



A study on proximate composition and minerals analysis of six different pre-pupa strains of *Samia ricini* (Eri silkworm)

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Abstract

Samia ricini, commonly known as Eri silkworm, is a widely reared species in Northeast India throughout the year. The silk insect that mostly generates Eri silk is also utilized as a food source more commonly by the Northeast tribes of India in comparison to the greater mass. In recent times, more people are becoming aware of the possibility that substituting edible insects in lieu of animal protein could improve the condition of the environment and lessen the issue of starvation in developing countries. However there still exists social taboo against entomophagy, and many pre-pupae are discarded as a sericulture by-product after the cocoon is formed completely. To make any kind of food acceptable and popular, it is necessary to understand its nutritional profile. Therefore, the aim of the study was to evaluate the nutritional value of the six different pre-pupa strains of eri silkworm so as to, eliminate entomophobia and popularise entomophagy owing to its nutritional significance. We anticipate that this study will not only reveal the nutritional status of pre-pupa, but will also open up new avenues for functional food creation.

Keywords: Entomophobia, pre-pupa, social taboo, entomophagy

Introduction

The term "entomophagy," derived from the Greek words "éntomos" meaning insect and "phāgein" meaning eating basically implies the habit of insect consumption among people. The ever-increasing population with an increasing demand for food with limited water and land resources are becoming a great pressure which might even result in a bigger problem due to lesser land availability for crop production and animal rearing. According to Van Huis *et al.* (2013) if no changes can be made in agricultural production, there are high chances of increased greenhouse emissions and environmental degradation. Such changes can lead to harmful consequences which require sooner attention by introducing alternative sources for animal protein. Edible insects are known to emit lesser greenhouse gases and ammonia than most other livestock and in addition, they require less area for rearing, less water for survival and even consume organic waste streams. Edible insects are also known for their wide variation in nutrient content including proteins, carbohydrates, and vitamins for both human and domestic animals thereafter contributing significantly to food security and livelihoods in many developing countries (Vantomme P *et al.*, 2010). Many ethnic groups, mostly in the Northeast India consume about more than 200 different types of edible insects (Thangjam, R., *et al.*, 2020). Because of its year-round availability, the Eri silkworm (*Samia ricinii*) is mostly consumed by the indigenous people of northeastern India in comparison to other insect species. As such its consumption is highest accounting for 87.7% of the total (Mishra, N., *et al.*, 2003). 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 (Sarkar, B.N., *et al.*, 2018). But, despite its availability and variability, only a small portion of the entire yearly production of Eri cocoons is consumed by the tribal people of Assam, with the majority being thrown away as waste. Another interesting characteristic of the Eri silkworm is that, in contrast to the muga silkworm, which loses its quality if the pupa is removed prior to reeling, the cocoon of Eri silkworm keeps its quality intact even after the pupa is removed. Hence the name "Ahimsa silk". Published works regarding chemical composition of *Bombyx mori* (Pereira, Ferrarese-Filho, Matsushita, & DeSouza, 2003; Rao, 1994) and *Antheraea pernyi* (Zhou & Han, 2006a) are available however no works have been published on the complete nutrient composition of the consumable pre pupa stages of Eri silkworm mentioning the strains. Even though, reports confirm Eri pupa be a potential source of protein along with dietary lipids and other nutrients (Mazumdar, M., 2019, Choudhury, K., *et al.*, 2020), but works published on yield contributing characters of the different strains are more prevalent (Sarkar, B.N., *et al.*, 2016) ^[12]. The present study was thus designed with an aim to enunciate the nutritional significance of the distinct pre-pupa stains of Eri silkworm by evaluating its macro-nutrient and micro-nutrient profiles.

Materials and Methods

Collection and indoor rearing of Eri silkworm

Based on the body color and marking, 5th instar larvae of six different strains of *S. ricini* were collected from traditional rearers (Figure I). The newly hatched larvae were fed with tender leaves of the Castor (*Ricinus communis*) plant. To limit the variations of nutrient contents due to environmental factors, the samples used for the following biochemical analysis were taken from the crop reared during the months of March-April when temperature was around 25±2 degree Celsius with humidity = 80±5%.

