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Husbandry and Nutrition

Review

State of the Art in the Utilization of Silk Worm Pupae for Animal Nutrition

Gabriel Gorrín Armas *

* The Sierra Maestra Science, Technology, and Innovation Institution, Complejo Barlovento, Ave 5ta y 246, Playa. La Habana, Cuba.

Correspondence: gabrielgorrinarmas77@gmail.com; ggorrin@bionaturasm.cu

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ABSTRACT

Background: Large-scale insect breeding is a sustainable practice that causes little environmental impact and significant quantities of residues. The silk worm pupa is one of the most commonly generated sub-products in sericulture. Several countries use it as an alternative source for animal nutrition due to its availability and low cost. It offers multiple nutritional benefits that result in better animal health and production. **Aim.** To conduct a review of state of the art utilization of silk worm pupae in animal nutrition, their nutritional value, uses in animal feeding, aviculture, aquaculture, and other domesticated species. **Development:** The scientific results have demonstrated that the pupae can be used satisfactorily as an ingredient in the diet of most domesticated species due to their high contents of protein, essential amino acids, and polyunsaturated fatty acids. It can meet the nutritional demands of animals to a large extent. The market offers attractive choices for animals, including pets (birds, reptiles, amphibians, fishes), based on lyophilized silk worm pupae, which are defatted, frozen, and sold by well-known international brands. **Conclusions:** The silk worm pupae constitute a cost-effective highly nutritional sub-product for the diet of domesticated species with favorable results in animal health and production indicators.

Keywords: silk worm, insects, pupae, sub-products (*Source*: *MeSH*)

INTRODUCTION

Large-scale domestic insect breeding has taken place for more than 7000 years in sericulture (silk production), apiculture (honey production), biological pest control, and the production of medications and shellac coating (Dobermann *et al.*, 2017). Insects can be reared using organic sub-products, which reduced pollution and makes them protein-rich foods. The impact of insect

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rearing on the environment is not greater than that of conventional livestocking, since the emission of greenhouse gases into the atmosphere is low (Kowalska *et al.*, 2020).

Sericulture is a well-established rural agro-business in India, producing around 40 000 metric tons of dry silk worms a year (MT/y) (Bag *et al.*, 2013). To make 1 kg of raw silk, 8 kg of humid pupae are needed, which means 2 kg of dry pupa (Makkar *et al.*, 2014). The pupae obtained after the winding of silkwarm cocoons are generally discarded, though they have high contents of amino acids, oils, carbohydrates, and minerals. Their nutritional value is compared to fish meal, and it is sold at a much lower price. They are highly degradable, and are often dumped into the environment or used as fertilizer (Raja *et al.*, 2019).

A large quantity of the wastes accumulated as a result of winding could be used with a high potential as raw material for several industries (Bandlamori *et al.*, 2012). Because of its high protein contents, the meal from silkworm pupae is suitable to feed livestock, especially monogastric species (poultry, pigs, and fish), and ruminants (Makkar *et al.*, 2014).

Aim: To conduct a review of the state of the art on the utilization of silk worm pupae in animal nutrition, its nutritional value, uses in animal feeding, aviculture, aquaculture, and other domesticated species.

DEVELOPMENT

Nutritional value

Table 1. Chemical values of the main nutrients in silkworm pupae meal (Raja *et al.*, 2019).

Nutrients (% de DM)	Silkworm pupae meal	Defatted silkworm pupae meal
Crude protein	60.7 (81.7)	75.6
Fats	25.7	4.7
Calcium	0.38	0.40
Phosphorus	0.60	0.87
Ca/P ratio	0.63	0.46

The proximate composition of fresh silkworm pupae contains 65-70% humidity, 12-16% CP, 11-20% ethereal extracts (EE), 1.2-1.8% carbohydrates, and 0.8-1.4% ashes. (Hassan, 2018).

Protein is an important component of edible insects that comprises 30%-65% of total dry matter (DM). Between 46% and 96% of all amino acids are present in the protein of insects, though there are limited quantities of tryptophan and lysine (Dobermann *et al.*, 2017).

