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Protein-sparing effect of dietary lipid: Changes in growth, nutrient utilization, digestion and IGF-I and IGFBP-I expression of Genetically Improved Farmed Tilapia (GIFT), reared in Inland Ground Saline Water

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ABSTRACT

Protein-sparing by lipid helps in conserving protein exclusively for growth with minimal inevitable protein breakage. It will subsequently reduce feed cost and nitrogenous pollutants in the habitat. In this context, an experiment of 60 days period was conducted to study the protein-sparing effect of lipid in the diet of Genetically Improved Farmed Tilapia (GIFT) (*Oreochromis niloticus* L) fingerlings reared in inland ground saline water (IGSW) at 10 g L^{-1} salinity. The experiment followed 4 × 2 factorial design with eight purified diets of four crude protein (CP) levels (30%, 35%, 40% and 45%), each with two lipid levels (6% and 10%). GIFT fingerlings of uniform size (3.00 ± 0.01 g) were acclimatized and distributed into eight treatment groups in triplicates corresponding to each of the prepared diets and fed to satiation three times daily. The results showed significantly ($p < 0.05$) higher percent weight gain (WG, 693.26%), apparent net protein utilization (ANPU, 34.03), protein efficiency ratio (PER, 2.35) and improved digestive enzymes (protease, amylase, lipase) activity with lower feed conversion ratio (FCR) for diets containing 35–45% CP and 10% lipid. According to two-way ANOVA, high dietary lipid (10%) resulted highest WG% (678.95), PER (2.02), ANPU (30.67) and lowest FCR (1.31) values. Increased whole body CP (15.54%) and lipid (7.61%) contents were observed in the fish fed high lipid (10%) diets. The viscerosomatic and hepatosomatic index also increased with increasing dietary lipid. The dietary CP levels have not significantly affected the body composition except total ash content. The highest insulin like growth factor-I (IGF-I) gene expression was observed in the liver of fish fed 35% CP with 10% lipid. Results revealed that increasing dietary lipid exhibited enhanced protein-sparing effect in GIFT fingerlings. Thus, the study concludes that a diet containing 35% CP and 10% lipid is optimal for GIFT fingerlings reared in IGSW of 10 g L^{-1} salinity.

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1. Introduction

Salinization of inland areas is in an increasing trend globally due to climate change and anthropogenic activities. These areas are characterized by high soil and ground water salinity (Inland Ground Saline Water, IGSW) and hence not suitable for traditional agriculture. Meanwhile, growing demand leads to expansion of aquaculture and looking for new production systems (Fitzsimmons et al., 2011). Thus, IGSW can be a promising new arena for aquaculture (Allan et al., 2009). As IGSW is unfit for drinking and desalinization is expensive, it is profitable to divert the salt-affected land and water towards aquaculture (Doupé et al., 2003). Several euryhaline species were introduced for culture in IGSW but genetically improved farmed tilapia (GIFT - *Oreochromis niloticus)* developed by WorldFish Centre in 1981 through systematic breeding has dominated these waters (Suresh and Lin, 1992; Kamal and Mair, 2005; Bhosle et al., 2018; Stickney, 2017; Singha et al., 2020, 2021). Eknath and Acosta (1998) described GIFT as the premiere version of Nile tilapia (*O. niloticus*) and set a milestone for the tilapia aquaculture industry with the strain showing significant improvement in growth *i.e.,* about 85% more than that of Nile tilapia. Luo et al. (2017) reported remarkable growth of GIFT tilapia with 100% survival when reared in salinity range between 0 and 20 g L^{-1}. It is also known for its high disease resistance, acceptability of different varieties of feed as well as the ability to grow in waters of wide salinity ranges (El-Sayed, 2006; Luo et al., 2017). Therefore, IGSW can be utilized for the culture of GIFT effectively with necessary dietary interventions.

The scope of tilapia as a candidate species is unlimited in India and elsewhere in the world (Prabu et al., 2019). When aquaculture grows expeditiously to meet the rising demands, it adds stress on availability of cost-effective feed (Tacon and Metian, 2015). Hence, the development of feed formulations with each nutrient ensuring minimal wastage without compromising the quality will be rewarding. Among the dietary nutrients, protein is the most important and preferred nutrient for fish but costlier than any other macro-nutrients (NRC, 2011). Protein provides various essential and non-essential amino acids for growth and energy production (De Silva and Anderson, 1995). When excess dietary protein is given, amino acids derived from them are catabolized (Trans-deamination) for energy production leading to ammonia production which can be lethal to fish (Abdel-Tawwab et al., 2010). Hence, protein inclusion in any feed must be judicious. Aklakur (2017) reported that inland saline water is known for its ionic imbalance which could cause impairment in health, physiological status and growth of fish. During such conditions, fish may demand more energy in their diets to compensate the energy loss. Moreover, fish feed on energy satiation, therefore it is very much crucial that the diet must be containing optimal combination protein and non-protein energy supplying nutrients which in turn helps in adequate intake of various essential nutrients within the stipulated quantity of feed intake (Guillaume et al., 2001). Lipids can more effectively conserve protein for development than carbohydrates because of its better bioavailability than any other nutrient in fish. This is referred to as the lipid's protein-sparing action. (Kaushik et al., 1995; Guillaume et al., 2001; Sagada et al., 2017). In the protein-sparing effect of lipids, major part of the energy would be obtained from dietary lipids, enabling protein to be utilized exclusively for growth with minimal inevitable catabolism thereby resulting improved growth and nutrient utilization (El-Sayed and Teshima, 1992; Kaushik et al., 1995; Nankervis et al., 2000; Guillaume et al., 2001; Ng et al., 2008; Sagada et al., 2017). Dietary lipid excess may cause fatty fish and deterioration of muscle quality (Lovell, 1979; El-Sayed and Kawanna, 2008). Hence, to promote optimal growth without excessive nutrient loss, the mix of protein and fat/lipid must be at an ideal level. The insulin like growth factors (IGF), its binding proteins (IGF-BP) and receptor systems are important growth indicators since they act as messengers between the production and targeted action of growth hormone in fish (Moriyama et al., 2000; Fuentes et al., 2013).

Even though there are studies on the requirement and role of particular nutrient in the diet of GIFT as well as tilapia in general *e.g.*, role and optimum dosage of protein (Qiang et al., 2012; Haidar et al., 2018; Singha et al., 2020, 2021; Zeng et al., 2020), lipid (Tian et al., 2015; Xu et al., 2019), carbohydrate to lipid ratio (Kabir et al., 2020), there are no reports to corroborate on the combined effects of protein and lipid *i.e.*, optimal combination of protein and lipid in the diet of GIFT explicitly in inland ground saline water. Considering the above facts, the feeding trial was conducted to study the protein-sparing effect of lipid thereby developing an optimal combination of protein and lipid in the diet of GIFT tilapia reared in IGSW in terms of growth, nutrient utilization, digestion and growth-related gene expressions.

2. Materials and methods

2.1. Location of the experimental trial

Rohtak regional centre (28°86′ N, 76°47′ E) of ICAR-Central Institute of Fisheries Education (ICAR-CIFE) located in the inland saline region of northwest India in Rohtak district.

2.2. Procurement and acclimation of GIFT fingerlings

Fingerlings of GIFT were purchased from the regional centre of Rajiv Gandhi Centre for Aquaculture (RGCA) located at Vijayawada, India. The fish were shipped in aerated water in double-layered plastic packets and delivered to the Rohtak Centre (ICAR-CIFE, Haryana, India). The fish were allowed to float on chlorine free freshwater of 28 ºC temperature in a cement tank (4 m × 3 m × 1 m, 10,000 L capacity) for 30 min for acclimatization. The dissolved oxygen (DO) content of the tank was maintained at 6 g L^{-1} through uninterrupted aeration. Crumbled commercial feed (28% CP and 4% lipid) was used to feed the fish after 24 h of stocking. After a week of initial acclimatization, salinity acclimation was done. The salinity of the water was increased gradually by adding IGSW to achieve an increment of 1 g L^{-1} salinity per day till the required salinity of 10 g L^{-1} was achieved and it was maintained. Feeding was skipped during the addition of IGSW and was continued only 5 h after salinity increment. Acclimation of fish and salinity increment of rearing

media to the desired salinity (10 g L^{-1}) was realized over a period of 17 days.

2.3. Experimental diets

Eight experimental diets were prepared following 4 × 2 factorial design (Table 1) using gelatin and casein, starch and dextrin, sunflower oil and cod liver oil as purified source for the corresponding nutrient *viz* protein, carbohydrate and lipid, respectively. All protein and carbohydrate sources along with heat stable additives (cellulose and carboxy methyl cellulose) were kneaded to make a dough by adding adequate amount of water. The dough was cooked in steam for 20 min. The remaining ingredients (butylated hydroxy toluene, vitamin-mineral mixture, choline chloride, oil, and Stay C) were mixed evenly into the cooked dough. Further, it was squeezed through a pelletizer to make pellets of 1 mm diameter. After drying, the pellets were filled into labelled airtight containers which were then stored in a refrigerator. Eight purified experimental diets were designated as follows: P30L6, P35L6, P40L6, P45L6, P30L10, P35L10, P40L10 and P45L10. Thus eight diets with four levels of protein and two levels of lipid were prepared for different treatments.

