Chemical Composition and Nutritional Evaluation of Spent Silk Worm Pupae

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Spent silk worm pupae were analyzed for their nutrient composition, and their protein quality was evaluated in weanling rats. Protein content of the pupae was found to be 48.7 g %, while fat content was 30 g %. Defatted spent silk worm pupae meal contained 75.2 g % protein. The essential amino acid content of the pupal protein was similar to that of whole egg protein with the exception of tryptophan (0.9 g/16 g of N). Tryptophan is the limiting amino acid of pupal protein. The chemical score of the protein was found to be 60, as compared to 100 for whole egg protein. Pupal fat contained 66.8% total unsaturated fatty acids, and linolenic acid accounted for 25.7 g % of the total fat. The protein quality of spent silk worm pupae was significantly lower than that of casein as judged by PER and NPU. The lowered food intake of animals on pupal diet may be due to the bad odor of the pupae meal or the presence of pupal hormone (ecdysone).

Keywords: *Silk worm pupae; nutrient composition; amino acid composition; fatty acid profile; protein quality*

Production of green mulberry cocoons in India increased from 31 864 tonnes in 1971–1972 to 116 672 tonnes during 1990–1991. Silk worm pupa constitutes 60% of dry cocoon weight. Hence, 23 334 tonnes of dry spent silk worm pupae is available in the country. Since pupae are reported to have 30 g % fat and 50 g % protein, around 7000 tonnes of fat and 12 000 tonnes of protein are likely to be available from the pupae, as byproducts of the silk industry (Datta *et al.*, 1993).

Most of the pupae are being used as fertilizer, and a small proportion is used as a constituent of chick and fish feed. The major difficulty in the utilization of spent silk worm pupae is that it cannot be stored for long periods and it has a bad odor.

Even though large quantities of spent silk worm pupae are available in India, data regarding its chemical composition and protein quality are scant. There are a few studies in which spent silk worm pupae were added to diets of chick (Icchponani and Malik, 1971; Fagoonee, 1983), pig (Rodrigues *et al.*, 1970), and sheep (Khan and Zubairy, 1971) at 5% or 10% level. Hence, a comprehensive investigation was undertaken to analyze spent silk worm pupae for its chemical composition, amino acid composition, and fatty acid profile. Pupal protein quality was evaluated in weanling rats.

MATERIALS AND METHODS

Spent silk worm pupae, 250 g of whole pupae, and 2.0 kg of defatted pupae from the same batch were obtained from Sreenivasa Oil and Fertilizers, Kallur, Ananthapur district. Whole pupae were used for chemical analysis, and the defatted pupal meal was utilized for the protein quality evaluation in rats.

Protein (N × 6.25), fat, ash, phosphorus, calcium, and iron were estimated according to AOAC (1970) methods. Trace elements such as zinc, copper, chromium, manganese, and magnesium were determined in an atomic absorption spectrophotometer (Varian Techtron, Model AAS 1000). Nicotinic acid content of the pupae was determined by a microbiological method using *Lactobacillus arabinoses* as the test organism (Rao and Ramasastri, 1969).

Moisture-free silk worm pupae were ground and extracted with solvent ether in a Soxhlet extractor for 18 h. After ether evaporation, an aliquot of fat was taken up for fatty acid analysis. Methyl esters of the pupal fat were prepared according to the method of Lowenstein *et al.* (1975). The fatty acid composition was determined by GLC on a Varian 3700 gas chromatograph equipped with a flame ionization detector with a column (12 ft × 0.5 in.) packed with 10% Silar 10C on chromosorb W-AW (80/100 mesh).

Fifteen milligrams of moisture- and fat-free sample of spent silk worm pupae powder was hydrolyzed in evacuated sealed ampules with 6 N hydrochloric acid at 110 °C for 20 h. Excess acid was removed in a flash evaporator under reduced pressure. The amino acid analysis was carried out by ion-exchange chromatography in an automatic amino acid analyzer (Moore *et al.*, 1958). One gram of moisture- and fat-free sample of spent silk worm pupae meal was hydrolyzed with 25 mL of 2 N sodium hydroxide for 6 h at 15 lb of pressure in an autoclave. After cooling, the pH was adjusted to 4.5, and the mixture was made up to a known volume and filtered. Then the tryptophan content was estimated from this alkaline hydrolysate by a microbiological method using *Leuconostoc mesenteroids* as the test organism (Barton-Wright, 1946).

