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FARM-MADE FEED FOR POLYUNSATURATED FATTY ACIDS (PUFAs) RICH CARP PRODUCTION IN INDIA: A CASE STUDY

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ABSTRACT

An 8 months feed demonstration program was conducted in the ponds of Ramakrishna Mission, Belur Math, West Bengal, India. The program aimed at providing hands-on training to trainees at Samaj Sevak Sikshan Mandir (SSSM) on farm-made feed formulation and use in aquaculture. Two ponds were selected, one at Shilpamandira of 0.1 h and fish were fed Feed-1, and another of 0.08 h at Samaj Sevak Sikshan Mandir (SSSM) and fed Feed-2. Indian major carps, *Catla catla* (catla) and *Labeo rohita* (rohu) were stocked at 4500 fish ha^{-1}. The stocking size of rohu was 80-90g and of catla was 220-245g. Locally available low cost feed ingredients, rice bran, mustard oil cake, til oil cake and linseed oil sludge were used to formulate and prepare two farm-made mash feeds (Feed-1 and Feed-2). The fish were fed at 2% of total fish biomass in the ponds and were fed through bag feeding. At the end of the study rohu grew to 1.07 kg and catla to 1.6 kg in Shilpamandira pond (Feed-1), and rohu to1.5 kg and catla to 2.2 kg in Samaj Sevak Sikshan Mandir pond (Feed-2). The net production of fish was 4.9 and 6.8 t ha^{-1} for 8 months with Feed-1 and Feed-2, respectively. Dietary inclusion of linseed-oil-sludge significantly increased the PUFA content in Indian major carps. The feed cost was substantially reduced by replacing mustard oil cake with til oil cake and incorporation of linseed oil sludge, a very low priced ingredient.

Keywords: Omega-3 fatty acids, carp poly culture, linseed oil, unconventional feed

INTRODUCTION

The fisheries sector occupies an important place in the socio-economic development of the country, which envisages livelihood, nutritional security, employment

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generation and export earnings. Indian fisheries occupy the second position in global fish production and second in aquaculture in the world with an annual growth rate of 4.7%, recording 3.2% growth in marine sector and 6.2% in inland sector (Paul and Giri, 2015). The fisheries sector has grown from traditional activity in the early fifties, when India initiated the first five year plan (1950-51 to 1955-56), and now transformed into significant commercial enterprise with impressive growth in production from 0.75 million tons in the 1950s to 10.4 million tons (MT) during 2015-16. The sector has emerged as the largest single employer in the country for more than 14.5million people (DAHAD, 2015-16). About 35% of the Indian population is fish eaters and the per capita consumption is 9.8 kg, whereas present global per capita fish intake is over 20 kg (FAO, 2016) (http://www.fao.org/news/story/en/item/icode/). Inland fisheries have emerged as a major contributor to the overall fish production in the country with a present share of 64.07 % in total fish production. Within inland fisheries there is great shift from captured fisheries to aquaculture and at present freshwater aquaculture shares 80% of total inland fish production in India (DAHD, 2015-16).

The rapid expansions of the aquaculture industry, along with the improvement and change in culture techniques, have increased the demand for fish feeds. Feed cost is considered as the major recurring expenditure in any fish-culture operation. Expenditure on feed alone amounts to 60% of total fish production cost (Paul and Giri, 2015).

Commercial feeds for carp culture are available in the market but small fish farmers are unable to use these feeds because of their high cost. Traditional practice for fish farmers is to use mixture of groundnut oil cake and rice bran or cereals as fish feed. Recently the price of both the ingredients are highly increased which farmers are unable to afford (Rath et al., 2014) to feed the fish.

