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Review Article

A Review – Bionutritional Science of Silkworm Pupal residue to Mine New ways for utilization

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Abstract

The review revealed that, annually India produces about 40,000 metric tons of silkworm pupae on dry weight. Lots of silkworm pupae have not been fully utilized. Recently, the chemical compositions of desilked silkworm pupae have attracted considerable attentions in the world and desilked silkworm pupae are considered to be a good source of a large number of bioactive substances. The silkworm pupal powder contains moisture (7.6%), crude protein (71.9%), lipid (34.4%), fat (20%) and ash (4%). Pupal oil is rich in unsaturated fatty acid. Fourteen fatty acids were identified with the extracts from silkworm pupae and steric acid was the predominant saturated fatty acid. Alpha linolenic acid is the major component of pupal oil (32-44%). Nutrition studies of silkworm pupal proteins revealed that, the essential amino acids to total amino acids high as 41.2% and 39.2% respectively. Glutamic acid (18.3%), histidine (14.6%) and alanine (10.2%) are common amino acids in silkworm pupae. The enzymatic activities *viz,* urease, phosphatase, dehydrogenase and cellulase were recorded during silkworm litter pupal waste (SLPW) composting. Replacement of fish meal by silkworm pupae in broiler diets indicated that the growth rate, feed conversion, meat yield and profitability increased almost linearly on increasing level of silkworm pupae (SWP).

Keywords: Bionutrient, silkworm pupa, protein, fatty acid.

Introduction

Biochemical composition of silkworm pupae

Lipid

Sridhara and Bhat (1965) studied the constituents of silkworm fat. Analyses of silkworm organs revealed that the fat body had the most fat and the least free fatty acids, where as haemolymph contained the least fat. Shoichi Nakasone and Toshioito (1967) studied the fatty acid composition of lipids of *Bombyx mori* L. from egg to adult of larval organs was determined by gas-liquid chromatography. Nandeesha *et al.* (1999) conducted a field experiment on common carp, *Cyprinus carpio* with dietary administration of three levels of pupa oil. Fish growth increased significantly with the increased level of both pupa and sardine oils.

The study demonstrated not only the usefulness of pupa oils, which is rich in unsaturated fatty acids. (n−3), as an equally potent energy source as sardine oil but also the possibility of using additional fat in the diet to economically promote the growth. Eiichi *et al.* (2002) reported that, the oil content of the silkworm, *Bombyx mori*, in the pupal stage was 4.8% for the male and 9.0% for the female (wet basis), respectively. Total lipid (TL) extracted from silkworm pupae mainly consisted of triacylglycerol (TG), phosphatidylethanolamine, and phosphatidylcholine. TL and TG contained approximately 40% α-linolenic acid as the predominant fatty acid. 20:3n-3 was also present in the pupa, but the quantities were very small. Total tocopherol in TL was 125.2 µg/g lipids for the male and 224.1 µg/g lipid for the female respectively.

