

## RESEARCH ARTICLE

# Rice bran in the diet as an enriching source of long chain Omega 3 poly unsaturated fatty acids (LC n-3 PUFAs) in fillets of genetically improved farmed Tilapia (GIFT)

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**Abstract:** Rice bran is a cheap, easily available, widely used fish feed ingredient in Asian countries. In this study, contribution of rice bran on the growth and fatty acid profile of fillets of genetically improved farmed Tilapia (GIFT) fish was evaluated. Two diets with 30 % protein and different lipid levels (7.70 % in Diet I and 12.55 % in Diet II) were formulated. Diet I had 9 % rice bran and 18 % maize whereas Diet II had 24 % rice bran and 3 % maize. Other ingredients were common and used in same percentages. GIFT fingerlings were randomly stocked in 8 cages (quadruplicate/treatment) at a density of 75 fingerlings/ m<sup>3</sup> and fed with the two test diets for 210 days. Growth performances were recorded monthly. At the end of the experiment proximate composition and fatty acid profile of GIFT fillets were determined. Crude protein and crude lipid percentages were higher in fillets of fish fed with Diet II. Total poly unsaturated fatty acids (PUFAs), total omega (n-3) fatty acids, especially eicosapentaenoic acid and docosahexaenoic acid in fish fed with Diet II were higher and significantly different from the fish fed with Diet I (p = 0.00). Mono unsaturated fatty acids were significantly low (p = 0.00) in fish fed with Diet II. No significant difference was observed in mean final weight, specific growth rate, feed conversion ratio, protein efficiency ratio or survival rate in fish fed with the two diets revealing that incorporation of rice bran into the diet at a higher percentage could improve the nutritive quality of GIFT fillets while maintaining the growth and survival.

**Keywords:** Fish consumption, fish fatty acids, fish LC n-3 PUFAs, HDL, rice bran feed.

## INTRODUCTION

Fish and fishery products are valuable components of a healthy human diet. In 2017, world total fish production was estimated about 172.6 million tonnes, from which 89 % (153 million tonnes) was used for direct human consumption (FAO, 2018). A 150 g portion of fish in adult diet can provide about 50 to 60 % of the daily protein requirement (FAO, 2014). Fish provide high-quality protein, essential n-3 poly unsaturated fatty acids (n-3 PUFAs), lipo-soluble vitamins and minerals, which are important for human well-being (Fallah *et al.*, 2011). Acceptance of fish or fishery products by the consumers depends on nutritive quality, cost and food safety. Functional characteristics related to the sensory properties of taste and appearance also has a major impact in consumer preference (Damez & Clerjon, 2008).

Long chain Omega 3 polyunsaturated fatty acids (LC n-3 PUFAs), especially docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are known as essential PUFAs. Proven great health benefits of n-3 Omega PUFAs on rheumatoid arthritis (Rennie *et al.*, 2003), coronary artery disease by improving the control of blood pressure (Freeman, 2000) and renal functions even in hypertensive heart transplant recipients have been reported (Holm *et al.*, 2001). They protect, and

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sometimes even enhance the effects of some medical treatments for diseases such as Alzheimer's disease, multiple sclerosis and cancer (Gogus & Smith, 2010). Their effects on different types of cancers (Terry *et al.*, 2004), immune disorders (Yaqoob, 2004) and diabetes (Nettleton & Katz, 2005) are also proven. As such n-3 PUFAs have become an accepted and widely used feed supplement in clinical nutrition and health of humans.

Many studies have reflected the positive influence of n-3 LC-PUFAs in fish on human health (Mozaffarian *et al.*, 2005). Sea fish species are rich in essential PUFAs (Karapanagiotidis *et al.*, 2006) while fresh water food fish also contain substantial amount of PUFAs (Trbovic *et al.*, 2013). Fresh water fish contain higher levels of n-6 fatty acids than marine fish. They also have the ability to produce PUFAs by desaturating and elongating linoleic (18:2n-6) and linolenic acids (18:3n-3) or both (Tocher, 2003). These fatty acids are converted to arachidonic acids (20:4n-6) and then later in to DHA (Goda *et al.*, 2007). Fish oil is rich in n-3 series fatty acids, especially EPA and DHA. It is an important ingredient in aqua feed that provides highly digestible polyunsaturated fatty acids (Choi & Lee, 2015). Oily vegetable protein is also commonly used as an ingredient in aqua feed due to low cost or as an alternative supplement source for fish oil. These oils however are expensive and increase the feed cost for the farmer. If this requirement could be replaced fully or partially by a substitute source with the same nutritive levels it could be beneficial for the small-scale farmers especially in developing countries.

