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Proximate and amino acid analysis of mulberry and non-mulberry silkworm pupae

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Abstract
The proximate and amino acid composition of both deoiled and non deoiled pupae 'eri' (*Samia ricini*) and 'muga' (*Antheraea assamensis*) and mulberry silkworm (*Bombyx mori*) were analyzed. Investigation revealed that silkworm pupae contained protein (40%-60%), fat (4%-20%), moisture (4%-8%), fibre (3%-6%) and ash (4%-6%). Overall protein content was highest in *Antheraea assamensis* pupae (63.53%) followed by *Samia ricini* (56.15%) and *Bombyx mori* (55.71%). Amongst the two types of pupae, the highest protein content was found in deoiled pupae. Amino acid analysis of silkworm pupae showed 17 amino acids, including all the essential amino acids. The total essential amino acid content is higher in mulberry pupae (50.2%) compared to Muga (46.5%) and ERI pupae (44%). The result of the study concluded that nutritive value of silkworm pupae could be utilized as a supplementary protein source in human and animal nutrition.

Keywords: Silkworm pupae, ERI, muga, mulberry, deoiled, protein, fats, amino acid

1. Introduction
The silkworm pupae is an important by-product of the silk reeling industry. It is also consumed as protein by the indigenous people of Northeast India, Japan, China and Korea [1]. Commercially 5 species of silkworm are reared *viz*., *Samia ricini* (Eri), *Bombyx mori* (Mulberry), *Antheraea assamensis* (Muga), *A. proylei* (Tasar) and *A. pernyi* (Tasar). The mulberry silkworm *Bombyx mori* belongs to family Bombycidae while the non-mulberry silkworm 'ERI' (*Samia ricini*) and 'Muga' (*Antheraea assamensis*) belongs to family Saturniidae [2].
Due to their richness in protein and fatty acid, the silkworm pupae are used in piggery, fishery, poultry, and dog feed [3]. It is also having scope as an alternate source of protein in human and animal nutrition. Though silkworm pupae are very popular as protein supplement in traditional food, the information available on the nutritional value is meagre.
The studies on nutritional profile of both deoiled and non deoiled mulberry (*Bombyx mori*), ERI (*Samia ricini*) and muga (*Antheraea assamensis*) silkworm pupae is very less. Therefore, the present study was undertaken to evaluate and compare the nutritional value among pupae of three species of silkworm, *B. mori* (Mulberry), *Samia ricini* (ERI) and *Antheraea assamensis* (Muga).

2. Materials and Methods
2.1 Sample preparation
Pupae of the silkworm *Samia ricini* and *Antheraea assamensis* were purchased from the villages in Assam and pupae of *Bombyx mori* was procured from a silk reeling industry located at Coimbatore, Tamil Nadu, India. The samples were washed with distilled water and dried in a hot air oven for 3 days at 60-70 ºC [4]. The dried samples were ground in a blender to obtain powder. Powdered samples were deoiled using n-hexane solvent in soxhlet apparatus and the defatted pupal powder was collected and stored in airtight container for further use.

2.2 Proximate composition
Proximate composition was determined by the methods of Association of the Official Analytical Chemists (AOAC). The moisture content was determined by drying at 135 °C for

2h (AOAC, 2005; method 930.15); the ash content by using the muffle furnace at 600 ℃ to constant weight (method 942.05); the crude fibre content by dilute acid and alkali hydrolysis (method 978.10); Nitrogen was determined using the Kjeldahl method [5]. The quantity of protein was calculated as 6.25 x N (method 7015, AOAC, 1984). Lipid content was determined using n-hexane by soxhlet extraction.

2.3 Amino acid analysis
The silkworm pupal powder is mixed with ethanol and stirred using an agitator. Samples were filtered and particles were collected. The particles were subjected to triple extraction with ethyl acetate. The extracts were dissolved in hexane and oil was extracted by soxhlet apparatus. The extract was hydrolysed with 6 N HCl at 110 ºC for 22 h [6]. After hydrolysis the amino acid analysis was done using high performance liquid chromatography (HPLC).