Percentage of fatty acid

Sridhara and Bhat (1965) observed the Silk glands contained the maximum phospholipids percentage. Stearic acid predominated in those tissues that had a high percentage of phospholipid. Stearic acid was the predominant saturated fatty acid in both the phospholipids and lecithin and it accounted for 35–50 per cent of the free fatty acids of all the tissues. Shoichi nakasone and Toshioito (1967) recorded fourteen fatty acids, including minor or trace elements, were identified with the extracts from the silkworm. Linoleic acid predominated throughout development, usually constituting one-third of the total acids and it was also in most of the larval organs. The fatty acid composition varied greatly during the larval period and the fatty acid pattern showed large variations among larval organs. Eiichi *et al*. (2002) suggested that, the silkworm pupa would be a good source of the functional fatty acid, α-linolenic acid and the functional pigments, lutein and neoxanthin. Tomotake *et al*. (2010) found that, the silkworm pupae possessed n-3 fatty acids, especially α-linolenic acid (36.3%), as a major component. Longvah *et al.* (2011) provided value addition to spent eri silkworm as an alternative source of edible oil for the food and feed industry by carrying out a short-term nutritional and toxicological evaluation of Eri silkworm pupae oil using Wistar NIN rats. Growth performance of rats fed either sunflower oil (Control) or eri silkworm pupae oil (Experimental) was comparable. Supanida Winitchai *et al*. (2011) studied that, the pupae oil obtained from Soxhlet extraction had unsaturated fatty acid content in the range 72–79% and alpha-linolenic acid content in the range 32–44%, whereas that obtained from the maceration extraction had unsaturated fatty acid content in the range 75–80% and alpha-linolenic acid content in the range 40–46%. Warsito *et al*. (2011) had done enrichment of alpha-linolenic acid (ALA) of basil seed oil, *Ocimum basilicum L.* by fractional crystallization and crystallization of fatty acid in urea inclusion complexes (UIC) methods. Mu-gil kwon *et al.* (2012) reported that, fatty acid composition of silkworm pupae oil was revealed by high-pressure liquid chromatography and gas chromatography – mass spectroscopy analyses. They contain a high ratio of essential fatty acids, [α-linolenic acid (ω-3 fatty acid]+ linoleic acid) (49.0%), and also contain non-essential fatty acids, oleic acid (19.9%), palmitoleic acid (2.5%), palmitic acid (19.7%), stearic acid (8.6%), and eicosapentaenoic acid (EPA) (0.3%).

Nutrient composition

Majumder *et al.* (1994) chemically analyzed the delipidated chrysalis powder and showed that DLCP (De Lipidated Chrysalis Pupae) is a nutritious food which contained fairly good amounts of moisture, chitin, water-soluble proteins, and carbohydrates, amino acids, minerals and Vitamin C, besides protein as the major constituent. Kanika Trivedy *et al.* (2008) reported that, the *Bombyx mori* pupal powder contains significantly higher amount of protein in female as compared to male in both the hybrids of PM x CSR2 and CSR2 x CSR4 studied. On an average, in the former hybrid 14.81% and in the later hybrid 14.58% soluble protein was present as compared to total protein 60.81 and 63.66%, respectively. Tomotake *et al.* (2010) evaluated the nutritional value of silkworm pupae (*Bombyx mori*). The percentages of total protein and lipid contents by dry weight were 55.6 and 32.2%, respectively.

Proximate composition and mineral contents

Mishra *et al.* (2003) reported that, 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%). The energy contents of the silkworm pupae were in the range of 706 to 988 KJ. Pereira *et al*. (2003) observed that chrysalis (worm) toast as a rich source of protein (51.1%), lipid (34.4%), essential fatty acids such as the linolenic acid (C18: 3n-3, 24.4%), zinc (244 $\mu g\ g^{-1}$) and potassium (4.77 $mg\ g^{-1}$). The composition of this sub-product showed good polyunsaturated / saturated (0.99) and n-6/n-3 (0.30) fatty acids ratios. Jun Zhou and Dingxian Han (2006) showed that, the pupal powder of *Antheraea pernyi* contained 7.6% moisture, 71.9% crude protein, 20.1% fat and 4.0% ash on a dry matter basis. The mineral analysis indicated high K content with a low Na/K ratio and low heavy metal content. Longvah *et al.* (2011) reported on proximate composition of Eri silkworm pre pupae and pupae as a good source of protein (16 g %), fat (8 g %) and minerals. 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.

Amino acid

Jun Zhou and Dingxian Han (2006) studied the pupal protein contained 18 known amino acids, including all of the essential amino acids and sulphur-containing amino acids. Compared with the amino acid profile recommended by FAO/WHO, the pupal protein was of high quality due to its high content of essential amino acids. Longvah *et al.* (2011) reported that, the amino acid scores of Eri pre pupae and pupae protein were 99 and 100 respectively, with leucine as the limiting amino acid in both cases. Net protein utilization (NPU) of pre pupae and pupae was 41 as compared to 62 in casein. Protein digestibility corrected amino acid score (PDCAAS) was 86.

