

Making aquatic weed as potential feed for Nile tilapia (*Oreochromis niloticus* L.) and its impact on fatty acid profile

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A study was conducted to evaluate the nutritional potentials of three commonly available, aquatic weeds namely, lemna (*Lemna minor*), water hyacinth (*Eichhornia crassipes Crassipes*) and azolla (*Azolla pinnata*) for ascertaining their suitability as complementary fish feed with the aim to reduce the cost of traditional feeds. Three isonitrogenous (30% crude protein approximately) and isocaloric (4.0 kcal g⁻¹ approximately) diets were formulated including lemna, water hyacinth and azolla as principal ingredient. Three groups of juvenile fish of fifty (50) number per group (Average wt. 5.5 g; Average length 4.5 cm) were fed with three different types of feeds with lemna meal (LNM), water hyacinth meal (WHM) and azolla meal (AZM) for 90 days duration. Significant difference ($P < 0.05$) in body weight gain, protein efficiency ratio and gonadosomatic index were observed among fish fed the LNM, WHM, AZM and control diets (CON). All treatments are significantly higher than control in respect to value of weight gain, PER and GSI. The lemna possibly contains better quality of protein which in turn influenced somatic as well as reproductive growth of those fish. Fish fed with three kinds of feed formulations exhibits no apparent variations with the control, which means that the feed provided to the fish has no impact on accumulation of PUFA especially n-3PUFA. The n-3/n-6 ratio also shows little or negligible variations.

Keywords: Aquatic weed, Complementary fish feed, Isonitrogenous, Isocaloric, Juvenile fish, Protein efficiency ratio

INTRODUCTION

Fish feed generally constitutes 60–70% of the operational cost in intensive and semi-intensive aquaculture system (Singh et al., 2006). The fish feed used in aquaculture is quite expensive, irregular and short in supply in many third world countries. These feeds are sometimes adulterated, contaminated with pathogen as well as containing harmful chemicals for human health. Naturally there is a need for the development of healthy, hygienic fish feed which influences the production as well as determines the

quality of cultured fish.

Considering the importance of nutritionally balanced and cost-effective alternative diets for fish, there is a need for research effort to evaluate the nutritive value of different non-conventional feed resources, including terrestrial and aquatic macrophytes (Edwards et al., 1985), (Wee and Wang, 1987) (Mondal and Ray, 1999). Aquatic and terrestrial macrophytes have been used as supplementary feeds in fish farming since the early times of freshwater fish culture (Bardach et al., 1972) and still play an important role as fish feed in extensive culture systems (Edwards, 1987). The aquatic weeds have been shown to contain substantial amounts of protein and minerals (Ray and Das, 1995). However,

Table 1. Biochemical composition of lemna, water hyacinth and azolla used for feed for Tilapia (*O. niloticus*)

Ingredient	Lemna (g/100g)	Water hyacinth (g/100g)	Azolla (g/100g)
Dry matter	92.81	92.68	92.56
Organic matter	82.92	82.20	82.80
Crude protein	24.24	19.70	20.56
Crude lipid	9.07	7.98	9.89
Ash	9.89	10.48	9.76
Nitrogen free extract	9.61	36.81	31.42
Crude fibre	9.58	9.58	9.24
Grass energy (Kcal g ⁻¹)	3.74	3.70	3.69

these materials are not evaluated for essential fatty acid content earlier. 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 lipid content particularly the fatty acid composition of fish feed, because it influences the growth rate as well as fat deposition in fish (Cengiz et al., 2003).

The test fish Nile tilapia (*O. niloticus* L.) is most favored in aquaculture due to its tolerances. These include ease of breeding in captivity, tolerance to both crowding and relatively poor water quality and low susceptibility to diseases (Suresh, 2003). The aim of this paper is to evaluate the growth and fatty acid content in the farmed fish using low cost feed materials.