Unlike plant proteins, animal proteins are rich in essential amino acids and more easily digested (Sasma *et al.*, 2018). The silkworm pupae meal contains a wide range of crude protein (CP) that varies between 52 and -72%, whereas the defatted meal could contain 80% (Makkar *et al.*, 2014). Tomotake *et al.* (2010) reported 55.6 % total dry protein in silkworm pupae. Moreover, after determining the proximate composition of wasted pupae from silkworms in different species, they found higher average protein values in Samia *Cynthia ricini* and *Antheraeaproeylei* (41% and 40%, respectively), compared to *Bombyxmori*con (31 %).

Table 2. Comparison of the protein, fat, and energy contents of some of the most commonly consumed insect species (Dobermann *et al.*, 2017).

Insect	Protein (DM%)	Fat (DM%)	Energy (kcal/100 g)
Rhynchophorus phoenicis	32.86	36.86	478.87
Tenebrio militor	48.35	38.51	557.12
Dipteran (flies)	49.48	22.75	409.78
Hemipteran (true bugs)	48.33	30.26	478.99
Oecophylla smaragdina (weaver ant)	46.47	25.09	484.45
Isopteran (termites)	35.34	32.74	
Bombyxmori (silkworm larva)	61.8	8.81	389.6
Cirinaforda (Cirina forda carterpillar)	47.48	11.5	359
Galleriamellonella (greater wax worm)	38.01	56.65	650.13
Samia cynthiaricinii	54.7	25.6	463.63
Achetadomesticus (adult home cricket)	65.04	22.96	455.19
Schistocercasp.	61.05	17	427
Sphenarium purpuracens (adult grasshopper)	61.33	11.7	404.22
Ruspoliadifferens	44.3	46.2	

The true protein of silkworm was found to correspond to 73% of CP contents, thus explaining the presence of chitin, since this component includes nitrogen (N). Moreover, the chitin content in the pupae meal is relatively low, approximately 3-4% DM (Makkar *et al.*, 2014). The low chitin content is favorable, because this structural carbohydrate can be considered to have anti-nutritional properties due to the possible negative effects on protein digestibility. A study of melliferous bees demonstrated that the removal of chitin improved the protein quality of the insect measured through the digestibility of the protein, amino acid contents, protein efficiency index, and net protein use (Dobermann *et al.*, 2017). The crude fiber of silkworm pupae meal is mainly chitin that animals barely use (Pai, 2017).

The silkworm pupae contain 18 known amino acids, including all the essential amino acids and sulphureous amino acids. Lysine (6-7% in 100 g of CP) and the levels of methionine plus cystine around 4% are particularly high (Makkar *et al.*, 2014). Many of these essential amino acids are found in considerable quantities (Pai, 2017).

Table 3. Amino acid composition of defatted silkworm pupae meal (Raja *et al.*, 2019).

Amino acids	g/16 g of Nitrogen
Alanine	5.6
Arginine	5.8
Aspartic acid	10.4
Cystine	1.0
Methionine	3.5
Lysine	7.0
Isoleucine	5.1
Leucine	7.5
Phenylalanine	5.1

Threonine	5.2
Tryptophan	0.9
Glutamic acid	13.9
Histidine	2.6
Proline	5.2
Serine	5.0
Glycine	4.8
Tyrosine	5.9
Valine	5.5

Fat is the second principal component of insects, after protein. The unsaturated fatty acid profile is similar to that of poultry and lean fish, but it contains more polyunsaturated fatty acids (PUFAs) than any poultry or red meat. Insects contain little or no trace of EPA (eicosatetraenoic acid) and DHA (docosahexaenoic acid), but contain linoleic acid ($C_{18:2}$), and linoleic acid ($C_{18:3}$) occasionally, which humans can synthesize to produce arachidonic acid ($C_{20:4}$), and EPA (Dobermann *et al*., 2017).

