Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2019; 7(4): 78-85

Received: 10-05-2019
Accepted: 12-06-2019

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Silkworm pupae meal as alternative source of protein in fish feed

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Abstract
The ever-increasing cost of fish meal and other ingredients for fish feed has led to the search for other alternatives for protein source by the industry. Insects, form part of the natural fish diet and they represent a good source of protein. Over the last decade, studies on the replacement of fishmeal with insect meal have emerged with promising results. Silkworm pupae, a waste of the silk reeling industry, is a suitable candidate as a Fish Meal replacement because of its high nutritional value. Dry pupae contain 50–70% crude protein and 24–33% crude lipid, and is a high-quality insect protein source with a rich, balanced content of essential amino acids. The works of various researchers, on various fish species have led to the development of recommended inclusion levels of silkworm pupae meal in the diet of the following aquaculture species, 30-50% for major and minor carps, 5-15% for trout, 50-60% for masher, 75-100% for catfish, 30-40% for ornamental fishes and 5-20% for shellfishes that is assured to give better growth performance compared to fish meal.

Keywords: Silkworm pupae, carp nutrition, fish meal replacement, fish feed

Introduction
Fishmeal (FM) is one of the major feed ingredient used in the preparation of fish feed due its balanced amino acid composition, high digestibility and palatability, which is enhances the uptake, digestion and absorption of nutrients in fish [55]. However, the steady decline in catches of wild fish and the increased demand for quality aquaculture feed resulted in a rapid decrease in the availability of fishmeal [18]. Shortage in fish meal results in high price of fish feed [2, 70] and new feed ingredients are necessary for sustainable development of aquaculture.

The use of plant protein in diets for aquaculture species has been very successful, but several inherent problems have been identified including the presence of anti-nutritional factors [19] and unbalanced amino acid composition [48]. Most animal protein resources are nutritionally more similar to FM and appear to be more suitable as a dietary replacement (Table.1)

Sericulture originated in China during the 27th century BC and later spread to other parts of the globe. The world production of reelable silkworm cocoons was about 485,000 tonnes in 2011 [17]. Presently India is the second largest producer of silk and also the world's largest consumer of silk. India produces 4 major types of silks, viz., Mulberry, Tassar, Muga and Eri, among these, the domesticated silkworm, (*Bombyx mori*) or mulberry silk is the most common contributing to 80% of India's production [22]. Domesticated silkworm (*B. mori*), reared on mulberry leaves, is used for production of silk yarn, from the heat killed pupae. One cocoon can supply about 800 m of silk-thread which is a fibroine, peculiar elongated molecule thread [66]. The pupae remaining after reeling of silk fibre, becomes a waste product of this industry and can serve as a feedstuff [67]. This spent SWP are highly degradable often discarded in the open environment or used as a fertilizer [93]. The utilization of this waste Silkworm pupae (SWP) for feed or for the production of valuable biological substances such as chitin, protein, oil and fatty acids (α-linolenic acid) can be an ecofriendly method to mitigate the environmental impact of silk production [54]

Nutritional aspects of silkworm pupae
Dried silkworm pupae's nutritional value is comparable with that of fishmeal and comes with a much lower price. Its crude protein content ranges from 52 to 72% while for the deoiled meal it can be upto 65 to 80% (Table. 2). SWP protein is rich in essential amino acids such as valine, methionine and phenylalanine. The contents of essential amino acids in SWP protein

were on par with the FAO/WHO/UNU suggested nutritional requirements for fish (2007). The lysine (6-7% in 100 g CP) and methionine plus cystine levels of approximately 4% are particularly high in silkworm pupae and the detailed amino acid levels are given in the table (Table 3 and 4).