2.4 Statistical analysis
All the measurements were performed in triplicate. Mean values ± standard deviations (SD) were calculated. The data were subjected to one way analysis of variance (ANOVA) following Tukey's honestly significant difference post hoc analysis. The significance of variance ($p<0.05$) between means was calculated using IBM SPSS 16 software.

3. Results and Discussion
3.1 Chemical composition of Silkworm pupae
The proximate composition of deoiled and non-deoiled pupae of three silkworm species is given in Table 1. The highest protein content was found in deoiled pupae (63.53%) in muga silkworm followed by ERI (56.15%) and mulberry silkworm (55.71%). In non-deoiled pupae, mulberry silkworm protein content was found to be 46.06% followed by deoiled pupae meal, 55.71%, which is in line with the findings of Kim *et al.* (2016) [7] *i.e.*, 47.87% protein. Significant variation was noticed in the protein content of pupae among the three species (Table 1). Generally, both deoiled and non deoiled silkworm pupa yielded good sources of protein. Mishra *et al.* (2003) [8] reported that non-deoiled mulberry pupae contained 34.38% crude protein. Since the inner material of the pupae was removed during the experiment by Mishra *et al*. (2002), the value obtained by them is lower than the observed value of the present experiment (46.06%).

A significant difference was observed in the lipid content between non deoiled pupae of the three species and it was comparatively higher in non-deoiled pupae than the deoiled pupae. However no significant difference is observed between the deoiled pupae of all the three species. Highest lipid content (27.33%) was recorded in non-deoiled pupae of the mulberry silkworm. Hu *et al*. (2017) [9] employed a microwave assisted extraction of lipids using a mixture of ethanol and n-hexane and found the lipid content of mulberry pupae was found to be 30.34%. Kim *et al*. (2016) [7] examined the lipid content of 19.2% using Soxhlet extraction method in non-deoiled pupae and 4.75% in deoiled pupae which confirmed the present findings.

The moisture content showed the difference between the deoiled and non deoiled pupae of the same species at 5% level of ANOVA. The highest moisture content was seen for non-deoiled ERI pupae (8.43%) followed by muga (7.80%) and mulberry (7.62) and least moisture content was observed in the deoiled mulberry pupae (4.02%). There was significant difference in deoiled pupae of mulberry (4.02), muga (4.36) and ERI (4.90).

The ash content of deoiled and non-deoiled pupae of three species of silkworm varied in the range between 3.63-6.93% (Table 1). Deoiled mulberry pupae exhibited the highest amount of ash (6.93). Felix *et al*. (2020) [10] found that the ash content in mulberry pupae was 7.94% as silkworm protein concentrate (SPC) was used.

The fibre content of all the three species of non-deoiled pupae is less than 4%. Among the deoiled pupae ERI silkworm had the highest amount (6.8%) of fibre followed by muga and mulberry (6.6% and 6.3% respectively) whereas non-deoiled pupae had the lowest fibre content (3.3%-Muga). Kim *et al*. (2016) [11] reported that fibre content of silkworm was 6.38% which supports the results of the present investigation.

Table 1: Proximate composition of silkworm pupae (g/100g dry weight)

Components	Mulberry pupae		ERI pupae		Muga pupae	
	Non deoiled	Deoiled	Non deoiled	Deoiled	Non deoiled	Deoiled
Moisture%	7.62± 0.458	4.02± 0.240	8.43± 0.614	4.90± 0.226	7.80± 0.386	4.36± 0.152
Crude protein %	46.06± 0.458	55.71± 1.927	54.56± 0.392	56.15± 0.282	61.63± 0.836	63.53± 0.087
Crude fat %	27.33± 0.541	4.70± 0.271	19.50± 0.624	5.30± 0.048	17.00± 1.185	4.70± 0.303
Crude Fibre %	3.05± 0.167	6.31± 0.048	3.50± 0.031	6.80± 0.166	3.30± 0.235	6.6± 0.209
Ash%	4.20± 0.303	6.93± 0.157	4.76± 0.130	5.96± 0.371	4.56± 0.018	3.63± 0.035

Values are expressed as Mean ± SD

Values with different alphabets in each column are significantly different at α = 0.05 by Turkey's post hoc test.

