Sericulture byproduct as feed for tilapia, *Oreochromis mossambicus* (Peters)

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ABSTRACT

Fingerlings of tilapia, *Oreochromis mossambicus* (Peters) with mean weight 5.0 ± 0.20g were randomly stocked at 20 fingerlings per tank in three replicates (tank size, L x B x H; 1m x 1m x1m) for 90 days to evaluate the effect of sericulture byproduct on growth and flesh fatty acid profile of tilapia. Two isonitrogenous pelleted feeds *viz.* silk worm pupae meal (SWM) and market available feed (MAF) were formulated and fed to the fish twice daily. The feed (SWM) containing fermented silk worm pupae as principal ingredient was the most efficient in terms of final average weight gain, specific growth rate, protein efficiency ratio and fatty acid profile (Poly unsaturated fatty acid and n3/n6 ratio). Fish PUFA, especially the n3fatty acids and n3/n6 ratio are affected positively when fed with SWM which is good for the quality of the fish produced with respect to the benefits of consumers' health. The results showed that fermented silk worm pupae can be used in the diet for tilapia which improves yield as well quality of cultured fish.

KEY WORDS: Fish PUFA**,** n3/n6 ratio, sericulture byproduct, tilapia

INTRODCUTION

Several studies in recent past to make supplementary feeding of fish cost-effective have been directed to substitute the high cost fish meal with less expensive protein sources. This aspect of feed development research is centered on the search for inexpensive, locally available and nutritious protein sources that can supply all the nutritional needs of the fish (Gabriel *et al*., 2007).Sericulture is a well established rural agribusiness in India and produces about 40,000 metric tonnes per year of silkworm pupae (SWP) on dry weight basis (Rangacharyulu *et al*., 2003). A small quantity of this SWP is sun-dried and used in animal feeds, while a considerable amount is discarded in open places though it contains significant amount of crude protein (34.40%) one of the major feed component, besides considerable amount of crude lipid (10.63%), carbohydrate (9.95%) etc. claiming as a promising ingredient of fish feed (Table 1). An appropriate fermentation ensiling process has been developed to prepare a pathogen free SWP (Yashoda *et al*., 2001) which is the key ingredient of SWM feed. Microbial fermentation and nutrient synthesis is typically important in organisms with a diet high in fibre (Annison, 1993). Fermentation is a simple and cheap process where there may be an increase in the nutrient level through microbial synthesis (Wee 1991), apart from microbial degradation. Many studies have been carried out to evaluate the effects of non-conventional ingredients used in diets as FM substitutes on fish fatty acid composition (Maina *et al.,* 2003; Williams et al. 2003). Surprisingly, such an important farm waste potential for making nutritionally balanced

fish feed formulation is remained unexplored. Research information on utilization of such alternative fish feed source is scanty. The beneficial fatty acid in fish body is synthesis from the feed materials they consumed (Horrobin and Manku 1990). The beneficial effects of fish lipids on human health have already been well established (Mukhopadhyay 2009). It is therefore of utmost importance to determine the influences of feed on growth as well as fat deposition in fish (Cengiz *et al.,* 2003).

Keeping the above facts in view, the investigation was carried out with the main objective to study the growth performance of *O. mossambicus* by using fermented silkworm pupae as alternative source of protein in fish feed and also to study the qualitative changes in fish flesh as human food.

MATERIALS AND METHODS

Experimental set up

Twenty fingerlings in triplicate groups were used in two different treatments. Altogether one hundred and twenty (120) Niletilapia (male and female ratio 1:1) fingerlings were usedin this experiment. The fishfingerlings were treated with potassium permanganatesolution (1 mg L^{-1}) to remove any external parasitesand were acclimatized in a big tank for five days.Experiments were carried out at the tanks of aquacultural engineering section of IIT-Kharagpur, Paschim Medinipur, West Bengal, India. Each group offingerlings also were initially weighed to record theinitial biomass. They were stocked in six rectangularcemented tanks (1000 L). The water system was static in nature andthe bottom of the tank was filled with local agriculturalsoil (pH 6.4 ± 0.05). The experiment was conducted for 90 days from June to July in the year 2011.Dechlorinated

well water (temperature 26 ± 3 °C, pH 7.0 ± 0.05, free CO2 0.4 ±0.01 mg L^{-1}, available nitrogen0.5 ±0.05 mg L^{-1} and dissolved oxygen (DO) 6± 0.5 mg L^{-1})was used in the experiment.

