Received: 4 August 2019 | Revised: 19 January 2020 | Accepted: 22 January 2020

DOI: 10.1111/anu.13050

ORIGINAL ARTICLE

Aquaculture Nutrition WILEY

Effects of dietary protein on growth performance, nutrient utilization, digestive enzymes and physiological status of grey mullet, *Mugil cephalus* L. fingerlings reared in inland saline water

Avinash Talukdar[1] | Ashutosh Dharmendra Deo[1] | Narrotam Prasad Sahu[1] | Parimal Sardar[1] | Muhammad Aklakur[1] | Satya Prakash[2] | Nazeema Shamna[1] | Sarvendra Kumar[3]

[1]Fish Nutrition, Biochemistry and Physiology Division, ICAR – CIFE, Mumbai, India

[2]ICAR-Central Institute of Fisheries Education, Rohtak Centre, Lahli, India

[3]Birsa Agricultural University, Ranchi, India

Correspondence
Ashutosh Dharmendra Deo, Principal Scientist, FNBP Division, CIFE, Mumbai 400 061, India.
Email: ashutosh@cife.edu.in

Funding information
Indian Council of Agricultural Research

Abstract

A 60 days feeding trial was conducted to illustrate the effect of graded levels of protein on the growth and metabolic enzymes of grey mullet (*Mugil cephalus* L.) fingerlings reared in inland saline water (ISW). Six isoenergetic (16 MJ/kg) and isolipidic (60 g/kg) diets containing 240, 260, 280, 300, 320 and 340 g crude protein (CP)/kg diet were formulated and fed to triplicate. Weight gain %, specific growth rate, protein utilizing efficiency, feed efficiency and RNA:DNA ratio were significantly higher ($p < .05$) in 320 and 300 g CP/kg diets. Fish fed with 240 g CP/kg diet showed significantly higher ($p < .05$) feed intake, whole-body lipid content, hepatosomatic index value and liver glycogen content. Transaminase enzymes and malate dehydrogenase activities were elevated in fish fed 340 g CP/kg diet. Protease activity increased with increasing dietary CP level, but amylase activities showed an inverse relationship. No significant ($p > .05$) variations were observed for lactate dehydrogenase, oxidative stress enzymes, blood parameters and serum osmolality among all the treatment groups, but red blood cell count increases with increasing dietary CP levels. Based on the results, feeding dietary protein level of 300 g CP/kg is economically viable for rearing of grey mullet in ISW.

KEYWORDS

dietary protein requirement, grey mullet, growth, inland saline water, physiological status, protein utilization

1 | INTRODUCTION

The world aquaculture production continues its pace to meet the projected aquaculture production of 109 million tonnes by 2030 (FAO, 2018). Alternate and sustainable use of available water resources has become a necessity due to the growing population pressure. Among such resources, the salt-affected land, which renders over more than 100 million hectares worldwide and expected to rise by 50% in 2050, could be a potential alternate site for aquaculture activity (Jamil, Riaz, Ashraf, & Foolad, 2011; Partridge, Lymbery, & George, 2008). The saline groundwater of salt-affected areas is neither being utilized for drinking purposes nor for agriculture purpose, but can be used for aquaculture. The variation in the ionic composition of inland saline water (ISW), particularly with lower K^+ and higher Ca^{2+} and Mg^{2+} ions than seawater at given salinity (Jana, Garg, & Patra, 2004; Saoud, Davis, & Rouse, 2003), can have physiological

and growth disturbance in the cultured fish species (Aklakur, 2017). Amelioration of ISW with fortification of K^+ ion has been used for culture of *Mugil cephalus* (Barman, Jana, Garg, Bhatnagar, & Arasu, 2005), *Chanos chanos* (Jana, Garg, Barman, Arasu, & Patra, 2006), *Sciaenops ocellatus* (Fielder, Bardsley, & Allan, 2001), *Argyrosomus japonicas* (Doroudi, Fielder, Allan, & Webster, 2006), *Pagrus auratus* (Fielder & Allan, 2003), *Lates calcarifer* and *Oncorhynchus mykiss* (Partridge, Sarre, Ginbey, Kay, & Jenkins, 2006). The suitability for culture of euryhaline fish using ISW has been successfully evaluated in many countries, including China, Thailand, Vietnam, Ecuador, Brazil, Mexico, United States, Australia and India (Allan, Fielder, Fitzsimmons, Applebaum, & Raizada, 2009; Forsberg, Dorsett, & Neill, 1996; Jana et al., 2004). In India, culture of euryhaline fish such as *M. cephalus* and *C. chanos* is successfully carried out in ISW (Barman et al., 2005; Jana et al., 2006).

The stripped grey mullet, *M. cephalus* L., is found worldwide in tropical and subtropical waters contributing to valuable fisheries along the coastal and estuarine regions of many countries (De Silva, 1980). If salinity increased gradually, *M. cephalus* can acclimatized to salinities exceeding 100 g/L (Hotos & Vlahos, 1998). Mullets are described as omnivorous fish (Whitfield, Panfili, & Durand, 2012), ecologically important species feeding at the lowest trophic level (Lupatsch, Katz, & Angel, 2003), thus suitable for mono and polyculture system (Abdel-Hakim, Hussein, Bakeer, & Soltan, 2001). In 2016, the mullet production in the world was 0.077 MMT of which only 23% was contributed from the culture fisheries (FAO, 2018). In recent years, there has been a growing economic interest for culture of grey mullets in India due to its high price, excellent meat quality and culturing flexibility particularly in coastal and ISW. Previous reports on the rearing of grey mullets in ISW were on culture methods (Biswas et al., 2012; Garg, Jana, & Arasu, 2006; Jana et al., 2004) and optimum salinity level (Barman et al., 2005). Therefore, information on its nutritional requirements particularly protein is a prerequisite for developing a nutritionally balanced cost-effective feed. Besides, no commercial feed is available for mullet species which could be a bottleneck in enhancing the mullet culture and production. The response to growth of fish in relation to its nutritional requirement (particularly protein) in challenged environment of ISW is the key concern to explore the optimum dietary formulation for cost-effective growth potential of the mullets. Further, the nutrient requirement may differ in ISW reared fish as the reorganization of physiological processes during salinity acclimatization may alter growth (Tseng & Hwang, 2008; Wootton, 1990).

Taking advantage of the feeding preference of grey mullets towards benthic and detritus microalgae, artificial low-cost feeds can be formulated using alternate plant protein source ingredients (De, Ghoshal, Biswas, Mukherjee, & Kumar, 2018; El-Dahhar, Salama, Moustafa, & Elmorshedy, 2014; Gisbert, Mozanzadeh, Kotzamanis, & Estévez, 2016; Jana, Sudesh, Garg, Sabhlok, & Bhatnagar, 2012; Kalla, Garg, Kaushik, Arasu, & Dinodia, 2003; Wassef, El Masry, & Mikhail, 2001). In this context, leaf meal or plant protein sources can be the cheapest source of protein in the fish diet, which is often economically and environmentally sustainable (Adewolu, Adeniji, & Adejobi, 2008). The leaf meal of terrestrial legume plant, *Sesbania bispinosa* having crude protein content of 22%–30% (Kaitho et al., 1998), can be an alternative plant ingredient in fish feed, subjected to its effect on growth performance and physiological status of fish.

For any culture initiative of fish, knowledge of the optimum dietary protein requirement is essential to formulate nutritionally and economically feasible aquafeed (Khan & Jafri, 1990; Sealey et al., 2013). Mostly, protein requirements studies are carried out using purified research diets (NRC, 1993). However, in practical terms, purified diets have no such applications in commercial level feeding practices due to its high price. In this context, locally available plant protein ingredients as protein source could be a practical approach to develop a low-cost feed. There are a few studies on protein requirement of grey mullet in brackish water condition (De, Ghoshal, & Kundu, 2012) and on different protein sources (Luzzana et al., 2005). However, optimal protein requirement of grey mullet may vary in ISW due to the difference in culture condition. Therefore, the present study was conducted to elucidate the effect of graded levels of dietary protein on growth performance, nutrient utilization, digestive enzymes and physiological status of *M. cephalus* fingerlings reared in ISW.

2 | MATERIALS AND METHODS

2.1 | Diet preparation

Six experimental diets were formulated to be isoenergetic (16 MJ/kg) and isolipidic (60 g/kg lipid) containing 240, 260, 280, 300, 320 and 340 g crude protein (CP)/kg diet (Table 1). Sesbania leaf meal, fish meal, groundnut oil cake and defatted soybean meal were used as a main protein source and deoiled rice bran and wheat flour as an energy source to maintain the isoenergetic diet. α-cellulose, carboxymethyl cellulose (CMC) and butylated hydroxytoluene (BHT) were used as filler, binder and antioxidant in the diets, respectively. All the practical ingredients were thoroughly mixed with water (70% W/V) to make dough and steam cooked in a pressure cooker for half an hour. Additives, oil and vitamins mineral mixture (Emix Plus) were incorporated into the dough after cooling, and the dough was pressed through a pelletizer (Ace Exports) of 2 mm diameter to prepare the sinking pellets. The feed pellets were air-dried followed by drying in hot air oven at 50°C until the moisture level was below 100 g/kg, labelled according to the treatments and stored at 4°C until use.

2.2 | Experimental design and feeding trial

Groundwater having a salinity of 10 g/L from bore well was pumped out into four cemented tanks (length 300 cm × width 200 cm × water depth 150 cm) following filtration through 100 μm

TABLE 1 Ingredients and proximate composition of the experimental diets

	Dietary protein levels (g CP/kg diet)					
Composition	**240**	**260**	**280**	**300**	**320**	**340**
Ingredients (g/kg)						
SLM[a]	100	100	100	100	100	100
Fish meal[b]	50	50	50	50	50	50
GNOC[b]	120	150	180	210	240	270
DSBM[c]	140	170	200	230	260	290
DORB[b]	120	120	120	120	120	120
Wheat flour[b]	340	280	220	160	100	40
Vit-Min mix[d]	15	15	15	15	15	15
Betaine[c]	5	5	5	5	5	5
Cellulose[c]	43	43	43	43	43	43
Fish oil:Veg oil (1:1)	50	50	50	50	50	50
BHT[c]	2	2	2	2	2	2
CMC[c]	15	15	15	15	15	15
Total	1,000	1,000	1,000	1,000	1,000	1,000
Proximate composition of the diet (g/kg; on dry matter basis; mean of triplicates)						
Moisture	74.0	76.0	73.8	74.2	73.7	74.1
Crude protein	242.0	262.6	283.7	302.6	321.1	341.9
Ether extract	62.4	61.9	62.6	62.8	63.6	62.9
Total ash	63.8	64.7	64.9	67.2	65.0	67.4
Crude fibre	55.3	53.6	53.0	52.7	51.4	52.3
Gross energy (MJ/kg)	16.07	16.05	16.07	16.04	16.10	16.05
P/E ratio (g/MJ)	15.0	16.3	17.7	18.9	20.1	21.3

Note: Proximate composition: GNOC (CP—430 g/kg, CL—35 g/kg, TA—106 g/kg, CF—65 g/kg) and DSBM (CP—520 g/kg, CL—8 g/kg, TA—65 g/kg, CF—43 g/kg).