The non-defatted pupae meal is rich in fats, reaching up to 37%. The oil contains a high percent of polyunsaturated fatty acids, especially linoleic acid, with values that vary between 11 and 45% of total fatty acids (Makkar *et al.*, 2014). According to Pai (2017), the silkworm pupae contain saturated fatty acids (20.7%), unsaturated fatty acids, such as palmitic acid (20.7%), oleic acid (70.1%), linoleic acid (14.0%), and linolenic acid (9.1%). Tomotake *et al.* (2010) found similar values when determining the composition of fatty acids in the silkworm pupae. In this case, the total lipid content was 32.2%, saturated fatty acids were 28.8%, monounsaturated fatty acids were 27.7%, PUFAs were 43.6%, with a predominance of $C_{18:3}$.

The energetic content of the silkworm pupae is within 706-988 kj/Kg of DM. The dry powder contained 71.9% CP, 20.1% EE, and 4.0% of ashes from dry matter. In another study, the values of CP and EE contents were 55.6% and 32.2%, respectively (Hassan, 2018). Likewise, the pupae meal has resulted in higher gross energy compared to the soy meal (Ullah *et al.*, 2017).

The corroboration of the vitamin-mineral content of the silkworm pupae was limited (Ullah *et al.*, 2017). Makkar *et al.* (2014) noted that compared to other insects, it has low levels of Ca and a low Ca/P ratio.

Table 4. Content of minerals and vitamins in different insect species Values in mg/kg (Akhtar and Isman, 2018).

Species	Calcium	Iron	Zinc	Potassium	Magnesium	Niacin	B12 µg	Thiamin	Riboflavin
Acheta domesticus	40.7	1.9	6.7	347	33.7	3.8	5.4	0.4	34.1
Tenebrio militor	23.1	2.2	4.6	340	60.6	5.6	0.5	2.1	8.1
Galleria mellonella	24.3	5	2.5	221	32.6	3.7	0.1	2.3	7.3
Hermetia illucens	934.2	6.6	13	453	40	7.1	5.5	7.7	16.2

Bombyx mori	17.7	1.6	3.1	326	49.8	2.6	0.1	3.3	9.4
Blaptica dubia	38	1.4	3.2	224	50	4.4	23.7	-	-
Musca domestica	76	12.5	8.5	303	80.6	9	0.6	11.3	77.2
Gonimbrasia belina	174	31	14	1032	160	-	-	-	-

However, Lokeshwari *et al.* (2019) said that pupae contain a remarkable quantity of minerals like calcium, iron, zinc, and magnesium. Therefore, they can provide the minerals needed by the human/animal body to perform its functions. The authors found the highest content of iron and magnesium in the pupae of butterfly *Samia Cynthia ricini* worms.

Table 5. Content of minerals and vitamins in silkworm pupae meal

Minerals	**(Pai, 2017)**	**(Pryadharshini *et al.*, 2017)**
Calcium (%)	0.63	0.65
Phosphorus (%)	1.25	1.22
Sodium (%)	0.03	0.30
Potassium (%)	1.07	0.80
Magnesium (mg/Kg)	-	0.325
Iron (mg/Kg)	-	230.00
Zinc (mg/Kg)	-	285.00
Vitamins (mg/100 g)		
E	1000	-
B1	15	-
B2	80.0	-
B12	0.5	-

Utilization of Silkworm Pupae in Animal Nutrition

In Japan, the silk worm pupae are being prepared and used as feed for livestock, pigs, and birds. Rabbits fed on silkworm pupae showed an increase in the excreta of fat and a significant hair growth rate (Priyadharshini *et al.*, 2017).

In 35 day-old White New Zealand rabbits, supplementation using 4% silkworm pupae meal in the diet produced greater carcass weight, without affecting the contents of essential amino acids in the meat. Likewise, the PUFAs underwent a significant increase, an opposite behavior to the index of atherogenic index in the muscles, which decreased significantly. The latter suggests that the inclusion of this meal in the diet has improved the composition of fatty acids in muscle tissue, which reduces the risk of cancer and atherosclerosis in consumers of this type of meat **(**Kowalska *et al.*, 2020).