Table 1: Main chemical constituents in insect meals vis-à-vis fishmeal and soymeal [54]

Constituents % in DM)	Black soldier fly larvae	Housefly maggot meal	Meal-worm	Locust meal	House cricket	Silkworm pupae meal	Silkworm pupae meal (deoiled)	Fishmeal	Soymeal
Crude protein	42.1 (56.9)	50.4 (62.1)	52.8 (82.6)	57.3 (62.6)	63.3 (76.5)	60.7 (81.7)	75.6	70.6	51.8
Lipid	26.0	18.9	36.1	8.5	17.3	25.7	4.7	9.9	2.0
Calcium	7.56	0.47	0.27	0.13	1.01	0.38	0.40	4.34	0.39
Phosphorus	0.90	1.60	0.78	0.11	0.79	0.60	0.87	2.79	0.69
Ca:P ratio	8.4	0.29	0.35	1.18	1.28	0.63	0.46	1.56	0.57

Table 2: Proximate composition of Silkworm pupae

Protein	Fat	Moisture	Fibre	NFE	Ash	Dry matter	Reference
55	25	-	3	-	-	-	Panda [73]
55	25-27	10	3	-	-	-	Panda [74]
68.7	2.5	-	4	-	-	-	Panda [74]*
50	25	10	3	-	5	-	BIS [4]
65	3	10	4	-	6.5	-	BIS [4]
76	-	-	-	-	-	-	Chopra *et al...* [11]
74.95	-	-	-	-	-	-	Sujatha [86]
73.41	-	-	-	-	-	-	Joshi *et al...* [41]
47.90	27	-	3.4	-	5.6	90.90	Fagoone, [15]
81.60	6	-	-	-	2.1	-	Lin *et al...* [50]
48.12	34.20	-	1.84	11.40	4.44	-	Majaonkar & Bjambure [52]
72.82	0.47	5.89	-	-	-	-	Bose and Majumder [8]
51.6	31.1		-	-	-	-	Koreleski *et al...* [46]
70.58	18.62	-	-	4.61	6.19	91.39	Hossain *et al...* [29]
84.32	2.8	-	-	6.27	7.61	94.45	Hossain *et al...* [29]
49.12	22.6	-	5.60	11.1	5.80	94.22	Debjani Majumder [13]
25.5	-	61.3	-	9.7	1.6	-	Rangayachalu *et al...* [70]
50.2	27.85	5.14	4.93		10.69	-	Nandeesha *et al...* [64])
72.3	5.6	-	-	-	6.3	-	Lee *et al...* [49]
51.28	4.71	-	3.67	-	11.76	91.79	Olayani and Babasanmi, [71]
69.52	11.72	-	5.01	-	7.88	87.50	Osa and Iwalaye, [72]
48.4	32.51	7.18	-	-	4.59	-	Nisha *et al...* [67]
60.7	25.7	-	3.9	-	5.8	-	Makkar *et al...* [53]
75.6	4.7	-	6.6	-	6.8	-	Makkar *et al...* [53]
52.3	27.8	-	-	-	-	-	Ji *et al...* [35]
57.6	23.3	-	5.55	-	11.2	92.10	Rafiullah [75]
57.21	31.29	6.65	2.39		4.01	-	Bhagat and Barat [4]

* Deoiled Silkworm pupae meal

Table 3: Amino acid composition of silkworm pupae meal (non-deoiled) [54]

Amino Acids	g/16 g 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

Table 4: Amino acid composition of silkworm pupae meal (deoiled) [51]

Amino Acids	g/16 g Nitrogen
Alanine	4.4 ±0.2
Arginine	5.1±0.3
Aspartic acid	7.8±0.7
Cystine	0.8±0.5
Methionine	3.0±0.4
Lysine	6.1±0.4
Isoleucine	3.9±0.2
Leucine	5.8±0.2
Phenylalanine	4.4±0.3
Threonine	4.8±0.3
Tryptophan	1.4±0.2
Glutamic acid	8.3±0.7
Histidine	2.6±0.1
Proline	5.20±0.1
Serine	4.5±0.2
Glycine	3.7±0.3
Tyrosine	5.5±0.2
Valine	4.9±0.2

Silkworm pupae as a dietary protein source in animal feed

Naidu [58] stated that Powdered SWP meal is a good protein supplement for poultry mashes. Later several researchers have carried out investigation on utilization of SWP meal as animal protein source for varied purposes and they are reported that silkworm pupae are a good source of proteins, amino acids, fat, carbohydrates and vitamins, besides being rich in oil. Nagaraj and Basavanna [57] reported that protein concentration of pupae can be used to supplement the poultry feed and in cattle feed and reported that this meal might be an important substitute for fish meal.

