and Promoting Entomophagy

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Authors' contributions

This work was carried out in collaboration among all authors. Author SD originated the research idea, analysed the data, drafted the manuscript, collected the data and conducted various experiments, developed the interpretations of the study. Author BD revised and improved the manuscript. Author TK helped in idea formulation, reviewed, edited and helped in data analysis. All authors read and approved the final manuscript.

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ABSTRACT

Samia ricini widely known as Eri silkworm is a commonly reared lepidopteran throughout the year. Six different strains of Eri silkworm are present, named according to their body pattern and color - greenish blue zebra (GBZ), greenish blue spotted (GBS), greenish blue plain (GBP), yellow zebra (YZ), yellow spotted (YS) and yellow plain (YP). Apart from being reared mostly for Eri silk, this lepidopteran insect is also considered as a popular delicacy among the tribes of Northeast India,

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along with it being moderately famous among the locals too. The proximate analysis of the lyophilized samples was carried out as per AOAC (2015) guidelines and mineral analysis was carried out in atomic absorption spectroscopy. Among the six different strains, YS contained highest moisture (7.82 gm) and crude fiber content (18.8 gm), whereas, YZ showed highest amount of total ash (4.32 gm) and crude fat content (22 gm). Furthermore, carbohydrate content was seen to be highest in GBS (14.41 gm). Hence, this study is aimed to unveil the nutritional worth of the six distinct pupa strains of Eri silkworm in an effort to dispel entomophobia, and promote entomophagy owing to the nutritional benefit it offers.

Keywords: Eri silkworm; lyophilized; evaluate; proximate analysis; mineral analysis.

1. INTRODUCTION

The word "entomophagy," comes from the Greek word "éntomos," meaning insect, and "phăgein," which means eating, refers to the practice of insect consumption among humans. The escalating human population is putting a great pressure on the existing resources of our planet including the demand for sustainable food sources. This humungous increase in population brings with it many new challenges along with the key challenge of feeding our ever-increasing population. Additionally, as per Food and Agricultural Organization (FAO), the global agricultural land area is approximately 5 billion hectares which represents nearly 38% of the total land area of our planet. Any further increase in the agricultural area can lead to ecological imbalance as land conversion from natural ecosystems to agriculture has been historically the largest cause of Greenhouse gas emissions, often linked to loss of Biomass and carbon in biomass above and below the ground (FAO 2020). As the arable land on earth is limited and the cost of rearing the traditional food animals is also increasing, promotion of entomophagy could be a solution to this herculean problem of food security. Consuming edible insect as a source of food can provide us with high nutritional value in our diet and rearing insects is much cost efficient as compared to other commonly reared organisms. India being the second largest producer of natural silk in the world produces a large number of silkworm pupae every year. According to a study by the United Nations (UN) the global human population has reached 8 billion in mid-November 2022 from an estimated 2.5 billion in 1950, which means we are adding 1 billion people on earth every year since 2010 and 2 billion since 1998. It is expected that the world population will reach to 9.7 billion people by 2050 and by mid- 2080 it will be nearly 10.4 billion (UN article: Peace, dignity and equality on a healthy planet). Farming of traditional food plants and animals like wheat and livestock is facing many new challenges in today's world: lack of land for further expansion of agriculture, shortage of water, decrease in the soil nutrients, emission of greenhouse gases etc. Thus, there is an urgent need for us to look towards newer sources of food and nutrition. Even though the consumption of Eri Silkworm (*Samia ricini*) pupae is considered a popular delicacy among the tribal communities of our country but its acceptance is still considered as a taboo among the masses. Thus, a large number of pupae are wasted every year and research indicates the necessity of its management in tackling the problem of food security in future. Another fascinating fact about the Eri silkworm is that six strains of *S. ricini* have been identified, namely Greenish blue plain (GBP), Yellow plain (YP), Greenish blue-spotted (GBS), Yellow-spotted (YS), Greenish blue zebra (GBZ), and Yellow-zebra (YZ), based on the body colour and markings present in the 5th instar larva [1]. Published works are present on the chemical composition on *Bombyx mori* [2] and *Antheraea pernyi* [3]. Though, reports confirm Eri pupa of being a potential source of protein along with dietary lipid and other nutrients [4,5], however limited works are published on the complete nutrient composition comparing the consumable stages of Eri silkworm - pre-pupa and pupa. Also, yield contributing characters of different strains of Eri silkworm reports are reported [1] but no works have been published till now mentioning the complete nutritional profiling on the strain specific Eri silkworm.