MATERIAL AND METHODS

Experimental set up

Fifty fingerlings in triplicate groups used in three different treatment. Altogether six hundred (600) Nile tilapia (male and female ratio 1:1) fingerlings were used in this experiment. Twelve groups of tilapia fingerlings comprising fifty (50) individuals each (average weight Weight 5.5 g and Average Length 4.5 cm) were obtained from Balarampur fish farm, 3 Km away from IIT-Kharagpur, West Bengal, India . Another batch of fish were fed with control feed (CON).The fish fingerlings were treated with potassium permanganate solution (1 mg L⁻¹) to remove any external parasites and 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 of fingerlings also were initially weighed to record the initial biomass. They were stocked in twelve rectangular cemented cement tanks (1000 L) separately and three different and one control feed were administered.

Triplicate tanks were allocated for each dietary treatment. The water system was static in nature and the bottom of the tank was filled with local agricultural soil (pH 6.4 ± 0.05). The experiment was conducted for 90 days from June to August in the year 2010. Dechlorinated well water (temperature 30 ± 4 °C, pH 7.2 ± 0.05, free CO₂ 0.5±0.01 mg L⁻¹, available nitrogen 0.6±0.05 mg L⁻¹ and dissolved oxygen (DO) 6 mg L⁻¹) was used in the experiment.

Feed formulation and preparation

The principal feed ingredients were collected from local pond at very low cost. These substances were economically cheap but contained significant amount of crude protein (above 20%). Biochemical composition of lemna, water hyacinth and azolla used in the for feed for tilapia are shown in Table 1. Diets used for growth trial were prepared that feed formulations remain almost isonitrogenous (30 g 100 g⁻¹) and isoenergetic (4Kcal 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 A control feed was made with out aquatic weeds which are the principal ingredients for other three feeds formulated. Mustard oil cake, wheat flour, rice bran, egg shell dust and vitamin premix were common ingredient in every feed tested. 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 1.2 mm die. The resulting pellets were dried in a hot air oven for 48 h at 50 °C and then

Table 2. Formulation and composition of the experimental diets (%)

Sl. no	Name of feed	Ingredients	% of ingredient in formulated feed	% of crude protein	% of lipid	% of carbohydrate *	Calorific value of feed (kcal/g)
1	LNM	Lemna dust Rice bran MOC Wheat flour Egg shell dust and vitamin premix (3:1)	46 09 34 10 01	30.10	9.2	11.2	4.1
2	WHM	Water hyacinth dust Rice bran MOC Wheat flour Egg shell dust and vitamin premix (3:1)	47 10 32 10 01	29.70	9.0	12.3	4.0
3	AZM	Azolla dust Rice bran MOC Wheat flour Egg shell dust and vitamin premix (3:1)	48 10 31 10 01	30.56	9.1	12.7	4.1
4	CON	MOC Rice bran Wheat flour Egg shell dust and vitamin premix (3:1)	34 10 55 01	29.65	8.5	14.5	4.0

* Carbohydrates calculated by difference.

packed in polythene bags for frozen.

Feeding

The feed was given ad libitum in a feeding bag hung from an iron rod in four locations in each tank. Unconsumed feed was removed after 1 hour from the beginning of feed administration and dried in a hot air oven at 50°C. Feed consumption was estimated by subtracting the weight of the unconsumed feed from the weight of the feed offered. Fish, feed samples, and unconsumed feeds were weighed on an electric balance to an accuracy of 0.1 mg. (Orpat, CZ-20)

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: (Chakrabarty et al., 2010)

FI (g fish⁻¹ day⁻¹) = Total feed intake per fish/number of days

SGR (% day⁻¹) = $100 \times (\ln[\text{final body weight}] - \ln[\text{initial body weight}]) / \text{no. of Days}$

FCR = feed intake/live weight gain

PER = live weight gain/crude protein intake

HSI (%) = $100 \times (\text{liver weight} / \text{total body weight})$

Gonad estimation

Gonad weight was measured at the end of the experiment. The ovaries were first removed, weighted and then gonadosomatic index (GSI) was computed according to the formula of Dahlgren (1979).