Abbreviations: BHT, butylated hydoxytoluene; CMC, carboxymethyl cellulose; DORB, deoiled rice bran; DSBM, defatted soybean meal; GNOC, groundnut oil cake; SLM, Sesbania leaf meal.

Vitamin A, 55,00,000 IU; vitamin D_3, 11,00,000 IU; vitamin B_2, 2,000 mg; vitamin E, 750 mg; vitamin K, 1,000 mg; vitamin B6, 1,000 mg; vitamin B_{12}, 6 µg; calcium pantothenate, 2,500 mg; nicotinamide, 10 g; choline chloride, 150 g; Mn, 27,000 mg; I, 1,000 mg; Fe, 7,500 mg; Zn, 5,000 mg; Cu, 2,000 mg; Co, 450 mg; L-lysine, 10 g; DL-methionine, 10 g; selenium, 125 mg; vitamin C, 2,500 mg.

[a]Collected from local village, Assam, India.

[b]Procured from local market, India.

[c]Procured from HImedia ltd.

[d]Composition of vitamin mineral mix (Premix Plus) (quantity/2 kg).

filter bag to remove any unwanted debris (Raizada et al., 2015). After settlement for a week, groundwater was diluted to 8 g/L salinity using freshwater and then transferred to six circular storage tanks (diameter 105 cm × water depth 89 cm, 770 L capacity) as per the experimental needs. Fingerlings of grey mullets *M. cephalus* were collected from the estuarine water of Sunderban area, West Bengal, India, by the local fish farmers and were safely transported to wet laboratory of Central Institute of Fisheries Education (CIFE), Rohtak, Haryana, India, in airtight oxygen-filled containers at 6 g/L salinity. The fish were randomly distributed into four circular tubs (diameter 60 cm × water depth 35.5 cm, 100 L capacity) followed by a gradual increase in water salinity from 6 to 8 g/L for 1 hr using ISW (8 g/L) from the storage tanks. After salinity adjustment, the fish were transferred to another circular tank (diameter105 cm × water depth 89 cm, 770 L capacity) having ISW salinity of 8 g/L and kept for 15 days for acclimatization to the experimental condition. Fingerlings of grey mullets (2.7 ± 0.5 g, 6.4 ± 0.06 cm) were randomly stocked into triplicate tanks (diameter 88.5 cm × water depth 53 cm, 325 L capacity) for each treatment groups following a completely randomized design (CRD) with 15 fish for each tank with 40/m^2 stocking density. Based on the initial observation of feed consumption, all groups of fish were hand-fed twice a day at 0900 and 1800 hr for 60 days. The tanks were kept in open sunlight inside the wet laboratory throughout the experimental period. About 70% of tank water

with excreta was siphoned every alternate day and replaced by an equal volume of well-aerated storage water. At every 15 days interval, all the fish from each tank were weighed to determine growth and feed requirement. Water quality parameters including water temperature, dissolved oxygen concentration, pH (Systronics, pH system 361), total alkalinity, ammonia, potassium, calcium and magnesium were recorded in 7 days interval following standard methods of APHA (2005). The salinity of water was recorded in every alternate day with handheld refractometer (Atago).

2.3 | Sampling procedure

Before commencing the trial, 20 fish were randomly selected and kept for whole-body proximate composition analysis. At the end of the experimental period (60 days), three fish per replicate were anaesthetized with clove oil (50 μl/L) for the collection of blood and serum. Blood samples were drawn from the caudal vein using a 1 ml hypodermic syringe (without anticoagulant) and transferred immediately into Eppendorf tubes and kept 2 hr at room temperature for clotting. After this, blood was centrifuged for 10 min at 4850 *g* to obtain serum. Subsequently, remaining blood sample was transferred to an Eppendorf tube containing EDTA, gently shaken in order to prevent haemolysis of blood and kept for analysis. Muscle sample was collected from the same fish and used for the analysis of glycogen and nucleic acid content. Again, following anaesthetized with clove oil (50 μl/L), remaining fish were sampled for enzyme assays (three fish per replicate) and whole-body composition (three fish per replicate). Dissected tissues of different organs (intestine, liver, muscle and gill) were immediately homogenized with cold 0.25 M sucrose solution in a glass tube using Teflon-coated mechanical tissue homogenizer (MICCRA D-9, ART Prozess & Labortechnik) to prepare a 5% tissue homogenate. The homogenate samples were centrifuged at 10,000 × *g* for 10 min in a cooling centrifuge (Thermo Scientific) at 4°C, and the supernatant was collected in sample vials and stored at −20°C until use. Liver and intestine from three fish per replicate are dissected out, and weight was taken for calculation of hepatosomatic index (HSI) and intestinal somatic index (ISI). Quantification of the protein of different tissues was carried out by Lowry, Rosebrough, Farr, and Randall (1951) method.

2.4 | Growth performance, protein utilization and body indices

The growth and nutrient utilization parameters were calculated based on following formulae (Ma et al., 2019):

$$\text{Weight gain \% (WG)} = (\text{Final weight} - \text{Initial weight}/\text{Initial weight}) \times 100,$$

$$\text{Specific growth rate (SGR,\%/day)} = [\text{Log}_e(\text{Final weight}) - \text{Log}_e(\text{Initial weight})]/[\text{Number of days}] \times 100,$$

$$\text{Feed efficiency (FE)} = \text{Net weight gain (in g on wet weight basis)}/\text{Feed consumed (in g on dry weight basis)},$$

$$\text{Feed intake (FI,\%/day)} = \text{Feed fed (g)}/[\text{days} \times (\text{initial body weight} + \text{final body weight}/2)] \times 100,$$

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

$$\text{Apparent net protein utilization \% (ANPU)} = (\text{Final body protein} - \text{Initial body protein})/(\text{total protein fed}) \times 100,$$

$$\text{Protein growth rate (PGR, \%/day)} = [\text{Log}_e(\text{Final body protein}) - \text{Log}_e(\text{Initial body protein})]/[\text{Number of days}] \times 100,$$

$$\text{Survival (\%)} = (\text{Final no. of fish harvested}/\text{Initial no. of fish stocked}) \times 100,$$

$$\text{Hepatosomatic index (HSI) (\%)} = (\text{wet weight of liver}/\text{whole body- weight of fish}) \times 100$$

$$\text{Intestinal somatic index (ISI) (\%)} = (\text{wet weight of intestine}/\text{whole body- weight of fish}) \times 100.$$

2.5 | Quantification of RNA and RNA:DNA ratio

Quantification of nucleic acids was performed by pentose analysis following the method of Schneider (1957). DNA and RNA concentration in the tissue were calculated based on the following formulae:

$$\mu\text{g DNA/ml} = \frac{1}{4}\,\text{OD at } 600\text{ nm} \times 0.019,$$

$$\mu\text{g RNA/ml} = \{(\text{OD at } 660\text{ nm} + 0.0081) - \mu\text{g DNA/ml} \times 0.013)\}/0.116.$$

2.6 | Proximate composition analysis

The proximate composition of the experimental diets and fish was determined using the standard methods of AOAC (1995) for moisture, crude protein (CP), ether extract (EE), crude fibre (CF) and ash. Moisture was determined by drying samples in an oven at 102°C till to get a constant weight. Crude protein content was determined using an automated Kjeldahl (Kelplus, PELICAN). Crude lipid was determined by the ether extraction method using the Soxhlet apparatus (Model SD2, 1045, PELICAN). Ash content was determined by burning the samples in a muffle furnace (WIT; C and L Tetlow) at 550°C for 18 hr. The crude fibre was determined by Fibre tech (Tulin Equipment).

2.7 | Intestinal digestive enzyme assays

The amylase activity was assayed as the reducing sugars produced due to the action of glucoamylase and a-amylase on carbohydrate

using dinitro-salicylic-acid (DNS) method (Rick & Stegbauer, 1974). The amylase activity was expressed as the mole of maltose released from starch min^{-1} mg^{-1} protein at 37°C. The protease activity was estimated as described by Drapeau (1974) using 1% casein as substrate. The reaction mixture comprising a substrate, 0.05 M Tris-phosphate buffer (pH 7.8) and 100 µl sample was stopped after 10 min by adding 10% trichloroacetic acid (TCA) followed by filtration. One unit of enzyme activity was defined as the amount of enzyme needed to release acid soluble fragments equivalent to Δ0.001 at 280 nm/min at 37°C and pH 7.8. Lipase activity was determined as described by Cherry and Crandall (1932) method. The reaction mixture was prepared with distilled water, sample, phosphate buffer solution (pH 7) and olive oil emulsion and incubated for 37°C for 24 hr. The reaction mixture was then titrated against 0.05 N NaOH until it showed a permanent pink colour. The milli-equivalent of alkali consumed was the activity of the enzyme and expressed as unit/mg protein.

2.8 | Metabolic enzyme assays

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assayed as described by Wooten (1964). The α-ketoglutarate and DL-aspartic acid were used as the substrate for AST and α-ketoglutarate and DL-alanine for ALT. The reaction mixture of sample homogenate and substrate was incubated at 37°C for 1 hr, and the reaction was stopped by adding of 2, 4-dinitrophenylhydrazine (DNPH). After adding 0.4 N NaOH (5 ml), the absorbance was taken at 540 nm. The activity of the enzyme was expressed as nanomoles oxaloacetate formed min^{-1} mg^{-1} protein at 37°C.

The lactate dehydrogenase (LDH) activity was assayed by the method of Wrobleiuski and Laude (1955). The reaction was started by adding sodium pyruvate to the reaction mixture comprising of 0.1 M phosphate buffer (pH 7.5), NADH solution (dissolved in 1 ml of phosphate buffer solution) and 100 µl of tissue homogenate. The OD was recorded at 340 nm, and enzymatic activity was expressed as unit mg^{-1} protein min^{-1} at 37°C where 1 unit was equal to Δ0.01 OD/min. The malate dehydrogenase (MDH) activity was assayed by the method of Ochoa (1955), and the specific enzymatic activity was expressed as unit mg^{-1} protein min^{-1} at 37°C where 1 unit was equal to Δ0.01 OD/min.

2.9 | Oxidative stress enzymes and osmolality

Superoxide dismutase (SOD) was assayed by using the method described by Misra and Frodovich (1972) based on the oxidation of epinephrine–adrenochrome transition by the enzyme, and absorbance was measured at 480 nm for 3 min. The activity of one unit of SOD was expressed as the amount of protein required for 50% inhibition of epinephrine auto-oxidation. Catalase (CAT) activity assayed according to the method described by Takahara et al. (1960) using phosphate buffer (50 mM, pH 7.0) and the reaction was started by the addition of H_2O_2 solution, and the decreasing absorbance was measured at 240 nm. One unit of CAT activity was the amount of protein required to decompose H_2O_2. The serum and water osmolality (mOsm/kg) were measured using a cryoscopic osmometer (Osmomat® 030; Gonotec GmbH).