The Indian Council of Agricultural Research is operating an outreach program on "Fish feeds" among six fisheries research institutes, ICAR-CIFA, Bhubaneswar; ICAR-CIFRI, Barrackpore; ICAR-CIBA, Chennai; ICAR-CMFRI, Kochi; ICAR-DCFR, Bhimtal and ICAR-CIFE, Mumbai. The program has been undertaken to create awareness among the fish farmers across the country on the use of farm-made fish feeds to enhance production and popularization of fish culture in the rural sectors as well besides supporting their livelihood. Being the network leader ICAR-Central Institute of Freshwater Aquaculture under took several farmers awareness programs on fish feeds (Rath et al., 2014), hands-on training on farm feed preparation and feeding demonstration in farmers ponds in Odisha, West Bengal and Karnataka. Use of farm-made feed with locally available feed ingredients in rural aquaculture sectors open a new era for feed based aquaculture. Linseed oil is rich in omega-3 fatty acids and in the present experiment linseed oil sludge has been used as by-product of oil milling industry. The incorporation of linseed oil sludge in farm-made feed would improve the omega-3 fatty acid profile of fish. Keeping in view of the above facts a

feed demonstration program was undertaken at the ponds of Ramakrishna Mission, Belur Math with a view to use the locally available feed ingredients for production of fish enriched with omega-3 fatty acid.

MATERIALS AND METHODS

Pond preparation

Initially ponds were netted out repeatedly for two days with nylon dragnet followed by fry net to take out fish. Both the ponds are located in such places where chances of entry of predator and weed fishes are not there. Pond fertilization was carried out with application of cow dung and single super phosphate at 3 ton ha^{-1} and 75 kg ha^{-1}, respectively as a basal dose one week prior to stocking, with alternating applications every fortnight at 1ton $ha^{-1}month^{-1}$ and 20 kg $ha^{-1}month^{-1}$, respectively (Jena et al., 1999).

Fish maintenance and feeding

Under the Outreach Activity on Fish Feeds, two ponds were selected at Rama Krishna Mission, Belur Math, to provide hands-on training to 30 trainees at Samaj Sevak Sikshan Mandir (SSSM) on farm-made feed. Selected ponds were a) 0.1 ha at Shilpamandira (Feed-1) and b) 0.08 ha at SSSM (Feed-2) under the Sarada Pith unit of Belur Math. Both the ponds were stocked with rohu and catla juveniles at a stocking density of 4500 juveniles ha^{-1} and at a stocking ratio of catla:rohu was 90:10.. The stocking size of rohu was 80-90 g and for catla was 220-245 g. The objective of the program was to train the vocational trainees of RKM Samaj Sevak Sikshan Mandir through learning by doing mode, with a view to focus the utilization of locally available feed ingredients as cheap resources for fish feed making for low cost fish production.

Locally available low-cost feed ingredients were identified by surveying the local oil mills and procured. For the demonstration program two different farm-made feeds were formulated as per nutrient requirement guidelines of NRC (2011) for carps. Two mash feeds were prepared using rice bran, till oil cake, mustard oil cake and linseed sludge in different proportions for feeding the fish (Table 1). Linseed oil sludge is cheaply available in the oil milling industry as one of the by-product of crushing unit. Suitable feed dispensing mechanism were also developed, in which nylon bags were suspended on galvanized wire and connected by a small-pulley so that trainees can provide the feed regularly by standing on the pond side without going into the pond. Fish were fed at 2% of the body weight daily in two divided meals for a period of 8 months. Monthly sampling was done to assess the fish growth, health and calculate the ration requirement. Regular racking of pond water was practiced to release the obnoxious gases from the pond bottom due to accumulation of metabolites.

Table 1. Feed formulations (% as such basis)and proximate composition (% DM basis)

Ingredients	Feed-1	Feed-2
Til oil cake	60	--
Mustard oil cake	--	60
Rice bran	30	30
Linseed oil sludge	10	10
	100	100
Proximate composition		
Dry matter	97.70	97.80
Crude protein	26.32	25.26
Crude Fat	4.61	5.20
Ash	17.78	18.02

Growth parameters

Fishes were weighed at monthly intervals and mortality of fish was recorded. Net weight gain, feed conversion ratio (FCR), specific growth rate (SGR) and percent survival were calculated (Castell and Tiews, 1980).