2.4. Design and maintenance of the experiment

The experiment was designed to study the protein sparing effect of lipid. Therefore, four protein levels (30%, 35%, 40% and 45%) and each protein level with two lipid levels (low (6%) and high (10%)) were taken. The two different lipid levels were used to help in

Table 1
Formulation and proximate composition of different experimental diets fed to GIFT fingerlings reared in IGSW for the period of 60 days.

Ingredients (g kg^{-1})	Diets[a]							
	P30L6	P35L6	P40L6	P45L6	P30L10	P35L10	P40L10	P45L10
Casein[b]	285.0	334.5	380.0	433.0	285.0	334.5	380.0	433.0
Gelatin[b]	70.0	80.0	94.0	100.0	70.0	80.0	94.0	100.0
Dextrin[b]	120.0	120.0	120.0	120.0	120.0	120.0	120.0	120.0
Starch[b]	320.0	270.0	220.0	170.0	354.8	305.0	255.0	205.0
Cellulose[b]	104.5	95.0	85.5	76.5	29.7	20.0	10.5	1.5
Cod liver oil[c]	30.0	30.0	30.0	30.0	50.0	50.0	50.0	50.0
Sunflower oil[d]	30.0	30.0	30.0	30.0	50.0	50.0	50.0	50.0
CMC[e]	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Vitamin-mineral mix[f]	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Stay C[g]	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
BHT[h]	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Choline Chloride[b]	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Proximate composition (on dry matter basis)								
Moisture (%)	7.28	7.11	7.66	7.37	6.80	6.59	6.69	6.51
Crude protein (%)	30.24	35.06	40.02	45.06	30.25	35.08	40.26	45.11
Ether extract (%)	5.96	5.99	5.96	5.96	9.94	9.88	9.98	9.96
Total ash (%)	2.59	2.57	2.80	2.43	2.76	2.68	2.62	2.55
Crude fibre (%)	9.69	9.89	9.11	10.09	2.55	2.93	2.79	3.29
Nitrogen free extract (%)	44.2	39.38	34.45	29.18	47.71	42.84	37.66	32.68
Gross energy (kJ g^{-1})	18.38	18.39	18.38	18.37	20.98	20.95	20.99	20.96
Available energy (DE[i]) (kJ g^{-1})	14.67	14.68	14.68	14.66	16.76	16.73	16.76	16.73
P:E[j](mg protein kJ^{-1})	20.61	23.87	27.26	30.74	18.05	20.97	24.01	26.95

[a] P30L6, 30% protein and 6% lipid; P35L6, 35% protein and 6% lipid; P40L6, 40% protein and 6% lipid; P45L6, 45% protein and 6% lipid; P30L10, 30% protein and 10% lipid; P35L10, 35% protein and 10% lipid; P40L10, 40% protein and 10% lipid; P45L10, 45% protein and 10% lipid.
[b] Purified ingredients procured from Hi-Media Ltd., India.
[c] Procured from Coastal Exports Pvt. Ltd., Mangalore, India.
[d] Fortune Refined Sunflower Oil procured from DMart, Mumbai, India.
[e] CMC, carboxymethyl cellulose.
[f] Composition of the vitamin-mineral mixture (quantity/kg): Vitamin A, 55,00,000 IU; Vitamin D_3, 11,00,000 IU; Vitamin B_2, 2000 mg; Vitamin E, 750 mg; Vitamin K, 1000 mg; Ascorbic acid, 2500 mg; Vitamin B_6, 1000 mg; Vitamin B_{12}, 6 mcg; Calcium Pantothenate, 2500 mg; Nicotinamide, 10 g; Mn, 27,000 mg; I, 1000 mg; Fe, 7500 mg; Zn, 5000 mg; Cu, 2000 mg; Co, 450 mg; Selenium, 125 mg.
[g] Stay C, ROVIMIX® STAY-C®35 (DSM in Animal Nutrition & Health).
[h] BHT, butylated hydroxytoluene.
[i] DE, digestible energy.
[j] P:E, Protein to energy ratio.

determining the protein sparing effect of lipid with respect to efficient protein utilization in the diet. Thus, the experiment followed 4 × 2 factorial design, where eight treatment groups were designed, triplicated into 24 circular fibre reinforced plastic (FRP) tanks. Three hundred and sixty (360) GIFT fingerlings (3.00 ± 0.01 g) were distributed into circular FRP tanks of 0.5 m^3 volume (diameter 96.4 cm × height 69 cm), tanks were filled to three fifty litres (350 L) of IGSW (10 g L^{-1} salinity). The fish were stocked at 15 fish per tank. Experimental units were aerated vigorously and fitted with thermostatic electric water heaters with set temperature of 28 ºC. The diet respective to the treatment group was fed manually thrice daily (08:00, 14:00, 20:00 h) until satiation of the fish.

2.5. Water quality analysis

Physico-chemical parameters of the rearing media such as, DO, pH, salinity and temperature, total alkalinity (TA), total hardness (TH), calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions were measured by methods suggested by APHA (2005). Water temperature was measured using a digital thermometer while DO and pH were measured using automated probe (Hanna instruments, USA). A refractometer (Atago S/Milli-E, Japan) was used to ascertain the water salinity. The concentration of sodium (Na^{2+}) and potassium (K^{+}) ions were estimated using a flame photometer (ESICO, Model 1382, India).

2.6. Sampling

Initial sampling of the fish was done after overnight starvation prior to stocking. 15 days interval samplings were carried to observe the progress of the experiment. The final growth sampling of different experimental groups was also carried out after overnight starvation on the 61st day of experiment. The whole-body composition was analysed using a group of five fish collected from each of the experimental tanks. Another set of randomly collected fish (3 in number) from each experimental unit were anaesthetized by using clove oil (50 μL L^{-1}) and the visceral organs were collected for enzymatic and gene expression studies. The intestines of the anaesthetized fish were dissected and used to analyse the activity of digestive enzymes. Screw-cap cryo-tubes (1 mL) filled with RNAlater™ solution were used to collect the liver tissues for analysis.

2.7. Growth, nutrient utilization and survival

Growth and nutrient utilization parameters of the fish were calculated as follows:

$$\text{Percent weight gain (WG\%)} = [\{\text{Final body weight (g)-Initial body weight (g)}\}/\text{Initial body weight (g)}] \times 100$$

$$\text{Feed conversion ratio (FCR)} = \text{Feed intake (dry weight in g)}/\text{Body weight gain (wet weight in g)}$$

$$\text{Protein efficiency ratio (PER)} = \text{Body weight gain (wet weight in g)}/\text{Protein intake (dry weight in g)}$$

$$\text{Apparant net protein utilization (ANPU)(\%)} = [\{\text{Final body protein (\%)-Initial body protein (\%)}\}/\text{Initial body protein (\%)}] \times 100$$

The survival % of the experiment was calculated as follows,

$$\text{Survival (\%)} = (\text{Total number of fish at the end of trial}/\text{Total number of fish stocked at the beginning}) \times 100$$

2.8. Analysis of proximate composition

The chemical composition (Table 1) of experimental diets and whole carcass of fish were done following the methods proposed by AOAC (1995). The crude protein (CP) was estimated multiplying total nitrogen value with 6.25. Diethyl ether was used as the solvent to get the ether extract (EE)/lipid of the dried samples in a Socs-plus (SCS 08 AS, Pelican Equipments, India). Total ash (TA) content was calculated by incinerating a known weight of the sample in a muffle furnace at 550 °C for a period of 6 h. Crude fibre (CF) portion of fat free dry samples of the ingredients and experimental diets was determined by digestion in an acid (1.25% hydrochloric acid) and alkali (1.25% sodium hydroxide) in FibroTRON (Tulin Equipments, India). The digested sample was oven dried at 100 ± 2 ºC, and placed in muffle furnace at 550 ºC for 6 h for incineration. Bomb calorimeter (5E-AC/PL, Changsha Kaiyuan Instruments Co., Ltd., China) was used to analyse the gross energy (GE) of feed ingredients and experimental diets following the manufacturer's protocol. The total available energy/digestible energy (DE) was calculated by means of their physiological fuel value of major nutrients such as protein (16.7 kJ g^{-1}), nitrogen free extract (16.7 kJ g^{-1}) and lipid (36.7 kJ g^{-1}) (Halver, 1976; Guillaume et al., 2001).