Eighteen weanling (21 days old) male rats, belonging to the Institute's colony (Wistar/NIN) and weighing around 43 g each, were randomly distributed into three groups of six each. Animals belonging to group I received protein-free diet; groups II and III animals were maintained on casein control diet and spent silk worm pupae diet, respectively. These two diets when analyzed contained 10 g % protien. The compositions of the experimental diets are given in Table 1. The diets were adequate with respect to all of the nutrients. All of the diets were steamed for 20 min after double the quantity of water was added, and then the diets were offered to the animals. Animals were housed individually in raised-bottom cages. Food and water were given *ad libitum*. Daily food intake and weekly body weight changes of individual animals were recorded throughout the experiment, and from the data PER was calculated (Campbell, 1963). Fecal samples of individual animals were collected during the last 3 days of the experiment. The nitrogen content of the dry fecal samples was estimated by the Kjeldhal method. From the data, dry matter and protein digestibilities were calculated. At the end of 28 days of feeding the experimental diets, all of the animals were sacrificed under ether anesthesia. The carcasses were hydrolyzed in 6 N hydrochloric acid by autoclaving at 15 lb of pressure for 2 h. Carcass nitrogen content was determined

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Table 1. Percent Composition of Experimental Diets

ingredient of the diet	group I	group II	group III
vitamin mixture[a]	1	1	1
salt mixture[b]	4	4	4
oil	10	10	10
casein		13	
defatted spent silk worm pupae			14
starch	85	72	71

[a] Vitamin mixture according to that of Campbell (1963). [b] Salt mixture according to that of USPXVII.

Table 2. Chemical Composition of Spent Silk Worm Pupae

constituent	spent silk worm pupae	constituent	spent silk worm pupae
moisture (g %)	18.9	zinc (mg/100 g)	23
protein (g %)	48.7	magnesium (mg/100 g)	207
fat (g %)	30.1	copper (mg/100 g)	0.15
ash (g %)	8.6	chromium (mg/100 g)	1.69
phosphorus (mg/100 g)	474	manganese (mg/100 g)	0.71
calcium (mg/100 g)	158	nicotinic acid (mg/100 g)	0.95
iron (mg/100 g)	26		

by the Kjeldhal method, and NPU was calculated from the data (Miller, 1963).

The results were tested for significance by the method of analysis of variance.

RESULTS AND DISCUSSION

Table 2 gives the nutrient composition of spent silk worm pupae. The pupae were rich in protein (48.7 g %) and fat (30 g %). The defatted pupal meal contained 75.2 g % protein. Phosphorus, iron, and magnesium contents of the pupae were also high, but nicotinic acid content of the pupae was low.

Rodrigues *et al.* (1970) observed 55 g % protein and 26.4 g % fat, while Khan and Zubairy (1971) reported 65.5 g % protein and 6.2 g % fat for silk worm pupae. Lin *et al.* (1983) gave values for protein and fat as 79.8 and 6.1 g %, respectively, for silk worm chrysalids. The results of the present investigation are similar to those reported by Rodrigues *et al.* (1970).

The amino acid composition of silk worm pupal protein is given in Table 3. Lysine, threonine, methionine, and tyrosine content of silk worm pupal protein were higher than that observed in whole egg protein. The tryptophan content of the protein was low (0.9 g/16 g of N) and is the limiting amino acid of the pupal protein. The chemical score of the protein was found to be 60 when compared to the whole egg protein score of 100. The second limiting amino acid of the protein is valine. Of 16 g of total nitrogen, amino acid nitrogen accounted for 13.8 g, and the remaining 14% of nitrogen may be due to non-protein nitrogen as well as destruction of amino acids during acid hydrolysis.

The essential amino acid content of the spent silk worm pupal protein agrees with that reported by Lin *et al.* (1983) for silk worm chrysalids with the exception of tryptophan. They reported the tryptophan content of the silk worm chrysalids as 1.5 g/100 g of protein as compared to 0.9 g/16 g of N for silk worm pupae observed in this study.

