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Silkworm pupae meal: An alternative protein source for livestock

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Abstract
Silkworm pupae meal is a nutrient-dense by product of the silk reeling industry and is available in significant quantity. It is a good source of protein, lipids, minerals, and vitamins and is considered a good source of nutrients. It is a rich source of limiting amino acids such as methionine and lysine and has an essential amino acid profile similar to that of a whole egg. Also, it is a good source of polyunsaturated fatty acids such as linolenic acid. The excellent nutrient profile makes silkworm pupae meal a valuable and versatile ingredient that can be used in animal feed as an alternative to conventional protein sources such as soybean meal, groundnut cake, etc. Thus, the use of silkworm pupae meal in livestock feed will lessen the dependency on conventional protein sources. In addition, the environmental pollution caused by the disposal of huge amounts of silkworm pupae will be significantly reduced.

Keywords: Amino acids, cocoon, linolenic acids, pupae

Introduction

Feed cost accounts for more than half of the cost of dairy production. The net deficit of 44% in concentrates (Singh *et al*., 2022) [49], high cost of oil cakes and decline in availability of land under forage production has led to inadequate supply of feeds for livestock. Due to the shortage of feed resources in the country, there is a huge gap in the availability and requirement of concentrate ingredients for feeding the livestock. Further hope of feeding millions of livestock and safeguarding their feed and nutritional security will depend on efficient utilization and identification of unconventional feed sources in addition to judicious utilization of available feed resources.

Silkworm pupae meal is one such alternate protein supplement, which can be explored and used as a livestock feed which otherwise the fresh pupae is thrown into open fields in some parts of the country, causing environmental pollution. India stands second in the world in raw silk production after China. This is due to a significant progress made in the production of raw silk, where production has reached 34903 metric tonnes during the end of 2021 (International Sericultural Commission, year 2021) [2]. Silkworm pupae is a by-product of the cocoon reeling process and contains 50-80 percent proteins (Rashmi *et al*., 2018; Ichim *et al.,* 2008) [40, 16], 25-35 percent fats (Rashmi *et al*., 2018; Ichim *et al.,* 2008) [40, 16], 8-10 percent sugars, E, B_1, B_2 vitamins, nicotinic acid, pantothenic acid, copper, iron and selenium (Ichim *et al*. 2008) [16]. The protein of silkworm pupae is superior to fish meal (Mathur *et al*. 1988; Mishra and Das, 1992) [30, 31] and beef protein (Mishra and Das, 1992) [31]. It is a rich source of limiting amino acids such as methionine and lysine (Longvah *et al*. 2011; Rao, 1994; Chandrasekaraiah *et al.* 2003b; Sampath *et al*. 2003) [27, 38, 8, 43]. The essential amino acid content of pupal protein is similar to that of whole egg protein with exception of tryptophan 0.9g per 16 g of nitrogen (Rao, 1994) [38]. The protein quality of spent silk worm pupae meal was significantly lower than casein (milk protein) as judged by protein efficiency ratio (PER) and net protein utilization (Ioselevich *et al*., 2004) [18]. Silkworm pupae meal fat contains 20.7 per cent saturated fatty acid and 70.1 per cent unsaturated fatty acids. It is a rich source of polyunsaturated fatty acids such as linolenic acid (Makkar, 2014) [29]. The silkworm pupae consisting of numerous biological constituents and have a multifaceted uses such as feed / food for animals, including human beings, medicine and manure for crops.

The higher protein and amino acid content in silkworm pupae meal compared to many of the conventional protein sources would reduce the dependency on conventional protein sources such as soybean meal and groundnut cake etc., which in turn partially fulfil the huge gap between the supply and demand for feed resources apart from reducing the environmental pollution. It is, therefore necessary to evaluate its replacement value in place of oil cakes and the effect on the nutrient utilization. One among such is silkworm pupae meal which is a by-product of silk reeling industry. The exploration of silkworm pupae meal can add on to feed basket, which will in turn reduce the demand for conventional feed resources.