2.10 | Haematological parameters

The total erythrocytes count was done using Neubauer's counting chamber of a haemocytometer as described by Hendricks (1952). A solution having 20 µl of blood with 3,980 µl of erythrocyte diluting fluid (Qualigens) was taken in a clean test tube and shaken gently. The counting was done by putting a drop of this mixture into the counting chamber of a haemocytometer. White blood cells (WBC) count was performed following the method of Shaw (1930). The haemoglobin (Hb) content was analysed following the cyanmethemoglobin method (Van Kampen & Zijlstra, 1961) using Drabkins Fluid (Qualigens, GlaxoSmithKline Pharmaceutical Ltd). The optical density (OD) was measured at 540 nm, and the final concentration was calculated based on the following formula; Haemoglobin (g/dl) = [OD(T)/OD(S)] × (251/1,000) × 60, where T is test, and S is standard.

2.11 | Statistical analysis

Statistical analysis of data was performed by using the software program SPSS 22.0 (IBM Inc.) for Windows 10, and the data were expressed as means ± standard error (*SE*). All data were subjected to one-way ANOVA, and post hoc analysis was carried out by Duncan's multiple range test (DMRT) at a 5% level of probability ($p < .05$). The second-order polynomial regression model (Robbins, Norton, & Baker, 1979) was used to estimate the dietary protein requirement for *M. cephalus* fingerlings on the basis of weight gain (WG%).

3 | RESULTS

3.1 | Water quality parameters

Water quality parameters such as water temperature, dissolved oxygen concentration, pH, salinity, total alkalinity, ammonia, calcium, magnesium and potassium were found in the range from 27°C to 30°C, 5.3 to 6.5 mg/L, 7.8 to 8.5, 8 g/L, 226 to 246 mg/L, 0.045 to 0.059 mg/L, 110 to 123 mg/L, 322 to 347 mg/L and 20 to 26 mg/L, respectively, throughout the experimental period.

3.2 | Growth performance, protein utilization and nucleic acid content

The effects of dietary protein levels on the growth performance, protein utilization and nucleic acid content of grey mullet

TABLE 2 Growth performance, protein utilization and nucleic acid content of grey mullet fingerlings fed diets containing different protein levels

	Dietary protein levels (g CP/kg diet)					
Parameters	**240**	**260**	**280**	**300**	**320**	**340**
FBW (g/fish)	5.23 ± 0.11^{a}	5.27 ± 0.08^{a}	6.26 ± 0.07^{b}	6.86 ± 0.12^{cd}	7.00 ± 0.22^{d}	6.69 ± 0.11^{c}
WG (%)	79.06 ± 2.4^{a}	85.75 ± 2.7^{a}	116.67 ± 2.7^{b}	138.34 ± 5.9^{cd}	145.12 ± 3.7^{d}	131.12 ± 2.5^{c}
SGR (%/day)	0.97 ± 0.02^{a}	0.99 ± 0.02^{a}	1.27 ± 0.02^{b}	1.44 ± 0.02^{cd}	1.50 ± 0.04^{d}	1.40 ± 0.02^{c}
FE	0.37 ± 0.01^{a}	0.38 ± 0.01^{a}	0.49 ± 0.01^{b}	0.58 ± 0.02^{cd}	0.60 ± 0.01^{d}	0.54 ± 0.01^{c}
FI (%/day)	1.88 ± 0.02^{b}	1.88 ± 0.02^{b}	1.85 ± 0.02^{ab}	1.81 ± 0.01^{a}	1.82 ± 0.02^{a}	1.82 ± 0.01^{a}
PER	1.54 ± 0.03^{a}	1.46 ± 0.05^{a}	1.75 ± 0.05^{b}	1.95 ± 0.07^{c}	1.87 ± 0.04^{bc}	1.59 ± 0.02^{a}
ANPU (%)	9.01 ± 0.81^{b}	8.82 ± 1.61^{ab}	8.25 ± 0.14^{b}	9.98 ± 0.83^{c}	8.71 ± 0.38^{bc}	7.79 ± 0.69^{a}
PGR (%/day)	0.16 ± 0.02^{a}	0.20 ± 0.01^{ab}	0.22 ± 0.01^{b}	0.28 ± 0.01^{c}	0.31 ± 0.02^{c}	0.21 ± 0.01^{b}
DNA (μg/ml)	0.65 ± 0.05^{a}	0.67 ± 0.08^{a}	0.61 ± 0.08^{a}	0.56 ± 0.02^{a}	0.57 ± 0.04^{a}	0.67 ± 0.12^{a}
RNA (μg/ml)	4.62 ± 0.12^{a}	4.79 ± 0.13^{a}	5.00 ± 0.19^{ab}	5.34 ± 0.14^{bc}	5.58 ± 0.17^{c}	5.33 ± 0.14^{bc}
RNA/DNA	7.19 ± 0.54^{a}	7.31 ± 0.56^{a}	8.46 ± 0.95^{b}	9.55 ± 0.50^{c}	10.03 ± 0.63^{c}	8.98 ± 0.23^{bc}
Survival (%)	100	100	100	100	100	100

Note: Data expressed as mean ± *SE*, $n = 3$; mean values in each row with different superscripts differ significantly ($p < .05$).

Abbreviations: ANPU, apparent net protein utilization; FBW (g/fish), final body weight; FE, feed efficiency; FI (%/day), feed intake; PER, protein efficiency ratio; PGR (%/day), protein growth rate; SGR, specific growth rate; WG (%), weight gain %.

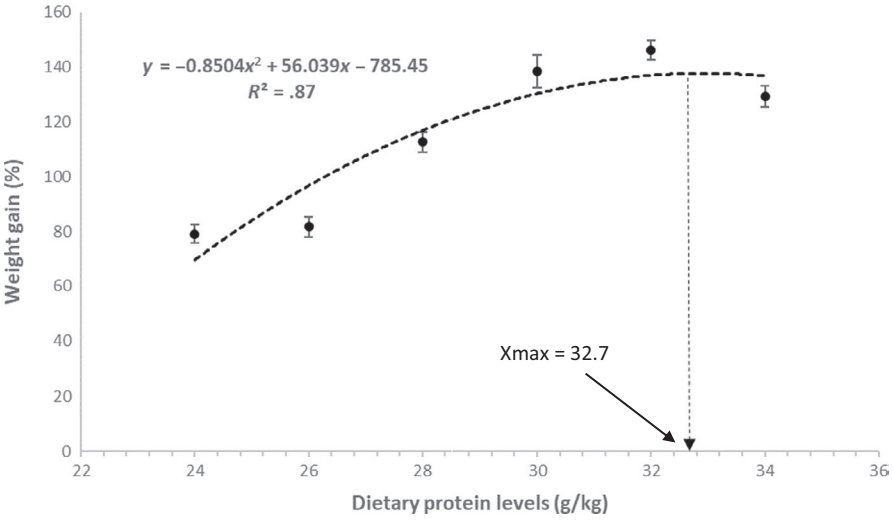

FIGURE 1 Second-order polynomial regression analysis based on weight gain (WG %) showing maximum growth of *Mugil cephalus* fingerlings in 327 g/kg dietary protein.

fingerlings were presented in Table 2. The WG (%), SGR and FE were significantly higher ($p < .05$) in the 320 g CP/kg diet than that of 240, 260, 280 and 340 g CP/kg diets except 300 g CP/kg diet. Whereas, WG (%), SGR and FE were significantly lower ($p < .05$) in 240 and 260 g CP/kg diets than the other treatment groups. Based on the second-order polynomial regression ($y = -0.8504x^2 + 56.039x - 785.45$, $R^2 = .87$) analysis of weight gain (%), the dietary protein level for maximum growth of grey mullet was estimated to be 327 g CP/kg diet (Figure 1).The PER and ANPU values were significantly higher ($p < .05$) in the 300 g CP/kg diet than that of 240, 260, 280 and 340 g CP/kg diets except 320 g CP/kg diet. Similarly, the protein growth rate (PGR) was significantly higher ($p < .05$) in the 320 and 300 g CP/kg diets than that of 240, 260, 280 and 340 g CP/kg diets. Whereas, the PGR was significantly lower ($p < .05$) in the 240 g CP/kg diet than the other treatment groups. The muscle RNA concentration was significantly higher ($p < .05$) in the 320 g CP/kg diet as compared to 240, 260 and 280 g CP/kg diets except 300 and 340 g CP/kg diets. The RNA:DNA ratio was significantly higher ($p < .05$) in 320 and 300 g CP/kg diets than that of 240, 260 and 280 g CP/kg diets except 340 g CP/kg diet. The muscle DNA content remains similar ($p > .05$) among all the treatment groups. No mortality occurred in any of the treatment groups throughout the feeding trial.

3.3 | Whole-body carcass composition and body indices

Dietary protein levels significantly affected ($p < .05$) whole-body carcass composition and HSI of mullet fingerlings (Table 3). The

TABLE 3 Whole-body proximate composition (g/kg wet weight basis) and body indices of grey mullet fingerlings fed diets containing different protein levels

	Dietary protein levels (g CP/kg diet)						
Parameters	**Initial**	**240**	**260**	**280**	**300**	**320**	**340**
Moisture	755.3 ± 4.5^{b}	731.7 ± 5.5^{a}	731.5 ± 4.2^{a}	732.4 ± 5.3^{a}	732.7 ± 3.6^{a}	733.1 ± 4.2^{a}	732.4 ± 5.3^{a}
CP	138.1 ± 1.2^{a}	149.8 ± 2.2^{b}	153.7 ± 3.1^{bc}	154.5 ± 2.4^{bc}	160.5 ± 1.7^{d}	161.3 ± 2.3^{d}	153.0 ± 2.1^{c}
EE	35.9 ± 1.5^{a}	55.5 ± 1.7^{d}	53.5 ± 1.5^{c}	52.7 ± 1.3^{c}	51.3 ± 1.4^{b}	49.6 ± 1.1^{b}	53.9 ± 1.8^{c}
Ash	54.4 ± 1.9^{b}	51.8 ± 1.4^{a}	51.3 ± 2.1^{a}	51.6 ± 1.4^{a}	51.0 ± 1.5^{a}	49.4 ± 1.3^{a}	52.4 ± 1.5^{a}
HSI		1.79 ± 0.11^{c}	1.65 ± 0.10^{bc}	1.55 ± 0.09^{b}	1.39 ± 0.07^{a}	1.36 ± 0.07^{a}	1.32 ± 0.08^{a}
ISI		4.17 ± 0.25^{a}	3.89 ± 0.23^{a}	3.80 ± 0.50^{a}	3.75 ± 0.33^{a}	3.67 ± 0.37^{a}	3.70 ± 0.20^{a}

Note: Data expressed as mean ± *SE*, $n = 3$; mean values in each row with different superscripts differ significantly ($p < .05$).
Abbreviations: CP, crude protein; EE, ether extract; HIS, hepatosomatic index; ISI, intestinal somatic index.

whole-body protein content was significantly higher ($p < .05$) in the 320 and 300 g CP/kg diets than that of 240, 260, 280 and 340 g CP/kg diets, whereas significantly lower ($p < .05$) value noticed in group fed 280 g CP/kg or less. Besides, fish fed 240 g CP/kg diet had significantly highest ($p < .05$) value for whole-body lipid than the other treatment groups, whereas significantly lower ($p < .05$) value found with 300 and 320 g CP/kg diets. Whole-body moisture and ash content remain unaffected ($p > .05$) by dietary protein levels. Initial protein and lipid content were significantly lower ($p < .05$) than the final, whereas moisture and ash content were significantly higher ($p < .05$) than the final in all the experimental groups. The HSI value decreased with the increase in dietary protein levels and was significantly highest ($p < .05$) in the 240 g CP/kg diet than the other treatment groups except 260 g CP/kg diet. The ISI value was found to be similar ($p > .05$) among the different treatment groups. However, a general decreasing trend of ISI value from lower to higher protein diet can be observed among the treatment groups.

