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Evaluation of Nutritional Composition of Hybrids of Waste Silkworm Pupa *Bombyx Mori* L As A Potential Raw Material For Poultry Feed- A Sustainable Technology For Future

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Abstract

The spent silkworm pupae (SSP) of Bombyx mori generated from reeling sectors can be substantial source of raw materials for poultry feed formulations. Two breeds of Bombyx mori silkworm pupa are majorly discarded from reeling industry viz.,. PM X CSR_2, CSR_2 X CSR_4. Present investigation found that there was no significant differences in the nutritional value between the two hybrids CSR_2 X CSR_4 and PM X CSR_2. The percentage of total protein contents by dry weight (defatted pupae) were 55.61% in CSR_2 X CSR_4 and 55.72% in PM X CSR_2 hybrids. The result showed that the hybrids of SSP were rich in protein containing 18 amino acids. Both the breeds were rich in sulphur containing amino acids viz, cystine and methionine which is important for the growth of broiler.

Keywords: amino acids, antioxidant, Poultry, Protein, Silkworm hybrids, Silkworm pupae

1. Introduction

Silkworms are well known as an efficient large-scale producer of silk threads. Among the various species of silkworms, the mulberry silkworm (*Bombyx mori* L.) and the non-mulberry silkworm (*Antheraea pernyi*) are of common use in sericulture. It is an economically important insect, being a primary producer of silk (cocoon). However, it is not well known among consumers that silkworm pupae are an interesting by-product obtained after the extraction procedure of silk threads. The pupae, which are obtained after reeling the silkworm cocoons, are generally thrown away though they are very rich in amino acids, oil, carbohydrate and minerals. The large quantity of wastes that accumulates in silk reeling process in India could be utilized as a high potential raw material for various industries including animal nutrition.

India is the second largest producer of Silk next to China. According to the Annual report of Central Silk Board of India, the annual production of raw mulberry silk for the year 2011 was 16380 Metric tons. The amount of silkworm pupal waste generated from the above would be 12270 metric tons. If this waste generated is effectively utilized it will provide 2147 metric tons of pupal oil and 4802 Mt of silkworm pupal protein which can be used for fortification animal/human feed as the amino acid composition of Silkworm pupa satisfies. (FAO/WHO/UNO of 2007).

Longvah et al., 2011 reported that the nutritional composition of Eri silkworm pre-pupae 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 proximate compositions for non-mulberry and mulberry silkworm pupae were in the range of: total protein (12 to 16%), total fat (11 to 20%), carbohydrate (1.2 to 1.8%), moisture (65 to 70%) and ash (0.8 to 1.4%) reported by Mishra et al 2003. Rao, P.U, 1994 reported that spent pupae contains 48.7 % protein and 30% fat whereas defatted spent silk worm pupae meal contained 75.2 g % protein. Bose and Majumder (1990) studied that the pupae powder contains 7.18% water, 29.57% fat, 48.98% protein 4.65% glycogen 3.37% chitin , 2.19% ash , vitamins (3.7%) etc showing that pupa is good source of protein and fat. The amino acid scores of Eri pre-pupae and pupae protein were 99 and 100, respectively, with leucine as a limited amino acids in both the cases. The high protein content in the defatted Eri silkworm meal (75%) with 44% of it accounting to essential amino acids makes it an ideal candidate for preparing protein concentrate isolates with enhanced protein quality that can be used in animal nutrition (Longvah et al., 2011). Jun Zhou and Dingxian Han (2006) reported that silkworm pupae contained eighteen known amino acids, including all of the essential amino acid (EAAs) and sulphur- containing amino acids. Methionine is an essential amino acid for animals particularly poultry.

The addition of methionine to the poultry diet has been correlated with the tendency to have less total body fat (Rostagno et al 1995), to improve growth performance and to reduce odor-related compounds in excr. eta (Chaver et al 2004). Consumption above 413 mg/day methionine resulted in significantly increased albumen total solids and protein, and yolk protein was significantly increased at and mg/day methionine compared to mg/day methionine (Shafer et al., 1998). Hence, the rich methionine component of SSP makes it an ideal candidate for poultry feed which could enhance the egg quality.

Currently two major types of commercial hybrids viz., CSR_2 X CSR_4 and PM X CSR_4 are reeled at reeling industries. Literature survey revealed that there are no comparative study for proximate analysis, amino acid and antioxidant for *Bombyx mori* pupae (after reeled) of CSR_2 X CSR_4 and PM X CSR_4 hybrid which can be used for poultry industry. Hence it was the major goal of this study to investigate if there is any difference in the nutritional value of these two hybrids.