Feed formulation and preparation

The principal feed ingredient (silkworm pupae) was collected from local sericulture farm after silk reeling at very low cost. These substances were economically cheap but contained significant amount (34–37%) of crude protein. Biochemical composition of silkworm pupae used in the feed for tilapia is shown in Table 1. Diets used for growth trial were prepared in such a manner, the feed formulations remain almost isonitrogenous (30 g 100 g^{-1}) and isoenergetic (4.0 Kcal g^{-1}) in nature. The choice of these nutrient levels, particularly protein, was intended to reflect the practical diets used in India. Diet formulations are presented in Table 2. Mustard oil cake, wheat flour, rice bran and egg shell dust were the ingredient of Market available feed (MAF). In addition to these ingredients the chief ingredient of SWM feed was fermented silkworm pupae. These ingredients were used to compensate lipid, protein and ash deficiency in formulated feed. Wheat flour was selected as binder. Each feed was fortified with egg shell dust which is available free of cost for calcium supplement. This was added keeping in mind that the developing fish needs huge quantity of calcium for its bone development. The different ingredients were thoroughly mixed using a food mixer (A200 Hobart Ltd). The proportion of different feed ingredients was determined by using Pearson's square method. The mixture was given the shape of pellets using a Pellet Mill (Model CL2) with a 12 mm die. The resulting pellets were dried in a hot air oven

for 48 h at 50 ºC, packed in polythene bags and kept in dry and cool place.

Feeding

The feed was given ad libitum in a feeding bag hungfrom an iron rod in four locations in each tank.Unconsumed feed was removed after 1hour from thebeginning of feed administration and dried in a hot airoven at 50°C. Feed consumption was estimated bysubtracting the weight of the unconsumed feed from theweight of the feed offered. Fish, feed samples, andunconsumed feeds were weighed on pan electricbalance to an accuracy of 0.1 mg.

Growth calculation

Growth and nutrient utilization were determined in terms of feed intake (FI), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), and hepatosomatic index (HSI) as follows (Bag *et al.*, 2011):

FI (g fish-1 day-1) = Total feed intake per fish/number ofdays

SGR (% day-1) = 100 × (ln[final body weight]- ln[initial body weight])/no. of days

FCR = feed intake/live weight gain

PER = live weight gain/crude protein intake

HSI (%) = 100 × (liver weight/total body weight)

GSI (%) =100 × (weight of gonad /total body weight)

Analysis

Feed and carcass samples were analyzed following standard procedures (AOAC 2000): dry matter (DM) after drying in a hot air oven (Gallenkamp, UK) at105°C for 24 h; crude protein (CP) by Kjeldahl method(N × 6.25) after acid hydrolysis, crude lipid (CL) afterextraction with petroleum ether for 7-8 h by Soxhlet method (40–60°C boiling range), total ash by igniting at550°C for 3 h in muffle furnace (Size 2,Gallenkamp,UK). Organic matter (OM) was calculated by subtractingtotal ash from DM (Giri et al. 2000). Crude fibre was determined using a moisture free defatted samplewhich was digested by a weak acid HCl (0.1N) followed by a weak base NaOH (0.1N) using the Fibertec System 2021 (FOSS, Denmark). Nitrogen-free extractwas determined by subtracting the sum of crudeprotein, crude lipid, crude fibre and ash from DM (Maynard et al. 1979). Gross energy was determinedusing a Bomb Calorimeter Model-DFU 24 following theprocess as described below. The sample was combusted in a chamber pressurized with pure oxygen and resulting heat measured by increase in the temperature of the water surrounding the bomb.