3.4 | Intestinal digestive enzyme assays

Dietary protein levels significantly affected ($p < .05$) protease and amylase activities, but not lipase activity ($p > .05$) in grey mullet fingerlings (Table 4). The proteases activity was significantly higher ($p < .05$) in 300, 320 and 340 g CP/kg diet fed groups than that of 240 and 260 g CP/kg diets fed group. However, 280 g CP/kg diet fed groups were not significantly different from 240 and 260 g CP/kg diets fed group. The amylase activity showed a decreasing trend in response to increase in dietary protein levels with a significantly higher ($p < .05$) activity in 240 and 260 g CP/kg diets fed groups than that of 300, 320 and 340 g CP/kg diets fed groups.

3.5 | Metabolic enzyme assays

The effect of dietary protein levels on muscle and liver aspartate transaminase (AST), alanine transaminase (ALT), malate dehydrogenase (MDH) and liver glycogen activities in grey mullet fingerlings is shown in Table 5. The liver ALT activity and AST activity of muscle and liver were significantly higher ($p < .05$) in the 340 g CP/kg diet than that of 240, 260, 280, 300 and 320 g CP/kg diets. Whereas, significantly lower ($p < .05$) values were obtained in 240 and 260 g CP/kg diets than the other treatment groups. Muscle ALT activity and LDH activities in liver and muscle were found to have no significant difference ($p > .05$) among all the different treatment groups. The liver MDH activity increased significantly ($p < .05$) in the 340 g CP fed group than that of other dietary protein levels, while muscle MDH activity remains similar ($p > .05$) among the treatment groups. The liver glycogen content was significantly higher ($p < .05$) in the 240 g CP/kg diet than that of 280, 300 and 320 g CP/kg diets except 260 and 340 g CP/kg diets.

3.6 | Oxidative stress enzymes, haematological parameters and osmolality

Activities of liver and gill superoxidase dismutase (SOD), catalase (CAT), haematological parameters and serum osmolality of grey mullet fingerlings fed experimental diets are presented in Table 6. The antioxidant enzyme activities, including SOD and CAT in liver and gill, were found to be similar ($p > .05$) among the treatment groups regardless of the dietary protein levels. The red blood cell (RBC) count was found to be significantly higher ($p < .05$) in 300, 320 and 340 g CP/kg diets than the 240 g CP/kg diet. However, 260 and 280 g CP/kg diets were not significantly different from 240 g CP/kg diet. The white blood cell (WBC) and haemoglobin (Hb) content recorded no significant difference ($p > .05$) to the dietary protein levels among the different treatment groups. Serum osmolality also remains similar ($p > .05$) among the different treatment groups.

4 | DISCUSSION

Generally, the growth rate is positively related to the dietary protein levels in many species. However, growth of fish decreases or remains

TABLE 4 Digestive enzymes activities of grey mullet fingerlings fed diets containing different protein levels

Parameters	Dietary protein levels (g CP/kg diet)					
	240	260	280	300	320	340
Proteases	6.13 ± 0.05^{a}	6.14 ± 0.08^{a}	8.72 ± 0.08^{bc}	10.22 ± 0.06^{c}	10.79 ± 0.09^{c}	11.32 ± 0.07^{c}
Amylase	11.41 ± 0.11^{a}	10.30 ± 0.18^{a}	9.11 ± 0.12^{ab}	8.44 ± 0.16^{b}	8.35 ± 0.18^{b}	8.26 ± 0.22^{b}
Lipase	6.83 ± 0.15^{a}	6.61 ± 0.11^{a}	8.05 ± 0.18^{a}	7.86 ± 0.06^{a}	7.54 ± 0.06^{a}	8.11 ± 0.19^{a}

Note: Data expressed as mean ± *SE*, $n = 3$; Mean values in each row with different superscripts differ significantly ($p < .05$). The protease activity was expressed as nanomole of tyrosine released min^{-1} g^{-1} protein. The amylase activity was expressed as the mole of maltose released from starch min^{-1} mg^{-1} protein at 37°C. The lipase activity was expressed as units/mg protein.

TABLE 5 Metabolic enzymes and glycogen content of grey mullet fingerlings fed diets containing different protein levels

Parameters	Dietary protein levels (g CP/kg diet)					
	240	260	280	300	320	340
AST (Muscle)	8.18 ± 0.69^{a}	8.25 ± 0.20^{a}	11.57 ± 0.78^{ab}	12.12 ± 0.32^{b}	12.16 ± 0.36^{b}	15.49 ± 0.20^{c}
AST (Liver)	20.84 ± 1.2^{a}	20.61 ± 1.3^{a}	19.72 ± 1.1^{a}	24.13 ± 1.0^{a}	23.67 ± 1.1^{a}	27.78 ± 1.1^{b}
ALT (Muscle)	6.84 ± 0.84^{a}	5.90 ± 0.35^{a}	7.79 ± 0.92^{a}	8.71 ± 0.87^{a}	9.10 ± 0.55^{a}	9.29 ± 0.75^{a}
ALT (Liver)	9.08 ± 0.87^{a}	8.59 ± 0.63^{a}	9.12 ± 0.26^{ab}	10.74 ± 0.53^{ab}	12.78 ± 0.46^{b}	16.24 ± 0.91^{c}
LDH (Muscle)	8.30 ± 0.31^{a}	6.49 ± 0.46^{a}	6.83 ± 0.17^{a}	6.55 ± 0.88^{a}	7.18 ± 0.96^{a}	6.93 ± 0.78^{a}
LDH (Liver)	9.61 ± 0.25^{a}	9.42 ± 0.32^{a}	8.92 ± 0.27^{a}	9.20 ± 0.21^{a}	8.84 ± 0.18^{a}	9.39 ± 0.11^{a}
MDH (Muscle)	1.15 ± 0.11^{a}	1.14 ± 0.14^{a}	1.16 ± 0.11^{a}	1.25 ± 0.13^{a}	1.34 ± 0.14^{a}	1.78 ± 0.26^{a}
MDH (Liver)	0.95 ± 0.09^{a}	0.98 ± 0.12^{a}	1.19 ± 0.20^{ab}	1.24 ± 0.11^{ab}	1.23 ± 0.08^{ab}	1.69 ± 0.16^{c}
Glycogen	2.16 ± 0.15^{b}	1.83 ± 0.09^{ab}	1.28 ± 0.05^{a}	1.24 ± 0.06^{a}	1.26 ± 0.08^{a}	1.55 ± 0.04^{ab}

Note: Data expressed as mean ± *SE*, $n = 3$; mean values in each row with different superscripts differ significantly ($p < .05$). AST activity was expressed as nanomoles oxaloacetate released min^{-1} mg^{-1} protein at 37°C, ALT activity was expressed as nanomoles Na pyruvate released min^{-1} mg^{-1} protein at 37°C, LDH activity was expressed as units min^{-1} mg^{-1} protein at 37°C, and MDH activity was expressed as units min^{-1} mg^{-1} protein at 37°C.

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; LDH, lactate dehydrogenase; MDH, malate dehydrogenase.

TABLE 6 Oxidative stress enzymes, haematological parameters serum osmolality of grey mullet fingerlings fed diets containing different protein levels

Parameters	Dietary protein levels (g CP/kg diet)					
	240	260	280	300	320	340
SOD (Gill)	27.74 ± 2.34^{a}	33.04 ± 5.77^{a}	28.69 ± 5.91^{a}	29.20 ± 5.21^{a}	28.28 ± 4.17^{a}	25.50 ± 5.19^{a}
SOD (Liver)	35.52 ± 1.38^{a}	34.45 ± 3.16^{a}	34.16 ± 2.77^{a}	32.20 ± 2.41^{a}	33.32 ± 1.89^{a}	33.61 ± 1.65^{a}
CAT (Gill)	6.75 ± 1.09^{a}	6.88 ± 1.38^{a}	4.89 ± 0.11^{a}	5.28 ± 0.55^{a}	5.46 ± 0.47^{a}	4.86 ± 0.80^{a}
CAT (Liver)	6.25 ± 0.56^{a}	5.21 ± 1.43^{a}	4.94 ± 0.43^{a}	5.27 ± 0.69^{a}	5.19 ± 0.70^{a}	5.35 ± 0.12^{a}
RBC ($\times 10^{6}$/μl)	2.34 ± 0.13^{a}	2.45 ± 0.14^{ab}	2.48 ± 0.15^{ab}	2.55 ± 0.17^{b}	2.62 ± 0.11^{b}	2.75 ± 0.12^{b}
Hb (g/dl)	35.17 ± 0.30^{a}	37.30 ± 0.22^{a}	36.33 ± 0.25^{a}	38.51 ± 0.14^{a}	39.56 ± 0.41^{a}	39.50 ± 0.13^{a}
WBC ($\times 10^{3}$/μl)	27.43 ± 1.90^{a}	26.46 ± 1.61^{a}	26.93 ± 2.40^{a}	29.32 ± 1.01^{a}	28.62 ± 2.32^{a}	30.53 ± 2.67^{a}
Osmolality (mOsm/kg)	347 ± 2.40^{a}	350 ± 2.20^{a}	351 ± 1.60^{a}	350 ± 1.20^{a}	352 ± 2.30^{a}	352 ± 1.40^{a}
Water osmolality (mOsm/kg)	237 ± 0.13^{a}	236 ± 0.15^{a}	235 ± 0.11^{a}	236 ± 0.08^{a}	237 ± 0.15^{a}	237 ± 0.12^{a}

Note: Data expressed as mean ± *SE*, $n = 3$; mean values in each row with different superscripts differ significantly ($p < .05$).

