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Nutrient composition and protein quality evaluation of eri silkworm (*Samia ricinii*) prepupae and pupae

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ABSTRACT

Eri silkworm (*Samia ricinii*) is a traditional source of food in northeast India, where it is grown primarily for silk and food uses. Nutrient analysis showed that the proximate composition of eri silkworm prepupae and pupae grown on either castor or tapioca were comparable and it was a good source of protein (16 g%), fat (8 g%) and minerals. The amino acid scores of eri prepupae and pupae protein were 99 and 100, respectively, with leucine as the limiting amino acid in both cases. Net protein utilisation (NPU) of prepupae and pupae was 41 as compared to 62 in casein. Protein digestibility corrected amino acid score (PDCAAS) was 86. The high protein content in the defatted eri silkworm meal (75%) with 44% total essential amino acids makes it an ideal candidate for preparing protein concentrate isolates with enhanced protein quality that can be used in animal nutrition.

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1. Introduction

Insects have played a very important role as a source of food in the history of human nutrition, especially in developing countries (Bodenheimer, 1951). Insects (on a dry weight basis) have very high crude protein content and insect proteins have been reported to be a good source of essential amino acids, equivalent or even superior to soy protein (Frinke, deFoliart, & Benevenga, 1989). Protein concentrates from insects have even been compared to that of casein (Ozimek et al., 1985). In general, insect protein tends to be low in sulphur-containing amino acids but high in lysine and threonine, one or both of which may be deficient in cereals. Insects are generally a rich source of fat, as well as being a good source of vitamins and minerals (Banjo, Lawall, & Songonuga, 2006; Malaisse & Parent, 1980; Oliveira, DeCavalho, DeSouza, & Simao, 1976).

The consumption of insects has been documented in Japan, Thailand, Africa, Latin America, Australia, Mexico and other parts of the developing world where they represent a cheap source of good quality protein (de Foliart, 1999; Mitsuhashi, 1997; YhoungAree, Puwastein, & Attig, 1997). The indigenous population in northeast India uses a variety of insects as food, one of which is the eri silkworm (*Samia ricinii*). The prepupae and pupae of eri silkworm are considered a delicacy in northeast India; however, the harvesting and sale of eri silkworm prepupae and pupae is still informal and it is sold only in local markets. In Assam, the consumption of silkworm pupae is not restricted to eri (*Attacus ricinii*) alone but others such as muga (*Antheraea assama*) and mulberry (*Bombyx mori*) are also consumed. The consumption was highest for eri (87.7%), followed by muga (57.4%) and mulberry (24.6%) (Mishra, Hazarika, Narain, & Mahanta, 2003).

Information on consumption of silkworm pupae in some countries is available, with limited data on the proximate composition. Published work includes chemical composition of the pupae of *B. mori* L. (Pereira, Ferrarese-Filho, Matsushita, & DeSouza, 2003; Rao, 1994) and *Antheraea pernyi* (Zhou & Han, 2006a). In China, the use of silkworm pupae as food has been practised since ancient times and silkworm has been recently approved as a new food source by the Ministry of Health of the Republic of China (Zhou & Han, 2006b). However, there appears to be no study on the nutrient composition or protein quality of eri silkworm (*S. ricinii*) prepupae or pupae. Therefore the study was undertaken to evaluate the nutritional potential of eri silkworm, based on its chemical composition and protein quality evaluation.

2. Materials and methods

2.1. Sample preparation

All the samples of eri silkworm prepupae and pupae grown on either castor or tapioca, required for the compositional analysis and animal experimentation, were supplied by the Central Silk Board, Government of India, Ministry of Textiles, Bangalore, through its local office in Hyderabad. The eri silkworm prepupae and pupae, grown on either castor or tapioca, were washed separately with deionised water and spread on filter paper sheets.

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After one hour, the samples of eri silkworm prepupae and pupae, grown either on castor or tapioca, were separately homogenised. Aliquots of the homogenised samples were taken for the determination of proximate composition, minerals and amino acids. The bulk homogenate samples were transferred into stainless steel trays and dried at 60–70 °C overnight in a hot air oven. Dried powder samples of eri silkworm prepupae and pupae were extracted with *n*-hexane using a Soxhlet apparatus, to obtain the defatted eri silkworm prepupae and pupae meals which were used for the protein quality evaluation *in vivo*.

2.2. Nutrient analysis

Moisture, ash, crude fibre and fat contents were assayed by the Association of the Official Analytical Chemists (AOAC, 2006) methods 934.01, 942.05, 962.09 and 920.39, respectively. Protein content (N X 6.25) was determined by the AOAC Kjeldahl method (984.13). Carbohydrate was obtained by difference (100 − sum of moisture, protein, fat, crude fibre and ash). Mineral estimation was carried out by dry-ashing the sample at 550 °C according to the AOAC (AOAC, 2006) procedure. Calcium, chromium, copper, iron, magnesium, manganese and zinc were determined in a Varian Techtron 100 Atomic absorption spectrophotometer (AAS). Phosphorus was determined by the Fiske and Subbarow method, as given in AOAC method 931.01.