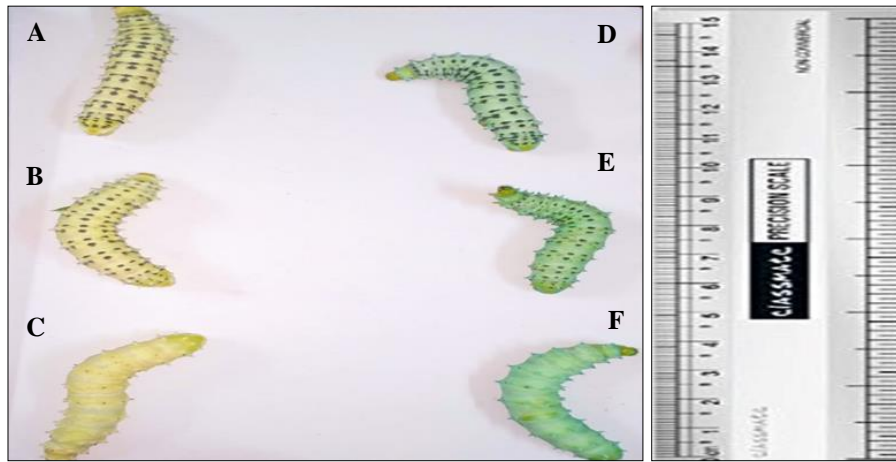


Fig 1: Photograph showing six different strains of *Samia ricini* - A. Yellow zebra
B. Yellow spotted C. Yellow plain D. Greenish blue zebra E. Greenish blue spotted
F. Greenish blue plain.

Sample Preparation

Pre-pupa of the Eri silkworm were collected from the cocoon within four days of its cocoon formation. The pre-pupa of Eri silkworm were taken out carefully from the cocoon and were lyophilized for removal of excess moisture and grinded in powdered form (Molnar, A., *et al.*, 2021). The powdered form was kept in closed containers under refrigerated conditions until further analysis.

Nutrient profiling

The following tests were performed to know the nutritional value of the six different pre-pupa strains of *S. ricini*. 100 g of each sample was used for analysis of the parameters studied. For each parameter, the experiments were repeated in triplicate to avoid experimental error. Proximate analysis for moisture, crude protein, crude fat, crude fibre, ash and carbohydrate was performed following standard protocols of AOAC (2015) [2].

Moisture content: The moisture content of the samples were determined by taking a known weight of the sample in a glass petridish of 125cm and drying it in a hot air oven at 100-105°C till a constant weight was achieved. The difference in weight of the sample indicated the moisture content, which was calculated by using the following formula:

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

Crude protein content: The protein content was determined by micro-kjeldahl method. 2 g of the powdered sample was digested in digestion unit for 45 minutes by concentrated H₂SO₄ and a digestion mixture comprising of finely powdered copper sulphate and potassium sulphate (mixed in the ratio 1:8). The digested sample was then distilled in distillation unit. Finally, it was titrated with 0.1 N HCl and the titration reading value was noted. Crude protein was obtained by multiplying the total nitrogen by a conversion factor of 6.25 (Jones's factor).

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

$$\text{Crude protein content (\%)} = N (\%) \times 6.25.$$

Crude fat: Fat content was estimated using Soxhlet method. For the estimation of fat content, about 2 g of the lyophilized powdered sample was kept in a thimble and placed in extraction apparatus. Extraction thimble was placed in extraction jars and fat was extracted using non-polar solvent, diethyl ether. The % fat was calculated using standard formula:

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

Ash: 4g of fish sample was weighed into an empty pre-weighed crucible and kept in a muffle furnace which was then ignited at 550°C till the residue become white. The furnace was turned off to cool and then the sample was weighted again. The ash content was calculated as follows:

$$\% \text{ Ash} = \frac{\text{Weight of crucible plus sample after ashing} - \text{Empty weight of crucible}}{\text{weight of the sample before adding}} \times 100$$

Carbohydrate: The percentage of carbohydrate was determined by subtracting the total percentage of moisture, protein, fat and ash from 100. The following equation was used to estimate the amount of carbohydrate (Narzari, S., *et al.*, 2013)

$$\text{Carbohydrate (\%)} = 100 - (\% \text{ moisture} + \% \text{ protein} + \% \text{ fat} + \% \text{ ash})$$

Mineral analysis

Mineral contents of the fish muscle were analysed using atomic absorption spectrometry (AAS).