Abbreviations: CAT, catalase; HB, haemoglobin; RBC, red blood cell; SOD, superoxide dismutase; WBC, white blood cell.

unaffected when fish are fed with dietary protein having lower or higher than optimum level (Kim & Lall, 2001; Shiau & Lan, 1996), because in such condition, protein synthesis is not favoured and a percentage of protein is catabolized resulting in reduction of protein conversion efficiency (Deng et al., 2014). Further, an insufficient supply of non-protein energy in feed also reduces growth performance despite having high protein in the diet due to preferential catabolism of protein (Winfree & Stickney, 1981). The unimproved growth of

grey mullets in 340 g CP/kg diet indicates that supplying the protein beyond optimum requirement does not give further added benefits. Similar result of growth rate stagnancy or decline beyond optimum protein level was reported in many other species (Abdel-Tawwab, Ahmad, Khattab, & Shalaby, 2010; Hossain, Almatar, & James, 2010; Jana et al., 2006; Mohanta, Mohanty, Jena, & Sahu, 2008; Ng, Soon, & Hashim, 2001; Papaparaskeva-Papoutsoglou & Alexis, 1986). Inferior growth in the 240 and 260 g CP/kg diet could be due to a lower P/E ratio in the diet (Moore, Hung, & Medranno, 1988). The good growth of mullet fingerlings when fed 320 or 300 g CP/kg diet can be attributed to the optimum P/E ratio (20.1 and 18.9 g/MJ, respectively) of the diets which indicate that the dietary protein was channelized for protein synthesis rather than preferential catabolism (Tucker, 1992). Hence, the present study partly showed that a P/E ratio of 18–20 g/MJ is optimum for the growth of mullet fingerlings reared in ISW. This value is closest to the optimum P/E ratio (20–25 g/MJ) reported for various omnivorous fish such as tilapia, magur and rainbow trout (NRC, 2011). Our results corroborate to that of De et al. (2012) who observed good growth of *M. cephalus* fry (0.5 g) at 300 g/kg dietary protein, but growth is decreased with further increase in dietary protein at 350 g/kg. Besides, Lin Jiang, Huang, and Shi (1998) concluded that 280 g/kg dietary protein is optimal for the maximum growth of grey mullets reared in seawater. The different responses could attribute to the methodology adopted, different environmental conditions (ISW), salinities and diet compositions (leaf meal-based diet) which can alter nutrient requirement of fish. Previous reports on dietary protein requirement value for mullet species are 260 g/kg for *Mugil capito* (Papaparaskeva-Papoutsoglou & Alexis, 1986), 316 g/kg for *Chelon labrosus* (Altunok & Özden, 2017), 350 g/kg for *Mugil platanus* (De Carvalho, Bianchini, Tesser, & Sampaio, 2010) and 400 g/kg for *Liza haematocheila* (Yoshimatsu, Furuichi, & Kitajima, 1992). Our findings on the optimal dietary protein requirement at 327 g CP/kg are in agreement to other authors, who concluded that dietary protein level between 300 and 400 g/kg influences good growth in mullet species and other omnivorous species under laboratory condition at various salinities (0–36 g/L) (Argyropoulou, Kalogeropoulos, & Alexis, 1992; De Carvalho et al., 2010; El-Sayed, 1991; Tacon, 1987). The values of growth performance in the present study further corroborate previous findings on grey mullets fed plant protein-based diet under laboratory condition (Abdel-Hakim et al., 2001; Barman et al., 2005; De et al., 2012; Gisbert et al., 2016; Kalla et al., 2003).

Generally, fish regulate feed intake in order to satisfy their metabolic energy requirement (Coutinho, Peres, Guerreiro, Pousão-Ferreira, & Oliva-Teles, 2012; Kim & Lall, 2001). However, lower dietary protein increases feed intake (FI) by fish in order to compensate for the deficient nutrient (Sá et al., 2014; Yang, Liou, & Liu, 2002). In this study, fish fed with 240 and 260 g CP/kg diets has higher FI value than those fed with higher protein diets probably to satisfy their energy needs. The FE was found to be maximum at 320 or 300 g CP/kg diets fed group thus indicates a good utilization of feed by grey mullet fingerlings at the optimum dietary protein level which can be a reason for the observed higher growth in the same group.

The protein utilizing efficiency, as described by PER, ANPU and PGR, decreases beyond the optimum dietary protein level found in this study. The maximum protein efficiency was observed in the 300 or 320 g CP/kg diets fed grey mullet fingerlings, and the PER significantly reduced in 340 g CP/kg diet fed group. The increased protein efficiency and retention could attribute to reduced preferential catabolism of protein because of sufficient non-protein energy available in the diet. The inferior protein utilization beyond 320 g CP/kg diet implies that some percentage of dietary protein being used as substrate for catabolism. Further, it has been reported that some amino acids play a role in osmoregulation (Ballantyne, 2001), and when the fish are exposed to lower salinity, the level of osmotically active amino acids in serum increases, which might be obtained from the catabolism of protein. Therefore, reduced protein accretion is expected as found in several fish species (Abdel-Tawwab et al., 2010 in Nile tilapia; Gangadhar, Nandeesha, Varghese, & Keshavanath, 1997 in rohu; Mohanta et al., 2008 in silver barband mullet species, Altunok & Özden, 2017; De et al., 2012; De Carvalho et al., 2010). The RNA:DNA ratio is a reliable parameter of protein synthesis hence signifies fish growth (Steinhart & Eckman, 1992). DNA is necessary for protein synthesis that remains constant in tissue (Mitra & Mukhopadhyay, 2002) as found in this study. RNA content and RNA:DNA ratios were corroborating to the good growth and protein utilization observed for grey mullet fingerlings in the 320 or 300 g CP/kg diets. Similar results of higher RNA:DNA ratio at 300 g CP/kg diet were also observed in *Labeo rohita* (Gangadhar et al., 1997).

Higher carcass protein retention found in 300 or 320 g CP/kg diets is possibly due to higher protein accretion, synthesis and nutrient utilization that corroborates to the higher growth performance found in this groups. However, De et al. (2012), De Carvalho et al. (2010) and Abdel-Hakim et al. (2001) found no influence of protein levels on carcass protein in mullet species. Similar rise in carcass protein to the increasing protein level was observed in grey mullet (Abdel-Maksoud, 2000), carps (Debnath et al., 2007), Malaysian catfish (Khan, Ang, Ambak, & Saad, 1993) and red grouper (Wang et al., 2016). Body lipid was higher in the lower protein fed group, possibly due to the conversion of carbohydrate to lipid as the dietary lipid was kept constant. Significantly lower body lipid was found in the 300 or 320 g CP/kg diets than those of other CP diets. A similar result has been reported in grey mullet (Abdel-Hakim et al., 2001; De et al., 2012; Papaparaskeva-Papoutsoglou & Alexis, 1986) and other species (Khattab, Ahmad, Shalaby, & Abdel-Tawwab, 2000; Mohanta et al., 2008). Body indices are used to evaluate available energy in fish, and excess availability of energy leads to the deposition of glycogen and lipid (Talukdar et al., 2019). The highest HSI in the lower CP diet can be related to an imbalance in P/E ratio as excess carbohydrate is being converted to liver glycogen and lipid, and thus the weight of the liver increased (Yang et al., 2002). Higher HSI value indicates poor growth (Deng, Ju, Dominy, Murashige, & Wilson, 2011; Wang et al., 2016) as observed in this study. In general, omnivorous fishes are well known to utilize a higher level of carbohydrate which in excess is stored as glycogen and lipid (Furuichi & Yone, 1981; Hemre, Mommsen, & Krogdahl, 2002). In this study, it seems that grey mullet prefers to store excess carbohydrate

which could also be an adaptive strategy to have energy reserve during the physiological adaptation in ISW and prioritizing protein for growth.

Prediction of feed utilization and growth performance is depending upon digestive enzymes activities that may alter with the variation of dietary protein level in fish (Sagada et al., 2017). The digestive capacity of fish is often negatively correlated with the plant protein-based diet (Estévez et al., 2011). However, omnivorous fish have a higher ability to use plant protein-based diet than carnivorous fish (Kuzmina, 1996). Intestinal proteases activity showed an increasing positive response to the dietary protein levels and found to be maximum in the higher CP diets. This could be due to that fish try to maximize the utilization of available protein by increasing the digestive capacity. Further, this also explains the good utilization capacity of plant protein-based diet by grey mullet fingerlings as previously demonstrated by Jana et al. (2012), Gisbert et al. (2016), Kalla et al. (2003) and De et al. (2018) in the same species. The increasing protease activity might have driven maximum protein synthesis in optimum protein level as supported by the highest RNA:DNA ratio and growth. Generally, intestinal amylase activity acts accordingly with dietary carbohydrate level, which is more prominent in omnivorous fish (Hidalgo, Urea, & Sanz, 1999). The amylase activity reduced with the increasing dietary protein which may be a response to the lower carbohydrate content in the higher CP diet as observed by Ma et al. (2019) in tilapia and Mohapatra, Sahu, and Chaudhari (2002) in rohu. However, in contrast, De et al. (2012) in grey mullet and Debnath et al. (2007) in rohu found no significant changes in amylase activity fed with different dietary protein levels. The difference could be due to the types of carbohydrate (NRC, 2011), fish size and season (Kuzmina, 1996) can affect amylase activity in fish. The higher activity of amylase enzyme in lower protein diets also corresponds to the higher HSI and glycogen value found in the lower protein diet. Lipase activity was found to be similar among the treatment groups, which could be due to the similar lipid content of the diets.

Aspartate transaminase (AST) and alanine transaminase (ALT) act to optimize growth and energy utilization by redistributing the amino group to form new amino acid, thus indicating protein availability. Amino acid derived from diet or tissue is first deaminated and then oxidized through Kreb's cycle (Knox & Greengard, 1965). Again, it has been reported that transaminase activities are either to rise or to remain unchanged to the dietary protein (Nagai & Ikeda, 1972). The elevated AST and ALT activities in 340 g CP/kg diet fed group related to the reduced protein efficiency and catabolism of protein which possibly due to lower carbohydrate content of the diet to support energetic demand; thus, an unimproved growth appears. Amino acids are the preferred substrate for gluconeogenesis in the fish liver with elevated gluconeogenesis observed for high protein diet (Ballantyne, 2001; Kumar et al., 2009). Dietary carbohydrate at the optimal level promotes growth which supports the metabolic demands by reducing gluconeogenic activity. In this study, it appears to be in 320 or 300 g CP/kg diets where transaminase enzymes supported protein synthesis, thus suggesting protein sparing action of carbohydrate for satisfying the energetic needs. Similar rise of AST and ALT activities with growth and dietary protein level was demonstrated in rohu (Kumar et al., 2009), *Rhamdia quelen* (Melo, Lundstedt, Metón, Baanante, & Moraes, 2006), Nile tilapia (Abdel-Tawwab et al., 2010) and Jian carp (Liu, Feng, Jiang, Liu, & Zhou, 2009). High carbohydrate level in the diet is associated with glycogen deposition in the liver due to elevated blood glucose that may impart metabolic stress, particularly in carnivorous fish (Pieper & Pfeffer, 1980). The elevated liver glycogen in the lower CP diet could possibly due to carbohydrate rich diet along with the higher amylase activity observed. Glycogen content gradually decreases with the enhancement of dietary protein upto 320 g CP/kg diet, thus suggesting mobilization of glycogen stores for physiological purposes and prioritizing protein for growth. Slight increase of glycogen content in 340 g CP/ kg diet fed group could be an effort to store surplus protein into energetic form (Stone, 2003). Similar results were reported by Shimeno, Shikata, Hosokawa, Masumoto, and Kheyyali (1997) in common carp, Vieira, Inoue, and Moraes (2005) in *Brycon cephalus*, Yang et al. (2002) in bluegill sunfish and Debnath et al. (2007) in *L. rohita*.