$GSI (\%) = 100 \times (\text{weight of gonad} / \text{total body weight})$

Analysis

Feeds and carcass samples were analyzed following standard procedures (AOAC, 1990): Dry dry matter (DM) after drying in a hot air oven (Gallenkamp, UK) at 105°C for 24 h; crude protein (CP) by Kjeldahl method ($N \times 6.25$) after acid hydrolysis, crude lipid (CL) after extraction with petroleum ether for 7-8 h by Soxhlet method (40-60°C boiling range), total ash by igniting at 550°C for 3 h in muffle furnace (Size 2 Gallenkamp, UK). Organic matter (OM) was calculated by subtracting total ash from DM (Giri et al., 2000) Crude fibre was determined using a moisture free defatted sample which 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 extract was determined by subtracting the sum of crude protein, crude lipid, crude fibre and ash from DM. (Maynard et al., 1979) Gross energy was determined using a Bomb Calorimeter Model-DFU 24 following the process 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-4, feed-3) 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 then again with the first solvent system viz., methanol – chloroform (2:1, v/v). Sample was ground with the solvent methanol-chloroform (2:1, v/v), filtered through Whatman no. 1 filter paper and residue was extracted with 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 with three volumes of water (100-200 ml, depending on the volume of pooled extracts) and layer was allowed to separate in a separatory funnel made by Pyrex glass Co.. 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 was evaporated 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 (butylated hydroxy toluene) was added at a level of 100 mg/L to the solvent as antioxidant.

Preparation of Methyl Ester of Fatty acids

Total lipid of various (fish flesh-4, feed-3) samples was dissolved in anhydrous methanol containing concentrated Sulfuric acid (1.0%, v/v) and the mixture was refluxed (Christie, 1982) for 2 hours. Methanol was evaporated to a small volume (1-3 ml) and cooled to 4°C, in a freezer. Distilled water 10-15 ml was added to the cooled mixture (1-3 ml) in hard glass test tubes by Pyrex and the methyl esters of fatty acids were extracted 3 times with aliquots (5-10 ml) of diethyl ether, vortexed in a Vortex mixer. The ethereal extracts were 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 methyl ester 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 bands corresponding to the standard were marked and then scrapped off the plate with a sharp razor blade. Methyl esters were recovered by extracting the silica gel bands containing the methyl ester samples in a mini glass column (10 cm length x 0.8 cm internal diameter, by Pyrex) with chloroform (30-50 ml), the later was evaporated 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 (by DataApex Ltd. 2006, Podoradská 1, 155 00 Prague 5, The Czech Republic, Code/Rev: MO58/24B–26 May,

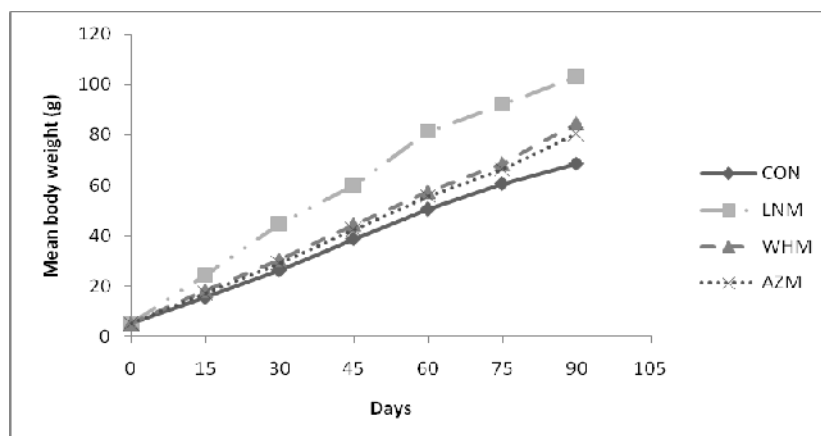


Figure 1. Mean body weight (g) evolution *O. niloticus* fed CON, LNM, WHM and AZM diets

Table 3. Growth performance and nutrient utilization of *O. niloticus* fed LNM, WHM and AZM diets (mean±SD)