About 4 g of the lyophilized powdered sample was taken in a conical flask, and to it a digestion mixture comprising of 3 ml HClO₄ + 21 ml HNO₃ + 1.5 ml H₂SO₄ were added and incubated overnight at room temperature. It was then further heated for 2–3 hour until colourless and then filtered with Whatman paper no. 42 making the volume up to 100 ml with 2 % HNO₃ (Gokoglu *et al.*, 2004). Minerals (Ca, Mg, Fe, Zn) were estimated using Atomic Absorption Spectrophotometer (Varian Spectra-220 AA, Australia). The mineral contents were expressed in mg/100 g of dry weight basis.

Results and Discussion

The present study has been aimed to carry out the complete proximate analysis along with the presence of minerals of one of the consumable life stages, which is the pre-pupa of six different strains *Samia ricini*. These lepidopteran insects meet the recommended intake of nutrients needed for a healthy body (WHO and FAO, 2004), but as no literature exists regarding the proximate analysis and mineral analysis of all the six strains available, hence this study has been conducted. According to the results of the proximate and mineral analysis carried out, the proximate analysis of all the strains of pre-pupa of Eri silkworm are depicted in Table 1. For, the different strains of pre-pupa, yellow plain had the highest crude fibre content of 16.98 gm/100 gm. Contrary to edible insects, animal meat typically has low fibre content (Orkus, A., *et al.*, 2021). Both the parameters, Crude protein content and crude fat was highest in yellow-spotted and yellow zebra respectively with values of 65.5 gm/100 gm and 39.1 gm/100gm respectively. Higher Crude protein content in yellow spotted is seen to be even higher than animal meat mostly common among people with chicken breast having 21.5 gm, turkey breast (19.2gm), pork (16.89gm), beef (20.1gm) of protein content per 100 gm of the meat which are much lesser than the amount of protein as per my finding indicating pre-pupa protein to be good contender for the available meat in the market especially in the developing countries where food scarcity often creates a great havoc. Also crude fat content is important as it is an important indication regarding the fatty acid content which the body cannot prepare itself requiring it to be consumed for body requirement. The strain as mentioned above with the highest amount of crude fat content is yellow zebra (39.1gm) whose value is much higher compared to the fat content analysis of chicken drumstick (6 gm), chicken breast (1.3gm), turkey drumstick (3.7gm) etc (Orkus, A., *et al.*, 2021). Moisture, total ash, and carbohydrate were highest in greenish blue plain having values of 9.17 gm/100 gm, 5.55 gm/100 gm, and carbohydrate 11.44 gm/100 gm respectively. Carbohydrates, a primary energy source and in most of the cases, carbohydrates in animal meat is either absent or present in a very negligible amount which is due to the fact that throughout the process of turning muscle into

meat, the main type of carbohydrate contained in muscle, glycogen, is broken down.