Rice bran is a by-product of rice milling industry. It is a cheap ingredient and a source of carbohydrate and lipid, which is widely used in fish feed formulation to reduce feed cost. Inclusion level of rice bran often ranges from 20 to 30 % in diets prepared for warm water fish (Suresh *et al.*, 2011). Rice bran is used as carbohydrate and lipid source in fish feed formulation. The principal fatty acid components of rice bran are 20 % palmitic (16:00) as saturated fatty acids (SFA) and 39 % oleic (18:1n-9) and 38 % linoleic (18:2n-6) as mono unsaturated fatty acids (MUFAs; Goda *et al.*, 2007). Approximate protein content of rice bran is 11-13 % (Oliveira *et al.*, 2011).

Over the past years Tilapia culture has been intensified and introduction of GIFT like strains has resulted in further development of aquaculture practices especially in South Asian countries. GIFT are widely used with other major Chinese and Indian carps in aquaculture in many Asian countries. Since its introduction, GIFT fillets have become popular in Asian countries and even in Europe and USA in the last two decades.

The present study was designed to evaluate the growth and flesh quality of cage-cultured GIFT under two dietary fat levels by incorporating two different percentages of rice bran, an inexpensive ingredient in formulating the diets. The specific objectives were to determine and compare the crude protein levels and fatty acid profile of GIFT fillets and growth rate of fish fed with two diets having two different levels of rice bran.

## METHODOLOGY

GIFT fingerlings ( $3.85 \pm 1.22$  g body weight) were randomly stocked in 8 net cages (each  $2 \text{ m}^3$ ) at a density of 75 fish per cubic meter. The eight cages were randomly divided into two groups to have four cages per treatment. Two different diets, Diet I ( $7.70 \pm 0.14$  % dietary crude fat) having 9 % rice bran and 18 % maize and Diet II ( $12.55 \pm 0.27$  % dietary crude fat) containing rice bran and maize 24 % and 3 %, respectively were formulated and used for the study. Other ingredients (fish meal 25 %, soya bean meal 27 %, coconut cake 20 % and vitamin and mineral premix 1 %) were common to both diets giving a protein content of 30 %. Feed rations were 10 % of body weight  $\text{day}^{-1}$  and was offered in two meals at 7.00 am and 17.00 pm (GMT +05:30) for 150 days and fed ad-libitum during the last 60 days.

A monthly random sample (30 %) of fish was taken from each cage for five (05) months and individual body weights were recorded to the nearest 0.01 g. After 150 days 75 fish from each cage were randomly caught and introduced into 08 new cages of the same size and reared for another 60 days for fattening. After the total culture period of 210 days a composite fish sample of 24 fish for each treatment was prepared by randomly selecting six fish (three from each sex) from each cage. Two fish from each sex were randomly selected from each composite sample for the analysis of fatty acid profile. Fish were sacrificed by immersing in chilled water, eviscerated, washed, skinned, beheaded and deboned. Fresh fillets (with average weight, length, width and thickness of 107 g, 14.35 cm, 8.35 cm and 0.94 cm, respectively) of each fish were packed separately and transported on the same day under chilled condition to the laboratory of the National Aquatic Resources Research and Development Agency (NARA), Colombo.

Homogenised muscle samples were subjected to lipid extraction as described by Bligh and Dyer (1959). Lipid content of each sample was determined as a percentage value. Extracted lipids were then trans-esterified by 0.25 M81-5550 Qualimix Fish S (Larodan Fine Chemicals, Malmo, Sweden) to form fatty acid methyl esters.