	Control	LNM	WHM	AZM
Initial weight(g)	5.10±0.10 ^a	5.11±0.12 ^a	5.11±0.11 ^a	5.12±0.10 ^a
Final weight(g)	68.56±0.75 ^d	103.33±0.89 ^a	84.66±0.75 ^b	80.66±0.73 ^c
Initial length (cm)	4.5±0.10 ^a	4.5±0.12 ^a	4.6±0.11 ^a	4.5±0.12 ^a
Final length (cm)	11.9±0.11 ^d	15.9±0.15 ^a	13.1±0.14 ^b	12.9±0.14 ^b
Feed intake(g fish ⁻¹ day ⁻¹)	2.00±0.20 ^c	2.49±0.23 ^a	2.08±0.21 ^b	2.01±0.20 ^c
Specific growth rate (%day ⁻¹)	0.70±0.06 ^d	1.09±0.08 ^a	0.88±0.09 ^b	0.84±0.07 ^c
Feed conversion ratio	2.85±0.08 ^d	2.28±0.08 ^a	2.35±0.06 ^b	2.40±0.06 ^b
Protein efficiency ratio	0.85±0.06 ^b	1.46±0.05 ^a	0.90±0.06 ^b	0.87±0.05 ^b
Hepatosomatic index	1.56±0.03 ^c	1.75±0.03 ^a	1.71±0.02 ^b	1.70±0.03 ^b
Gonado somatic index	0.95±0.07 ^c	1.64±0.06 ^a	1.04±0.08 ^b	1.01±0.0 ^b

Values are mean±SD, n=3

Values in the row superscripted by different alphabets are significantly different from each other (P<0.05, Duncan's new multiple range test (Duncan, 1955)

Separate analysis was done for each row.

2006).

Analysis of fatty acid methyl esters (FAME)

GLC of FAME was done on a BPX-70 megabore capillary column of 30 mt length and 0.53 mm internal diameter i.d. obtained from SGE, Australia. Oven temperature was programmed from 150°C - 240°C with a rate of 8°C/min. Initial and final temperatures were kept isothermal for 1 minutes and 20 minutes respectively. Injection port and detector temperatures were 250°C and 300°C respectively. Nitrogen gas was used as carrier gas and its flow rate was 6.18ml/min.

Statistical analysis

Data are presented as means ± SD. One-way ANOVA was used to find the significant effects of feed type and rearing period on the feed and growth rates and also to test the significance level of feed type on production of fingerlings (Zar, 1974).

RESULTS

The highest growth weight of (103.33g) was observed in the LNM applied feed series followed by WHM (84.66g) AZM (80.66g) and CON (68.56g) (Figure 1) applied feed series. The length of fish was maximum (15.9cm) in LNM fed treatment and minimum (12.9cm) in AZM fed treatment. The amount of feed intake was

Table 4. Fatty acid compositions of Total Lipids (TL) of Fish feeds as determined by GLC of methyl esters. (% w/w of each component in total fatty acids)

Components ^a	CON	LNМ	WHM	AZM
Saturated				
14:00	0.1	0.1	0.2	0.3
15:00	0.2	ND	ND	0.4
16:00	7.5	7.4	7.4	7.3
17:00	0.6	0.7	1.6	1.3
18:00	2.0	2.3	2.3	2.1
20:00	0.5	0.3	0.8	0.2
22:00	1.6	1.5	1.1	0.9
24:00:00	0.9	1	0.5	0.4
ΣSFA	13.6	13.3	13.9	12.9
Monoene				
14:01	0.3	ND	0.4	0.5
15:01	0.02	0.01	ND	ND
16:01	1.0	0.8	0.9	0.9
17:01	ND	ND	0.01	ND
18:1ω9	23.5	22.6	22.5	23
20:1ω9	8.0	7.5	8	7.6
22:1ω11	27.5	27.9	27.2	27.8
24:01:00	0.8	1	0.6	0.6
ΣMUFA	60.12	59.81	59.61	60.4
Diene				
16:02	0.01	ND	ND	0.02
18:2ω6	21.0	21.2	20.9	21
20:02	ND	ND	ND	ND
ΣDUFA	21.01	21.2	20.9	21.02
Polyene				
18:3ω6	0.01	ND	ND	ND
18:3ω3	5.2	5.5	5.3	5.4
20:3ω6	0.02	0.1	0.1	0.2
20:3ω3	ND	0.1	0.2	0.1
20:4ω6	0.01	ND	ND	0.02
20:5ω3	ND	ND	0.01	ND
21:5ω3	ND	0.03	ND	ND
22:5ω6	ND	ND	ND	ND
22:5ω3	0.03	ND	ND	ND
22:6ω3	ND	ND	ND	ND
ΣPUFA	5.27	5.73	5.61	5.7
Total -w3	5.23	5.63	5.51	5.5
Total -w6	21.04	21.3	21	21.22
n-3/n-6	0.24	0.26	0.26	0.25