The activity of lactate dehydrogenase (LDH) is related to oxygen debt during a stress condition where pyruvate is converted to lactate (Murray, Granner, Mayes, & Rodwell, 2000). As the carbohydrate content was higher in the lower CP diet, we expected that fish would be in metabolic stress as higher dietary carbohydrate is associated with stress in fish (Kumar et al., 2005). The unchanged LDH activity in liver and muscle among the treatment groups implies that anaerobic metabolism was inactive and dietary energy demand was satisfied for grey mullet fingerlings. The conversion of malate into oxaloacetate or vice versa involved the action of malate dehydrogenase (MDH) enzyme, which also takes part during gluconeogenesis (Hemre et al., 2002). Increased activity of MDH along with increased transaminase enzyme activity in higher protein diet indicates the availability of gluconeogenic substrate (pyruvate/oxaloacetate) which accordingly can be converted to glucose and used for other physiological activities. Melo et al. (2006) in *Rhamdia quelen* observed increased gluconeogenic activity due to higher protein in the diet. Lower MDH activity at the lower protein fed group could be an indication of reduced metabolic activity which may reduce the availability of oxaloacetate as a substrate due to lower transaminase enzyme activities (Verma et al., 2007; Yengkokpam et al., 2013).

Continuous cellular metabolic activity leads to the production of reactive oxygen species (ROS) or free radicals which in excess cause oxidative stress and damage biomolecules (Storey, 1996). However, catalase and superoxide dismutase (SOD) are endogenous antioxidant enzymes that convert the free radicals to more degradable form (Martínez-Álvarez, Morales, & Sanz, 2005). Dietary protein and plant-based diet are known to alter endogenous antioxidant enzyme activities in fish (Olsvik, Torstensen, Hemre, Sanden, & Waagbo, 2011; Pérez-Jiménez et al., 2009). In this study, antioxidant enzyme activities were not affected by the experimental diets, thus indicating that dietary protein levels or diet composition does not impart any oxidative stress in grey mullet fingerlings. Similar results of unchanged activity of stress enzymes have been reported in *Mugil* (Gisbert et al., 2016) and olive flounder (Seong et al., 2018) when

fed with plant protein-based diet. Nevertheless, plant or leaf protein-based diet is known to have radical scavenging capacity due to the presence of phenolic and flavonoid compounds (Adom & Liu, 2002; Fitriansyah, Fidrianny, & Ruslan, 2017; Gowri & Vasantha, 2010). Generally, the energetic cost for osmoregulation is minimized in fish near to its isotonic salinity by maintaining the gradient between blood and water hence sparing energy for the optimum growth (Boeuf & Payan, 2001; Morgan & Iwama, 1991). The salinity level (8 g/L) in the present study is closest to the isotonic salinity reported for grey mullets and other euryhaline species (9–13 g/L, Brett, 1979; Lee, Tamaru, Kelley, Moriwake, & Miyamoto, 1992; Lisboa, Barcarolli, Sampaio, & Bianchini, 2015; Nordlie, Szelistowski, & Nordlie, 1982). Thus, the tested salinity level does not influence the different responses of growth performance observed for grey mullets. The serum osmolality remains similar among the different treatment groups because osmolality is more a function of water salinity, which was constant in the experiment. Moreover, grey mullet fingerlings of the different experimental groups were maintaining hyperosmotic state. The previous study by Barman et al. (2005) reported good growth performance of grey mullets at 10 g/L reared in ISW; however, they did not consider lower salinity than 10 g/L in their study.

Alterations in blood haematological parameters express the adaptability of fish to different dietary compositions and environmental conditions which reflects the immunological potential of fish (Bahmami, Kazemi, & Donskaya, 2001; De et al., 2018). In this study, the RBC count is positively related to the dietary protein level, which may be due to release of erythrocytes from spleen as dietary protein does affect spleen activity (Abdel-Tawwab, 2012). A similar increase in RBC value with the increasing dietary protein level is observed in *Paralichthys dentatus* (Daniels & Gallagher, 2000), Nile tillapia (Abdel-Tawwab et al., 2010) and *Megalobrama amblycephala* (Habte-Tsion, Liu, Ge, Pan, & Chen, 2013). The Hb and WBC values remain unchanged to the dietary protein levels, which are supported by Kumar et al. (2005) and Baruah et al. (2009) in *Labeo rohita*. Antinutritional factors (ANF's) present in the plant ingredients usually reduced RBC synthesis and Hb formation in the fish body (Akinleye, Kumar, Makkar, Angulo-Escalante, & Becker, 2012; Francis & Becker, 2001). In this study, it seemed that dietary treatments or environmental condition does not influence the immune potential of grey mullets as no apparent changes were observed in haematological parameters nor in stress marker enzyme activities. Further, the haematological parameter values were also within the normal range reported for grey mullets (Fazio et al., 2013).

In summary, the present study concluded that the diet having 300 or 320 g CP/kg (18.9 or 20.1 g/MJ P/E ratio, respectively) is optimum for the maximum growth of *M. cephalus* fingerlings reared in ISW. However, 300 g CP/kg diet is economically more feasible than the 320 g CP/kg diet as higher protein utilizing efficiency is found with the former. Based on the second-order polynomial regression analysis, the dietary protein level for maximum growth of *M. cephalus* fingerlings is 327 g CP/kg under the experimental condition of this study. Diets with a low protein lead to glycogen and lipid deposition, while surplus protein beyond requirement does not improve growth. Furthermore, our study explored the possibility of incorporation of leaf meal-based diet for grey mullets as nutrient utilization and immunity of grey mullets remains unhindered. Therefore, the result of the present study will help to develop cost-effective practical feed for *M. cephalus* fingerlings reared in ISW.

ACKNOWLEDGEMENTS

The authors are thankful to Indian Council of Agricultural Research (ICAR) (Ph.D. student grant 2016–19) for the financial support, Director of ICAR-CIFE (Indian Council of Agricultural Research-Central Institute of Fisheries Education) for providing the necessary infrastructural facilities for completing the experiment.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Avinash Talukdar https://orcid.org/0000-0002-3356-1096

REFERENCES

Abdel-Hakim, N. F., Hussein, M. S., Bakeer, M. N., & Soltan, M. A. (2001). Effect of protein level and stocking density on growth performance of Nile tilapia (*Oreochromis niloticus*) cultured in tanks. *Egyptian Journal of Nutrition and Feeds*, *4*, 763–780.

Abdel-Maksoud, A. M. S. (2000). Response of grey mullet (*Mugil cephalus* L.) cultured in earthen ponds, to diets of varying protein levels. *Egyptian Journal of Animal Production*, *37*, 57–66.

Abdel-Tawwab, M. (2012). Effects of dietary protein levels and rearing density on growth performance and stress response of Nile tilapia, *Oreochromis niloticus* (L.). *International Aquatic Research*, *4*, 3. https://doi.org/10.1186/2008-6970-4-3

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* (L.). *Aquaculture*, *298*, 267–274. https://doi.org/10.1016/j.aquaculture.2009.10.027

Adewolu, M. A., Adeniji, C. A., & Adejobi, A. B. (2008). Feed utilization, growth and survival of *Clarias gariepinus* (Burchell 1822) fingerlings cultured under different photoperiods. *Aquaculture*, *283*, 64–67. https://doi.org/10.1016/j.aquaculture.2008.07.020

Adom, K. K., & Liu, R. H. (2002). Antioxidant activity of grains. *Journal of Agricultural and Food Chemistry*, *50*, 6182–6187. https://doi.org/10.1021/jf0205099

Akinleye, A. O., Kumar, V., Makkar, H. P. S., Angulo-Escalante, M. A., & Becker, K. (2012). Jatropha platyphylla kernel meal as feed ingredient for Nile tilapia (*Oreochromis niloticus* L.): Growth, nutrient utilization and blood parameters. *Journal of Animal Physiology and Animal Nutrition*, *96*, 119–129. https://doi.org/10.1111/j.1439-0396.2011.01137.x

Aklakur, M. D. (2017). Nutritional intervention for sustainable production in inland saline aquaculture a budding perspective in India. *Journal of Aquactic Marine Biology*, *6*, 172. https://doi.org/10.15406/jamb.2017.06.00172

Allan, G. L., Fielder, D. S., Fitzsimmons, K. M., Applebaum, S. L., & Raizada, S. (2009). Inland saline aquaculture. In G. Burnell,& G. Allan (Eds.), *New technologies in aquaculture* (pp. 1119–1147).Sawston, UK: Wood Head Publishing. https://doi.org/10.1533/9781845696474.6.1119

Altunok, M., & Özden, O. (2017). Effect of dietary protein on the growth of mullet, *Chelon labrosus*, reared in sea cages. *Archives of Polish Fisheries*, *25*, 157–164. https://doi.org/10.1515/aopf-2017-0015

AOAC. (1995). *Official methods of analysis* (16th ed.). Arlington, VA: Association of Official Analytical Chemists.

APHA, A. (2005). *Standard methods for the examination of water and wastewater* (21st ed., pp. 258–259).Washington DC: WEF.

Argyropoulou, V., Kalogeropoulos, N., & Alexis, M. N. (1992). Effect of dietary lipids on growth and tissue fatty acid composition of grey mullet (*Mugil cephalus*). *Comparitive Biochemistry and Physiology*, *101*, 129–135. https://doi.org/10.1016/0300-9629(92)90640-C

Arshad Hossain, M., Almatar, S. M., & James, C. M. (2010). Optimum dietary protein level for juvenile silver pomfret, *Pampus argenteus* (Euphrasen). *Journal of the World Aquaculture Society*, *41*, 710–720. https://doi.org/10.1111/j.1749-7345.2010.00413.x

Bahmami, M., Kazemi, R., & Donskaya, P. (2001). A comparative study of some haematological features in young reared sturgeons (*Acipenser persicus* and *Huso huso*). *Fish Physiology and Biochemistry*, *24*, 135–140. https://doi.org/10.1023/A:1011911019155

Ballantyne, J. S. (2001). Amino acid metabolism. *Fish Physiology*, *20*, 77–107. https://doi.org/10.1016/S1546-5098(01)20004-1

Barman, U. K., Jana, S. N., Garg, S. K., Bhatnagar, A., & Arasu, A. R. T. (2005). Effect of inland water salinity on growth, feed conversion efficiency and intestinal enzyme activity in growing grey mullet, *Mugil cephalus* (Linn.): Field and laboratory studies. *Aquaculture International*, *13*, 241–256. https://doi.org/10.1007/s10499-004-2479-5

Baruah, K., Pal, A. K., Sahu, N. P., Debnath, D., Yengkokpam, S., Norouzitallab, P., & Sorgeloos, P. (2009). Dietary crude protein, citric acid and microbial phytase interacts to influence the hemato-immunological parameters of rohu, *Labeo rohita*, juveniles. *Journal of the World Aquaculture Society*, *40*, 824–831. https://doi.org/10.1111/j.1749-7345.2009.00304.x

Biswas, G., De, D., Thirunavukkarasu, A. R., Natarajan, M., Sundaray, J. K., Kailasam, M., … Sarkar, A. (2012). Effects of stocking density, feeding, fertilization and combined fertilization-feeding on the performances of striped grey mullet (*Mugil cephalus* L.) fingerlings in brackishwater pond rearing systems. *Aquaculture*, *338*, 284–292. https://doi.org/10.1016/j.aquaculture.2012.02.004

Boeuf, G., & Payan, P. (2001). How should salinity influence fish growth? *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *130*, 411–423. https://doi.org/10.1016/S1532-0456(01)00268-X

Brett, J. R. (1979). Environmental facts and growth. In W. S. Hoar, & D. J. Randall (Eds.), *Fish physiology* (vol. I., pp. 1–89). New York, NY: Academic Press.