The nitrogen free extract (NFE) of the ingredients as well as the experimental diets and the total carbohydrate (TC) of the fish were calculated by the following formula-

$$\text{NFE} = 100\text{-}(\text{CP\%} + \text{EE\%} + \text{CF\%} + \text{TA\%})$$

$$\text{TC} = 100\text{-}(\text{CP\%} + \text{EE\%} + \text{TA\%})$$

The following formula was used to calculate the protein to energy ratio (P:E) of various diets-

$$\text{P:E (mg CP/kJ DE)} = \{(\text{CP\%} \times 1000)/\{\text{DE (kJ/g)} \times 100\}$$

2.9. Determination of body indices

The weight of whole viscera and liver (hepatic) was measured and used for calculating body indices such as viscerosomatic index (VSI) and hepatosomatic index (HSI). The values were calculated as follows;

$$\text{VSI}\ (\%) = (\text{Wet weight of the viscera}\ (\text{g}))/(\text{Wet weight of the fish}\ (\text{g})) \times 100$$

$$\text{HSI}\ (\%) = (\text{Wet weight of the liver}\ (\text{g}))/(\text{Wet weight of the fish}\ (\text{g})) \times 100$$

2.10. Enzyme assessment

2.10.1. Tissue homogenate preparation

The intestinal samples collected from the fish at the final sampling were homogenized using a mechanical homogenizer. This homogenate was prepared as a 5% tissue homogenate by adding adequate amount of refrigerated (4 °C) sucrose solution (0.25 M). To prevent the occurrence of any enzymatic activity, the entire homogenization was set up in ice cold condition by keeping the sample tubes in flake ice throughout the process. After centrifugation, the supernatant was separated and transferred into 2 mL eppendorf tubes. These tubes were stored at −20 °C until further use.

2.10.2. Tissue protein analysis

The method of Lowry et al. (1951) was employed to ascertain the protein in the intestinal tissue.

2.10.3. Analysis of activities of protease, amylase and lipase

Method elaborated by Drapeau (1974) following the casein digestion principle was employed to study protease activity and the result was expressed in release of tyrosine (micromole) per minute per milligram protein. Method suggested by Rick and Stegbauer (1974) was applied for amylase activity estimation. The activity of lipid digesting enzymes (lipase) was estimated by using the method of Cherry and Crandall (1932) and expressed as unit/hour/milligram protein.

2.11. Gene expression studies

2.11.1. Preparation of cDNA

The total RNA from the liver tissue was extracted by using TRIzol™ reagent, (Thermofisher scientific, USA). A Nano-Drop spectrophotometer (Thermofisher scientific, USA), was used to analyse the purity (260/280). Electrophoresis was also done to ensure the quantity and integrity of the RNA.

2.11.2. Primer designing

The reported primers of IGF-I, IGF-BPI, and β-actin (Singha et al., 2020) were used for the Real-Time qRT-PCR (Table 2). The house keeping gene was β-actin (reference gene).

2.11.3. mRNA expression using real time qRT-PCR

A Real-Time qRT-PCR System (AriaMx Real-Time PCR System, Agilent Technologies, USA) was used to quantify the relative expression of IGF-I and IGF-BPI genes. The 10 μL reaction mixture was prepared by mixing 5 μL SYBR Green, qPCR Master Mix, 1 μL of cDNA, 3 μL of nuclease free water (NFW) and 1 μL of each gene specific primer and used for quantification. Each qRT-PCR cycle consists of denaturation (95 °C for 15 s), annealing (59.3 °C for 15 s) and extension (59.3 °C for 60 s). The Ct value (Threshold) gave the amount of mRNA in the sample melting curve analysis was performed for each cycle. IGF-BPI and IGF-I mRNA expression was calculated by using method of Livak and Schmittgen (2001) ($2^{-\Delta\Delta CT}$ method).

Table 2
Primers used for gene expression study in real-time PCR for GIFT fingerlings reared in IGSW and fed with different experimental diets for the period of 60 days.

Target gene	Accession number	Primer sequence	Amplicon size
β-actin[a]	KJ126772.1	FP 5′-AATCCTGCGGAATCCACGAAAC-3′ RP 5′-CTCCTTCTGCATCCTGTCAGCG-3′	140
IGF-I[b]	EU272149.1	FP 5′-GGACGAGTGCTGCTTCCAAAGC-3′ RP 5′-TGCTCTTGGCATGTCTGTGTGC-3′	121
IGF-BPI[c]	XM_003438121.3	FP 5′-CCACTGGCGTTTCCTCAATGG-3′ RP 5′-GATGAGCAACCCATCCCAAACC-3′	128

[a] β-actin as housekeeping or reference gene.
[b] IGF-I, Insulin like growth factor-I.
[c] IGF-BPI, Insulin like growth factor- binding protein I.

$$\Delta Ct = Ct(\text{gene of target})\text{-}Ct\ (\beta\text{-actin gene} *)$$

$$\Delta\Delta Ct = \Delta Ct(\text{Treatment group})\text{-}\Delta Ct \quad (\text{Control group} **)$$

Where, TG, Treatment Group; CG, Control group; *Reference gene; **In present study, T1 (30% CP and 6% lipid) was the control group.

2.12. Statistical analysis

Duncan's multiple range test was used to compare the means when they showed significant difference ($p < 0.05$) at 95% confidence level in the ANOVA. Duncan's multiple range test was also applied to study the effects and interaction of different variables.

3. Results

3.1. Physico-chemical parameters of water

The water temperature ranged from 27.5 to 28.8 ^{o}C. Total hardness and total alkalinity were measured to be 2800-32002800–3200 mg L^{-1} and 240–280 mg L^{-1}, respectively. The salinity of the water ranged from 9.5 to 10.5 g L-1. Throughout the experiment, no CO_2 was discovered in the water. Ca^{2+} and Mg^{2+} concentrations were in the range 320–350 mg L^{-1} and 480–520 mg L^{-1}, respectively. The K^{+} concentration was found to be between 8.5 and 12 mg L^{-1}. The pH of the water was determined to be between 7.5 and 8.2.

3.2. Growth and nutrient utilization

In the present study, the two-way ANOVA analysis showed that individual effect of dietary lipid and dietary protein had significant ($p < 0.05$) effect on the growth and nutrient utilization of fish (Table 3). However, the interaction effect was not observed in weight

Table 3
Growth and nutrient utilization of GIFT fingerlings reared in IGSW and fed with different experimental diets for the period of 60 days.

Treatments[1]	WG[2] (%)	FCR[3]	PER[4]	ANPU[5]
P30L6	584.88^{a}	1.53^{b}	2.15^{c}	30.90^{bc}
P35L6	631.05^{b}	1.41^{b}	2.16^{c}	28.80^{b}
P40L6	624.89^{ab}	1.42^{b}	1.73^{ab}	25.98^{b}
P45L6	606.67^{ab}	1.47^{ab}	1.51^{a}	23.69^{a}
P30L10	629.87^{b}	1.41^{b}	2.35^{c}	31.43 ^{cd}
P35L10	712.37^{c}	1.25^{a}	2.22^{c}	34.03^{bcd}
P40L10	693.26^{c}	1.28^{a}	1.86^{b}	31.656 ^{cd}
P45L10	680.31^{c}	1.31^{a}	1.67^{ab}	25.56^{a}
Pooled SEM[6]	13.41	0.03	0.08	0.34
p value	0.00	0.00	0.00	0.00
Effect of dietary lipid (%)				
6	611.87^{a}	1.46^{b}	1.89^{a}	27.34^{a}
10	678.95^{b}	1.31^{a}	2.02^{b}	30.67^{b}
SEM[6]	6.71	0.02	0.04	0.42
p value	0.00	0.00	0.02	0.00
Effect of dietary protein (%)				
30	607.38^{a}	1.47^{b}	2.25^{c}	31.17^{c}
35	671.71^{b}	1.33^{a}	2.19^{c}	31.41^{c}
40	659.08^{b}	1.35^{a}	1.79^{b}	28.82^{b}
45	643.49^{b}	1.39^{a}	1.59^{a}	24.63^{a}
SEM[6]	9.48	0.02	0.054	0.59
p value	0.00	0.00	0.00	0.00
Combined effect of dietary lipid and dietary protein				
p value	0.58	0.86	0.82	0.02

Data are expressed as Mean (n = 3). Mean values in the same column bearing different superscripts differ significantly ($p < 0.05$), Duncan's multiple range test was used to compare means.

[1] P30L6, 30% protein and 6% lipid; P35L6, 35% protein and 6% lipid; P40L6, 40% protein and 6% lipid; P45L6, 45% protein and 6% lipid; P30L10, 30% protein and 10% lipid; P35L10, 35% protein and 10% lipid; P40L10, 40% protein and 10% lipid; P45L10, 45% protein and 10% lipid.

[2] WG (%), Percent weight gain.

[3] FCR, Feed conversion ratio.

[4] PER, Protein efficiency ratio.

[5] ANPU, Apparent net protein utilization.

[6] SEM, Standard error of mean.

gain %, feed conversion and protein efficiency ratio. But, effect of dietary lipid and protein had a significant interaction effect on ANPU. Feeding of 10% dietary lipid improved the weight gain %, PER and ANPU compared to 6%. Among the dietary protein levels, 35%, 40% and 45% showed a significantly higher ($p < 0.05$) weight gain% and a lower ($p < 0.05$) FCR. However, the fish fed with 30% and 35% dietary protein showed a significantly higher PER values.