Table 4 gives the fatty acid composition of pupal fat. Unsaturated fatty acids constituted 66.8% of total fat, while polyunsaturated fatty acids accounted for 29.9% of the fat. It is unusual to find 25.7% linolenic acid in fat of animal origin and equally rare to find such a high percentage of linolenic acid even among fat from vegetable origin, the exceptions being perilla and linseed fat (Longvah and Deosthale, 1991).

Data on the protein quality of evaluation of spent silk worm pupae are presented in Table 5. All of the parameters studied such as food intake, gain in body weight, PER, dry matter, and protein digestibilities as well as NPU for spent silk worm pupae diet were significantly lower than those of casein control diet, indicating the poor quality of the pupal protein.

Weekly average food intakes by animals on spent silk worm pupae diet were 50.5, 60.2, 62.5, and 60.0 g per animal during weeks 1−4 of the experiment, respectively, as compared to 55.9, 74.1, 84.7, and 86.0 g per animal for casein control diet. The food intake of animals on the pupae diet was static during the last 3 weeks of the experiment. Silk worm pupae meal had a bad odor. Whether this bad odor is responsible for the lowered food intake by the animals maintained on the pupae diet is not known. Fagoonee (1984) observed an adverse effect on growth performance of chicks fed on

Table 3. Amino Acid Composition of Spent Silk Worm Pupae[a]

amino acid	silk worm pupae	Lin *et al.* (1983)	whole egg protein[b]
lysine	7.5	6.7	7.0
histidine	2.5	2.8	
arginine	6.8	5.3	
aspartic acid	10.9	8.9	
threonine	5.4	5.2	5.1
serine	4.7	4.7	
glutamic acid	14.9	8.6	
proline	4.0		
glycine	4.6	4.1	
alanine	5.5	4.6	
valine	5.6	5.1	6.8
cystine	1.4	1.4	2.4
methionine	4.6	3.0	3.4
isoleucine	5.7	4.1	6.3
leucine	8.3	6.0	8.8
tyrosine	5.4	5.7	4.2
phenylalanine	5.1	4.4	5.7
tryptophan	0.9	1.5	1.5

[a] Values given are g/16 g of N. [b] FAO (1970).

Table 4. Fatty Acid Composition of Spent Silk Worm Pupae Fat[a]

fatty acid	silk worm pupae	perilla[b]	sesame[c]	soy[c]	safflower[c]
16:0	26.2	8.9	9.7	9.8	7.8
18:0	7.0	3.8	3.4	2.4	2.1
18:1	36.9	12.9	41.2	28.9	17.7
18:2	4.2	17.6	44.5	50.7	78.5
18:3	25.7	56.8		6.5	
total saturates	33.2	17.7	13.1	13.1	10.7
total unsaturates	66.8	87.3	86.5	86.1	89.2
polyunsaturates	29.9	74.4	44.5	57.2	78.2

[a] Values are percent of total fat. [b] Longvah and Deosthale (1991). [c] Gopalan *et al.* (1971).

Table 5. Protein Quality of Spent Silk Worm Pupae[a]

	group II	group III	lsd[b]
food intake (g/4 weeks)	301 ± 7.2	233 ± 7.0	21.5
gain in body wt (g/4 weeks)	14 ± 3.8	47 ± 3.7	10.6
PER	3.71 ± 0.043	2.06 ± 0.125	0.25
adjusted PER	2.50	1.39	
dry matter digestibility (%)	93 ± 0.5	88 ± 0.9	3.2
protein digestibility (%)	84 ± 1.5	67 ± 2.3	5.6
NPU	72 ± 1.7	45 ± 11.0	8.5

[a] Values given are mean ± SE. [b] Least significant difference.

5% and 10% dried spent silk worm pupae diets, and this growth depression was correlated to the pupal hormone, ecdysone, level in the diet.

Lin *et al.* (1983) reported PER values of 2.27 and 1.63 for silk worm chrysalid diet and casein plus 1% DL-methionine diet, respectively, as against 2.06 and 3.71 for pupae and casein diet observed in the present study. These differences are probably due to the different stages of development of the silk worm.

Defatted silk worm pupae meal had 75 g % protein. It would be interesting to isolate the protein, which may be devoid of the bad odor and the pupal hormone (ecdysone), and study its protein quality.

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