Heptadecanoic acid (C 17:0) methyl ester solution 0.05 mL (10 mgmL<sup>-1</sup>) was added prior to transesterification as an internal standard for quantification process. The fatty acid methyl esters of extracted lipids in each sample were determined using gas chromatography according to the protocol of manufacturer (GC-2014 Shimadzu, Kyoto, Japan). Fatty acid content was expressed as a percentage per total gram of fat in fish fillets. A portion of the same sample of fish flesh used for fatty acid profile analysis was taken to determine the proximate nutritional composition of the GIFT fillets according to standard procedures (AOAC, 1993). Water quality parameters in each cage used for the two feeding treatments were recorded biweekly.

Mean final weight (MFW), specific growth rate in weight (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and survival rate (SR) were estimated as growth indices of GIFT fish reared under the two feeding treatments using the equations given below.

FCR = Total weight of the feed consumed by fish (g) / weight gain of the fish (g)

SGR (% body weight gain in grams day<sup>-1</sup>) = Ln (final weight in grams) - Ln (initial weight in grams) x100 / Experimental duration in days (Cook *et al.*, 2000).

PER = Weight gain of fish (g) / crude protein intake from diet (g) (Zeitoun *et al.*, 1976).

SR (%) = Initial number of fish stocked - mortality / Initial number of fish stocked x 100

In determining the above values, monthly sample obtained from a single replicate was considered as one sample and as there were four replicates per treatment, n = 4).

Data of lipid and protein content of fish fillets, percentage fatty acid composition, and final mean body weight of fish were checked for normality and analysed by t-test, one-way ANOVA and Student Newman-Keuls test using SPSS -Version 17. All tests were carried out at 5 % of probability level (p < 0.05).

## RESULTS AND DISCUSSION

After 150 days of culture period average weight of fish were 117.14 ± 5.86 g and 113.74 ± 20.08 g for the fish fed with Diets I and II, respectively. All growth indices of fish, namely, mean final weight, specific growth rate, feed conversion ratio, protein efficiency ratio and survival rate were not significantly different (p > 0.05) between the two treatments (Table 1).

The mean values of water quality parameters recorded for the two treatments were not significantly different (p > 0.05) and they remained within the safe limits of the growth of Tilapia. Water temperature, transparency, dissolved oxygen and pH varied between 29.63–29.89 °C, 68.7 – 68.8 cm, 5.13 – 5.56 mgL<sup>-1</sup> and 7.12 – 7.64 respectively. Phosphate, nitrate - nitrogen and ammonia- nitrogen of culture water ranged between 0.56 – 0.7 mgL<sup>-1</sup>, 0.24 – 0.28 mgL<sup>-1</sup>, and 0.12 – 0.14 mgL<sup>-1</sup>, respectively.

Abdel-Warith *et al.* (2013) stated that complete or higher substitution of fish protein or oil by plant protein or oil associates with poor growth performances of cultured fish due to interference of certain anti-nutritional factors with the activities of digestive enzymes. Anti-nutritional factors that inhibit proteases are found in plant derived fish feed ingredients especially in legumes and oil seed (Sriket *et al.*, 2011). Tilapia does not tolerate a high level of crude fat (CF) in its diet, and higher CF levels in the diet depress its growth unlike carnivorous fish species (Lim & Webster, 2006). Diet containing 10-15 % CF demonstrated good growth and feed utilisation for hybrid Tilapia (Chou & Shiau, 1996). They suggested that Tilapia needs 5 % CF for minimum growth and 12 % CF for maximum growth in diet. As such, balance between animal and plant feed ingredients in aqua feed formulated for Tilapia is very important.

In the present study percentages of animal protein ingredients and plant protein ingredients were maintained around 25 % per each category in both diets. Dietary

**Table 1 :** Growth indices of GIFT fed with Diet I and Diet II (mean ± SE, n = 4)

Diet	MFW (g)	SGR (%/d)	FCR	PER	SR (%)
I (30 CP/7.70 CF)	117.1 ± 2.93 <sup>a</sup>	2.5 ± 0.01 <sup>a</sup>	1.6 ± 0.07 <sup>a</sup>	2.0 ± 0.09 <sup>a</sup>	83.5 ± 4.77 <sup>a</sup>
II (30 CP/12.55 CF)	113.7 ± 10.04 <sup>a</sup>	2.4 ± 0.05 <sup>a</sup>	1.5 ± 0.04 <sup>a</sup>	2.2 ± 0.06 <sup>a</sup>	89.0 ± 3.77 <sup>a</sup>

Mean final weight (MFW), specific growth rate in weight (SGR) feed conversion ratio (FCR), protein efficiency ratio (PER) and survival rate (SR); Means in the same column having different superscripts are significantly different (p ≤ 0.05)

energy supplementation through proportionate inclusion of lipid to protein levels may have promoted efficient utilisation of protein by GIFT for their growth.