^a First and second figures represent carbon chain length: number of double bonds

The ω values represent the methyl end chain from the centre of double bond furthest from the carboxyl end.

SFA = saturated fatty acids; MUFA = monounsaturated fatty acids;

DUFA = Diunsaturated fatty acids; PUFA = polyunsaturated fatty acids

ND= Not detected (<0.01)

Table 5. Fatty acid compositions of Total Lipids (TL) of Fish flesh as determined by GLC of methyl esters. (% w/w of each component in total fatty acids).

Components ^a	Control	LNM fed fish	AZM fed fish	WHM fed fish
Saturated				
14:0	5.2	3.6	3.4	4.8
15:0	1	1.3	1.2	1.5
16:0	31.1	29	29	30.5
17:0	0.9	2.2	2.9	2.4
18:0	8	9.1	8.8	8.3
20:0	0.4	0.1	ND	0.4
22:0	4.1	7.9	7.7	6.7
24:0	0.8	1	1.5	0.4
Σ SFA	51.5	54.15	54.54	55
Monoene				
14:1	0.9	0.7	0.8	0.6
15:1	0.3	0.2	0.4	0.5
16:1	7.7	7.9	8	8
17:1	0.3	1	1.1	1.1
18:1ω9	12.8	12.2	12.5	13.9
20:1ω9	1.3	1.2	1.5	0.9
22:1ω11	1.2	0.5	0.8	0.6
24:1	1.5	2	2	1.3
Σ MUFA	26	25.7	27.3	24.9
Diene				
16:2	0.2	ND	0.2	0.3
18:2ω6	6.2	5.9	5.6	5.8
20:2	-	-	-	0.1
Σ DUFA	6.4	5.9	5.7	6.2
Polyene				
18:3ω6	0.3	0.3	0.2	0.4
18:3ω3	3.1	2.9	2.5	3
20:3ω6	1.3	1	0.9	1.2
20:3ω3	0.07	0.02	0.02	0
20:4ω6	0.9	0.8	0.6	0.9
20:5ω3	1.6	1.7	1.3	1
21:5ω3	0.5	0.2	0.2	0.2
22:5ω6	0.2	0.2	0.1	0.2
22:5ω3	2.6	2.2	2	2.2
22:6ω3	5.6	5	4.7	4.8
Σ PUFA	16.17	14.32	12.52	13.9
Total -ω3	13.47	12.02	10.72	11
Total -ω6	8.9	8.2	7.4	8.1
n-3/n-6	1.51	1.46	1.44	1.35

^a First and second figures represent carbon chain length: number of double bonds
 The ω values represent the methyl end chain from the centre of double bond furthest from the carboxyl end.

SFA = saturated fatty acids; MUFA = monounsaturated fatty acids;