Proximate composition

Proximate composition of feed and fish tissue samples was analyzed as per AOAC (2005). Water quality parameters such as pH, DO, free CO_2, total alkalinity and temperature were measured employing the method of APHA (2005).

Fatty acid analysis

Pooled samples were extracted for fatty acid analysis by following method of Folch et al. (1957). Fatty acid methyl esters (FAMEs) were prepared as per Metcalfe et al. (1966). The FAMEs were quantified by injected 1 µl (50:1 split ratio) into (Gas Chromatograph/GC) Perkin Elmer; (CLARUS 480). Fatty acid was quantified through "Total Chrome" software in GC as reported earlier (Paul et al., 2015).

RESULTS AND DISCUSSION

Feed formulation and proximate composition of feed are presented in table 1. The proximate compositions of the feeds are alike ($P>0.05$). The nutrient composition of linseed oil sludge is presented in table 2. The proximate compositions (% DM basis) of linseed sludge was 4.4% crude protein, 19.00% fat and 40.83 % ash. The saturated fatty acids (SFA) was determined as 9.42 % of total fatty acids of the sludge and palmitic acid was the dominating SFA. The MUFA content was 16.83 and oleic acid was the leading fatty acid. The polyunsaturated fatty acid (PUFA) content was 73.76 % of the total fatty acids, where linolenic and linolelaidic acid were the dominant

PUFAs. The water quality parameters are presented in table 3. The water temperature ranged from 21-30^{o}C in two ponds. The water quality parameters like pH, CO_2, total alkalinity (mg l^{-1}) and dissolved oxygen (mg l^{-1}) ranges from 7.4 to 7.8, 4-8, 130-160 and 6.0-8.5 respectively in two different ponds, which is ideal for carp culture (Yadava and Garg, 1992; Hajek and Boyd, 1994, Azad et al. 2004; Swingle, 1969).

Table 2. Proximate composition (% DM basis) and fatty acid composition (% of total fatty acid) of Linseed sludge

Particulars	Linseed sludge
Crude protein	4.40
Crude fat	19.10
Ash	40.80
Fatty acid composition	
C:16 Palmitic acid	9.39
Others	0.03
∑ SFA	9.42
Oleic acid	14.08
Elaidic acid	1.20
Erucic acid	1.45
Others	0.10
∑ MUFA	16.83
Linolelaidic acid	16.83
Linolenic acid	46.05
Others	0.96
∑ PUFA	73.76

Table 3. Hydro biological parameters of ponds with different feed treatments

Particulars	Feed-1	Feed-2
Temperature	21-29^{o}C	22-30 ^{o}C
pH	7.5-7.8	7.4-7.6
CO_2 (ppm)	6-8	4-6
Total alkalinity (ppm)	150-160	130-140
Dissolved oxygen (ppm)	6-8	6-8.5

In the present demonstrated program the variations of total alkalinity were within the productive range for aquaculture ponds which also corroborate with the observations of Wahab et al. (1995) and Kohinoor et al. (1998). Good water quality is characterised by adequate oxygen levels in pond (Chiu (1988). Rahman et al. (1982)

reported that dissolved oxygen content of a productive pond should be 5.0 mg l^{-1} or more.

The fish production performance of different ponds are presented in table 4. The initial stocking size of rohu in two ponds ranged between 83.5 and 87.5 g while catla was 220.0 to 245.5 g. The final average weight of catla and rohu was 1.61 kg and 1.07 kg respectively in the pond of Shilpamandira (Feed-1) whereas respective average weight of catla and rohu in the pond of SSSM (Feed-2) was recorded as 2.20 and 1.51 kg respectively. Survival rate of catla in the two ponds at the time of harvest was recorded to be 88 to 92% while rohu to be 93.3 to 93.91%. The final fish biomass in the pond of Shilpamandira (Feed-1) was registered as 536.30 kg against 586.04 kg in the pond of SSSM (Feed-2), the net weight gain (kg) being 486.44 kg and 544.85 kg respectively. The feed conversion ratio (FCR) was 1.81and while 1.61 in Feed-1 and 2 respectively. The demonstration resulted net production rate of 4.9