Mean weight of the fish at the end of the experiment (210 days) was 371.5 g while weight gain during the 60-day fattening period is approximately 250 g. Fish (75 in number) selected for fattening were introduced into same size cages used for the growth experiments. Reducing the stocking density to half from the initial density seems to have positively influenced their growth. In addition, maturing gonads also may have contributed to a certain extent to this weight gain.

Fish subjected to fatty acid analysis were 210 days old. Mean body weights of males and females fed with Diet I were  $415.00 \pm 63.63$  g and  $394.50 \pm 64.34$  g, respectively while respective values of those, that were fed with Diet II were  $344.50 \pm 36.06$  g and  $333.33 \pm 79.10$  g. No significant difference ( $p = 0.54$ ) was observed in the mean body weights of the fish.

Percentage of total fat was higher in female GIFT fish in both treatments (Table 3). Biro *et al.* (2009) also demonstrated that fat content of flesh varies with the type of feed and sex of Tilapia. The distinct difference in FAs in males and females may be due to the differences in maturation processes between sexes including egg formation and development.

**Table 2 :** Fatty acid composition of fillets of fish (pooled male and female) fed with Diet I and Diet II (n=3)

Fatty Acid*	Diet I (30 P/7.70 CF)	Diet II (30 P/12.55 CF)
ΣSFA	$40.97 \pm 1.69^a$	$39.11 \pm 0.91^a$
ΣMUFA	$28.61 \pm 0.59^a$	$23.99 \pm 0.62^b$
Σn-6	$10.95 \pm 0.04^a$	$10.53 \pm 0.47^a$
Σn-3	$18.23 \pm 0.44^a$	$26.24 \pm 1.99^b$
ΣPUFA	$29.18 \pm 0.46^a$	$36.77 \pm 1.51^b$
C20:5n-3	$2.57 \pm 0.49^a$	$5.13 \pm 0.15^b$
C22:6n-3	$10.5 \pm 0.51^a$	$15.32 \pm 2.47^b$
n-3/n-6**	$1.66 \pm 0.03^a$	$2.5 \pm 0.3^b$

Means in the same row having different superscripts are significantly different ( $p \leq 0.05$ )

\* (% FA/ g total fat), \*\* calculation of n3/n6 based on the major fatty acids detected by the protocol supplied by the manufacturer.

ΣSFA- total saturated fatty acids, ΣMUFA- total mono unsaturated fatty acids, ΣPUFA- total poly unsaturated fatty acids, DHA- docosahexaenoic acid and EPA- eicosapentaenoic acid

Percentage of total saturated fatty acids (ΣSFA) per gram of total fat in fish flesh was not significantly different in the two treatments (Table 2). Palmitic acid (C 16:0) was the predominant SFA found in both males and female fillets tested (Table 3). Rice bran and maize powder are plant products having 13 % (Kahloh *et al.*, 1992) and 18 % (Adeoti *et al.*, 2013) palmitic acid respectively, from the total fatty acid content of those two ingredients used in preparing two experimental diets for the present study. No marked reduction of palmitic acid content in fillets was observed by the reduction of maize percentage in Diet II. It seems that the palmitic acid balance has been maintained by both maize and rice bran. In contrast, certain fish meals contain more palmitic acid compared with plant-based feed ingredients. When Tilapia fed with herring rich fish meal, a significantly higher palmitic acid content was found in fillets compared with that of the fish fed with sunflower cake. According to Maina *et al.* (2003), palmitic acid which is naturally available in large quantities in herring fish meal might have deposited in Tilapia fish fillets. During the present work, percentage of myristic acid content recorded in fillets of GIFT fish fed with Diet II was lower than that of the fish fed with Diet I. In contrast, stearic acid content in fillets of fish fed with the Diet II was higher (Table 3).