The amount of nutrients found in animal meat and insects varies depending on the species and are essential for the healthy operation of the human body. This information can help balance the mineral composition of daily food rations when there are anomalies associated with inadequate mineral content. Calcium and iron are the two minerals that are specifically needed for the normal growth and operation of the human body (Awuchi, C.G.I., *et al.*, 2020 and Sunycz, J., 2008). Cardiovascular disease development is also influenced by dietary calcium deficiencies (Reid, I., *et al.*, 2017). Middle-aged women may also be at higher risk of stroke if they consume insufficient amounts of calcium (Elders, P.J., *et al.*, 1994). Also, low iron content contributing to anaemia is one of the most common problem seen mostly in women (Sturtzel, B., *et al.*, 2018). Also, other two minerals magnesium and zinc are also important in human body for proper functioning of human body. Table:2 depicts the presence of minerals in the different strains of pre-pupa with values expressed in milligrams(mg) per 100 gram(gm) of lyophilized powder sample. Among pre-pupa strains, Calcium was highest in yellow spotted with a value of 1096.6 mg/100 gm which is seen to be higher than most other animal protein including chicken breast (0.11mg), turkey breast (0.05 mg), duck (0.05 mg), beef (0.08 mg) and pork (0.07 mg)etc, and zinc with a value of 1.55 mg/100 gm is highest in greenish blue spotted pre-pupa which was higher than some animal meat including chicken breast (1.31 mg), and duck (1.20 mg) but lesser than others like chicken drumstick (2.97 mg), turkey (2.63 mg) and beef (6.87 mg)etc (Orkus, A., *et al.*, 2021). Whereas, greenish blue zebra has the highest values for two different types of minerals iron (30.32 mg/100gm) the value of which is higher than iron content most commonly provided to children and adults egg white (2mg/100gm), chicken (1.4mg/100gm) and milk(0.3mg/100gm) (Wu,X., *et al.*, 2020) and magnesium (68.88 mg/100 gm) is much higher when compared to the values of chicken drumsticks(1.37 mg), chicken breast(2.21 mg), turkey(1.77 mg), pork (0.78 mg) etc(Orkus, A., *et al.*, 2021).

Table 1: Proximate analysis of the pre-pupa of different strains of Eri silk worm. Values in dry weight basis (g/100g) are expressed as Mean±SD, n=3

Pre-pupa strains	Moisture	Total Ash	Crude Fibre	Crude protein	Crude Fat	Carbohydrate
YP	5.21±0.21	3.18±0.37	16.98±0.24	46.23±0.21	10.23±0.26	7.36±0.29
YS	7.85±0.34	3.32±0.30	5.14±0.34	65.5±0.60	7±0.50	7.46±0.57
YZ	8.07±0.20	3.95±0.52	4.67±0.64	42.5±0.37	39.1±0.38	6.89±0.43
GBP	9.17±0.24	5.55±0.33	3.92±0.40	43.32±0.36	7.4±0.44	11.44±0.42
GBS	6.12±0.30	3.10±0.39	5.87±0.60	32.25±0.67	9.5±0.65	7.37±0.45
GBZ	5.32±0.36	3.66±0.35	7.02±0.35	40.56±0.48	11.77±0.47	8.76±0.54

Table 2: Presence of minerals in the pupa of different strains of Eri silkworm in mg/100 gms of a sample along with recommended values per day for human body. Values in dry weight basis (g/100g) are expressed as Mean±SD, n=3

Pre-pupa strains	Iron 16-18 mg per day	Magnesium 420 mg per day	Calcium 1000 mg per day	Zinc 11 mg per day
YP	8.98±0.29	36.87±0.33	48.87±0.27	0.43±0.33
YS	0	20.78±0.36	1096.6±0.25	0.36±0.35
YZ	0	48.59±0.31	130.5±0.29	0.57±0.30
GBP	13.65±0.32	52.88±0.24	108.98±0.34	0.68±0.36
GBS	15.66±0.34	56.32±0.22	56.34±0.31	1.55±0.33
GBZ	30.32±0.26	68.88±0.39	87.72±0.32	0.19±0.32

Conclusion

Planning and preparing meals with the right amount of nutrients analysis can be achieved by having proper knowledge of the nutrients and their relative quantities. It encourages dietary modifications and results in an overall improvement in human health. Such information can also improve the daily allowance of those nutrients whose availability falls short of suggested levels. The present study highlights the nutritional significance of the six different pre-pupa strains available as potential contender for the animal meat. The designed study is believed to increase the consumption of these pre-pupa stages of Eri- silkworm on a larger scale suggesting them as valuable sources of food in the human diet that will also subsequently offer solution to burning issues like malnutrition especially in the developing countries.

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