2. MATERIALS AND METHODS

2.1 Collection and Indoor Rearing of Eri Silkworm

Six distinct strains of *S. ricini* were identified based on body colour and markings, and larvae in their fifth instar were collected from traditional rearers (Fig. 1). The newly hatched eri silkworm pupa, utilised for the biochemical examinations, were fed the delicate leaves of the Castor

(*Ricinus communis*) plant that was cultivated during the months of March and April, when the temperature was approximately 25±2 °C and the humidity was approximately 80±5%, in order to minimize changes in nutrient contents caused by external influences.

2.2 Sample Preparation

Within seven days of the Eri silkworm's cocoon formation, pupae were extracted. The Eri silkworm pupa were carefully removed from the cocoon, lyophilized to remove excess moisture, and grinded into a powder [6]. Until further analysis, the powdered form was stored in airtight containers in a refrigerator.

2.3 Nutrient Profiling

To determine the nutritional worth of the six distinct *S. ricini* pupa strains, the following tests were carried out. For the analysis of the parameters, 100 g of each sample was taken. To prevent experimental error, the trials were performed three times for each parameter. Proximate analysis was carried out in accordance with AOAC (2015) standard protocols for moisture, crude protein, crude fat, crude fibre, ash, and carbohydrates.

Moisture Content: The moisture content of the sample was ascertained by placing the wet sample in a 125-cm glass petridish and then again weighing was carried out after removing excess moisture from the wet sample in a lyophilizer. The moisture content was determined by using the following calculation to the sample weight difference:

$$\text{Moisture content (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Divided by initial weight of the sample taken}} \times 100$$

Fig. 1. Photograph showing indoor rearing of six different strains of *Samia ricini*

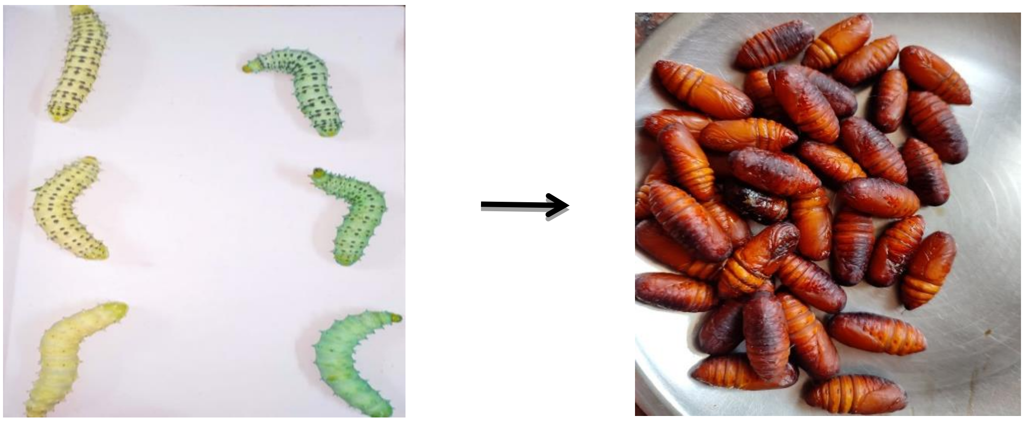

Fig. 2. Photograph showing different pre-pupa strains of Eri silkworm based on body color and marking which gets converted to same colored pupa form inside the cocoon after seven days of cocoon formation

Crude Protein Content: The micro-Kjeldahl technique was used to determine the protein content. For 45 minutes, 2 g of the powdered sample were broken down in a digestion unit using concentrated H_2SO_4 and a digestion mixture made up of finely ground potassium and copper sulphate (combined in a 1:8). In a distillation apparatus, the digested sample was then distilled. After which, 0.1 N HCl was used for titration, and the value was recorded. The total nitrogen obtained was then multiplied by a conversion factor of 6.25 (Jone's factor) to obtain crude protein.

$$N (\%) = \frac{\text{Titration reading} - \text{blank reading}) \times \text{Strength of acid} \times 14 \times 100}{\text{Weight of the sample}} \times 100$$

Crude protein content (%) = N (%) ×6.25.

Crude Fat Content: The Soxhlet technique was used to estimate the fat content. About 2 g of the lyophilised powdered sample was stored in a thimble and added to the extraction device in order to estimate the fat content. Diethyl ether, a non-polar solvent, was used to extract fat from the extraction thimble that was placed in the extraction jars. The % fat was calculated using standard formula:

$$\% \text{ crude fat} = \frac{\text{Weight of the residue}}{\text{Sample weigght}} \times 100$$

Ash Content: An empty, pre-weighed crucible was filled with 4g of pupa powder sample and kept in a muffle furnace which was then ignited at 550°C till the residue become white. The sample was weighed once more after the furnace was turned off and allowed to cool down . The ash content was calculated as follows:

$$\% \text{ Ash} = \frac{\text{weight of crucible plus sample} + \text{ash} - \text{empty weight of crucible}}{\text{weight of the sample}} \times 100$$

Carbohydrate Content: The carbohydrate percentage was determined by subtracting the total percentage of protein, fat and ash and moisture, from 100. The following equation was used to estimate the amount of carbohydrate [7].