Global scenario of silk

Silk constitute a small percentage of the global textile market about less than 0.2%. Yet its production base is spread over 60 countries in the world. The major producers are in Asia (90% of mulberry production and almost 100% of non-mulberry silk). Today China and India are the two worlds leading silk producers. China and India contributes about 80% and 15% of total silk produced in the world, respectively (Table 1).

Table 1: Silk production status of top eight countries (in metric tonnes)

Country	2015	2016	2017	2018	2019	2020	2021
China	170000	158400	142000	120000	68600	53359	46700
India	28523	30348	31906	35261	35820	33770	34903
Uzbekistan	1200	1256	1200	1800	2037	2037	2037
Thailand	698	712	680	680	700	520	503
Brazil	600	650	600	650	469	377	373
Vietnam	450	523	520	680	795	969	1067
North Korea	350	365	365	350	370	370	370

Source: International Sericultural Commission, (2021) [2]

Scenario in India

India, apart from second largest producer of silk, it is being the world's largest silk consumer. India is unique to produce all four varieties of silk namely Mulberry, Eri, Tasar, Muga. Karnataka accounts for the largest producer of silk in India, about 11,191 metric tonnes followed by Andhra Pradesh, West Bengal and others (Table 2).

Table 2: Production status of top ten states in India during 2021

State	Production (in metric tonnes)
Karnataka	11,191
Andhra Pradesh	8834
West Bengal	1632
Assam	5700
Tamil Nadu	2373
Jharkand	1052
Meghalaya	1234
Manipur	462
Chattisgarh	224
Nagaland	59

Source: Central Silk Broad, Ministry of textiles, GOI year (2021) [1]

Silkworm pupae

Silkworm pupae is a major by-product of silk reeling industry, obtained from unwinding of cocoons (Datta, 2007) [9]. Production of one kg raw silk yields 8 kg of wet pupae or 2 kg of dry pupae (Patil *et al*., 2013) [36]. After reeling silkworm pupae are a waste material which is generally discarded in the open environment without any utilization. Fresh spent silkworm pupae are highly degradable product due to its high moisture content. Its disposal in large quantities can be a serious threat to the environment (Wang *et al*., 2010) [54]. The conversion of these secondary and waste products into valuable biological active substances with their important uses in pharmaceutical, cosmetic, paper and cellulose, and organic agricultural food industries will not only reduce environmental pollution but also ensure profitable sericulture.

Processing Silkworm pupae as feed for livestock

Prior to reeling, the pupae are killed by boiling, drying or soaking in NaOH (Datta, 2007; Jintasataporn, 2012) [9, 21]. This softens the outer chitinous covering, which is then removed manually. The fresh spent silkworm pupae are highly perishable due to high moisture content. The degradation of silkworm pupae results in bad odour which associate with palatability issues (Rao, 1994; Finke, 2002) [38,13]. Therefore after washing the pupae they are pressed to remove excess of moisture and dried in sun or in mechanical driers and ground (Usub *et al*., 2008; Wijayasingh *et al*., 1977; Jintasataporn, 2012) [52, 56, 21]. Due to high fat content these dried pupae are prone for rancidity. Therefore, to ensure longer storage the dried pupae are solvent extracted. The extracted pupae withstand longer storage on account of the low level of fat content. Finally obtained defatted silkworm pupae meal has a higher protein content and longer shelf life than undefatted meal (Blair, 2008) [5]. Ensiling is an another way to increase the shelf life of silkworm pupae meal and ensiling with molasses, propionic acid, curd results in good quality silage. (Yashoda *et al*., 2008; Rangacharyulu *et al*., 2003) [58, 37].