Silkworm pupae as a dietary protein source for fish

Silkworm pupae (*B. mori*) has been an important fish feed ingredient in the Indo-Pacific region [25]. Increased raw pupa incorporation for a long period leads to off-odour [27] and unpleasant taste [77]. The dead SWP and moths could also be used as fish feed [83]. Studies with deoiled SWP meal revealed, it has a higher protein content than the non-deoiled SWP meal and suitable as a dietary protein source for fish [6]

Silkworm pupae as a feed ingredient for carp farming

Silkworm pupae are an important component in the diet of carp in Japan and China [26]. The processed silkworm pupae is an excellent source of protein in fish feed [59]. Traditionally in Asian aquaculture, particularly in China and India, animal by-products like SWP have been used as a feedstuff either singly or in compounded diets. Feeding and performance of common carp and their crosses in Cifteler, Central Anatolia in Turkey and reported that SWP meal was an important feed ingredient and also showed good growth and feed conversion rate [15]. However, it was observed that the taste of fish fed with raw SWP was unpleasant, compared with the taste of fish fed with dried SWP. The quality of protein and the presence of appetite stimulants in the silkworm pupae [88] and some attractants [56] could have been responsible for the comparable growth of fish obtained with deoiled silkworm pupae. Non-deoiled dried SWP due to its low cost and high protein content, is one of the best substitutes for fishmeal in the diets of several carp species [62, 63, 22, 50, 69].

Silkworm pupae as feed ingredient for Indian major carps

Indian major carp fingerlings fed with SWP incorporated feed, showed significant growth than control animals fed with mustard oil cake and rice bran [9]. Catla fed with 30% non-deoiled silkworm pupae diet showed higher growth rate on contrary rohu fed with SWP exhibited poor growth which was attributed to the low fat-requirement of the fish [31]. Investigation on keeping quality of the diet [32], influence of diet on body composition [33], digestibility of nutrients [34] and the influence of the diet on the organoleptic quality [35, 63] showed that SWP meal is equivalent to fish meal in all respects.

A study conducted to evaluate effect of cheaper proteins through supplementary diets in the culture of carps revealed that pelleted feed incorporated with SWP, in combination with clam meat and shrimp waste showed significantly enhanced growth in mrigal, but no significant growth in rohu [7]. Further studies on the effect of SWP and faecal matter in the diet of the catla and rohu proved the significant influence of SWP on the growth performance and revealed that it was a potential ingredient in carp diet [60]. Further studies with catla-rohu hybrid, which had a food habit similar to that of rohu revealed that pupa when used at a 15% level in combination with fish meal (@10%) resulted in superior growth of fish. Hence, pupa which costs much less than fish meal and available in large quantities, can be profitably utilized in the formulation of low-cost, nutritionally balanced diets for carps [61].

The use of SWP as dietary protein source for catla (*Catla catla*) fingerling and reported that a diet prepared with 100% inclusion of SWP exhibited a better growth performance and it could completely replace fish meal as protein source for Catla (*Catla catla*) fingerling [25]. A diet prepared with 50% inclusion of SWP in a diet with fish meal, showed improved growth rate and feed utilization by rohu (*Labeo rohita*) fingerlings [3]. The apparent protein digestibility (APD), true protein digestibility (TPD), apparent lipid digestibility (ALD) of rohu (*Labeo rohita*) fed with SWP and reported significant difference between APD (85.21%), TPD (88.99%), ALD (92.29%) of non-deoiled SWP compared to fish meal diet [30].

Fermented silkworm pupae (SWP) silage incorporated feed, resulted in appreciable body weight gain, feed conversion ratio (FCR) and specific growth rate (SGR) and proved superior to untreated fresh SWP pastes and fishmeal in the diets of carp species (IMC). Inclusion of SWP @ 43.75% in diet of rohu (*Labeo rohita*) yielded better growth, feed utilization and survival rate compared with other experimental diets with other oil cakes. This confirmed the possibility that SWP could be a suitable low-cost animal protein rich alternative source and can be successfully used as supplementary feed for rohu fingerlings [71]. Protein digestibility SWP diets showed no difference with that of the control, while fat and NFE digestibility were higher above 20 and 30% pupa incorporation, respectively in catla [89]. Thus, it could be concluded that Indian major carps can be reared with SWP, replacing fish meal upto 30-50%, without affecting the growth performance.