Carbohydrate (%) = 100 – (% moisture + % protein + % fat + % ash)

Mineral Analysis: Atomic absorption spectroscopy was used to examine the pupa mineral constituents (AAS). Dry weight of 100 gms pupa powder was taken for the analysis. A conical flask containing about 4 g of the lyophilized powdered sample was filled with a digestion combination (3 ml $HClO_4$ + 21 ml HNO_3 + 1.5 ml H_2SO_4), and the mixture was allowed to sit at room temperature for the entire night. After heating it for an additional two to three hours until it became colourless, it was filtered using Whatman paper number 42 to increase the volume to 100 millilitres with 2% HNO_3 [8]. Using an atomic absorption spectrophotometer (Varian Spectra-220 AA, Australia), the amounts of minerals (Ca, Fe, and Zn) were estimated. The mineral contents were given as milligrams per 100 grams on a dry weight basis.

Statistical Analysis: Mean and standard deviations were calculated for the proximate parameters and significant differences among the samples were analysed by one way analysis of variance (ANOVA) followed by tukey's HSD post hoc test. The data analysis were performed using SPSS software (IBM SPSS Statistics 20.0).

3. RESULTS AND DISCUSSION

The current study evaluates the the nutritional profiles of six different pupa strains of *Samia ricini* in context to determine the necessary amount of nutrients needed to maintain a healthy body, according to WHO and FAO (2024). On a usual basis, different types of food sources such as animal meat, plants, fish etc are consumed on a greater scale. However in this context, nutritional significance of insects remains neglected due to the concept related to entomophobia and also regarding the viability of its different nutritive components. Thereafter, it is necessary to examine and estimate the nutritional composition of insects such that, they can serve as alternative sources of food together with the commonly consumed food sources. Upon reviewing previous literatures, no research works on the full nutritional profile and mineral analysis of the six strains of pupa was documented. Hence the present study was aimed for the aforesaid purpose.

3.1 Proximate Analysis

The detailed record of macronutrient content of all the pupa strains of Eri silkworm shown in Table 1 provides a knowledge on its richness. The records hereby provide evidence regarding the lepidopteran insects providing high nutritional value for being rich in crude protein, crude fibre, crude fat etc. The records of nutritional values of these lepidopteran insects primarily depend upon its diet, stage consumed along with its metamorphosis [9]. The analysis conducted among the different pupa strains of Eri silkworm, reveals the presence of highest crude protein content in GBZ strain with a value of 54.55 gm/10 gm of sample. Among all the different pupa strains, YP and GBP showed no significant difference in the crude protein content, however significant change was observed in other pupa strains ($P<0.05$). This highest crude protein content is comparable to the protein content in beef and pork containing protein values of 40-75 gm/100 gm dry weight as per reports [10]. Thus, this finding suggests pupa protein to be a good substitute to the commonly available animal meat requiring more space along with it being a boon in developing countries where food scarcity cause havoc every now and then. Moisture and crude fibre content is found to be in greater amount in yellow spotted pupa with values of 7.82 gm/100 gm and 18.80 gm/100 gm respectively unlike animal meat which usually has a low fibre content [11]. No significant difference was observed in the moisture content for YZ and GBZ pupa strains, but others strain showed significant difference. Again for crude fibre content, YZ and GBS showed no significant difference, while other strains did ($P<0.05$). Ash content, which is mostly an indication of mineral content along with crude fat is found to be predominant in yellow zebra pupa, among the different pupa strains recording values of 4.32 gm/100 gm and 22 gm/100 gm respectively. Among the pupa strains, no significant difference was observed between YP and GBS, along with the strains YS and GBP for the ash content ($P<0.05$). Whereas for the crude fat content, GBS and GBZ showed no significant difference. The crude fat amount of the analysed pupa strains is higher than the recorded values of some of the readily available animal meat namely beef (3.5 gm/100 gm), mutton (15.12 gm/100 gm) and duck (18.12 gm/100 gm) and vegetable oil [11,12].

The body gets a lot of energy and necessary fatty acids from the fats in insects, and some fatty acids, such linoleic and α-linolenic acid, are crucial for human health maintenance. Thus, the nutrition and well-being of humans benefit from fats present in edible insects [13]. Another important source of nutrition is carbohydrate, the main energy source, which are either completely absent or present in very small amounts in majority of animal meats. This could be because of the fact that, the primary form of glucose found in muscle, glycogen, gets broken down during the process of converting muscle into meat. The requirement of low carbohydrate content in animal meat is consequently satisfied by the greater carbohydrate content found in nearly all six pre-pupa strains of which greenish blue spotted confirms its highest presence with an amount of 14.41 gm/100 gm of dry weight. No significant difference was observed among the strains YP, YZ and GBZ, alongwith YS and GBP strains ($P<0.05$).