Chemical composition of silkworm pupae

Silkworm pupae meal is a rich source of protein having CP around 50% DM to more than 80% DM. This protein is a good source of essential amino acids. The limiting amino acids in cattle such as methionine and lysine are found in appreciable percentage in the silkworm pupae protein (Chandrasekaraiah *et al*., 2002, 2003b) [7, 8]. Truly digestible protein accounts for only 73% of total crude protein content due to the contribution of chitin to the crude protein. However, the chitin constitutes only 3-4% DM (Finke, 2002; Suresh *et al*., 2012) [13, 50]. Silkworm pupae protein contains 18 amino acids making it a good nutritional source of high-quality protein. The extract of silkworm pupae is a rich source of polyunsaturated fatty acid especially α linolenic acid around 27.99% and has more than 68% total unsaturated fatty acids (Wei *et al*. 2009) [55]. It contains NDF around 11% DM (Finke, 2002) [13] and ADF around 6 -12% DM (Finke, 2002; Ioselevich *et al*., 2004) [13, 18]. It is a poor source of minerals around 3-10% DM. Silkworm being a good nutritional source has gained an attention (Tables 3, 4, 5) and found to be suitable for livestock feeding, especially in monogastric species (poultry, pigs and fish), and also in ruminants (Trivedy *et al*., 2008; Makkar *et al.*, 2014; Rashmi *et al*., 2022) [51, 29, 39].

Table 3: Chemical composition of fresh silkworm pupae, fatted silkworm pupae and defatted silkworm pupae

Component	Fresh silkworm pupae	Dried, fatted silkworm pupae	Dried, defatted silkworm pupae
Dry matter (% as fed)	26.2	91.4	93.8
Crude protein (% DM)	58.8	60.7	75.6
Crude fibre (% DM)	5.8	3.9	6.6
Ether extract (% DM)	28.5	25.7	4.7
Ash (% DM)	4.9	5.8	6.8
GE (MJ/kg DM)	26.5	25.8	22
Calcium (g/kg DM)	1.5	3.8	4.0
Phosphorus (g/kg DM)	9.0	6.0	7.0

(Hossain *et al*., 1997; Jintasataporn. 2012; Narang and Lal, 1985; Rao, 1994; Ioselevich *et al*., 2004) [15, 21, 33, 38, 18]

Table 4: Comparison with conventional sources like soybean, fish meal (% DM)

Constituent	Silkworm pupae meal	Silkworm pupae meal (defatted)	Fish meal	Soybean meal
Crude protein	60.7	75.6	70.6	51.8
Lipids	25.7	4.7	9.9	2.0
Calcium	0.38	0.40	4.34	0.39
Phosphorus	0.60	0.87	2.79	0.69
Ca:P ratio	0.63	0.46	1.56	0.57

(Chandrasekaraiah *et al*., 2003a; Makkar *et al*., 2014) [6, 29]

Table 5: Essential amino acid composition (g/16g nitrogen) and its comparison with fish meal and soya meal

Amino acid	Silkworm pupae meal	Silkworm pupae meal (defatted)	Fishmeal	Soya meal
Methionine	3.5	3.0	2.7	1.32
Cystine	1.0	0.8	0.8	1.38
Valine	5.5	4.9	4.9	4.5
Isoleucine	5.1	3.9	4.2	4.16
Leucine	7.5	5.8	7.2	7.58
Phenylalanine	5.2	4.4	3.9	5.16
Histidine	2.6	2.6	2.4	3.06
Lysine	7.0	6.1	7.5	6.18
Threonine	5.1	4.8	4.1	3.78
Tryptophan	0.9	1.4	1.0	1.36

Source: (Chandrasekharaiah *et al*., 2002, 2003b; Makkar *et al*., 2014) [7, 8, 29]

Utilization of silkworm pupae meal in livestock feeding

Ruminants

The research work conducted on the utilization of silkworm pupae meal in ruminants is either scanty or very limited.

A digestibility study was conducted by Khan and Zubairy (1971) [23] in adult male sheep using Tusser silk worm pupae as the sole protein source assuming that 50% of its CP is digestible and concluded that CP digestibility of silkworm pupae is around 70%. Narang and Lal (1985) [33] observed an increase in average daily body weight gain of calves when they replaced one third of groundnut cake by silkworm pupae in the milk replacer of Jersey calves.