3.2 Amino acid composition of silkworm pupae
The amino acid composition of pupae of different silkworm is given in Table 2. It was observed that 17 known amino acids including all the essential amino acids were present in the silkworm pupae. The total essential amino acid content is higher in mulberry pupae (50.2%) when compared to Muga (46.5%) and ERI pupae (44%). The mulberry pupae contained higher level of important essential amino acids including leucine (8.2g/100g), isoleucine (5.4g/100g) and lysine (7.2g/100g). The level of sweet amino acid (Glycine and alanine) is higher in ERI pupae (4.74g/100g and 6.0g/100g). Kwon *et al*. (2012) [6] reported that the amino acid content is a function of pupal age. With the increase in age of the pupae, the amino acid content also increases.

The essential amino acid score of silkworm pupae is given in table 3. Amino acid score is the ratio of amount of amino acid in 1g protein to the amount of amino acid required by humans. The comparison showed that the silkworm pupae satisfied the FAO/WHO/UNU recommendations for essential amino acids requirement by adults. Silkworm pupae contained high amount of important essential amino acids (Lysine and isoleucine). In case of Muga and ERI pupae, leucine is the limiting amino acid with a score of 93 and100 respectively.

Table 2: Amino acid composition (g/100 g of crude protein) of different silkworm pupae

Amino Acids (g/100g of protein)	Category	Mulberry silkworm pupae	ERI silkworm Pupae	Muga silkworm pupae
Alanine	N	5.4	6.0	5.5
Arginine	N	6.75	4.25	4.5
Aspartic acid	N	10.8	9.7	6.4
Cystine	N	1.4	0.5	1.6
Methionine	E	4.5	2.28	1.7
Lysine	E	7.2	6.4	6.6
Isoleucine	E	5.4	4.37	6.3
Leucine	E	8.2	6.6	6.2
Phenylalanine	E	5.1	5.13	7.3
Threonine	E	5.3	4.5	4.3
Glutamic acid	N	14.7	13.0	12.8
Histidine	E	2.4	2.53	2.9
Proline	N	4.1	6.38	10.3
Serine	N	4.63	5.13	4.3
Glycine	N	4.6	4.74	4.45
Tyrosine	N	5.3	6.41	4.2
Valine	E	5.4	5.28	6.1
Total amino acid	-	101.18	93.2	90.45
Total essential amino acid	-	50.2	44	46.5

Table 3: Essential amino acid scores of mulberry and non-mulberry silkworm pupae

Amino acid(mg/g crude protein)	EAA WHO/FAO/UNU (1985)	Mulberry pupae EAA score	ERI pupa EAA score	Muga pupa EAA score
Histidine	19	126	133	152
Isoleucine	28	194	156	225
Leucine	66	124	100	93
Lysine	58	124	110	113
Methionine +cystine	25	236	111	132
Phenylalanine+ tyrosine	63	165	183	182
threonine	34	155	132	126
Valine	35	154	150	174

4. Conclusion

Proximate analysis of silkworm pupae showed that they are a good source of high quality protein, fat, fibre, moisture and amino acid. Mulberry silkworm pupae contains protein of 45%-55%, ERI pupae 50%-56% and muga pupae 60%-63%. Thus amino acid profile showed the presence of high content of essential amino acid like Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, threonine and Valine. Thus silkworm pupae could be utilized as alternative source of dietary protein and amino acid in human and animal feed.

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