Cherry, I. S., & Crandall, L. A. (1932). The specificity of pancreatic lipase: Its appearance in the blood after pancreatic injury. *American Journal of Physiology*, *100*, 266–273. https://doi.org/10.1152/ajplegacy.1932.100.2.266

Coutinho, F., Peres, H., Guerreiro, I., Pousão-Ferreira, P., & Oliva-Teles, A. (2012). Dietary protein requirement of sharp snout sea bream (*Diplodus puntazzo*, Cetti 1777) juveniles. *Aquaculture*, *356*, 391–397. https://doi.org/10.1016/j.aquaculture.2012.04.037

Daniels, H., & Gallagher, M. (2000). Effect of dietary protein level on growth and blood parameters in summer flounder, *Paralichthys dentatus*. *Journal of Applied Aquaculture*, *10*, 45–52. https://doi.org/10.1300/J028v10n01_05

De Carvalho, C. V., Bianchini, A., Tesser, M. B., & Sampaio, L. A. (2010). The effect of protein levels on growth, postprandial excretion and tryptic activity of juvenile mullet *Mugil platanus* (Günther). *Aquaculture Research*, *41*, 511–518. https://doi.org/10.1111/j.1365-2109.2009.02340.x

De, D., Ghoshal, T. K., Biswas, G., Mukherjee, S., & Kumar, S. (2018). Evaluation of growth performance in *Mugil cephalus* L. juveniles fed diets incorporated with fermented plant feedstuffs replacing fishmeal or diets supplemented with fish gut bacteria. *Journal of Food Processing Technology*, *9*, 728.

De, D., Ghoshal, T. K., & Kundu, J. (2012). Effect of feeding different levels of protein on growth performance, feed utilization and digestive enzyme of grey mullet (*Mugil cephalus* L). *Animal Nutrition and Feed Technology*, *12*, 179–186.

De Silva, S. S. (1980). The biology of juvenile grey mullet: A short review. *Aquaculture*, *19*, 21–36. https://doi.org/10.1016/0044-8486(80)90004-6

Debnath, D., Pal, A. K., Sahu, N. P., Yengkokpam, S., Baruah, K., Choudhury, D., & Venkateshwarlu, G. (2007). Digestive enzymes and metabolic profile of *Labeo rohita* fingerlings fed diets with different crude protein levels. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *146*, 107–114. https://doi.org/10.1016/j.cbpb.2006.09.008

Deng, D. F., Ju, Z. Y., Dominy, W., Murashige, R., & Wilson, R. P. (2011). Optimal dietary protein levels for juvenile Pacific threadfin (*Polydactylus sexfilis)* fed diets with two levels of lipid. *Aquaculture*, *316*, 25–30. https://doi.org/10.1016/j.aquaculture.2011.03.023

Deng, J., Zhang, X., Han, X., Tao, L., Bi, B., & Kang, B. (2014). Dietary protein requirement of juvenile dianchi golden-line barbell, *Sinocyclocheilus grahami*. *Journal of World Aquaculture Society*, *45*, 421–429. https://doi.org/10.1111/jwas.12137

Doroudi, M. S., Fielder, D. S., Allan, G. L., & Webster, G. K. (2006). Combined effects of salinity and potassium concentration on juvenile mulloway (*Argyrosomus japonicus*, Temminck and Schlegel) in inland saline groundwater. *Aquaculture Research*, *37*, 1034–1039. https://doi.org/10.1111/j.1365-2109.2006.01525.x

Drapeau, G. (1974). Protease from *Staphylococcus aureus*. *Methods in Enzymology*, *45*, 469–475.

El-Dahhar, A. A., Salama, M. A., Moustafa, Y. T., & Elmorshedy, E. M. (2014). Effect of using equal mixture of seaweeds and marine algae in striped mullet (*Mugil cephalus*) larval diets on growth performance and feed utilization. *Journal of the Arabian Aquaculture Society*, *9*, 145–158. https://doi.org/10.12816/0026642

El-Sayed, A. F. M. (1991). Protein requirements for optimum growth of *Liza ramada* fry (Mugilidae) at different water salinities. *Aquatic Living Resources*, *4*, 117–123. https://doi.org/10.1051/alr:1991011

Estévez, A., Treviño, L., Kotzamanis, Y., Karacosatas, I., Tort, L., & Gisbert, E. (2011). Effects of different levels of plant proteins on the on growing of meagre (*Argyrosomus regius)* juveniles at low temperatures. *Aquaculture Nutrition*, *17*, 572–582. https://doi.org/10.1111/j.1365-2095.2010.00798.x

FAO. (2018). *The state of the World fisheries and aquaculture*. Rome, Italy: Food and Agricultural Organization of the United Nations.

Fazio, F., Marafioti, S., Torre, A., Sanfilippo, M., Panzera, M., & Faggio, C. (2013). Haematological and serum protein profiles of *Mugil cephalus*: Effect of two different habitats. *Ichthyological Research*, *60*, 36–42. https://doi.org/10.1007/s10228-012-0303-1

Fielder, D. S., & Allan, G. L. (2003). *Improving fingerling production and evaluating inland saline water culture of snapper*, Pagrus auratus. Australia: NSW Fisheries, Port Stephens Fisheries Centre.

Fielder, D. S., Bardsley, W. J., & Allan, G. L. (2001). Survival and growth of Australian snapper, *Pagrus auratus*, in saline groundwater from inland New South Wales, Australia. *Aquaculture*, *20*, 73–90. https://doi.org/10.1016/S0044-8486(01)00555-5

Fitriansyah, S. N., Fidrianny, I., & Ruslan, K. (2017). Correlation of total phenolic, flavonoid and carotenoid content of *Sesbania sesban* (L. Merr) leaves extract with DPPH scavenging activities. *International Journal of Pharmacognosy and Phytochemical Research*, *9*, 89–94.

Forsberg, J. A., Dorsett, P. W., & Neill, W. H. (1996). Survival and growth of red drum *Sciaenops ocellatus* in saline groundwaters of West Texas, USA. *Journal of the World Aquaculture Society*, *27*, 462–474. https://doi.org/10.1111/j.1749-7345.1996.tb00631.x

Francis, G., Makkar, H. P. S., & Becker, K. (2001). Anti-nutritional factors present in plant derived alternate fish feed ingredients and their effects in fish. *Aquaculture*, *199*, 197–227. https://doi.org/10.1016/S0044-8486(01)00526-9

Furuichi, M., & Yone, Y. (1981). Change of blood sugar and plasma insulin levels of fishes in glucose tolerance test. *Bulletin of the Japanese Society for the Science of Fish*, *47*, 761–764. https://doi.org/10.2331/suisan.47.761

Gangadhar, B., Nandeesha, M. C., Varghese, T. J., & Keshavanath, P. (1997). Effect of varying protein and lipid levels on the growth of rohu, *Labeo rohita*. *Asian Fisheries Science*, *10*, 139–147.

Garg, S. K., Jana, S. N., & Arasu, A. R. T. (2006). Determination of fertilization rate for optimum pond productivity and fish growth in inland saline groundwater ponds: Monoculture of grey mullet and milkfish. *Asian Fisheries Science*, *19*, 165–176.

Gisbert, E., Mozanzadeh, M. T., Kotzamanis, Y., & Estévez, A. (2016). Weaning wild flathead grey mullet (*Mugil cephalus*) fry with diets with different levels of fish meal substitution. *Aquaculture*, *462*, 92–100. https://doi.org/10.1016/j.aquaculture.2016.04.035

Gowri, S. S., & Vasantha, K. (2010). Free radical scavenging and antioxidant activity of leaves from Agathi (*Sesbania grandiflora*) (L.) Pers. *American-Eurasian Journal of Scientific Research*, *5*, 114–119.

Habte-Tsion, H. M., Liu, B., Ge, X., Pan, L., & Chen, R. (2013). Effects of dietary protein level on growth performance, muscle composition, blood composition and digestive enzyme activity of wuchang bream (*Megalobrama amblycephala*) fry. *Israeli Journal of Aquaculture – Bamidgeh*, *65*, 9.

Hemre, G. I., Mommsen, T. P., & Krogdahl, Å. (2002). Carbohydrates in fish nutrition: Effects on growth, glucose metabolism and hepatic enzymes. *Aquaculture Nutrition*, *8*, 175–194. https://doi.org/10.1046/j.1365-2095.2002.00200.x

Hendricks, L. J. (1952). Erythrocytes counts and haemoglobin determinations for two species of suckers, genus *Catostomas*, from Colorado. *Copeia*, *4*, 265–266.