According to study conducted by Chandrasekaraiah *et al.* (2002) [7] effective *in situ* nitrogen degradability of silkworm meal was as low as 29% and 20% for non defatted meal and defatted meal at 5% /h outflow rate. In another study conducted by Sampath *et al.* (2003) [38] revealed that *in situ* disappearance for lysine and methionine as 26% at 5% /h outflow rate for 24 h incubation.

The study conducted by Chandrasekaraiah *et al*. (2003a) [6] revealed the chemical composition of both fatted and defatted silkworm pupae and also their *in vitro* dry matter and organic matter digestibility. The OM, CP, EE, CF, TA, AIA, NDF, ADF for fatted silkworm pupae and de-oiled silkworm pupae were reported as 94.32, 55.95, 22.74, 3.50, 5.68, 5.18, 49.34, 6.42 and 93.83, 72.07, 8.03, 4.71, 6.17, 0.10, 53.73, 9.66 respectively. In addition, *in vitro* dry matter and organic matter digestibility of silkworm pupae (as such) and de-oiled silkworm pupae were reported around 71%, 72% and 65%, 65.6% respectively. Replacement of potato protein with silkworm pupae iso-nitrogenously in a diet containing barley and hay (75:25) increased the retention of nitrogen and energy (Ioselevich *et al*., 2004) [18].

Further, an *in vitro* experiment conducted by Rashmi *et al*. (2018) [40] demonstrated that defatted silkworm pupae meal could completely replace soybean meal in concentrate mixture without any negative effect on rumen fermentation and digestibility. In addition Rashmi *et al*. (2022) [39] reported that defatted silkworm pupae meal could safely substitute soybean meal up to 30% in concentrate mixture of cattle without any adverse effects on their health and performance.

Poultry

An extensive research has been carried out on utilization of silkworm pupae in poultry diets and fish diets.

A study was carried out by Ijaiyo *et al.* (2009) [17] in finishing broiler chicken, where chicken were fed varying level of silkworm pupae replacing fish meal in the di*et al*ong with a iso-caloric (3000 kcal/kg) and iso-nitrogenous diet (CP 20%) for 4 weeks. There was no significant difference in feed intake, body weight gain, feed conversion efficiency and protein efficiency ratio in the treatment groups. This revealed that silkworm caterpillar meal can completely replace fish meal resulting in cheaper ration.

The broiler chicks were fed iso-nitrogenous and iso-energetics diet along with varying level of silkworm pupae meal replacing fish meal for 42 days. It was observed that there was significant increase in the growth rate, feed conversion, livability, meat yield and profitability with increasing level of silkworm pupae (Khatun *et al*., 2003) [25].

Processed silkworm pupae at 25% and 50% of inclusion in the diet of broiler has shown increase in body weight gain, feed conversion ratio with no effect on dressing percentage, eviscerated yield percentage, giblet percentage, proving its inclusion in poultry diet for a profitable poultry rearing without any adverse effect (Mohd *et al.*, 2009) [32]. No significant effect was observed in the broilers fed with 50%, 75% and 100% silkworm pupae based diet. Therefore complete replacement of fish meal by silkworm is possible without any adverse effect (Virk *et al.*, 1980) [53]. However, Fagoonee (1983) [12] reported adverse effect on the growth of broilers fed on 100% silkworm pupae based diet. A negative correlation was reported between feed intake and level (50% to 100%) of silkworm pupae meal in diets of layers and the decreased growth in layers was ascribed to reduced feed intake (Deshpande *et al.*, 1996) [10].