The one-way ANOVA analysis showed that ANPU was significantly increased in P30L10, P35L10 and P40L10 groups, whereas P45L6 recorded the lowest ANPU value. However, higher weight gain % and lower FCR were recorded in P35L10, P40L10 and P45L10 groups.

3.3. Whole body composition of GIFT fingerlings

According to two-way ANOVA, dietary lipid content had significant effect ($p < 0.05$) on body moisture, CP and total lipid. There was an inverse relationship between body moisture and dietary lipid levels. Feeding of 6% dietary lipid increased the body moisture level compared to 10% dietary lipid level. Similarly, with the increase in dietary lipid level the body lipid levels and protein levels also increased considerably ($p < 0.05$).

The different levels of dietary protein had significant ($p < 0.05$) effect only on total ash levels. The 30% dietary protein reduced the total ash. The interaction effect was also observed only in total ash values and it was noted that P30L6, P35L6, P30L10, P40L10 and P45L10 groups showed lower total ash levels (Table 4).

Table 4
Whole body proximate composition (on % wet weight basis) of GIFT fingerlings reared in IGSW and fed with different experimental diets for the period of 60 days.

Treatments[1]	Moisture	CP[2]	TL[3]	TC[4]	TA[5]
P30L6	75.29^{c}	14.19	5.74^{a}	2.50	2.28^{a}
P35L6	75.13^{c}	14.76	5.85^{a}	1.84	2.42^{ab}
P40L6	73.92^{bc}	15.60	6.02^{a}	1.80	2.66^{b}
P45L6	74.13^{bc}	15.14	5.79^{a}	2.25	2.69^{b}
P30L10	72.90^{ab}	15.18	7.24^{b}	2.47	2.21^{a}
P35L10	72.37^{a}	16.24	7.36^{b}	1.37	2.65^{b}
P40L10	72.02^{a}	15.78	7.98^{b}	1.89	2.33^{a}
P45L10	71.78^{a}	15.37	7.86^{b}	2.53	2.46^{ab}
Pooled SEM[6]	0.46	0.46	0.32	0.48	0.09
p value	0.00	0.15	0.00	0.62	0.01
Effect of dietary lipid (%)					
6	74.62^{b}	14.92^{a}	5.85^{a}	2.09	2.51
10	72.27^{a}	15.64^{b}	7.61^{b}	2.07	2.41
SEM[7]	0.23	0.23	0.17	0.05	0.05
p value	0.00	0.04	0.00	0.93	0.14
Effect of dietary protein (%)					
30	74.10	14.68	6.49	2.49	2.25^{a}
35	73.75	15.50	6.60	1.61	2.54^{b}
40	72.97	15.69	7.01	1.85	2.49^{b}
45	72.96	15.26	6.82	2.39	2.58^{b}
SEM[6]	0.32	0.23	0.23	0.34	0.06
p value	0.06	0.19	0.41	0.24	0.01
Combined effect of dietary lipid and dietary protein					
p value	0.83	0.45	0.72	0.88	0.03

Data are expressed as Mean (n = 3). Mean values in the same column bearing different superscripts differ significantly ($p < 0.05$), Duncan's multiple range test was used to compare means.

[1] P30L6, 30% protein and 6% lipid; P35L6, 35% protein and 6% lipid; P40L6, 40% protein and 6% lipid; P45L6, 45% protein and 6% lipid; P30L10, 30% protein and 10% lipid; P35L10, 35% protein and 10% lipid; P40L10, 40% protein and 10% lipid; P45L10, 45% protein and 10% lipid.

[2] CP, crude protein.

[3] TL, total lipid.

[4] TC, total carbohydrate.

[5] TA, total ash.

[6] SEM, Standard error of mean.

Table 5
Body indices and digestive enzymes activity of GIFT fingerlings reared in IGSW and fed with different experimental diets for the period of 60 days.

Treatments[1]	VSI[2] (%)	HSI[3] (%)	Protease[4]	Amylase[5]	Lipase[6]
P30L6	3.15^{a}	2.25	0.25^{d}	0.33	0.11^{a}
P35L6	3.73^{a}	2.38	0.28^{e}	0.33	0.12^{ab}
P40L6	5.05^{b}	2.34	0.31^{f}	0.32	0.12^{ab}
P45L6	5.40^{b}	2.43	0.34^{g}	0.38	0.16^{ab}
P30L10	6.09^{bc}	2.39	0.21^{a}	0.34	0.13^{ab}
P35L10	6.54^{cd}	2.47	0.22^{b}	0.33	0.19^{bc}
P40L10	7.56^{de}	2.46	0.24^{c}	0.34	0.24^{c}
P45L10	8.28^{e}	2.59	0.26^{d}	0.31	0.17^{bc}
Pooled SEM[7]	0.27	0.08	0.01	0.04	0.03
p value	0.00	0.08	0.00	0.948	0.021
Effect of dietary lipid (%)					
6	4.33^{a}	2.35^{a}	0.30^{b}	0.34	0.12^{a}
10	7.12^{b}	2.48^{b}	0.23^{a}	0.33	0.19^{b}
SEM[7]	0.17	0.04	0.01	0.02	0.01
p value	0.00	0.02	0.00	0.08	0.01
Effect of dietary protein (%)					
30	4.62^{a}	2.32	0.23^{a}	0.34	0.12
35	5.13^{a}	2.43	0.25^{b}	0.33	0.16
40	6.31^{b}	2.40	0.27^{c}	0.33	0.18
45	6.84^{b}	2.51	0.30^{d}	0.35	0.16
SEM[7]	0.24	0.14	0.02	0.03	0.00
p value	0.00	0.22	0.00	0.07	0.14
Combined effect of dietary lipid and dietary protein					
p value	0.02	0.03	0.00	0.58	0.15

Data are expressed as Mean (n = 3). Mean values in the same column bearing different superscripts differ significantly ($p < 0.05$), Duncan's multiple range test was used to compare means.

[1] P30L6, 30% protein and 6% lipid; P35L6, 35% protein and 6% lipid; P40L6, 40% protein and 6% lipid; P45L6, 45% protein and 6% lipid; P30L10, 30% protein and 10% lipid; P35L10, 35% protein and 10% lipid; P40L10, 40% protein and 10% lipid; P45L10, 45% protein and 10% lipid.

[2] VSI, Viscero-somatic index.

[3] HSI, Hepato-somatic index.

[4] Protease activity expressed as millimole tyrosine released per mg protein/min.

[5] Amylase activity expressed in the terms of micromole maltose released per mg protein/min.

[6] Lipase activity expressed in units per mg protein per h.

[7] SEM, Standard error of mean.

3.4. Body indices and digestive enzymes activity

Feeding of different dietary lipid levels, protein levels and the interaction between lipid and protein levels showed a significant ($p < 0.05$) effect on viscerosomatic index while only the lipid levels and interaction between lipid and protein levels had a significant ($p < 0.05$) effect on hepato-somatic index. VSI and HSI were significantly increased due to higher lipid levels in the diet, while feeding of 40% and 45% protein levels increased the VSI values. One-way ANOVA showed dietary treatments had significant effect on VSI with the highest value in P40L10 and P45L10 groups. However, HSI was not affected by dietary treatments.

The protease activity was significantly ($p < 0.05$) affected by the dietary lipid and protein levels and the interaction between lipid and protein levels (Table 5). However, amylase was neither affected by interaction of dietary lipid and protein levels nor protein or

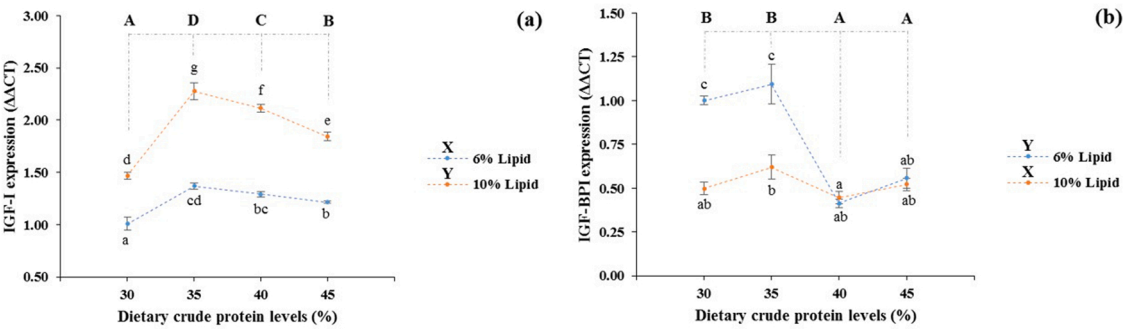

Fig. 1. (a) Hepatic IGF-I and (b) IGF-BPI expression of GIFT fingerlings reared in IGSW and fed with different experimental diets for the period of 60 days. Data presented as interaction plots between dietary crude protein (CP) levels (%) and lipid (%) (n = 3; mean ± standard error). A significant interaction effect of dietary CP × lipid was observed for both IGF-I (*p* values for effect of dietary CP levels, < 0.001; lipid, < 0.001; and interaction, < 0.001) and IGF-BPI (*p* values for effect of dietary CP levels, < 0.001; lipid, < 0.001; and interaction, < 0.001).

lipid levels. Similarly, Lipase was unaffected by the dietary protein and interaction of dietary lipid and protein levels. Activity of protease enzyme was directly proportional to the protein content of the diets *i.e.*, as it increased, the protease activity also increased and it was highest (6.84) in the diet with 45% dietary protein. Furthermore, increasing dietary lipid resulted decrease in the protease activity. Lipase activity increased significantly ($p < 0.05$) with the increasing dietary lipid. According to one way ANOVA, the dietary treatments had significant effect on protease and lipase activity and didn't affect the amylase activity. Highest protease activity was observed in P45L6 group and lowest (0.21) in P30L10 group. Whereas, highest lipase activity was observed in P45L10 (0.24) and lowest in P30L6 group.