2.Materials and methods:

Raw silkworm pupae were collected from a reeling center in Ramanagaram District which were discarded after silk-reeling. Pupae were cleaned properly and were sundried until constant weight was obtained and moisture percentage was calculated. The dried pupae were defatted . This was further subjected to biochemical analysis such as crude protein, carbohydrate, amino acid profile, ash content and moisture content (as per ASTM procedures).

2.1 Extraction of pupal oil

The dried pupae were defatted by using chloroform, briefly 100 grams of dried silkworm pupae were immersed in 500ml of Chloroform for about 5 minutes and filtered. The filter cake was again dried and immersed in Chloroform for 5 minutes and filtered. Chloroform was recovered by distillation in a rotary evaporator, The amount of oil obtained was measured.

2.2 Amino acid profile of defatted silkworm powder:

The amino acid composition was analysed by Hitachi amino acid analyzer . Before analysis, the filter cake was dried until standard weight was obtained. Hydrolyzed pupal powder was prepared using the filter cake by following the procedure of Hyun *et al.* (2004).Briefly, 1 gm of CSR_2 x CSR_4 and PM x CSR_2 pupal powder were dissolved in 80-fold volume of HCl (2 mol/lit) and boiled for 4 h. After boiling, the solution was brown in colour. The brown-coloured hydrolysed was neutralized to pH 7.4 with NaOH (2 mol/lit) and dialyzed against distilled water. Hydrolyzed sample was further analyzed for its amino acid content. The unhydrolyzed powder was removed by centrifuging at 1000 rpm for 15 minutes.

3. Result and Discussion:

3.1 Moisture:

In the present study the moisture content of dried pupae were 75% in CSR_2X CSR_4 and 75.3% in PM X CSR2. Tomotake et al (2010) reported that the moisture of silkworm pupae was 51.9%. Longvah et al., 2011 reported that the moisture content for non-mulberry and mulberry silkworm pupae were in the range of 65 to 70%.

3.2 Pupal oil:

In the present study pupal oil was extracted by using chloroform. The oil content in PM X CSR_2 was 18.6 % and CSR_2 X CSR_4 18.2%. There were no significant difference between the two types of hybrids. Winitchai et al (2011) extracted oil from five native Thai silkworm varieties, Keaw Sakon, Nangnoi Srisaket,Sam Rong, Nang Luang and None Ruesee. The yields of the oils by the Soxhlet and maceration methods were in the ranges 24–29%. According to Shiva Shanker et al., 2006 the oil content in Eri pupae was estimated to be in the range of 18-20% (dry basis).

The pupal oil was found to be enriched with α-linolenic acid (ALA) with palmitic acid as the second major fatty acid. Pupal fat contained 66.8% total unsaturated fatty acids, and linolenic acid accounted for 25.7 g % of the total fat (Rao, P.U, 1994). Majumder (1990) extracted the pupal oil from dried and cleaned silkworm pupae by solvent extraction methods like hexane, benzene, alcohol, chloroform, ether, petroleum ether, etc. in a soxhlet apparatus, followed by filtering and evaporating the solvent. The study showed that the hot extraction method gives more percentage of oil than that obtained by cold extraction . The study also confirmed that chloroform is the best extracting agent among the solvents. Sreekantaswamy and Siddalingaiah (1981) extracted the pupal oil by using chloroform : methanol = 2:1 (v/v). Zhao-Jun Wei et al., (2009) extracted pupal 29.73% by Supercritical carbon dioxide extraction (SC-CO_2) methods.