An experiment was conducted by Khatun *et al*. (2005) [24] to ascertain the effect of supplementation of silkworm pupae (at 0%, 6%, and 8%) in diet of layer. The live weight gain, egg production, feed efficiency were appreciable with increased level (up to 6%) of silkworm pupae in diet. The significant yield in growth performance and egg production proves silkworm pupae as a good protein source and its incorporation in poultry diets is paramount for economical poultry rearing. The improved growth performance with silkworm pupae supplemented diet has also been reported by Saikia (1972) [42], and Panda (1968) [35]. An unidentified growth factor in silkworm pupae has been assumed for better growth performance (Horie and Watanabe, 1980) [14]. In contrast, Joshi *et al.* (1979) [22] and Virk *et al.* (1980) [53] observed decline in growth performance with different inclusion level of silkworm pupae in diet. Likewise, drop in egg production with graded level of silkworm in diets was also reported by Virk *et al.* (1980) [53].

Decline in growth performance and feed conversion efficiency were reported with increased inclusion level of silkworm (5%) in diet, while a better feed conversion efficiency with addition of salt (0.25%) and mineral mixture (1%) was observed (Reddy *et al*., 1991) [41]. Sapcota *et al.* (2003) [44] reported a significant improvement in the productive performance of broilers by 100% inclusion of silkworms in the broiler diet. Thus the study concluded that silkworm pupae can replace fish meal completely without any adverse effect.

No significant effect was evident on growth performance, muscle characteristics and on its sensory evaluation with higher feed conversion ratio in group with 10% replacement of fish meal by silkworm pupae. Thus silkworm pupae can replace fish meal successfully at 10% without any adverse effect in broiler (Jintasatapon, 2012) [21].

An experiment was conducted by Sheikh *et al*. (2005) [47] to assess the effect of dietary inclusion of silkworm pupae on carcass characteristics in broilers. The dressed yield, eviscerated yield, Giblet yield did not differ significantly in addition to other parameters like blood loss and feather loss. Thus silkworm pupae meal can replace fish meal completely without any adverse effect.

According to Mahanta *et al*. (2004) [28], dietary supplementation of Muga silkworm pupae meal in breeders had reduced the ejaculate volume of semen, percent of live spermatozoa and sperm motility with rise in percent of live abnormal spermatozoa. However, other parameters like pH of semen, sperm concentration did not differ significantly. It concludes that incorporation of silkworm pupae in breeders' diet has undesirable effects on the breeding performance.

An experiment was conducted in finishers fed on different level of silkworm pupae (0%, 25%, 50%, 75% and 100%) by ljaiya *et al*. (2009) [17]. There was no significant difference in feed intake, average daily gain, feed conversion ratio, protein efficiency ratio, weight of carcass, body cuts, blood profile except albumin. The significant difference in albumin was attributed to protein content of silkworm pupae. It concludes that silkworm pupae meal can be incorporated up to 100% in the diet of finisher without any ill effect. Sheikh *et al*. (2010) [48] reports for marked retention in nitrogen, calcium and phosphorus in broilers fed on 100% silkworm pupae diet, which has been attributed to better utilization of dietary protein.

Rabbits

Rabbits fed with the silkworm pupae have shown increased fat deposition and fur growth rate significantly (Aruga 1994) [3]. Traditionally in China silkworm pupae meal has been a part of diet in rabbits.

Fish

Considerable amount of research has been carried out for exploring silkworm pupae as protein source in aquaculture.

An experiment was conducted in fingerlings fed on 40% CP at 5% live fish body weight for 40 days by Kurbanov *et al*. (2015) [26]. They observed that fish growth rate and feed utilization were higher in fingerlings fed the diet containing equal proportion of fish meal and silkworm pupae (50:50) and found to be lower in case of 100% silkworm pupae or fish meal. Also there was significant difference in weight gain, relative growth rate, food conversion ratio and protein efficiency ratio.