3.5. Hepatic IGF-I and hepatic IGF-BPI expression

Dietary treatment significantly ($p < 0.05$) affected the expression of both IGF-I and IGF-BPI (Fig. 1a and 1b, respectively). According to one way ANOVA the highest and lowest expression of IGF-I was recorded in liver of P35L10 and P30L6 groups, respectively. Whereas, the expression of hepatic IGF-BPI was highest and lowest in P30L6 and P40L6 groups, respectively. However, the hepatic IGF-BPI expression of P40L6 was similar to P45L6, P30L10, P40L10 and P45L10 groups.

According to two-way ANOVA, there was an inverse relation in hepatic IGF-I and IGF-BPI expression due to effect of dietary lipid. The expression of hepatic IGF-I was higher in high lipid (10%) fed group than low lipid (6%) fed group and it was inverse for the expression of hepatic IGF-BPI. The hepatic IGF-I expression was highest in 35% dietary protein fed group; this was observed from the effect of dietary protein. In case of IGF-BPI, the expression was decreased while dietary protein level increased. Combined effect of dietary lipid and dietary protein on both hepatic IGF-I and IGF-BPI expression was found to be significant ($p < 0.05$).

4. Discussion

4.1. Physico-chemical parameters of water

Physico-chemical quality of water plays a predominant role in the well-being of the animals reared in the water; hence, it is inevitable that the various parameters are maintained within acceptable limits for aquaculture. None of the parameters showed any significant ($p > 0.05$) difference among the treatments. Throughout the experimental period, the temperature ranged from 27.5 to 28.8 °C which is within optimum range for tilapia culture (Rakocy, 1989). Dissolved oxygen was maintained within ideal range (> 3.5 mg L^{-1}) proposed for rearing of tilapia (DeLong et al., 2009). The fish were gradually acclimated to a salinity of 10 g L^{-1} and this was in coherence with the suggestions of Lawson and Anetekhai (2011) and Suresh and Lin (1992). The alkalinity, hardness, Ca^{2+}, Mg^{2+} and K^{+} concentrations were higher than the normal rearing environment as the experiment was carried out in IGSW. The pH and osmolality of the water was in the tolerable limits by tilapia found in the highly alkaline waters in many parts of the world (Bergman et al., 2003). In conclusion, the water quality of the IGSW used in this experiment was suitable for rearing of GIFT fingerlings.

4.2. Growth and nutrient utilization

In the present study, feeding of 35%, 40% and 45% protein had shown similar values of growth and nutrient utilization and at 30% level the growth was significantly reduced. Improvement of growth and nutrient utilization of tilapia was observed when dietary protein was incremented up to 35% level beyond which there was no further improvements. Consistent with our observation, Kaushik et al. (1995) reported that Nile tilapia exhibited improved growth rate and nutrient utilization with the diet containing 35% CP. This is substantiated by Hidalgo and Alliot (1988) and Kim et al. (1991) who pointed out that excess dietary protein beyond the optimum level would lead to increase in the breakdown of amino acids as fuel for various metabolic reactions, thus reducing their availability for growth. This can be the reason for the reduced growth in the treatments with higher dietary CP in the present experiment. On the contrary, in the case of lipids, compared to low dietary lipid (6%), fish fed high dietary lipid (10%) groups exhibited significantly ($p < 0.05$) higher WG%, PER, ANPU and decreased FCR. This is corroborated by Ng and Chong (2004) who assessed minimum lipid requirement of tilapia and found the optimum lipid requirement to be between 10% and 15%. Additionally, Mohanta et al. (2008a) reported better growth performance of silver barb when the dietary lipid content increased from 4% to 9%. Interpretation of the PER and ANPU values in the present study shows that high lipid (10%) fed groups showed higher utilization of dietary protein than the low lipid fed groups. This high lipid would have spared protein effectively helping to enhance the nutrient utilization and growth of GIFT.

4.3. Body composition

The whole-body chemical composition of fish is critically influenced by the nutrient profile of the diet it consumes (Chowdhury et al., 2021; De Silva et al., 1991; Singha et al., 2021). In the present study, the increasing dietary lipid content had shown to increase body CP and lipid content but decreased the body moisture content. The increase in the body protein content might be due to the protein-sparing effect of lipid in relation to energy production leading to higher accretion of protein in the body of the fish (Kaushik et al., 1995). But, the increasing trend in total body lipid content might be due to feeding of diet with higher lipid resulting in deposition of excess lipid in the body (Ali et al., 2008; Sagada et al., 2017). El-Sayed and Teshima (1992) also reported that increase in dietary protein beyond 35% with increasing dietary lipid results in increased body fat content in Nile tilapia. Further, in the present study an inverse relationship between body moisture and lipid was observed. Similar findings were also reported by Cho et al. (2005) in juvenile turbot; Ali et al. (2008) in Nile tilapia; Singh et al. (2009) in *Clarias batrachus* fry and Phumee et al. (2009) in *Pangasius*

hypophthalmus fry. Dietary protein beyond 30% (35–45% CP) significantly ($p < 0.05$) enhanced the body TA content of GIFT. Similar results were also observed in brown trout (Elliott, 1976) and *Puntius gonionotus* (Mohanta et al., 2008b). However, body moisture, protein, lipid and carbohydrate contents were unchanged.

4.4. Body indices and digestive enzyme activities

Commonly, HSI and VSI are closely related to dietary lipid and are eventual indicators of the lipid deposition in fish (Ma et al., 2014; Sagada et al., 2017). In our research also VSI and HSI values were influenced by dietary lipid level. Increasing dietary lipid led to increased HSI and VSI values and lipid deposition. This is evident in the total lipid (TL) content in the proximate composition of the whole body of fish. The findings reported by Ma et al. (2014) in *Monopterus albus* and Sagada et al. (2017) in *Channa argus* confirms the same. Dietary protein was not exhibiting any significant effect in HSI. Similar results were reported in sunshine bass (Gallagher, 1999); in Nile tilapia (Abdel-Tawwab et al., 2010) and in *Channa argus* (Sagada et al., 2017).

Digestion and nutrient absorption are the most crucial factors that influence the fish growth. Digestion is carried out by various substrate specific digestive enzymes (Sagada et al., 2017; Singha et al., 2021). The highest protease activity was seen in P45L6 group and the least activity in P30L10 group. This may be due to the excess protein intake in the groups fed higher dietary protein with lower dietary energy (Guillaume et al., 2001). Whereas, in higher energy fed groups *i.e.*, P30L10 group, high energy and less protein resulted in less protein intake, consequently less protein digestion. Effect of dietary protein displayed an increasing trend in the protease activity and had no relation with the lipase activity. As the dietary protein level increased, the protease activity also increased. This coincides with the activity observed in *Clarius batrachus* (Mukhopadhyay et al., 1978) and *Pangasianodon hypothalamus* (Jayant et al., 2018). However, the groups fed diet with high lipid showed comparatively less protease activity. Lipase activity was increased due to dietary lipid increase. This can be attributed to high lipid intake in the high lipid (10%) fed groups than the low lipid (6%) fed group. Lipase activity was studied in milk fish (Borlongan and Benitez, 1990); sharptooth catfish (Uys and Hecht, 1987) and *Channa argus* (Sagada et al., 2017) which also exhibited increased enzymatic activity with increase in the lipid content in the diet, just as the results obtained in this set of experiments.