Antioxidant activity of silkworm pupae oil

Supanida Winitchai *et al.* (2011) extracted oil from five native Thai silkworm varieties. The oil extracted by the Soxhlet method from silkworm varieties showed free radical scavenging activity. Deori *et al.* (2014) investigated antioxidant activity of pupae of the muga and Eri silkworm and concluded that, the pupae could be used as natural antioxidants on food products.

Silkworm pupal biochemical analysis

Chitin , Chitosan and protein in silkworm pupae

Suresh *et al.* (2012) reported that, the pupae of silkworm are an alternative source of chitin which consequently yields chitosan. Among the different races of mulberry silkworm, multivoltine pure races contain higher chitin of 3.225 percent in male pupae and 3.078 per cent in female pupae. Similarly higher chitosan per cent was observed in male 2.449 per cent and 2.354 per cent in female pupae. Priyadarshini and Revanasiddaiah (2013) estimated the crude and purified protein percentage from de-oiled pupae powder at the different hours (0 hours, 72 hours, 144 hours, and 216 hours) of pupae development of Eri silkworm. It was found that crude protein and protein concentrate was gradually increased from 0 hours to 216 hours both in male and female pupae. However, female pupae exhibited 45.1g of protein concentrate at 216 hours of development when compared to male pupae (39.3g).

Fatty acids of silkworm pupae oil

Sharma and Ganguly (2011) showed that, the waste pupae contain 30% or more lipids. The lipid part of the waste Eri silkworm pupae can be transesterified with methanol to produce methyl esters of fatty acids. The present study reports a quick and efficient transesterification method to convert the lipid to fatty acid methyl ester. The physicochemical properties of the methyl ester are reported. The parameters fulfill the international requirement for biodiesel. Jun wang *et al.* (2015) designed an effective method for the determination of the fatty acid composition in silkworm pupae oils, five methylation methods were evaluated for use in the gas chromatographic (GC) quantification of fatty acid methyl esters (FAMEs). The two-step methylation effectively improved the synthesis yield of FAMEs.

Amino acid and urea in the silkworm

Studies of Shamitha and Rao (2008) revealed that, amino acids decreased in the fat body in outdoor and indoor reared larvae in contrast to that in the haemolymph where it has gradually increased from first to third crops. Secondly, in the fifth instar the excretory products are more compared to fourth instar in the indoor reared worms.

Alpha –linolenic acid and linoeic acid in edible oil

Wang *et al.* (2010) developed enrichment process of ALA, indicated desilked silkworm pupal oil as a potential raw material for producing ALA.

Enzymatic activities during composting

Sangeetha *et al.* (2012a) found the activity of urease (266.4 µg N g-1) and phosphatase (34.3 µg of PNPP g-1) SLPW + microbial consortium treatment. The maximum of dehydrogenese activity of 37.7 µg of TPF g-1 24 h-1 was determined in the SLPW + *Cellulomonas cellulans* and the cellulose activity was maximum (346.4 µg of reducing sugars g-1) in the Silkworm litter + microbial consortium.

Value addition of silkworm pupae

Silkworm pupae as food and Histopathological studies

Majumder *et al.* (1994) concluded that, DLCP (De Lipidated Chrysalis Pupae) had great promise as a future supplementary protein food source for human nutrition because of its greater nutritive value and for developing a low- cost protein food. Nandeesha *et al.* (1999) revealed that, fish growth increased significantly with the increased level of both pupa and sardine oils. Specific growth rate, feed gain ratio and biomass production improved significantly with increased level of oil incorporation. The non defatted silkworm pupae could be used to completely replace fishmeal and could be included up to 50% in the diet. Khatun *et al*. (2003) demonstrated that, the growth rate, feed conversion, livability, meat yield and profitability increased almost linearly on increasing level of SWP (Silkworm pupa). Mishra *et al*. (2003) suggested that unconventional food items (pupae) could be a good source of protein and fat. Pereira *et al*. (2003) observed that, chrysalis (worm) toast could balance human nutrition and could be used as an alternative dietary supplement of proteins and fatty acids or could be used as animal feed. Cheaper SWP (Silkworm pupa) could be an excellent substitute of costly protein concentrate in formulating diets for layers leading to increase profitability. Khatun *et al.* (2005).