3.2 Crude protein:

In present investigation crude protein percentage in defatted pupae in CSR_2 X CSR_4 and PM X CSR_2 were 55.61 and 55.72 respectively whereas crude protein percentage were less in undefatted pupae CSR_2 X CSR_4 (49.12%) and PM X CSR_2 (48.01%). Tomotake et al (2010) evaluated the nutritional value of silkworm pupae and the content of α-glucosidase inhibitor. The percentage of total protein by dry weight was 55.6%. 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 (Longvah et al., 2011). The chemical composition and the nutritional quality of protein of pupae of the silkworm Antheraea pernyi were investigated. Investigations showed that the pupal powder contained 71.9% crude protein, on a dry matter basis (Zhou and Han 2006). Nandeesha et al., (1990) reported that the digestibility of protein and fat from pupa diets was better than that from the fish meal control diet. Ichhponania and Malika (1971) evaluated the of deoiled silkworm pupae meal and corn steep fluid as protein sources in chick rations. A growth and metabolic study was conducted on chicks fed containing different levels of de-oiled silkworm pupae meal and corn-steep fluid as a replacement of fish meal and groundnut cake respectively. The results revealed that half of the fish meal (5 per cent) and half of the groundnut cake (10 per cent) can be safely replaced by de-oiled silkworm pupae and corn-steep fluid respectively on equal protein basis in chick rations.

3.3 Carbohydrate:

In the present study, carbohydrate percentage of defatted pupae were 16.2 (CSR_2 X CSR_4) and 15.99 (PM X CSR_2) however un-defatted pupae contained 10.64% (CSR_2 X CSR_4) and 12% (PM X CSR_2) .

3.4 Ash content:

Ash content of defatted pupae were 7.3% and 6.97% (CSR_2 X CSR_4 and PM X CSR_2) and crude fiber were 0.82% and 1% in CSRX CSR_2. Mishra et al (2003) study the Nutritive value of non-mulberry and mulberry silkworm pupae. The result showed that ash content of mulberry and non-mulberry pupae were range from 0.8 to 1.4% .

3.5 Amino acids profile:

The amino acid composition analysis showed there were no differences between the two hybrids pupae. Both of the silkworm pupae contents eighteen known amino acid including all of the essential amino acids and sulphur containing amino acids.

Methionine is an essential amino acid for animals particularly in poultry. Methionine plays several roles in poultry. The percentage of savoury amino acids (asparagines and glutamic acid) were high (9.91 and 15.12 in CSR_2 X CSR_4 and 9.98 and 14.97 in PM X CSR_2).

Jun Zhou and Dingxian Han (2006) reported the amino acid compostion of *Bombyx mori* Lin but author did not mentioned the type of silkworm breed used in the study. In the present study Cystine percentage were same in both the hybrids. It is known that Cystine can substitute for part of the requirement for Methionine, FAO/WHO/UNU (1985) does not give any indication of the proportion of total sulphur containing amino acids which can be made by Cystine. Most animal proteins are low in Cystine in contrast , many vegetable proteins contain more Cystine than Methionine. Adeyeye and Afolabi (2004) reported that for animal protein diet or mixed diets containing animal protein, Cystiene is unlikely to contribute more than 50 % of the total sulphur containing amino acid. (FO/WHO,1991) Rostango et al 1995 Chavez et ak 2004 reported that the Methionine plays several roles in poultry. The addition of Methionine in poultry diet has been correlated with the tendency to have less total body fat (Rosttango et al,1995), to improve growth performance and odour related compounds in exreta (Chavez et al, 2004) Consumption above ----mg/day methione resulted in significantly increased albumin, total solid protein and yolk protein was significantly increased at 556 mg/day Methionine compared to 413 mg/day Methionine (Shafer et al 1998)

Table: 1 Amino acid composition of pupae of the silkworm *Bombyx mori* L (mg/g crude protein)

Amino acids	**Amino acid % (CSR_2 x CSR_4)**	**Amino acid % (PM x CSR_2)**
Aspertic acid	9.91	9.98
Threonine	5.21	5.09
Serine	4.30	4.29
Glutamic acid	15.12	14.97
Glycine	4.20	4.29
Alanine	5.71	5.59
Cystine	1.60	1.60
Valine	5.01	4.99
Methionine	4.30	4.39
Isoleucine	5.51	5.59
Leucine	7.91	7.88
Tryrosine	5.11	4.99
Phenylamine	4.90	4.89
Lysine	7.31	7.39
Histidine	2.20	2.30
Arginine	6.61	6.69
Proline	3.90	3.99
Tryptophan	1.20	1.10

4. Conclusion

The results suggested that both the hybrids of *Bombyx mori* pupae were good sources of protein because of their high protein content. In addition, both silkworm pupae contains all 8 Essential amino acids. The amino acid composition were highly comparable .Both the hybrid pupae were rich in Sulphur containing amino acids viz Cystine and Methionine which were most important for the improvement of the quality of eggs . The present study concludes that any of the two hybrids may be used indiscriminatingly as raw material source for the formulation of poultry feed

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