Silkworm pupae as feed ingredient for Exotic carps

The digestibility of *(Cyprinus carpio)* common carp was not altered when fed with SWP incorporated feed [46]. Common carp *(Cyprinus carpio)* fingerlings fed with SWP meal, showed significant growth difference than fishes fed with mustard oil cake and rice bran [10]. Common carp fed with pelleted feed prepared with SWP, prawn waste or fish meal and tapioca flour showed an increased growth performance without affecting the flesh quality of fish [36].

Study on the effectiveness of silkworm pupae-based diet in common carp fingerlings revealed that 30% non-deoiled SWP diet showed higher growth performance [31]. Further Investigations on keeping quality of the feed [32], influence of diet on body composition [33], digestibility of nutrients in the feed [34] and the influence of the diet on the organoleptic quality [35, 63] showed that SWP meal was equivalent to fish meal in all respects.

Pelleted feed prepared with SWP in combination with clam meat and shrimp waste yielded better growth in silver carps [7]. In another study, common carp and silver carp fed with SWP faecal matter showed significant growth performance also reported that SWP were a potential ingredient in carp diet [60].

Common carp (*Cyprinus carpio*) fed on a diet prepared with SWP, at 30% protein, showed better growth rate and suggested SWP could replace fish meal in the diet of common carp, without affecting growth or quality. Further they also observed that common carp (*Cyprinus carpio*) had increased protein and fat digestibility with increased in level of pupae in their diets [63]. Swamy and Devaraj [86] evaluated the digestion of nutrient and nutrient accretion by common carp fry (*Cyprinus carpio*) fed dried SWP meal-based diet and observed common carp fry had better digestibility and also assimilated such diets better than the control diet. Nandeesha *et al..*. [65] reported that feed containing 50% crude protein, prepared with dried SWP as a sole source of protein could be used to completely replace fishmeal, without significant difference in the final weight gain, food conversion ratio, and protein efficiency ratio of common carp.

Rahman *et al..*. [69] conducted a study on replacement of fish meal by SWP and stated that mirror carp (*Cyprinus carpio* var *specularis*) fed with a diet containing 40% of SWP meal had better growth performance, with increased body lipid and protein levels. Ji *et al..*. [38] reported that 50% substitution of fish meal protein with SWP in the diet of mirror carp fingerlings *(Cyprinus carpio var. specularis)* improved whole-body protein deposition. Similarly, replacement of 50% of the fish meal protein with SWP in feed for Jian carp (*Cyprinus carpio var. Jian*) did not affect the growth performance or fish health and recorded that substitution levels above 60% significantly lowered growth [39]. The protein digestibility of SWP in finge-lipped carp (*L. fimbriatus*), increased at 20% incorporation and decreased at 40%, while fat and Nitrogen Free Extract (NFE) digestibility was higher at 20-40% incorporation respectively. Common carp *(Cyprinus carpio)*, exhibited higher digestibility of protein and fat at 10-30% inclusion levels of SWP in their diets [21]. Thus, it could be concluded that *cyprinus carpio* can be reared with SWP, replacing fish meal upto 30-50%, without affecting the growth performance.

Silkworm pupae as feed ingredient in other fish species

Silkworm pupae as feed ingredient in trout

Chum salmon (*Oncorhynchus keta*) fed with SWP (@ 5%), exhibited better feed efficiency ratio, however there was no significant difference in growth performance (1). Watanabe [92] conducted a study on digestible crude protein contents at various temperature and observed that digestibility of SWP based diet was highest at 15°C for rainbow trout (*Oncorhynchus mykiss*) and 20°C for ayu (*Plecoglossus altivelis*). According to Dheke and Gubhaju [13], SWP meal could be used as alternate to shrimp meal in rainbow trout fry (*Oncorhynchus mykiss*) diet without adverse effects on growth performance, feed conversion ratio and specific growth rate. Substituting fish meal with different levels of SWP 5%, 10% and 15% respectively showed increase in mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), and white blood cell values of rainbow trout (*Oncorhynchus mykiss*) with increased percent of SWP in their diet [79]. The incorporation of more than 10% SWP adversely reduced growth and increased the FCR in the diet of rainbow trout (*Oncorhynchus mykiss)* [80]. Cost-effective silkworm pupae diet could be used as better alternative to completely replace shrimp meals in the diet of rainbow trout fingerlings (*Oncorhynchus mykiss*) without compromising survival and growth [4]. Thus, it could be concluded that trout can be reared with SWP, replacing fish meal upto 5-15%, without affecting the growth performance.