4.5. Hepatic IGF-I and IGF-BPI gene expression

Fish growth is largely regulated by growth hormone (GH) which is controlled by mitogenic peptide called IGF-I (Humbel, 1990; Magdeldin et al., 2007). The IGF-I is produced by various organs in vertebrates such as gut, liver and kidney. However, hepatic IGF-I is considered as primary mediator of growth hormone-dependent growth in fish (Etherton, 1993; Kumar et al., 2019; Singha et al., 2020). In this study, IGF-I expression in liver (hepatic IGF-I) was directly proportional to protein level up to 35% protein level but further increment in protein level exhibited decreasing trend of IGF-I. These findings are similar to those reported in GIFT (Singha et al., 2020) however, the IGF-BPI results of this experiment were contrary. Similar to the influence of protein, increase in dietary lipid also resulted in increased IGF-I expression reaffirming the role of IGF-I in fish growth as observed by Kumar et al. (2019). Experiments in several fishes such as seabream (Pérez-Sánchez et al., 1995); salmonids (Beckman et al., 1998); *O. niloticus* (Vera Cruz et al., 2006); GIFT strain (Singha et al., 2020, 2021) and *Cirrhinus molitorella* (Jiang et al., 2010) proved significant correlation between growth rate and IGF-I. The growth parameter (WG %) of this experiment also supports the results of IGF-I expression. Similar results were reported that the IGF-I expression was found to be similar with the trend of WG% and SGR (Kumar et al., 2019). Combined effect of crude protein and dietary lipid was found to significantly ($p < 0.05$) influence the IGF-I and IGF-BPI expression. The role of IGF-I in protein, lipid, carbohydrate and minerals metabolism in fish was previously established by Moriyama et al. (2000). However, it must be noted that the IGF-BPI results are in agreement with the results of Kumar et al. (2019) but in contrary to the results of Singha et al. (2020). IGF-BP influence growth in fish as they are found to bind tightly to the IGF making it unavailable in the process of growth (Mir et al., 2019). There are six types of IGF-BP (1–6) reported and some of them were found to be positive and some negative in regulation of IGF in fish (Wood et al., 2005; Triantaphyllopoulos et al., 2020). Among the binding proteins, IGF-BPI is prominently expressed in unfavourable conditions such as fasting (Duan, 2002), hypoxia (Kajimura et al., 2005), nutrient deprivation (Shimizu et al., 2006), stress/cortisol injections (Picha et al., 2008). Thus, it can be inferred that IGFBP-I has an inverse relationship with IGF-I expression as observed in tilapia (Breves et al., 2014) and flounder (Safian et al., 2012). This correlates with the results of this experiment which had inverse relationship of IGF and IGFBP-I proving that higher protein and lipid content in diet promoted growth by increasing IGF-I expression. Furthermore, the authors of this study suggest that mRNA expression of peptide transporter (PEPT), cathepsinD (CatD), cathepsinF (CatF), calpain 2, glucosidase, triglyceride lipase (ATGL), fatty acid synthetase (FAS), and acetyl co-A carboxylase (ACC) could provide more information about fish growth and metabolism.

5. Conclusion

The findings demonstrate that protein sparing effect of lipid exhibits better growth, nutrient utilization and digestive enzymes activity in GIFT fingerlings reared in IGSW of 10 g L^{-1}. It is also further supported by enhanced hepatic IGF-I gene expression. Thus, it may be concluded from these interpretations that a diet with combination of 35% CP and 10% lipid is optimal for the digestion, nutrient utilization, growth and growth-related gene expression of GIFT fingerlings reared in IGSW of 10 g L^{-1} salinity.

CRediT authorship contribution statement

R. Thirunavukkarasar: Conceptualization, Data curation, Formal analysis, Methodology, Software, Writing – original draft, Writing – review & editing. **Pankaj Kumar:** Conceptualization, Data curation, Supervision, Validation, Writing – original draft, Writing – review & editing. **Narottam Prasad Sahu:** Conceptualization, Data curation, Writing – original draft, Writing – review & editing. **Parimal Sardar:** Conceptualization, Data curation, Writing – original draft, Writing – review & editing. **V. Harikrishna:** Methodology, Writing – original draft, Writing – review & editing. **Krishna Pada Singha:** Formal analysis, Software, Writing – original draft, Writing – review & editing. **N. Shamna:** Formal analysis, Software, Writing – original draft, Writing – review & editing. **Jane Jacob:** Formal analysis, Software, Writing – original draft, Writing – review & editing. **Gopal Krishna:** Conceptualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Abdel-Tawwab, M., Ahmad, M.H., Khattab, Y.A., Shalaby, A.M., 2010. Effect of dietary protein level, initial body weight and their interaction on the growth, feed utilization and physiological alterations of Nile tilapia, *Oreochromis niloticus*. Aquaculture 298 (3–4), 267–274. https://doi.org/10.1016/j.aquaculture.2009.10.027.

Aklakur, M.D., 2017. Nutritional intervention for sustainable production in inland saline aquaculture a budding perspective in India. J. Aquac. Mar. Biol. 6, 172. https://doi.org/10.15406/jamb.2017.06.00172.

Ali, A., Al-Ogaily, S.M., Al-Asgah, N.A., Goddard, J.S., Ahmed, S.I., 2008. Effect of feeding different protein to energy (P/E) ratios on the growth performance and body composition of *Oreochromis niloticus* fingerlings. J. Appl. Ichthyol. 24 (1), 31–37. https://doi.org/10.1111/j.1439-0426.2007.00897.x.

Allan, G.L., Fielder, D.S., Fitzsimmons, K.M., Applebaum, S.L., Raizada, S., 2009. Inland saline aquaculture. In: New Technologies in Aquaculture. Woodhead Publishing, pp. 1119–1147. https://doi.org/10.1533/9781845696474.6.1119.

AOAC, 1995. Animal feed. In: Cunniff, P.A. (Ed.), Official Methods of Analysis of AOAC International. AOAC International, Arlington, USA, pp. 5–15.

APHA, 2005. Standard Methods for the Examination of Water and Wastewater, twenty-first ed. American Public Health Association,, Washington DC, p. 1220.

Beckman, R.B., Donald, A.L., Moriyama, S., Beeda, Lee-Pawlak, Walton, W.D., 1998. Insulin-like growth factor-I and environmental modulation of growth during smoltification of Spring Chinook Salmon (*Oncorhynchus tshawytscha*). Gen. Comp. Endocrinol. 109 (3), 325–335. https://doi.org/10.1006/gcen.1997.7036.

Bergman, A.N., Laurent, P., Otiang'a-Owiti, G., Bergman, H.L., Walsh, P.J., Wilson, P., Wood, C.M., 2003. Physiological adaptations of the gut in the Lake Magadi tilapia, *Alcolapia grahami*, an alkaline- and saline-adapted teleost fish. Comp. Biochem. Physiol. Part A. Mol. Integr. Physiol. 136, 701–715. https://doi.org/10.1016/S1095-6433(03)00223-X.

Bhosle, R.V., Chavan, B.R., Meshram, S.J., Dhamagaye, H.B., Wasave, S.S., Pai, R., 2018. Growth performance of gift tilapia (*Oreochromis niloticus*) fry in nursery rearing by using brackish water. In: “Empowerment Rural Communities Aquaculture”(ERCA-2018), p. 31.

Borlongan, I.G., Benitez, L.V., 1990. Quantitative lysine requirement of milkfish (*Chanos chanos*) juveniles. Aquaculture 87 (3–4), 341–347. https://doi.org/10.1016/0044-8486(90)90071-T.

Breves, J.P., Tipsmark, C.K., Stough, B.A., Seale, A.P., Flack, B.R., Moorman, B.P., Lerner, D.T., Grau, E.G., 2014. Nutritional status and growth hormone regulate insulin-like growth factor binding protein (igfbp) transcripts in Mozambique tilapia. Gen. Comp. Endocrinol. 207, 66–73. https://doi.org/10.1016/j.ygcen.2014.04.034.

Cherry, I.S., Crandall, L.A., 1932. The specificity of pancreatic lipase: its appearance in the blood after pancreatic injury. Am. J. Physiol. Leg. Content 100 (2), 266–273. https://doi.org/10.1152/ajplegacy.1932.100.2.266.

Cho, S.H., Lee, S.M., Lee, S.M., Lee, J.H., 2005. Effect of dietary protein and lipid levels on growth and body composition of juvenile turbot (*Scophthalmus maximus* L) reared under optimum salinity and temperature conditions. Aquac. Nutr. 11 (4), 235–240. https://doi.org/10.1111/j.1365-2095.2005.00338.x.

De Silva, S.S., Gunasekera, R.M., Shim, K.F., 1991. Interactions of varying dietary protein and lipid levels in young red tilapia: evidence of protein sparing. Aquaculture 95, 305–318. https://doi.org/10.1016/0044-8486(91)90096-P.

Chowdhury, D.K., Sahu, N.P., Sardar, P., Deo, A.D., Bedekar, M.K., Singha, K.P., Maiti, M.K., 2021. Feeding turmeric in combination with ginger or garlic enhances the digestive enzyme activities, growth and immunity in Labeo rohita fingerlings. Anim. Feed Sci. Technol. 277, 114964 https://doi.org/10.1016/j.anifeedsci.2021.114964.

De Silva, S.S., Anderson, T.A., 1995. Fish Nutrition in Aquaculture, Vol. 1. Chapman & Hall, New York.

DeLong, D.P., Losordo, T., Rakocy, J., 2009. Tank culture of tilapia. SRAC Publication No. 282. North Carolina State University, Southern Regional Aquaculture Center.