Percentage of ΣMUFA was significantly lower in fish fed with rice bran rich feed than the fish fed with maize rich feed (Diet 1; Table 2). Reduction in palmitoleic acid and oleic acid content was observed in fillets of fish fed with Diet II (Table 3). Trbovic *et al.* (2013) had observed unfavourable fatty acid profile in carps fed with maize rich feed and pointed out the importance of combining cereal and oil seed in diet. Csengeri (1996) also had observed an increase of ΣMUFA content in flesh of common carps when fed with maize, which could be related to metabolic processes of fresh water fish. According to Henderson (1996), when energy rich diets are offered, freshwater fish can de-saturate endogenously-synthesised SFAs to MUFA especially oleic acid by A9 desaturase enzyme.

Much difference was not observed in the content of n-6 PUFAs between flesh of fish reared under the two treatments except male fish fed with Diet II. Linoleic acid (C18:2n-6) was the dominant n-6 PUFA and the percentage of it was higher in fish fed with Diet I (Table 3).

Significantly higher percentages of ΣPUFAs, Σn-3 PUFAs especially docosahexaenoic acid (DHA, C22:6n-3) and eicosapentaenoic acid (EPA, C20:5n-3) were observed in fillets of fish fed with Diet II containing

**Table 3:** Total fat content and fatty acids as a percentage of total fat (% FA/ g total fat) in fillets of GIFT fish with respect to Diet I and Diet II (n = 6)

Fatty Acids	Diet I (30 CP/7.70 CF)		Diet II (30 CP/12.55 CF)	
	Male	Female	Male	Female
Total fat content (%)	1.25 ± 0.00	1.57 ± 0.00	1.07 ± 0.01	1.58 ± 0.01
Saturated fatty acids				
Myristic (C14:00)	8.75 ± 0.01	8.69 ± 0.01	5.39 ± 0.00	5.08 ± 0.15
Palmitic (C16:00)	22.64 ± 0.00	26.14 ± 0.05	23.53 ± 0.04	26.21 ± 0.07
Stearic (C18:00)	7.14 ± 0.01	6.62 ± 0.06	9.4 ± 0.02	8.61 ± 0.08
Mono unsaturated fatty acids				
Palmitoleic (C16:1n-7)	7.94 ± 0.01	7.37 ± 0.03	5.91 ± 0.05	6.75 ± 0.04
Vaccenic (C18:1n-7)	3.25 ± 0.0	3.14 ± 0.02	3.83 ± 0.02	3.38 ± 0.01
Oleic (C18:1n-9)	17.45 ± 0.49	16.99 ± 0.00	13.71 ± 0.02	14.39 ± 0.06
Poly unsaturated omega n-6 fatty acids				
Linoleic (C18:2n-6)	10.15 ± 0.04	10.28 ± 0.02	9.13 ± 0.04	9.86 ± 0.01
Arachidonic (C20:4n-6)	0.79 ± 0.01	0.68 ± 0.02	0.98 ± 0.02	1.08 ± 0.02
Poly unsaturated omega n-3 fatty acids				
Linolenic (C18:3n-3)	2.0 ± 0.00	1.78 ± 0.01	1.00 ± 0.04	1.48 ± 0.28
Eicosapentaenoic (C20:5n-3)	2.61 ± 0.02	2.53 ± 0.02	5.00 ± 0.02	5.26 ± 0.03
Docosapentaenoic (C22:5n-3)	3.18 ± 0.00	3.34 ± 0.04	3.89 ± 0.02	3.99 ± 0.00
Docosahexaenoic (C22:6n-3)	10.05 ± 0.06	10.94 ± 0.07	18.07 ± 0.03	13.78 ± 0.02

higher percentage of rice bran when compared to Diet I (Tables 2 and 3). Amount of a fatty acid in mg/100 g of wet fillet is important when considering the nutritional aspect of fish used for human consumption. When the percentages of fatty acids were converted to milligrams, the amount of  $\Sigma$ n-3 omega PUFAs in fish fed with Diet II was twice (0.53 g/100 g) as the value recorded for Diet I (0.26 mg/100 g).