Silkworm pupae as feed ingredient in mahseer

Raw SWP incorporated at 60% in feed improved growth rate in Deccan Mahseer. Similarly, 50% inclusion of de-oiled silkworm pupae in the diet significantly increased the growth performance of mahseer [81]. Sawhney [78] observed that feed prepared with SWP for masheer (*Tor putitora*) fingerlings showed a significant growth, it was suggested that silkworm pupae was cheaper and could improve the economic returns of the fish farmers by partial replacement of fishmeal in rearing of masheer fingerlings. Thus, it could be concluded that mahseer can be reared with SWP, replacing fish meal upto 60%, without affecting the growth performance.

Silkworm pupae as feed ingredient in Catfish

A study to understand the influence of diets with different protein sources on the growth and metabolism of the tropical catfish (*Clarias batrachus*) revealed that dried SWP are a better source of protein in the diet providing satisfactory growth [91]. Hossain *et al..*. [28] suggested that SWP meal could be used as a substitute for fish meal up to 75% without affecting growth rate, feed conversion ratio, protein efficiency ratio and apparent net protein utilization in the diet of catfish (*Heteropneustes fossils*) fingerlings.

SWP meal is a least expensive and alternative protein source in diet of Asian catfish (*Clarias batrachus*) fingerlings. A diet containing 100% SWP meal yielded better growth rate, feed conversion and protein utilization in catfish (*Clarias batrachus*) fingerlings [23] and African catfish (*Clarias gariepinus*) fingerlings [72, 73]. Further there was no significant different on the protein digestibility of catfish (*Clarias batrachus*) fed with non-deoiled SWP [8]. A diet prepared with 80% inclusion of non-deoiled gave better result in terms of growth in Asian catfish, (*Clarius batrachus*) fry [24]. Kurbanavo *et al..*. [49] reported that SWP can be used as an alternative source of protein (upto 50%) for African catfish (*Clarias gariepinus*) fingerlings. Thus, it could be concluded that catfish can be successfully reared with SWP, replacing fish meal upto 75-100%, without affecting the growth performance.

Silkworm pupae as feed ingredient in mariculture

Japanese seabass (*Lateolabrax japonicus*) had an energy digestibility of 73% and CP digestibility of 85%, for non-deoiled silkworm pupae meal which was lower than that of poultry by-product meal, feather meal, blood meal and soymeal but comparable to that of meat and bone meal [37]. Lee *et al..*. [50] assessed the response of dietary substitution of fishmeal with SWP meal, promate meal, meat and bone meal and/or their combination on the performance of juvenile Olive flounder *Paralichthys olivaceus*). They observed that dietary substitution of fishmeal with 10% SWP and 10% SWP + 20% promate meal (PM) had no detrimental effect on growth and feed utilization of Olive flounder. Thus, it could be concluded that marine fishes can be successfully reared with SWP, replacing fish meal upto 10%, without affecting the growth performance.

Silkworm pupae as feed ingredient in ornamental fish species

The ornamental fish industry can also satisfactorily use silkworm pupae meal in feed [85]. Silver barb (*Barbonymus gonionotus*) fingerlings performed better than control, when fed with a diet replacing fish meal with 38% SWP [52]. Jintasatapom *et al..*. [40] studied the effect of substitution of SWP for fish meal in broodstock diet for Snakeskin Gourami (*Trichogaster pectoralis*) and revealed that SWP can be used to replace fish meal upto 50% in broodstock diets without any adverse effect on egg quality in terms of fry number fingerling number and survival rate during the first month of nursery rearing. SWP meal could be effectively utilized in rearing of Red zebra fingerlings (*Maylandia estherae*) diets up to 60% without any adverse effects on growth performance and feed utilization. The supplementation of SWP meal not only enhanced the growth of Red zebra fingerlings (*M. estherae*) but also reduced the cost of feed formulation [68]. A study on the growth performance of Rainbow shark fed with SWP, revealed that SWP at 30% level of replacement provided significant change in growth as against the control animals fed with FM alone [43]. Thus, it could be concluded that ornamental fish species can be successfully reared with SWP, replacing fish meal upto 30-40%, without affecting the growth performance.