Doupé, R.G., Lymbery, A.J., Starcevich, M.R., 2003. Rethinking the land: the development of inland saline aquaculture in Western Australia. Int. J. Agric. Sustain. 1, 30–37. https://doi.org/10.3763/ijas.2003.0104.

Drapeau, G., 1974. Protease from *Staphylococcus aureus*. Methods Enzymol. 45, 469–475. https://doi.org/10.1016/S0076-6879(76)45041-3.

Duan, C., 2002. Specifying the cellular responses to IGF signals: roles of IGF-binding proteins. J. Endocrinol. 175, 41–54.

Eknath, A.E., Acosta, B.O., 1998. Genetic improvement of farmed tilapias (GIFT) project: final report, March 1988 to December 1997. ⟨https://doi.org/10.1093/jn/125.3.546⟩.

Elliott, J.M., 1976. Energy losses in the waste products of brown trout (*Salmo trutta*). J. Anim. Ecol. 45, 561–580. https://doi.org/10.2307/3891.

El-Sayed, A.F.M., 2006 Tilapia culture in salt water: environmental requirements, nutritional implications and economic potentials. In: Eighth Symposium on Advances in Nutritional Aquaculture, pp. 15–17.

El-Sayed, A.F.M., Teshima, S.I., 1992. Protein and energy requirements of Nile tilapia, *Oreochromis niloticus* fry. Aquaculture 103 (1), 55–63. https://doi.org/10.1016/0044-8486(92)90278-S.

El-Sayed, A.F.M., Kawanna, M., 2008. Effects of dietary protein and energy levels on spawning performance of Nile tilapia (Oreochromis niloticus) broodstock in a recycling system. Aquaculture 280 (1–4), 179–184.

Etherton, T.D., 1993. Insulin-like growth factors: role in growth and development. In: The Endocrinology of Growth, Development, and Metabolism in Vertebrates, pp. 197–217.
Fitzsimmons, K., Martinez-Garcia, R., Gonzalez-Alanis, P., 2011. Why tilapia is becoming the most important food fish on the planet? In: Liu, L.P., Fitzsimmons, K. (Eds.), Proceedings of the 9th International Symposium on tilapia in Aquaculture, Shanghai, pp. 1–8.
Fuentes, E.N., Valdés, J.A., Molina, A., Björnsson, B.T., 2013. Regulation of skeletal muscle growth in fish by the growth hormone–insulin-like growth factor system. Gen. Comp. Endocrinol. 192, 136–148. https://doi.org/10.1016/j.ygcen.2013.06.009.
Gallagher, M.L., 1999. Growth responses, tissue composition, and liver enzyme changes in juvenile sunshine bass, Morone chrysops× M. saxatilis, associated with dietary protein and lipid level. J. Appl. Aquac. 9 (4), 41–51.
Guillaume, J., Kaushik, S., Bergot, P., Metailler, R., 2001. Nutrition and Feeding of Fish and Crustaceans. Springer, New York.
Haidar, M.N., Bleeker, S., Heinsbroek, L.T.N., Schrama, J.W., 2018. Effect of constant digestible protein intake and varying digestible energy levels on energy and protein utilization in Nile tilapia. Aquaculture 489, 28–35. https://doi.org/10.1016/j.aquaculture.2017.12.035.
Halver, J.E., 1976. The nutritional requirements of cultivated warm water and cold water fish species. Paper No. 31. FAO Technical Conference on Aquaculture. 26, 9–12.
Hidalgo, F., Alliot, E., 1988. Influence of water temperature on protein requirement and protein utilization in juvenile sea bass, *Dicentrarchus labrax*. Aquaculture 72 (1–2), 115–129. https://doi.org/10.1016/0044-8486(88)90152-4.
Humbel, R.E., 1990. Insulin-like growth factors I and II. Eur. J. Biochem 190, 445–462. https://doi.org/10.1111/j.1432-1033.1990.tb15595.x.
Jayant, M., Muralidhar, A.P., Sahu, N.P., Jain, K.K., Pal, A.K., Srivastava, P.P., 2018. Protein requirement of juvenile striped catfish, *Pangasianodon hypophthalmus*. Aquac. Int. 26, 375–389. https://doi.org/10.1007/s10499-017-0216-0.
Jiang, J., Zhang, D., Qiu, L., Lin, H., Jiang, S., 2010. Research on assessing effects of diets of mud carp (*Cirrhinus molitorella*) using IGF-I mRNA expression level. South China Fish. Sci. 6 (2), 66–72.
Kabir, K.A., Verdegem, M.C., Verreth, J.A., Phillips, M.J., Schrama, J.W., 2020. Effect of dietary carbohydrate to lipid ratio on performance of Nile tilapia and enhancement of natural food in pond aquaculture. Aquac. Res. 51 (5), 1942–1954. https://doi.org/10.1111/are.14546.
Kajimura, S., Aida, K., Duan, C., 2005. Insulin-like growth factor-binding protein-1 (IGFBP-1) mediates hypoxia-induced embryonic growth and developmental retardation. Proc. Natl. Acad. Sci. USA 102, 1240–1245. https://doi.org/10.1073/pnas.0407443102.
Kamal, A.H.M.M., Mair, G.C., 2005. Salinity tolerance in superior genotypes of tilapia, Oreochromis niloticus, Oreochromis mossambicus and their hybrids. Aquaculture 247 (1–4), 189–201. https://doi.org/10.1016/j.aquaculture.2005.02.008.
Kaushik, S.J., Doudet, T., Médale, F., Aguirre, P., Blanc, D., 1995. Protein and energy needs for maintenance and growth of Nile tilapia (*Oreochromis niloticus*). J. Appl. Ichthyol. 11 (3–4), 290–296. https://doi.org/10.1111/j.1439-0426.1995.tb00029.x.
Kim, K.I., Kayes, T.B., Amundson, C.H., 1991. Purified diet development and re-evaluation of the dietary protein requirement of fingerling rainbow trout (*Oncorhynchus mykiss*). Aquaculture 96 (1), 57–67. https://doi.org/10.1016/0044-8486(91)90139-X.
Kumar, S., Sahu, N.P., Ranjan, A., Gupta, S., Sardar, P., Kumar, M., Gupta, G., 2019. Optimization of de-oiled rice bran inclusion level in the diet of *Labeo rohita*: Effect on growth performance, nutrient utilization, insulin-like growth factors I and II gene expression. Aquac. Nutr. 25 (2), 477–485. https://doi.org/10.1111/anu.12873.
Lawson, E.O., Anetekhai, M.A., 2011. Salinity tolerance and preference of hatchery reared Nile tilapia, *Oreochromis niloticus* (Linneaus, 1758). Asian J. Agric. Sci. 3 (2), 104–110.
Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25 (4), 402–408. https://doi.org/10.1006/meth.2001.1262.
Lovell, R.T., 1979. Factors affecting voluntary food consumption by channel catfish [Ictalurus punctatus]. In: Proceedings of the Annual Conference Southeastern Association of Fish and Wildlife Agencies.
Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.
Luo, G., Li, W., Tan, H., Chen, X., 2017. Comparing salinities of 0, 10 and 20 in biofloc genetically improved farmed tilapia (*Oreochromis niloticus*) production systems. Aquac. Fish. 2, 220–226. https://doi.org/10.1016/j.aaf.2017.10.002.
Ma, X., Hu, Y., Wang, X.Q., Ai, Q.H., He, Z.G., Feng, F.X., Lu, X.Y., 2014. Effects of practical dietary protein to lipid levels on growth, digestive enzyme activities and body composition of juvenile rice field eel (Monopterus albus). Aquac. Int. 22 (2), 749–760. https://doi.org/10.1007/s10499-013-9703-0.
Magdeldin, S., Uchida, K., Hirano, T., Grau, E.G., Abdelfattah, A., Nozaki, M., 2007. Effects of environmental salinity on somatic growth and growth hormone/insulin-like growth factor-I axis in juvenile tilapia *Oreochromis mossambicus*. Fish. Sci. 73, 1025–1034. https://doi.org/10.1111/j.1444-2906.2007.01432.x.
Mir, I.N., Srivastava, P.P., Bhat, I.A., Jaffar, Y.D., Sushila, N., Sardar, P., Kumar, S., Muralidhar, A.P., Jain, K.K., 2019. Optimal dietary lipid and protein level for growth and survival of catfish Clarias magur larvae. Aquaculture. https://doi.org/10.1016/j.aquaculture.2019.734678.
Mohanta, K.N., Mohanty, S.N., Jena, J.K., Sahu, N.P., 2008a. Optimal dietary lipid level of silver barb, *Puntius gonionotus* fingerlings in relation to growth, nutrient retention and digestibility, muscle nucleic acid content and digestive enzyme activity. Aquac. Nutr. 14 (4), 350–359. https://doi.org/10.1111/j.1365-2095.2007.00542.x.
Mohanta, K.N., Mohanty, S.N., Jena, J.K., Sahu, N.P., 2008b. Protein requirement of silver barb, *Puntius gonionotus* fingerlings. Aquac. Nutr. 14 (2), 143–152. https://doi.org/10.1111/j.1365-2095.2007.00514.x.
Moriyama, S., Ayson, F.G., Kawauchi, H., 2000. Growth regulation by insulin-like growth factor-I in fish. Biosci., Biotechnol. Biochem. 64 (8), 1553–1562. https://doi.org/10.1271/bbb.64.1553.
Mukhopadhyay, P.K., Dehadrai, P.V., Banerjee, S.K., 1978. Studies on intestinal protease: isolation, purification and effect of dietary proteins on alkaline protease activity of the air-breathing fish, *Clarias batrachus* (L). Hydrobiologia 57 (1), 11–15. https://doi.org/10.1007/BF00018622.
Nankervis, L., Matthews, S.J., Appleford, P., 2000. Effect of dietary non-protein energy source on growth, nutrient retention and circulating insulin-like growth factor I and triiodothyronine levels in juvenile barramundi, *Lates calcarifer*. Aquaculture 91 (4), 323–335. https://doi.org/10.1016/S0044-8486(00)00436-1.
Ng, W.K., Chong, C.Y., 2004. An overview of lipid nutrition with emphasis on alternative lipid sources in tilapia feeds. Aquaculture 233, 423–437.
Ng, W.K., Abdullah, N., De Silva, S.S., 2008. The dietary protein requirement of the Malaysian mahseer, Tor tambroides (Bleeker), and the lack of protein-sparing action by dietary lipid. Aquaculture 284 (1–4), 201–206.
NRC, 2011. In: National Research Council (Ed.), Nutrient Requirements of Fish and Shrimp. The National Academies Press, Washington, DC, USA, p. 70. https://doi.org/10.17226/13039.
Phumee, P., Hashim, R., Aliyu-Paiko, M., Shu-Chien, A.C., 2009. Effects of dietary protein and lipid content on growth performance and biological indices of iridescent Shark (*Pangasius hypophthalmus*, Sauvage 1878) fry. Aquac. Res. 40 (4), 456–463. https://doi.org/10.1111/j.1365-2109.2008.02116.x.
Picha, M.E., Turano, M.J., Beckman, B.R., Borski, R.J., et al., 2008. Endocrine biomarkers of growth and applications to aquaculture: a minireview of growth hormone, insulin-like growth factor (IGF)-I, and IGF-binding proteins as potential growth indicators in fish. N. Am. J. Aquac. 70 (2), 196–211. https://doi.org/10.1577/A07-038.1.
Prabu, E., Rajagopalsamy, C.B.T., Ahilan, B., Jeevagan, I.J.M.A., Renuhadevi, M., 2019. Tilapia–an excellent candidate species for world aquaculture: a review. Annu. Res. Rev. Biol. 1–14. https://doi.org/10.33745/ijzi.2019.v05i02.005.
Qiang, J., Yang, H., Wang, H., Kpundeh, M.D., Xu, P., 2012. Interactive effects of temperature-dietary protein level on somatotropic gene expression and its interrelationship with growth in juvenile GIFT tilapia Oreochromis niloticus. Aquaculture 364, 263–271. https://doi.org/10.1016/j.aquaculture.2012.08.021.
Rakocy, J.E., 1989. Tank Culture of Tilapia. Southern Regional Aquaculture Center, pp. 523–529.
Rick, W., Stegbauer, H.P., 1974. α-Amylase: measurement of reducing groups. Methods Enzym. Anal. 2, 885–915. https://doi.org/10.1016/B978-0-12-091302-2.50074-8.
Safian, D., Fuentes, E.N., Valds, J.A., Molina, A., 2012. Dynamic transcriptional regulation of autocrine/paracrine igfbp1, 2, 3, 4, 5, and 6 in the skeletal muscle of the fine flounder during different nutritional statuses. J. Endocrinol. 214 (1), 95–108. https://doi.org/10.1530/JOE-12-0057.