Jun Zhou and Dingxian Han (2006) The results of the present study provide some technical information and suggestions for the food industry for more effective utilization of pupae of the silkworm *A. pernyi.* Karthikeyan and Sivakumar (2007) utilized the silkworm pupal waste for mass cultivation of biopesticide bacterium, *Bacillus thuringiensis.* Where viable spore count (VSC) was taken as a criteria for evaluating the efficiency of pupal waste medium. A very high VSC of $369x10^9$ was obtained in the present investigation. Banday *et al.* (2009) reported that, the birds fed with processed SWP (Silkworm pupa) at both 25 and 50% levels showed an increase ($P<0.05$) in body weight gain. Feed conversion showed improvement ($P<0.05$). Wang *et al*. (2010) studied that alpha Linolenic acid (ALA) was widely used in food and pharmaceutical industry, as an important medical material. The developed enrichment process of ALA indicated desliked silkworm pupal oil as a potential raw material for producing ALA. Histopathological examination of the various tissues of rats showed no signs of toxicity even after feeding the eri silkworm oil for 18 weeks. Serum cholesterol and triglyceride was significantly reduced ($P < 0.05$) while high-density lipoprotein cholesterol was significantly increased ($P < 0.05$) which is attributed to the high α-linolenic acid content of eri silkworm oil. Longvah *et al*. (2011a). Jagannatha rao *et al. (*2011) concluded that, the fermented SWP silage produced better growth /performance in broiler chicken compared to the conventionally used expensive fish meal. Longvah *et al*. (2011a&b) indicated that, to provide value addition to spent Eri silkworm as an alternative source of edible oil for the food and feed industry by carrying out a short-term nutritional and toxicological evaluation of Eri silkworm pupae oil using Wistar NIN rats. The high protein content in the defatted Eri silkworm pupae 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. Supanida Winitchai *et al.* (2011) indicated that oil from native Thai silkworm pupae could be used as an alternative in the food and cosmetic industries.

Silkworm pupae as compost

Heenkende and Parama (2010) opined that seri compost could be used to obtain higher yields of French bean. Sharma and Ganguly (2011) showed that, the silkworm pupal bio-waste can be converted to good quality fuels which may be used as biodiesel additives. The results of the experiment by Sangeetha *et al.* (2012) clearly indicated that the application of SLPW (Silkworm litter-pupal waste) + Vermicompost recorded significantly higher leaf yield (32,098.5 kg) and NPK content (3.11%, 0.39% and 2.48 %) respectively.

Demographic characteristic study and consumption pattern of silkworm pupae

Mishra *et al.* (2003) reported that, overall consumption was highest for Eri (87.7%) followed by Muga (57.4%) and mulberry silkworm pupae (24.6%) irrespective of age group and gender. Amongst the three major communities predominant in the villages, the highest consumption was in the Ahom community (Eri 91% and Muga 63%). In conclusion these unconventional food items with high cultural acceptability and nutritive value may be utilized in formulating potential alternate recipe for malnourished population as well as nutritious delicacy for others.

Conclusion

The review revealed that, the silkworm pupae is very rich source of proteins, lipids and minerals so could be used as an alternative dietary supplement in animal feed. Hence, the silkworm pupae may be utilized in formulating potential alternate recipe for malnourished population. It is also useful in pharmaceutical industry, as an important medical material. Silkworm pupal bio-waste can be converted to good quality fuels which may be used as biodiesel additives. Silkworm pupae are the only one animal source which contains high amount of alpha linolenic acid, which is having a high medicinal property can be exploited for the welfare of mankind. Further, there is scope for the utilization of silkworm pupal bioproteins. After the extraction of lipids and proteins from silkworm pupae, the residue can be used for the preparation of enriched compost with high nutrients. So that, the major byproduct generated in silk reeling industry, the pupae can be effectively used to raise the returns of silk cocoon reelers in particular and cocoon growers at large.

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