Silkworm pupae as feed ingredient in shellfish species

Feeding experiments with shrimp, (*Metapenaeus monoceros*) revealed that digestive efficiency was reduced when silkworm pupae meal was used to replace fish meal [90]. However, in giant freshwater prawn (*Macrobrachium rosenbergii*) replacement of fishmeal with SWP did not produce any adverse effect on productive performance and the recommended level of SWP was 8.6% by weight [41]. Cho [11] studied the dietary effect of substitution of animal and/or plant protein sources for fishmeal on the growth and body composition of juvenile abalone and suggested that a combination of soymeal (29%, DM basis) and SWP meal (16.9%, DM basis) could totally replace fishmeal and also result in better survival and growth performance in Abalone juveniles *(Haliotis discus).* Thus, it could be concluded that shellfish species can be successfully reared with SWP, replacing fish meal upto 30-40%, without affecting the growth performance.

Deoiled silkworm pupae in fish feed

In domestic animals the feeding value of SWP meal is unsatisfactory, due to the high fat content that causes negative responses. Thus, solvent extracted de-oiled SWP meal is a more suitable protein feedstuff for land animals [20, 85]. Protein concentration of SWP makes it a suitable fish meal substitute in formulated diets [57].

Srikanth and Keshvanath [84] studied growth response of *Tor khudree* fed pelleted feeds containing de-oiled SWP and observed it to provide better growth than fish meal. Catla showed significant growth with deoiled SWP feed, while common carp showed significant growth with both earth worm meal and deoiled SWP based feed [44]. On contrary in another study, inclusion of deoiled SWP, either alone or in combination with fish meal at various levels, indicated their relatively poor ability to induce growth in carps as compared to fish meal [62]. The survivality of common carp (*Cyprinus carpio*) was maximum (86.66%) with deoiled SWP meal [45]. Shyama and Keshavanath [82] observed significantly better growth rate and survival rates in Mahseer fingerlings (*Tor khudree*) fed (@ 5% BW) with a diet, wherein 50% of fish meal was replaced with deoiled SWP**.**

Hossain *et al.* [29] evaluated the protein digestibility of (*Oreochromis mossambicus*) and observed no significant difference between protein digestibility of non-deoiled SWP (85.74%) and deoiled SWP meal (84.95%). Hossain *et al.*. [30] evaluated the apparent protein digestibility (APD), true protein digestibility (TPD), apparent lipid digestibility (ALD) of rohu (*Labeo rohita*) fed with de-oiled SWP and observed significant difference between APD (83.04%), TPD (86.87 %), ALD (91.21%) of de-oiled SWP compared to fish meal diet.

Inclusion of de-oiled SWP in fish feed showed better growth and conversion, as compared to conventional feed mixture of rice bran and the mustard oil cake (1:3) [62]. In a similar study of partial or complete replacement of fishmeal by 10, 15, 20 and 25% de-oiled pupae in a feeding trial on (*Cyprinus carpio*) showed relatively better growth with a diet containing a combination of 10% pupae and 15% fishmeal [63].

Conclusion

Insect meals are one of the best alternatives to partially or completely replace fish meal, which is mainly due to the versatility and ability of insects to change their amino acid and fatty acid profiles. Moreover, insects are natural food sources for fish, especially continental species. This study reveals that, the SWP is very rich source of proteins, lipids and minerals so could be used as an alternative dietary supplement in fish feed. The cost of SWP are less compared to fish meal and due to their unique composition can be one of the best alternative to fish meal to reduce the cost of production without much effect on the growth performance farmed fish species.

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