Table 4. Fish production in two ponds at Belur math fed on farm-made feeds

Particulars	Feed-1	Feed-2
Pond size (ha)	0.10	0.08
Fish stocked (4500 fish ha^{-1})	450.00	360.00
Initial av. wt.(g) of catla	245.50	220.00
Intial av wt.(g) of rohu	83.50	87.50
Survival at harvest (%): catla	92.00	88.00
Survival at harvest (%) : rohu	93.30	93.910
Final Av. wt.(kg): catla	1.61	2.20
Final Av. wt.(kg) :rohu	1.07	1.51
Total quantity at harvest (kg):		
catla :	74.06	96.80
rohu :	462.24	489.24
Net biomass gain(kg)	486.44	544.85
Total feed intake (kg 8 $month^{-1}$)	880.00	878.00
FCR	1.80	1.60
SGR (%)	1.01	1.13
Net production (t ha^{-1}8 $month^{-1}$)	4.90	6.80
Feed cost (Rs.) kg^{-1}	15.70	18.70
Feed cost (Rs.) kg^{-1} fish production	28.26	29.92

Av. wt = Average weight

and 6.8 t ha^{-1}8 $months^{-1}$ respectively in Feed-1 and 2. At the end of the demonstration the trainees of RKMSSSM developed skill with self confidence to adopt aquaculture practice as one of the avenues to upgrade their livelihood status and scope for self-reliance as well.

Table 5 represents the proximate composition (%w/w basis) of rohu and catla of initial and final samples of both the ponds. The moisture content varies from 69.92 to 74.18 %. The protein content varies from 14.13 to 17.70 %, fat from 2.92 to 4.43% and ash content from 2.62 to 6.05% in catla and rohu of different ponds fed with farm-made feed. The fish carcass composition was higher in both rohu and catla after 8 months of experimental period. The data reported in this experiment is in agreement with the earlier report by Paul et al. (2016)

Table 5. Carcass composition (% w/w basis) of rohu and catla fed with farm-made feed

Particulars	Initial sample		Feed-1		Feed-2	
	Rohu	Catla	Rohu	Catla	Rohu	Catla
Moisture	78.65±0.96	74.44±0.38	74.13±0.94	74.18±0.67	75.29±0.22	69.92±1.11
Protein	12.69±0.30	12.60±0.29	14.13±0.33	14.64±0.04	14.57±0.14	17.70±0.06
Fat	1.77±0.28	2.27±0.06	3.25±0.04	3.85±0.01	2.92±0.07	4.43±0.08
Ash	2.12±0.04	2.41±0.05	3.42±0.07	2.62±0.06	3.82±0.0005	6.05±0.09

Data are presented as Mean± S.E

The fatty acid profile of rohu and catla of initial and final samples of two ponds are presented in table 6. The saturated fatty (SFA) acid content of carcass of rohu at the beginning of the demonstration was 65.88 %, which decreased to 53.57 % and 52.23 % of the total fatty acids, at the end of demonstration, on feeding Feed-1 and Feed-2, respectively. Similarly, in catla the carcass SFA at the beginning was 33.87 % and increased to 61.54 % and 40.72 % with Feed-1 and 2, respectively in the final harvest samples. The predominate fatty acid among SFA was palmitic acid. The palmitic acid was considered as a key to many metabolic processes in fish and other aquatic animals as reported by Ackman and Eaton (1966). In the muscle of rohu, palmitic acid is higher which is in agreement with the earlier report by Paul et al. (2013).