Extraction of lipids

The total lipids were extracted from all the samples, (fish flesh-2, feed-2) following the method of Bligh and Dyer (1959) using methanol-chloroform (2:1, v/v),methanol-chloroform-water (2:1:0.8, v/v/v), and thenagain with the first solvent system viz., methanol-chloroform (2:1, v/v). Sample was ground with thesolvent methanol-chloroform (2:1,v/v), filtered throughWhatman no. 1 filter paper and residue was extractedwith the next solvent system, consisting of methanol-chloroform-water (2:1:0.8, v/v/v). The process was repeated once again with methanol-chloroform (2:1, v/v). Finally, the three extracts were pooled, diluted withthree volumes of water (100-200 ml, depending on thevolume of pooled extracts) and layer was allowed to separate in a separatory

funnel made by Pyrex glassCo.. The chloroform layer at the bottom of the separatory funnel was withdrawn and dried over anhydrous sodium sulphate in glass stoppered conical flasks, by Pyrex. The chloroform solution of lipid wasevaporated in a rotary vacuum evaporator by Rotavap under a pressure of 40-50 mm of Mercury, weighed on a micro-balance by Sartorius and redissolved in distilled n-hexane (10-20 ml) and kept at -20°C for future use. BHT (butylatedhydroxy toluene) was added at a levelof 100 mg/L to the solvent as antioxidant.

Preparation of methyl ester of fatty acids

Total lipid of various (fish flesh-2, feed-2) samples wasdissolved in anhydrous methanol containingconcentrated Sulfuric acid (1.0%, v/v) and the mixturewas refluxed (Christie, 1982) for 2 hours. Methanol wasevaporated to a small volume (1-3 ml) and cooled to 4°C, in a freezer.Distilled water 10–15 ml was added tothe cooled mixture (1-3 ml) in hard glass test tubes byPyrex and the methyl esters of fatty acids wereextracted 3 times with aliquots (5-10 ml) of diethyl ether, vortexed in a Vortex mixer. The ethereal extractswere taken out by Pasteur pipettes, pooled and dried over anhydrous sodium sulphate, (1-2 gm) in conical flasks (25-50 ml capacity) with glass stopper, filtered through Whatman no. 1 filter paper, vacuum dried, redissolved in n-hexane (1-2 ml volume) and kept in a freezer at 4 °C for future use.

Purification of Fatty Acid Methyl Ester (FAME) by Thin Layer Chromatography (TLC)

Fatty acid methyl esters were purified (Mangold 1969; Misra *et al.,* 1984) by TLC using a solvent system of n-hexane-diethyl ether (90:10, v/v). A standard methylester was also run on the same plate in a separate lane, for identification of the methyl ester bands in the samples.

The location of methyl ester bands were indicated by placing the TLC plate in an iodine vapour chamber by Pyrex glass co.. The methyl ester bandscorresponding to the standard were marked and then scrapped off the plate with a sharp rajor blade. Methylesters were recovered by extracting the silica gel bandscontaining the methyl ester samples in a mini glasscolumn (10 cm length x 0.8 cm internal diameter, byPyrex) with chloroform (30-50 ml), the later wasevaporated and the methyl esters were kept in n-hexane(1-2 ml) in a freezer at 4 °C till analyzed by Gas Liquid Chromatography (GLC).

Gas Liquid Chromatography (GLC)

GLC of fatty acid methyl esters were done on a Chemito 1000 instrument, equipped with Flame Ionization Detector (FID). Quantifications were done by computer using specific Clarity Lite software.

Analysis of fatty acid methyl esters (FAME)

GLC of FAME was done on a BPX-70 mega bore capillary column of 30 mt length and 0.53 mm internaldiameter obtained from SGE, Australia. Oventemperature was programmed from 150 °C – 240 °C with a rate of 8 °C/min. Initial and final temperatures werekept isothermal for 1 minute and 20minutesrespectively. Injection port and detector temperatures were 250 °C and 300 °C respectively. Nitrogen gas was used as carrier gas and its flow ratewas 6.18ml/min.