DUFA = Diunsaturated fatty acids; PUFA = polyunsaturated fatty acids

ND = Not detected (<0.01)

highest (2.49g) in LNM provided treatment followed by WHM (2.08g), AZM (2.01g) and CON (2.00g) provided

treatment. This was 19.71 % higher than WHM, 23.88 % higher than AZM and 24.5% higher than CON and as

expected the feed conversion ratio (FCR) was lowest (2.28) in LNM followed by WHM (2.35), AZM (2.40) and CON (2.85) (Table 3). The specific growth rate was highest (1.09) in LNM fed treatment and lowest (0.70) in CON fed treatment. The protein efficiency ratio (PER) was significantly differed ($P < 0.05$) different treatments. The PER value is highest (1.46) in LNM fed treatment and lowest (0.85) in CON feed treatments. The hepatosomatic index (%) (HSI) was highest (1.75) in LNM fed treatment series and lowest (1.56) in CON feed treatment series (Table 3). The highest (1.64) value of gonadosomatic index (%) (GSI) was observed in LNM feed treatment and the GSI values varied significantly ($P < 0.05$) with WHM (1.04), AZM (1.01) and CON (0.95) feed treatment (Table 3).

The Saturated Fatty Acid (SFA) content (%) was highest (55.0) in WHM fed treatment and lowest (51.5) in CON fed fish. The Mono Unsaturated Fatty Acid MUFA content (%) was highest (27.3) in AZM fed treatment followed by CON (26.0), LNM (25.7) and WHM (24.9) fed fish. The Poly Unsaturated fatty acid (PUFA) content (%) was the highest (16.17) in CON fed fish followed by LNM (14.32), WHM (13.9) and AZM (12.52) fed fish. Fish fed with three kinds of feed formulations exhibits no apparent variations with the CON fed fishes (Table 5), which means that the feeds (Table 4) provide to the fish has no impact on accumulation of PUFA especially n-3 PUFA. The n-3/n-6 ratio also shows little or negligible variations (Table 5).

DISCUSSION

Throughout the experiment, water quality in all treatments remained within the favourable range required by tilapias (Ballarin and Hatton 1979) indicating that these feed could be utilized in tilapia farming pond. Water quality parameters such as temperature, pH, specific conductance, sechi disc transparency, dissolved oxygen and TAN as were found to be very similar in all the tanks and hence their effects on growth of the experimental fish were ignored in evaluating the efficiency of the feeds.

The highest weight gain was observed in the treatment administered with LNM feed. This indicates that fish can consume the feed well. The results show high acceptability for the LNM among cultured tilapia. This was possibly due to their higher palatability and preference of the fish to take it as their potential food. The low FCR of LNM indicates that fish can easily digest the feed and convert these feed into their body mass. The tested value of FCR showed a lower magnitude (2.28) indicating an encouraging effect on economic involvement in fish farming. The protein efficiency ratio was significantly ($P < 0.05$) higher in LNM fed fish than WHM and AZM feed treatment. This indicates the better quality of protein in the feed produced from lemna (LNM). The higher value of GSI

indicates that the LNM has better impact on the reproductive function. The meal prepared from Azolla has the lowest impact on reproductive function when compared to other two meals.

Only 14 acids are really needed to describe the fatty acids of fish' (Ackman, 2000), though he listed 16 fatty acids. In the present study 28 fatty acids were recorded from the fish flesh and feeds. The result is more or less similar to those reported from other tropical and certain temperate zone freshwater fish (Zenebe et al., 1998; Ackman et al., 2002). A great deal of variation is observed in percent lipid among the Indian carps studied and this correlates with other reports for tropical freshwater and marine fish (Andrade et al., 1995 and Zenebe et al., 1998). Lipid components also exhibit variation between the organs and among the fish species.

Fatty acid deficiency in fish species is indicated by the presence of 20:3n-9 acid (Watanabe 1982). Thus, the absence of eicosatrienoic acid in these fish indicates that these fish are not suffering from fatty acid deficiency. This observation corroborates that of Nematipour and Gatlin (1993) for hybrid striped bass in the USA.

A very low n-3/n-6 may increase the thrombogenicity through increased production of arachidonic acid and thromboxane A₂. This is because both linoleic acid and linolenic acid use the same set of enzymes for desaturation and chain elongation (Li et al., 1999). 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 atherogenic (Dewailly et al., 2001; Christensen et al., 2001). The n-3/n-6 ratio proves that the fish under discussion may be classified as Type II lean fish as their n-3/n-6 ratio in most cases is between 1 and 2. In the present study n-3 is seen to increase in LNM fed fish.

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