The monounsaturated fatty acid content ranges from 23.67 to 38.76 % in initial carcass sample of catla as well as rohu. However, the MUFA content in harvested sample of rohu and catla were 24.80 to 26.40 and 12.54 to 36.53 %, respectively and the dominating fatty acid of MUFA class is Oleic acid. The polyunsaturated fatty acid (PUFA) content in initial samples were 10.47 to 27.36 % in rohu and catla. The

Table 6. Fatty acid profile of (% of total fatty acid) of rohu and catla

Fatty Acid	Initial Sample		Feed-1		Feed-2	
	rohu	catla	rohu	catla	rohu	catla
Lauric Acid	-	0.22	0.05	0.23	10.23	0.11
Myristic Acid	3.67	5.12	1.10	6.16	2.92	1.89
Pentadeconoic Acid	1.94	1.51	0.24	3.34	2.50	0.92
Palmitic Acid	46.87	19.80	46.08	31.51	25.60	23.63
Heptadeconoic Acid	2.44	2.59	0.22	1.11	4.27	0.90
Stearic Acid	8.16	0.01	4.68	14.27	0.05	10.96
Heneicosanoic Acid		1.81	0.36	2.15	4.25	1.33
Tricosanoic Acid		1.49	0.64	0.31	1.57	0.14
Others	2.80	1.32	0.02	2.46	0.84	0.90
∑ SFA	65.88	33.87	53.57	61.54	52.23	40.72
Palmitoleic Acid	6.21	0.91	0.98	9.89	7.52	4.12
Oleic Acid	15.01	22.67	22.75	-	-	30.4
Eladic Acid	-	11.03	-	-	17.89	-
Eicosanoic Acid	-	2.16	-	-	-	-
Erucic Acid	0.57	0.09	0.78	1.01	0.21	0.91
Others	1.89	1.90	0.29	1.64	0.78	1.10
∑ MUFA	23.67	38.76	24.80	12.54	26.40	36.53
Linoleic Acid	4.66	7.56	12.86	7.97	9.87	13.73
Linolenic Acid	2.70	14.19	6.91	7.09	4.71	4.12
Arachidonic Acid	0.37	1.06	0.02	0.64	0.76	0.92
EPA	1.36	3.95	0.06	2.35	0.99	0.85
DHA	0.70	-	0.89	3.54	3.09	2.15
Others	0.68	0.60	0.91	1.27	1.96	0.97
∑ PUFA	10.47	27.36	21.65	22.86	21.38	22.74
∑ ω3	4.83	17.72	7.96	12.82	9.36	6.30
∑ ω6	5.64	9.64	13.42	10.04	11.57	15.59
ω3: ω6	0.85	1.84	0.59	1.28	0.80	0.40

SFA- Saturated Fatty Acid, MUFA- Monounsaturated Fatty Acid, PUFA-Polyunsaturated Fatty Acid, EPA-Eicosapentaenoic Acid and DHA-Docosahexaenoic Acid.

PUFA content in both the species of the two treatment groups range from 21.38 to 22.86 %. Here the predominant fatty acids are linolenic, linoleic, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The EPA content of initial samples was 1.36 to 3.95(%)**.** The EPA content of rohu from both the feed treatment groups ranges from 0.06 to 0.99 % while DHA content in the same species varies from 0.89

to 3.09 (%) in Feed-1 and 2. Value of EPA content in harvested catla of both ponds ranges from 0.85 to 2.35% and DHA content of 2.15 to 3.54 %. Steffens and Wirth (2005) reported that freshwater fish are rich in essential polyunsaturated fatty acid of the n-3and n-6 series, which is in agreement with fillet composition of experimental catla and rohu. Compared to beef and chicken, fish meat contains higher levels of n-3 PUFAs Calder (2004), which are known to be cardio-protective (Sanderson *et al*., 2002) and antithrombotic (Calder, 2004). Fish oils are known to be rich source of essential PUFA of the omega-3 family (Kenari et al., 2009 and Giri et al., 2010). The data on fatty acid profile of catla and rohu as reported in the paper are in agreement with earlier reports (Memon et al., 2011 and Paul et al., 2015). This indicates that the fish reared with farm-made omega-3 feed tailored the fatty acid profile of harvested fish tissue. The feed cost was substantially reduced by replacing mustard oil cake with til oil cake and with incorporation of linseed oil sludge, a very low priced ingredient.

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