Statistical analysis

Data from each treatment were subjected to one-way Analysis of Variance

(ANOVA). The data are presented as mean±SE of three replicate groups; statistical analysis was performed using the SPSS 11.0 for windows. Duncan multiple range test was used to compare the mean values between individual treatments (Duncan, 1955). Differences were considered significant at p<0.05.

RESULTS AND DISCUSSION

The highest weight gain (75.81g) was observedin the SWM applied feed. The growth rate was always faster in SWM fed fish than fish fed with MAF (Figure 1). This indicates that fish can assimilate theSWM feed well. This was possibly due to their higher palatability andpreference of the fish to take it as their potential food. Amount of feed intake was highest (2.49g) in SWM. The feed conversion ratio (FCR) was differed significantly and lowest value (2.29) was recorded fromSWM fed fish indicating an encouraging effect oneconomic involvement in fish farming. The specific growth rate (0.90) and protein efficiency ratio (1.65) were highest in SWM fed treatment. This indicates the better quality of protein in the feed produced from fermented silkworm pupae (SWM). The hepatosomatic index (HSI) and gonadosomatic index were not differed significantly between two treatments (Table 3).

In two feeds, SFA was less and MUFA was high in amount. But in all fed fish the situation was just reverseindicating that fish may be converted MUFA of feed into the SFA of its flesh. The amount of $22:1\omega11$ fatty acid was reduced absolutely in fed fish which is the principal reason for such reduction. Amount of DUFA was reduced nearly 3times in fish than in feed (table 4). The value of linoleic acid ($18:2\omega6$) of formulated feed was nearly 15% which

has been found to be much lower (nearly half) in both the fed fishes. The mechanisms behind this conversion depend on the efficiency of the experimental fish by adopting desaturation and chain elongation process.

This result indicates that fish may convert DUFA to SFA. The amount of PUFA and n3 fatty acids (EPA and DHA) was more or less similar in feed and fed fish. But n6 PUFA was reduced significantly in all fed fish in comparison to the feed offered. The n3/n6 ratio showed an increasing tendency in all fed fish and SWM fed fish exhibited notable enhancement of n3/n6 ratio (1.25). Because amount of n6 fatty acid was lowest in SWM fed fish indicating transformation of n6 fatty acid to n3.The most interesting aspect of the present experiment is that the fishes provided with high level of n6 fatty acids in feeds offered however, the fishes were able to covert n6 fatty acids to n3 fatty acids efficiently to a very low n3/n6 ratio to comparative higher value of n3/n6. The mechanism of this conversion could not be explained at present. However, it proves that these fishes have the ability for such a conversion for the maintenance of physiological homeostasis. Ackman (2000) stated that only 14 fatty acids are really needed to describe the fatty acids of fish. However, Ackman et al. (2002) listed 64 fatty acids from 5 fresh water fishes of West Bengal, India. The fish under discussion recorded 28 fatty acids of the total lipid (TL) and the result is more or less similar to those reported from other tropical and certain temperate zone fresh water fishes.

According to Ackman et al. (2002), dominant fatty acids in lipids of all the fishes were myristic (14:0), palmitic (16:0), stearic (18:0), palmitoleic ($16:1\omega7$), oleic ($18:1\omega9$), linoleic ($18:2\omega6$), linolenic

(18:3ω3), arachidonic (20:4ω6), eicosapentaenoic (20:5ω3) and docosahexaenoic (22:6ω3) acids. The present results corroborate with the above findings. The total SFA of the experimental fish was nearly double than the amount reported by Ackman *et al.* (2002).