Fresh water fish require dietary fatty acids such as C18:2n-6 and or C18:3n-3 (0.5-2 %) which are converted in to PUFA (NRC, 1993). Studies have demonstrated that Tilapia also has this ability to desaturate and elongate the chain C18:2n-6 and C18:3n-3 to longer chains of n-6 and n-3 LC-PUFA, respectively (Visentainer *et al.*, 2005). Rice bran being a rich source of linoleic acid (C18:2n-6) (Kahloh *et al.*, 1992), inclusion of higher percentage of rice bran in Diet II seems to have a favourable influence on PUFA profile of GIFT fillets by efficient conversion of C18:2n-6 or C18:3n-3 into EPA or DHA, respectively.

Similarly, a better n-3/n-6 ratio was recorded for the Diet II (Table 2) in this study which was formulated by incorporating a higher percentage of rice bran. A Higher n3/n6 value is an indication of better nutritive quality of

fish. Ratio of n3/n6 recorded for Diet II of the present study was higher than that was reported by Strobel *et al.* (2012) for freshwater farmed rainbow trout (2.06 ± 0.91) and iridescent shark (*Pangasius hypophthalmus*; 0.24 ± 0.03) while it was lower than the values observed for marine fishes such as tuna (4.97 ± 2.17) and herring (6.42 ± 1.7). Food chains of marine fishes depend on the primary producers such as marine phytoplankton (microalgae like diatoms) which can effectively synthesise LC PUFA from linoleic acid and alpha-linolenic acid via a series of desaturation and elongation reactions.

During the present study, the highest n-3/n-6 ratios were recorded for fillets of male (2.76) and female (2.24) fish fed with Diet II while it was 1.63 and 1.69, respectively in male and female fish fed with Diet I. Biro *et al.* (2009) had demonstrated that fillets of male Tilapia have higher n-3/n-6 ratio compared with their counterparts when fed with diets rich in soya bean oil, linseed oil and fish oil.

Crude protein, fat, ash and dry matter content in fillets were significantly higher in fish fed with Diet II (Table 4). Results highlight the importance of higher fat levels (12 %) in the diet provided to cultured GIFT in

**Table 4:** Proximate nutritional composition of male and female fillets and pooled samples of fillets of fish fed with Diets I and II (mean  $\pm$  SD)

% proximate composition	Diet I (n=3)		Diet II		Diet I (n = 6) (pooled male and female)	Diet II
	Male	Female	Male	Female		
Crud protein	19 $\pm$ 0.0	18.24 $\pm$ 0.1	20.19 $\pm$ 0.0	20.11 $\pm$ 0.0	18.62 $\pm$ 0.4 <sup>a</sup>	20.15 $\pm$ 0.1 <sup>b</sup>
Crude fat	2.02 $\pm$ 0.0	2.5 $\pm$ 0.0	2.46 $\pm$ 0.0	2.39 $\pm$ 0.0	2.27 $\pm$ 0.3 <sup>a</sup>	2.43 $\pm$ 0.4 <sup>b</sup>
Crude ash	1.11 $\pm$ 0.0	0.88 $\pm$ 0.0	1.38 $\pm$ 0.0	1.01 $\pm$ 0.0	1.0 $\pm$ 0.1 <sup>a</sup>	1.2 $\pm$ 0.2 <sup>b</sup>
Dry matter	22.16 $\pm$ 0.0	21.63 $\pm$ 0.0	24.05 $\pm$ 0.0	23.53 $\pm$ 0.0	21.89 $\pm$ 0.3 <sup>a</sup>	23.79 $\pm$ 0.3 <sup>a</sup>

Means in the same row having different superscripts are significantly different ( $p \leq 0.05$ )

increasing crude protein levels of fillets. Kasheif *et al.* (2011) and Kheir (1997) have also revealed that higher dietary fat could increase the body protein content in *Oreochromis niloticus* and *Oreochromis aurius*, respectively.

No significant difference ( $p \leq 0.05$ ) was recorded in growth indices between GIFT fed with the two diets. Fish fed with Diet II had significantly higher ( $p \leq 0.05$ )  $\Sigma$ PUFAs,  $\Sigma$ n-3 PUFAs, n3/n6 and essential fatty acids (DHA and EPA) with comparatively low  $\Sigma$ MUFAs concluding that rice bran used at correct percentage in formulating fish diet could positively influence the nutritional quality of GIFT fish without negative effect on the growth indices of fish. Rice bran is much cheaper than maize and the results of the present work could be applied in formulating a less expensive commercial feed for culturing GIFT which is a good source of proteins and essential fatty acids for human.

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