According to a study conducted by Oso *et al.* (2014) [34], in *Claris garienpinus* juveniles, the diets containing different inclusion level of silkworm pupae significantly improved weight gain, specific growth rate, and protein efficiency ratio. Best results were observed at 25% silkworm pupae in the diet. Sawhney (2014) [45]. studied the growth promoting role of silkworm pupae at inclusion level of 25% in the diet of *Tor putitora* fingerlings and concluded that inclusion of silkworm pupae in the fish diets promises an economical fish production.

According to an experiment conducted by Ji *et al*. (2013) [19]. in juvenile *Cyprinuscarpio* var. *Specularis* no significant difference was observed on body weight (FBW), weight gain rate (WGR), special growth rate (SGR), protein efficiency ratio (PER), feed conversion ratio (FCR), survival rate (SR) between silkworm pupae supplemented group and fish meal supplemented group. Also reported for lowered tissue lysine, tyrosine content in silkworm supplemented group. The study proves silkworm pupae as an alternative protein source at 50% replacement.

Jianlu *et al*. (2013) [20] conducted a study in Jian carp to evaluate the effect of different inclusion level of silkworm pupae in the diet and they observed negative correlation between replacement level and final body weight, feed intake and feed utilization. They concluded that silk worm pupae can successfully replace fish meal at 50%.

A similar study was conducted to assess the optimum level of inclusion of silkworm pupae in the diet of juvenile Jian carp. A negative relation was observed between level of inclusion of silkworm pupae and the growth, protein efficiency ratio, feed intake in Jian carp. Reduction in feed intake with increasing level of inclusion of silkworm pupae due to poor palatability results in poor growth. Decreased protein efficiency ratio at higher level is attributed to decreased protease activity at higher level of silkworm pupae inclusion. In addition at higher level of silkworm pupae increased expression of heat shock protein 70, oxidation stress and lipid peroxidation has been reported. The study concludes 50% is the optimum level of inclusion of silkworm pupae in diet (Ji *et al*., 2013) [19].

Higher level of silkworm pupae in the diet of rainbow trout *Oncorhynchus* has reported for reduction in RBC and HB level and rise in WBC. The decrease RBC and HB is attributed to hemopoeitic effect of unknown component of silkworm pupae. Elevation of WBC is ascribed to the immune system triggering factor present in silkworm pupae. It was concluded that inclusion of silkworm pupae had negative effect on haematological parameters in rainbow trout (Shakoori *et al*.,

2014) [46].

A comparative study between fish meal and silkworm meal was conducted in Rohu by Hossain *et al*. (1997) [15]. The apparent digestibility was found to be higher for silkworm pupae when compared to fish meal, but no difference was observed for true digestibility of silkworm pupae and fish meal fed groups. Rangacharyulu *et al*. (2003) [37] and Yashoda *et al*. (2008) [58] demonstrated that ensiling increases the shelf life of silkworm pupae meal and good quality silages are obtained when ensiling with molasses, propionic acid or curd as lactic acid culture.

Similarly, Bag *et al.* (2013) [4] reported for improvement in growth performance, nutrient utilization, PUFA, and n3/n6 ratio in fish fed on fermented silkworm pupae which have been attributed to good assimilation of fermented silkworm pupae meal in fish. Thus substitution of fish meal by inexpensive fermented silkworm pupae results in cost effective fish rearing with good yield and quality fish.

In another study, Rangacharyulu *et al*. (2003) [37] reported an increase in survival rate, feed conversion ratio and specific growth rate in fermented silkworm fed group against the untreated fresh silkworm fed group. It was concluded that ensiling or fermentation improves the nutritive value of silkworm pupae.

Conclusion

Silkworm pupae meal has a huge potential to be an alternative protein source for livestock. Besides, it is available in significant quantities. The higher protein and amino acid content in silkworm pupae meal compared to many of the conventional protein sources would reduce the dependency on conventional protein sources such as soybean meal and groundnut cake etc., which in turn partially fulfil the huge gap between the supply and demand for feed resources apart from reducing the environmental pollution. Thus, silkworm pupae meal could be an excellent alternative to conventional protein sources for livestock in terms of both nutrients and cost.

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