The degradation of pupae produces an unpleasant smell attributed to the presence of compounds in the leaves of white mulberry trees that could be sequestered by the worms, including essential oils, flavonoids, and terpenoids. This undesirable smell has been associated with the palatability of animals. Silage can increase the useful life of pupa meal. The defatted pupae must be ground to produce a more uniform mix in the rations (Makkar *et al.*, 2014).

Brands and products

The bromatological qualities and benefits of the silkworm pupae for human consumption have favored the existence of a broad range of commercial products and brands in the market, for ornamental animals, especially fish. Supa (Supa Koi Silkworm Pupae) sells 5 L buckets containing highly nutritional pupae, which are easily digestible by the Koi fish as treatment and nutritional supplement to improve their growth and skin pigmentation. Petifool (Germany) offers Petifool Koi Silkworm Pupae for Koi fish, 100% natural, colorant-free and preservative-free supplement with a high percent of proteins (53.0% CP) and a low level of fiber (3.0 % CF).

Natures Gruby Chubby Mealworms (the United Kingdom) is another brand that produces feed for Koi fish based on silkworm pupae, with very similar nutritional characteristics (53.0% PC and 4.0% CF) to Petifool. This feed also provides better protection to the slime layer that helps create resistance against bacterial and parasitic diseases, thus producing large and healthy fish. In this region, the Cotswold Koi Company trades the Kusuri Silkworm Pupae also to improve Koi's nutrition and skin pigmentation.

Hikari (Japan) from manufacturer Kyorin Co. Ltd. (Kamihata Fish Industory Group) has been producing specific balanced fish diets for years. They are leaders in premium fish nutrition. Among their fish productions are Silkworms Selects to stimulate growth, with no negative effects on the fish. The levels of advanced technology and formulation allows them to use whole silkworm pupae to prevent common problems like oxidation.

In Chewy, an online store in the USA, which sells feeds and other products for pets, the silkworm pupae are sold as the main ingredient in several choices, from the Exotic Nutrition Pet Supply Company in the USA. One of them is the Exotic Nutrition Silkworm Pupae Hedge Hog Treats, for the diet of hedgehogs. They trade easily digestible, soft foods, which upon cooking can be ideal for opossum, squirrels, birds, amphibians, and reptiles. These criteria confirm the potentialities of the silkworm pupae in animal nutrition.

Aviculture

Most research studies on farm animals using insect meals and oils have been conducted in birds (Kowalska *et al.*, 2020). In poultry nutrition, the contribution of essential amino acids for short-term growth is a critical factor (Hassan, 2018).

Methionine is an essential amino acid for animals, especially poultry. Its supplementation in the diet has been associated with the tendency to have less body fat, improving growth and performance, and to reduce the compounds associated with the smell of excreta. Therefore, the rich component of methionine in the pupae make it the ideal candidate for poultry nutrition, which might improve egg quality (Bandlamori *et al.*, 2012).

In 8-week-old Rhode Island broiler chicks, there was an increase of the maximum weight and better feed conversion rate using diets made of 50% fish meal, plus 50% pupa meal. No deaths were associated to the inclusion of the meal of silk worm pupae, or due to the toxicological effects on the chicks (Dutta *et al.*, 2012).

Broilers fed on 25% inclusion and 50% processed pupa meal underwent an increase in body weight and greater feed conversion rate compared to the meal of pupae without processing (raw). The fish meal in the finishing diet has been replaced in 25, 50, 75, and 100% with pupae meal (Hassan, 2018).

In layers, the feed conversion using insect meal in their diet was better; however, a variation in egg size was observed. Similar results were observed in partridge ducks fed on insect meal, compared to a standard soy diet (Dobermann *et al.*, 2017). In White Leghorn layers, using 50% replacement based on silkworm pupae meal in the diet, better results were observed in egg production, compared to other substitution levels and the control. Feed conversion was not affected by the addition of the pupa meal to the diet. No mortality was observed. Likewise, the hematological parameter did not suffer adverse effects (Ullah *et al.*, 2017). Moreover, the hens improved their response capacity in the yolk color of eggs when the pupa meal was used as a nutritional supplement (Priyadharshini *et al*, 2017).