3.2 Mineral Analysis

Despite the fact that minerals are regarded as micronutrients, their absence results in adverse health consequences. Statistics shows that micronutrient deficiencies result in around one million preventable deaths annually, highlighting the need to enhance and understand the importance of minerals [14]. Additionally, minerals found in insects, particularly iron and zinc, may be very important for nutrition [15]. Two billion people worldwide still suffer from micronutrient deficiencies, which are mostly due to low levels of iron, zinc [16]. One most frequent micronutrient deficiency in underdeveloped nations is anaemia, which is particularly common in girls attaining puberty and nursing mothers. Another important mineral is dietary calcium deficiency which greatly influences the development of cardiovascular disease [17]. Women in their middle years may also be more susceptible to stroke if they don't get enough calcium in their diet [18]. Table 2 records both iron(1096.6mg/100gm) and calcium(56.40 mg/100 gm) is found to be highest in yellow spotted pupa which is much higher than the recorded values of most other commonly consumed edible insects including *Tenebrio molitor* having calcium amount 184 mg/kg and iron content of 21.5 mg/kg and *Bombyx mori* with values of calcium (177 mg/kg) and iron (16.5 mg/kg) [19] along with animal proteins like beef (0.08 mg/100 g), and pork (0.07 mg/100 g) [11]. Coming to the zinc content, greenish blue spotted records the highest amount with a value of 4.32 mg/100 gm which is comparatively higher than most other edible insects *Bombyx mori* (30.7 mg/kg), *Tenebrio molitor* (44.5 mg/kg) as per reports [19] and also when compared to animal meats as in turkey (2.63 mg/100 g), and beef (6.87 mg/100 g) [20,11].

Table 1. Proximate analysis of the pupa of different strains of Eri silkworm. Values in dry weight basis (g/100 g) are expressed as Mean ± SD, n=3. Different mean followed by different superscripts in a column differ significantly

Pupa strains	Moisture	Total Ash	Crude Fibre	Crude protein	Crude fat	Carbohydrate
YP	5.78±0.24^{a}	1.87±0.22^{a}	7.32±0.24^{a}	30.52±0.27^{a}	4.8±0.24^{a}	5.07±0.33^{a}
YS	7.82±0.34^{b}	2.01±0.25^{b}	18.80±0.28^{b}	36.46±0.23^{b}	9.24±0.28^{b}	7.41±0.28^{b}
YZ	3.57±0.28^{c}	4.32±0.25^{c}	6.62±0.26^{c}	43.46±0.29^{c}	22 ±0.32^{c}	5.65±0.32^{a}
GBP	2.22 ±0.30^{d}	2.12±0.23^{b}	10.89±0.28^{d}	30.42±0.28^{a}	11.72±0.34^{d}	9.87±0.3^{b}
GBS	4.52±0.24^{e}	1.75±0.29^{a}	6.84±0.25^{c}	32.53±0.30^{d}	8.61±0.3^{e}	14.41±0.22^{c}
GBZ	3.44±0.26^{c}	3.29±0.21^{d}	5.29±0.28^{e}	54.55±0.26^{e}	8.62±0.29^{e}	5.06±0.37^{a}

Table 2. Table showing mineral analysis of different strains of Eri silkworm in dry weight basis expressed in mg/100 gm

Pupa strains	Iron	Calcium	Zinc
YP	47.76	35.12	2.98
YS	1096.6	56.40	2.18
YZ	6.79	47.35	0.77
GBP	956.22	32.27	2.16
GBS	122.3	40.61	4.32
GBZ	25.52	30.52	0.68

4. CONCLUSION

As the global population continues to rise, the challenge of providing sufficient, nutritious and sustainable food becomes increasingly urgent. As per 2023 reports of Global Hunger Index (GHI), India ranks 111 out of 125 countries with a score of 28.7 that basically implies a serious level of hunger. Thereafter, alternative sources of food addressing hunger issues like malnutrition must be quested on an immediate basis. The present study focuses on the rich nutritional profile of the different pupa strains of Eri silkworm suggesting them as promising solution to the existing nutritional gap. Also, when compared to conventional food sources, the pupa strains are recorded to have high nutritional value in context of protein, lipid and carbohydrate contents. Additionally, these pupa strains were also found to contain sufficient levels of minerals needed to maintain a balanced body. However, though some local communities of the north-eastern region of India have long recognised the nutritional benefits of consuming Eri silkworm, yet, a larger mass disregards their consumption due to myths associated to their nutritional composition. Thus the present study was hence designed to promote the consumption of eri silkworm pupa strains, among a wider segment of society by emphasizing their rich nutritional profile.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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