Fatty acid deficiency in fish species is indicated by the presence of eicosatrienoic acid (20:3ω9) (Watanabe, 1982). Thus, the absence of eicosatrienoic acid in these fish indicates that these fish are not suffering from any fatty acid deficiency and the formulated feeds fulfill the requirement of fatty acids for tilapia. This observation corroborates that of Nematipour and Gatlin (1993) for hybrid striped bass in the USA. The n-3 PUFA is the chief group of components through which the beneficial effects of fish are mediated. The principal effects of n-3 PUFA are antithrombogenic and antiarrhythmic, whereas that of n-6 PUFA is antiatherogenic (Dewailly *et al.,* 2001; Christensen *et al.,* 2001). Ackman et al. (2002) stated that n3/n6 ratio should range 1–2 for fresh water fish. The n3/n6 ratio of SWM fed fish was within the same range. The fish fed with SWM accumulates more n3 fatty acids than n-6 fatty acids which increase n3/n6 ratio (1.25) in return. Fish oil, lipids and their constituent fatty

acids have high digestibility and energy value. Fish lipids contain high levels of n3 PUFA which may be essential for worm blooded animals. Fish is more beneficial than fish oil but for CAD patients prescribed amount of n3 PUFA (EPA and DHA) is required as the best insurance against sudden death (Bag, 2012). Low fat and easy digestibility of the two batches of experimental fed fishes under investigation together with its EFA (n3 fatty acid) resource and other attributes discussed above can be recommended as a better diet on par with the recognized fish diet.

CONCLUSION

The feed prepared from fermented silkworm pupae enhance growth and thereby yield of *O. mossambicus*. It improves quality of fish by accumulating more n3 PUFA in the flesh of the fish as well as increasing the n3/n6 ratio which is beneficial for human health. Moreover, the feed can be formulated at local level leading to employment generation in rural areas.

Table 1: Biochemical composition of silkworm pupae used as feed for tilapia

Ingredient (%)	Silkworm pupae
Dry matter	91.45
Crude protein	34.40
Crude lipid	10.63
Carbohydrate	9.95
Ash	13.41
Nitrogen free extract	13.39
Crude fibre	9.67
Gross energy (Kcal g^{-1})	3.51

Table 2: Detailed calculations of SWM and MAF diet

Name of feed	Ingredients	% of CP in ingredient	% of ingredient in formulated feed	% of crude protein in feed	% of lipid in feed	% of carbohydrate in feed	Calorific value of feed (kcal/g)
SWM	SWP	34.4	36.9	30.10	9.3	9.6	4.0
	MOC	34.65	22.6				
	Wheat flour	9.08	30.8				
	Rice bran	8.4	7.5				
	Egg shell dust	1.8	2.2				
MAF	MOC	34.65	42.0	29.75	8.3	10.0	3.9
	Wheat flour	9.08	46.0				
	Rice bran	8.4	10.5				
	Egg shell dust	1.8	1.5				

SWP = Silkworm pupae; CP =Crude Protein; MOC= Mustard Oil Cake

Table 3: Growth performance and nutrient utilization of *O. mossambicus* fed with SWM and MAF

Particulars	SWM	MAF
Initialweight (g)	5.10 ±0.01[a]	5.10 ±0.02[a]
Finalweight (g)	75.81 ±0.13[a]	58.26 ±0.24[b]
Initiallength (cm)	4.50 ±0.03[a]	4.50 ±0.01[a]
Finallength (cm)	14.60 ±0.12[a]	12.00 ±0.10[b]
Feed intake (g fish^{-1} day^{-1})	2.21 ±0.05[a]	1.80 ±0.02[b]
Specific growth rate (% day^{-1})	0.90 ±0.19[a]	0.72 ±0.05[b]
Feedconversion ratio	2.29 ±0.23[a]	2.65 ±0.05[b]
Protein efficiency ratio	1.65 ±0.12[a]	1.25 ±0.04[b]
Hepatosomatic index	1.55 ±0.06[a]	1.17 ±0.07[b]
Gonado somatic index	1.62 ±0.07[a]	1.12 ±0.06[b]

MAF = Market available feed

SWM = Silkworm pupae meal

Table 4: FA profiles of *O. mossambicus* fed with SWM and MAF feeds (% w/w of each component in total fatty acids)