Sagada, G., Chen, J., Shen, B., Huang, A., Sun, L., Jiang, J., Jin, C., 2017. Optimizing protein and lipid levels in practical diet for juvenile northern snakehead fish (*Channa argus*). Anim. Nutr. 3 (2), 156–163. https://doi.org/10.1016/j.aninu.2017.03.003.

Shimizu, M., Beckman, B.R., Hara, A., Dickhoff, W.W., 2006. Measurement of circulating salmon IGF binding protein-1: assay development, response to feeding ration and temperature, and relation to growth parameters. J. Endocrinol. 188, 101–110. https://doi.org/10.1677/joe.1.06475.

Singh, R.K., Desai, A.S., Chavan, S.L., Khandagale, P.A., 2009. Effect of water temperature on dietary protein requirement, growth and body composition of Asian catfish, *Clarias batrachus* fry. J. Therm. Biol. 34 (1), 8–13. https://doi.org/10.1016/j.jtherbio.2008.08.005.

Singha, K.P., Shamna, N., Sahu, N.P., Sardar, P., HariKrishna, V., Thirunavukkarasar, R., Kumar, M., Krishna, G., 2020. Feeding graded levels of protein to genetically improved farmed Tilapia (GIFT) juveniles reared in inland saline water: Effects on growth and gene expression of IGFI, IGF-IR and IGF-BPI. Aquaculture 525, 735306. https://doi.org/10.1016/j.aquaculture.2020.735306.

Singha, K.P., Shamna, N., Sahu, N.P., Sardar, P., Harikrishna, V., Thirunavukkarasar, R., Chowdhury, D.K., Maiti, M.K., Krishna, G., 2021. Optimum dietary crude protein for culture of genetically improved farmed tilapia (GIFT), *Oreochromis niloticus* (Linnaeus, 1758) juveniles in low inland saline water: effects on growth, metabolism and gene expression. Anim. Feed Sci. Tech. 271, 114713 https://doi.org/10.1016/j.anifeedsci.2021.114713.

Stickney, R.R., 2017. Tilapia feeding habits and environmental tolerances. In: Tilapia in Intensive Co-culture. John Wiley & Sons, New Jersey, pp. 25–35.

Suresh, A.V., Lin, C.K., 1992. Tilapia culture in saline waters: a review. Aquaculture 106 (3–4), 201–226. https://doi.org/10.1016/0044-8486(92)90253-H.

Tacon, A.G., Metian, M., 2015. Feed matters: satisfying the feed demand of aquaculture. Rev. Fish. Sci. Aquac. 23 (1), 1–10. https://doi.org/10.1080/23308249.2014.987209.

Tian, J., Wu, F., Yang, C.G., Jiang, M., Liu, W., Wen, H., 2015. Dietary lipid levels impact lipoprotein lipase, hormone-sensitive lipase, and fatty acid synthetase gene expression in three tissues of adult GIFT strain of Nile tilapia, Oreochromis niloticus. Fish. Physiol. Biochem. 41 (1), 1–18. https://doi.org/10.1007/s10695-014-0001-1.

Triantaphyllopoulos, K.A., Cartas, D., Miliou, H., 2020. Factors influencing GH and IGF-I gene expression on growth in teleost fish: how can aquaculture industry benefit? Rev. Aquac. 12 (3), 1637–1662. https://doi.org/10.1111/raq.12402.

Uys, W., Hecht, T., 1987. Assays on the digestive enzymes of sharptooth catfish, *Clarias gariepinus* (Pisces: Clariidae). Aquaculture 63, 301–313. https://doi.org/10.1016/0044-8486(87)90080-9.

Vera Cruz, E.M., Brown, C.L., Luckenbach, J.A., Picha, M.E., Bolivar, R.B., Borski, R.J., 2006. Insulin-like growth factor-I cDNA cloning, gene expression and potential use as a growth rate indicator in Nile tilapia, *Oreochromis niloticus*. Aquaculture 251, 585–595. https://doi.org/10.1016/j.aquaculture.2005.06.039.

Wood, A.W., Duan, G., Bern, H.A., 2005. Insulin-like growth factor signaling in fish. Int. Rev. Cytol. 243 (1), 215–285. https://doi.org/10.1016/s0074-7696(05)43004-1.

Xu, F., Xu, C., Xiao, S., Lu, M., Limbu, S.M., Wang, X., Du, Z., Qin, J.G., Chen, L., 2019. Effects of α-lipoic acid on growth performance, body composition, antioxidant profile and lipid metabolism of the GIFT tilapia (Oreochromis niloticus) fed high-fat diets. Aquac. Nutr. 25 (3), 585–596. https://doi.org/10.1111/anu.12881.

Zeng, N.N., Jiang, M., Wen, H., Liu, W., Wu, F., Tian, J., Yu, L.J., Lu, X., Guo, Z.B., 2020. Effects of water temperatures and dietary protein levels on growth, body composition and blood biochemistry of juvenile GIFT tilapia (Oreochromis niloticus). Aquac. Nutr. https://doi.org/10.1111/anu.13181.