The cost of the feed was lower in the diets that included 100% pupa meal, with greater income as the level of inclusion was increased. A gradual replacement of fish meal by the cheaper pupa meal can reduce the total cost of poultry production (Dutta *et al.*, 2012). These results are encouraging, since in the economy of poultry production, feeds take 60-80% of the total cost of production, whereas the costs of protein account for approximately 15% the feeding costs (Ullah *et al.*, 2017).

No negative effects have been reported on poultry growth fed on insect meal. However, most documents have described similar growth rates or even better in chicks, compared to soy meal or soy meal plus fish meal (Hassan, 2018).

Aquaculture

In aquaculture, feeds are the most expensive inputs, accounting for 57%-87% of the total recurrent cost (Sasmal *et al.*, 2018). Fish meal is one of the most important sources of fish nutrition, improving growth through greater palatability of feeds, nutrient consumption, digestion, and absorption (Samaddar, 2018). However, the utilization of insects in fish nutrition is not new, but a general practice of small farms in Africa and Asia. Predominantly, the black soldier fly, the domestic fly larvae, silkworms, and mealworms have been used as feeds in aquaculture (Dobermann *et al.*, 2017).

The defatted silkworm pupae meal is one of the best substitutes of fish meal in the diet of several carp species due to the low costs and high protein content. *In Turkey it was reported to be an important ingredient in the nutrition and performance of the common carp and their crossbreds, resulting in proper growth and feed conversion rate. Nevertheless, the taste of fish fed on raw pupae meal was unpleasant compared to the taste of fish fed on dried pupae meal* (Raja *et al.*, 2019).

The digestibility of the common carp (*Cyprinuscarpio*) is not affected when fed on silkworm pupae. The fries showed a significant growth difference compared to the fish fed on oil and mustard cakes and rice bran (Raja *et al.,* 2019).

The levels of survival found are 84.16%, with greater production and better feed conversion rate in rohu fish (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*) carps fed on fermented silkworm pupae silage, compared to diets based on the silk worm pupae without treatment, plus fish meal (Rangacharyulu *et al*., 2003).

The diets based on fermented silkworm pupae enabled greater weight gain, growth speed, n3 polyunsaturated fatty acid accumulation, and higher n6/n3 ratio in *Tilapia mossambica* fries, which indicated that the fish can assimilate this feed well. It owed, possibly, to a greater palatability and preference of fish to take it as their potential feed (Bag *et al.*, 2013).

Shakoori *et al.* (2016), in rainbow trouts (*Oncorhynchus mykiss*) demonstrated that 10% of fish meal can be replaced with silkworm pupae without adverse effects on the values of feed conversion, specific growth speed, weight gain, condition factor, and survival. On the contrary, an inclusion above 10% reduced growth and feed conversion.

The recommendation for optimum growth and satisfactory protein use in African catfish (*Clarias gariepinus*) fries, is a diet containing 50% protein substitution, using the silkworm pupae in the fish meal. Fries fed on a 25%-75% inclusion have shown better growth performance that using diets with a single source of animal protein, since the protein ingestion is greater (Kurbanov *et al.*, 2015).

A study to understand the influence of diets with different sources of protein on the growth and metabolism of the tropical catfish (*Clarias batrachus*) revealed that the dry pupa of silkworm is the best source of protein, producing satisfactory growth (Raja *et al.,* 2019).

In Japanese sea bass (*Lateolabrax japonicus*), energy and CP digestibility was 73% and 85%, respectively, for the pupa meal with oil, which was lower than the meal of poultry sub-products, feather meal, blood meal, and soy meal, but comparable to the meat and bone meal (Raja *et al.*, 2019).

CONCLUSIONS

The silkworm pupae constitute a cost-effective highly nutritional sub product included in the diet of domesticated species with favorable results in terms of health and production indicators.

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AUTHOR CONTRIBUTION

Conception and design of research: GGA; analysis and interpretation of data: GGA; redaction of the manuscript: GGA.

CONFLICT OF INTERESTS

The author declare the existence of no conflicts of interests.