Components	Feed		Fed fish	
	MAF feed	SWM feed	MAF fed fish	SWM fed fish
Saturated				
14:0	0.7	0.6	5.0	4.4
15:0	0.2	0.5	0.9	1.3
16:0	5.3	5.4	30.5	27.9
17:0	0.4	0.2	2.8	2.5
18:0	2.0	1.9	7.8	8.0
20:0	0.6	0.6	0.5	0.3
22:0	0.8	0.1	3.9	4.0
24:0	1.1	2.0	0.9	1.8
∑SFA	**11.1**	**11.3**	**52.3**	**50.2**
Monoene				
14:1	0.0	0.0	1.0	0.3
15:1	0.0	0.0	0.3	0.2
16:1	1.2	1.4	7.5	7.6
17:1	0.0	0.0	0.4	0.7
18:1ω9	22.6	21.9	14.6	12.0
20:1ω9	7.0	8.1	1.5	2.1
22:1ω11	27.2	27.9	1.6	0.8
24:1	1.2	1.5	1.8	1.7
∑MUFA	**59.2**	**60.8**	**28.7**	**25.4**
Diene				
16:2	0.0	0.0	0.2	0.5
18:2ω6	16.2	14.6	8.7	7.7
20:2	0.0	0.0	0.0	0.0
∑DUFA	**16.2**	**14.6**	**8.9**	**8.2**
Polyene				
18:3ω6	0.5	0.4	0.3	0.4
18:3ω3	2.6	2.9	2.3	3.0
20:3ω6	1.1	1.1	1.4	1.0
20:3ω3	0.00	0.02	0.07	0.02

20:4ω6	0.9	1.0	1.0	0.9
20:5ω3	1.2	1.7	1.1	1.6
21:5ω3	0.5	0.5	0.5	0.4
22:5ω6	0.2	0.5	0.4	0.6
22:5ω3	2.0	2.6	2.2	2.7
22:6ω3	4.5	5.6	4.0	5.6
∑PUFA	**13.5**	**13.32**	**10.17**	**16.22**
Total -ω3	**10.8**	**9.82**	**12.67**	**13.32**
Total -ω6	**17.8**	**17.6**	**11.8**	**9.1**
n3/n6	**0.60**	**0.75**	**0.86**	**1.25**

MAF = Market available feed

SWM = Silkworm pupae meal
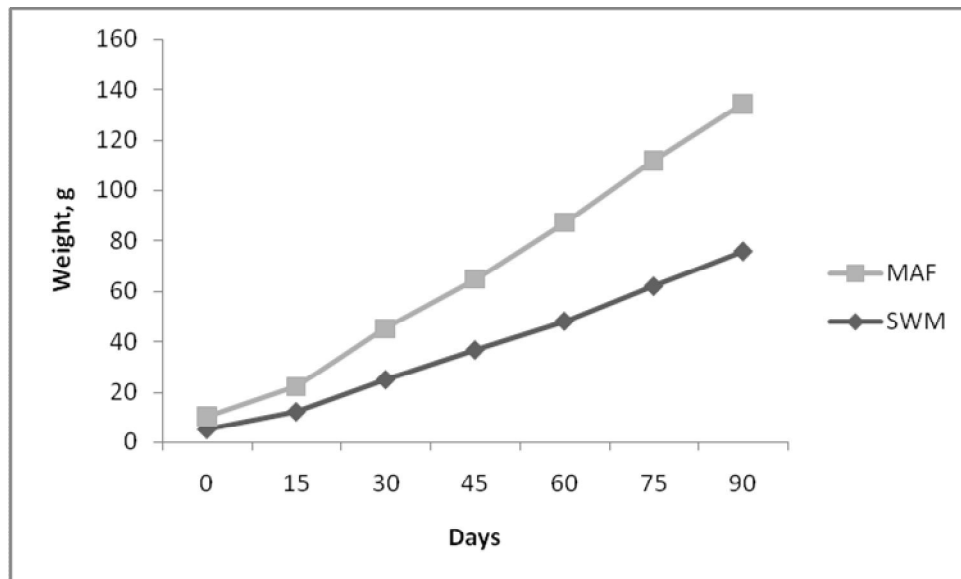
Figure 1: Growth rate of *O. mossambicus* fed with SWM and MAF

MAF = Market available feed

SWM = Silkworm pupae meal

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