2.3. Amino acid analysis

Amino acid analysis was carried out by hydrolysing the samples in sealed ampoules in *vacuo* with 6 N HCl at 110° for 22 h (Moore, 1963). Excess acid was removed in a flash evaporator under reduced pressure at a temperature of less than 40 °C. The sample was then dissolved in buffer and amino acid analysis carried out in a Biochrome30 Amino Acid Analyzer (Peace & Gilani, 2005). Cystine and methionine were estimated separately after performic acid oxidation. Amino acid score was calculated using the FAO/WHO/UNU (1985) suggested pattern of amino acid requirement for preschool children (2–5 years).

2.4. Net protein utilisation (NPU)

Net protein utilisation was carried out, using Wistar NIN rats, after the experimental protocol was approved, by the institutional animal ethics committee. Diet was formulated according to the procedure of Reeves, Nielsen, and Fahey (1993), consisting of 83.3% cornstarch, 10% sunflower oil, 4% mineral mixture, 1% vitamin mixture, 0.2% choline chloride, 1.5% cellulose. The protein (casein or defatted eri prepupae or pupae meal) replaced cornstarch at 10% protein level in the diets. Each group, consisting of six animals each (21 day-old Wistar NIN male rats), was randomly assigned to one of the different diet groups and fed the respective diets for 14 days *ad libitum* as described earlier (Longvah & Deosthale, 1998). Towards the end of the study, animals were transferred to metabolic cages and faeces collected for three days for nitrogen determination. Animals were sacrificed and nitrogen content of each animal was determined by the Kjeldahl method (N X 6.25).

2.5. Protein digestibility corrected amino acid score (PDCAAS)

The PDCAAS value was determined according to the formula:

$$\text{PDCAAS} = \frac{\text{mg of the limiting amino acid in 1 g of test protein}}{\text{mg of same amino acid in 1 g of reference protein}} \times \text{faecal true digestibility } (\%) \times 100$$

3. Results and discussion

3.1. Chemical composition of eri silkworm

The nutrient compositions and mineral contents of eri silk worm prepupae and pupae grown on either castor or tapioca were comparable (Table 1). Protein content of eri silkworm prepupae and pupae was around 16% which is higher than the 10.3% reported for an edible insect *Acheta domesticus* (Pennino, Dierenfeld, & Behler, 1991) but not as high as the 24.4% reported for another edible insect *Euxoa auxiliaries* (White & Kendall, 1993). The protein content of eri silkworm prepupae and pupae, on a dry weight basis, was around 54% which is higher than the 48.7% reported for spent silk worm pupae (Rao, 1994) or the 49.1–53.5% in *B. mori* L. chrysalis toast (Pereira et al., 2003). Generally, insects are good sources of nutrients and protein content as high as 79% has been reported in the edible insect *Gonimbrasia richelmanii* (Ramos-Elorduy et al., 1997). Protein content in the defatted eri silkworm pupae meal was as high as 75%.

Fat content of 8.0–8.6%, observed in eri prepupae and pupae, was lower than that found in larva of *Rhynchophorus phoenicus* (25%), reported by Ekpo and Onigbinde (2005). However, on a dry weight basis, the fat content of eri prepupae and pupae was more than 25% (Table 1). The fat content of eri silkworm prepupae and pupae in the present investigation represents a good source of oil though it was slightly lower than the fat content of 30.1% in spent silk worm pupae (*B. mori*) reported by Rao (1994). On a dry weight basis, eri silkworm pupae have as much as 1923 kJ/100 g of energy which is comparable to the average value of 1919 kJ/100 g found in 23 species of edible caterpillar in Zaire (Malaisse & Parent, 1980). The high protein and fat content of eri silkworm prepupae and pupae offer immense potential for mitigating the calorie and protein deficiency in a developing country such as India if rearing and consumption of eri pupae is introduced wherever there is large scale cultivation of its host plant.

3.2. Minerals and trace element contents of eri silkworm

Minerals and trace element contents of eri silkworm prepupae and pupae, grown on either castor or tapioca leaves, were comparable (Table 1). Total mineral content of 1.25/100 g in eri prepupae was comparable to that of other insect larvae, such as Mopane worm (Dreyer & Wehmeyer, 1982). The eri silkworm prepupae and pupae appear to be a good source of phosphorus, calcium and magnesium. Iron content of 24 mg/100 g in eri silkworm prepupae and pupae, on a dry weight basis, was lower than the 40 mg/100 g reported in *Antherea pernyi* (Zhou & Han, 2006a). The phosphorus content of eri silkworm prepupae and pupae was 570–585 mg/100 g on a dry weight basis which was slightly higher than the 474 mg/100 g reported by Rao (1994) in spent silkworm pupae. The high zinc content of 7.24 mg/100 g in dried eri pupae is of significance, especially considering the importance of this trace element in health and nutrition. Many attempts have been made to make nutritious food supplements available to the population, using staple foods to ensure nutritional security. However, nonconventional sources of nutrients, such as silkworm pupae, which offer immense potential, are yet to be explored for such uses.

3.3. Net protein utilisation of defatted eri silkworm

Data on the food intake, gain in body weight and net protein utilisation of eri silkworm prepupae and pupae are presented in Table 2. Food intake and percent dry matter digestibilities of

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