Hidalgo, M. C., Urea, E., & Sanz, A. (1999). Comparative study of digestive enzymes in fish with different nutritional habits. Proteolytic and amylase activities. *Aquaculture*, *170*, 267–283. https://doi.org/10.1016/S0044-8486(98)00413-X

Hotos, G. N., & Vlahos, N. (1998). Salinity tolerance of *Mugil cephalus* and *Chelon labrosus* (Pisces: Mugilidae) fry in experimental conditions. *Aquaculture*, *167*, 329–338. https://doi.org/10.1016/S0044-8486(98)00314-7

Jamil, A., Riaz, S., Ashraf, M., & Foolad, M. R. (2011). Gene expression profiling of plants under salt stress. *Critical Reviews in Plant Sciences*, *30*, 435–458. https://doi.org/10.1080/07352689.2011.605739

Jana, S. N., Garg, S. K., Barman, U. K., Arasu, A. R. T., & Patra, B. C. (2006). Effect of varying dietary protein levels on growth and production of *Chanos chanos* (Forsskal) in inland saline groundwater: Laboratory and field studies. *Aquaculture International*, *14*, 479–498. https://doi.org/10.1007/s10499-006-9050-5

Jana, S. N., Garg, S. K., & Patra, B. C. (2004). Effect of periphyton on growth performance of grey mullet, *Mugil cephalus* (Linn.), in inland saline groundwater ponds. *Journal of Applied Ichthyology*, *20*, 110–117. https://doi.org/10.1046/j.1439-0426.2003.00530.x

Jana, S. N., Sudesh, X., Garg, S. K., Sabhlok, V. P., & Bhatnagar, A. (2012). Nutritive evaluation of lysine-and methionine-supplemented raw vs heat-processed soybean to replace fishmeal as a dietary protein source for Grey Mullet, *Mugil cephalus*, and Milkfish, *Chanos chanos*. *Journal of Applied Aquaculture*, *24*, 69–80. https://doi.org/10.1080/10454438.2012.652032

Kaitho, R. J., Tegegne, A., Umunna, N. N., Nsahlai, I. V., Tamminga, S., Van Bruchem, J., & Arts, J. M. (1998). Effect of *Leucaena* and *Sesbania* supplementation on body growth and scrotal circumference of Ethiopian highland sheep and goats fed teff straw basal diet. *Livestock Production Science*, *54*, 173–181. https://doi.org/10.1016/S0301-6226(97)00152-8

Kalla, A., Garg, S. K., Kaushik, C. P., Arasu, A. R. T., & Dinodia, G. S. (2003). Effect of replacement of fish meal with processed soybean on growth, digestibility and nutrient retention in *Mugil cephalus* (Linn.) fry. *Indian Journal of Fisheries*, *50*, 509–518.

Khan, M. S., Ang, K. J., Ambak, M. A., & Saad, C. R. (1993). Optimum dietary protein requirement of Malaysian freshwater catfish, *Mystus nemurus*. *Aquaculture*, *112*, 227–235. https://doi.org/10.1016/0044-8486(93)90448-8

Khan, M. A., & Jafri, A. K. (1990). On the dietary protein requirement of *Clarias batrachus* Linnaeus. *Journal of Aquaculture in the Tropics*, *5*, 191–198.

Khattab, Y. A. E., Ahmad, M. H., Shalaby, A. M. E., & Abdel-Tawwab, M. (2000). Response of Nile tilapia (*Oreochromis niloticus* L.) from different locations to different dietary protein levels. *Egyptian Journal of Aquatic Biology and Fisheries*, *4*, 295–311.

Kim, J., & Lall, S. P. (2001). Amino acid composition of whole body tissue of Atlantic halibut (*Hippoglossus hippoglossus*), yellowtail flounder (*Pleuronectes ferruginea*) and Japanese flounder (*Paralichthy solivaceus*). *Aquaculture*, *187*, 367–373. https://doi.org/10.1016/S0044-8486(00)00322-7

Knox, W. E., & Greengard, O. (1965). The regulation of some enzymes of nitrogen metabolism—An introduction to enzyme physiology. *Advances in Enzyme Regulation*, *3*, 247–313. https://doi.org/10.1016/0065-2571(65)90059-2

Kumar, S., Sahu, N. P., Pal, A. K., Choudhury, D., Yengkokpam, S., & Mukherjee, S. C. (2005). Effect of dietary carbohydrate on haematology, respiratory burst activity and histological changes in *L. rohita* juveniles. *Fish and Shellfish Immunology*, *19*, 331–344. https://doi.org/10.1016/j.fsi.2005.03.001

Kumar, S., Sahu, N. P., Pal, A. K., Sagar, V., Sinha, A. K., & Baruah, K. (2009). Modulation of key metabolic enzyme of *Labeo rohita* (Hamilton) juvenile: Effect of dietary starch type, protein level and exogenous α-amylase in the diet. *Fish Physiology and Biochemistry*, *35*, 301–315. https://doi.org/10.1007/s10695-008-9213-6

Kuzmina, V. V. (1996). Influence of age on some digestive enzyme activity in some freshwater teleosts. *Aquaculture*, *148*, 25–37. https://doi.org/10.1016/S0044-8486(96)01370-1

Lee, C. S., Tamaru, C. S., Kelley, C. D., Moriwake, A., & Miyamoto, G. T. (1992). The effect of salinity on the induction of spawning and fertilization in the striped mullet, *Mugil cephalus*. *Aquaculture*, *102*, 289–296. https://doi.org/10.1016/0044-8486(92)90155-E

Lin, H. Z., Jiang, Q., Huang, J. N., & Shi, H. (1998). A preliminary study on the optimum protein level and protein to energy ratio of the diet for *Mugil cephalus*. *Journal of Shanghai Fisheries University*, *3*, 187–192.

Lisboa, V., Barcarolli, I. F., Sampaio, L. A., & Bianchini, A. (2015). Acclimation of juvenile *Mugil liza* Valenciennes, 1836 (Mugiliformes: Mugilidae) to different environmental salinities. *Neotropical Ichthyology*, *13*, 591–598. https://doi.org/10.1590/1982-0224-20140123

Liu, Y., Feng, L., Jiang, J., Liu, Y., & Zhou, X. Q. (2009). Effects of dietary protein levels on the growth performance, digestive capacity and amino acid metabolism of juvenile Jian carp (*Cyprinus carpio* var. Jian). *Aquaculture Research*, *40*, 1073–1082. https://doi.org/10.1111/j.1365-2109.2009.02201.x

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, *193*, 265–275.

Lupatsch, I., Katz, T., & Angel, D. L. (2003). Assessment of the removal efficiency of fish farm effluents by grey mullets: A nutritional approach. *Aquaculture Research*, *34*, 1367–1377. https://doi.org/10.1111/j.1365-2109.2003.00954.x

Luzzana, U., Valfrè, F., Mangiarotti, M., Domeneghini, C., Radaelli, G., Moretti, V. M., & Scolari, M. (2005). Evaluation of different protein sources in fingerling grey mullet *Mugil cephalus* practical diets. *Aquaculture International*, *13*, 291–303. https://doi.org/10.1007/s10499-004-3099-9.x

Ma, R., Liu, X., Meng, Y., Wu, J., Zhang, L., Han, B., … Li, C. (2019). Protein nutrition on sub-adult triploid rainbow trout (1): Dietary requirement and effect on anti-oxidative capacity, protein digestion and absorption. *Aquaculture*, *507*, 428–434. https://doi.org/10.1016/j.aquaculture.2019.03.069

Martínez-Álvarez, R. M., Morales, A. E., & Sanz, A. (2005). Antioxidant defenses in fish: Biotic and abiotic factors. *Reviews in Fish Biology and Fisheries*, *15*, 75–88. https://doi.org/10.1007/s11160-005-7846-4

Melo, J. F. B., Lundstedt, L. M., Metón, I., Baanante, I. V., & Moraes, G. (2006). Effects of dietary levels of protein on nitrogenous metabolism of *Rhamdia quelen* (Teleostei: Pimelodidae). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *145*, 181–187. https://doi.org/10.1016/j.cbpa.2006.06.007

Misra, H. P., & Frodovich, I. (1972). The role of superoxide anion in the auto oxidation of epinephrine and a simple assay for SOD. *Journal of Biological Chemistry*, *247*, 3170–3175.

Mitra, G., & Mukhopadhyay, P. K. (2002). Growth, nutrient utilization and tissue biochemical changes in rohu, *Labeorohita* fed with natural and prepared diets. *Journal of Applied Aquaculture*, *12*, 65–78. https://doi.org/10.1300/J028v12n03_07

Mohanta, K. N., Mohanty, S. N., Jena, J. K., & Sahu, N. P. (2008). 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. *Aquaculture Nutrition*, *14*, 350–359. https://doi.org/10.1111/j.1365-2095.2007.00542.x

Mohapatra, M., Sahu, N. P., & Chaudhari, A. (2002). Utilization of gelatinized carbohydrate in diets in *Labeo rohita* fry. *Aquaculture Nutrition*, *8*, 1–8. https://doi.org/10.1046/j.1365-2095.2003.00243.x

Moore, B. J., Hung, S. S. O., & Medranno, J. F. (1988). Protein requirement of hatchery produced juvenile white sturgeon (*Acipenser transmontanus*). *Aquaculture*, *71*, 235–245. https://doi.org/10.1016/0044-8486(88)90262-1

Morgan, J. D., & Iwama, G. K. (1991). Effects of salinity on growth, metabolism, and ion regulation in juvenile rainbow and steelhead trout (*Oncorhynchus mykiss*) and fall chinook salmon (*Oncorhynchus tshawytscha*). *Canadian Journal of Fisheries and Aquatic Sciences*, *48*, 2083–2094. https://doi.org/10.1139/f91-247

Murray, R. K., Granner, D. K., Mayes, P. A., & Rodwell, V. W. (2000). Glycogen metabolism. In P.A. Mayes, & R.K. Murray, (Eds.)*Harper's biochemistry* (25th ed., pp. (199–207).Stanford, CT: Appleton and Lange

Nagai, M., & Ikeda, S. (1972). Carbohydrate metabolism in fish. III. Effect of dietary composition on metabolism of glucose U 14C and glutamate in carp. *Bulletin of the Japanese Society for the Science of Fish*, *38*, 137–143.

Ng, W. K., Soon, S. C., & Hashim, R. (2001). The dietary protein requirement of a bagrid catfish, *Mystus nemurus* (Cuvier and Valenciennes), determined using semipurified diets of varying protein level. *Aquaculture Nutrition*, *7*, 45–51. https://doi.org/10.1046/j.1365-2095.2001.00160.x

Nordlie, F. G., Szelistowski, N. A., & Nordlie, W. C. (1982). Ontogenesis of osmotic regulation in the striped mullet, *Mugil cephalus* L. *Journal of Fish Biology*, *20*, 79–86. https://doi.org/10.1111/j.1095-8649.1982.tb03896.x

NRC. (1993). *Nutrient requirement of fish*. Washington, DC: National Academy Press.

NRC. (2011). *Nutrient requirements of fish and shrimp*. Washington, DC: National Academies Press.

Ochoa, S. (1955). Malic dehydrogenase from pig heart: l-Malate + DPN + ⇆ Oxalacetate + DPNH + H+ In S. P. Colowick, & N. O. Kapalan (Eds.), *Methods of enzymology* (vol. *1*, pp. 735). New York, NY: Academic press Inc.

Olsvik, P. A., Torstensen, B. E., Hemre, G. I., Sanden, M., & Waagbo, R. (2011). Hepatic oxidative stress in Atlantic salmon (*Salmo salar* L.) transferred from a diet based on marine feed ingredients to a diet based on plant ingredients. *Aquaculture Nutrition*, *17*, 424–436. https://doi.org/10.1111/j.1365-2095.2010.00778.x

Papaparaskeva-Papoutsoglou, E., & Alexis, M. N. (1986). Protein requirements of young grey mullet, *Mugil capito*. *Aquaculture*, *52*, 105–115. https://doi.org/10.1016/0044-8486(86)90030-X

Partridge, G. J., Lymbery, A. J., & George, R. J. (2008). Finfish mariculture in inland Australia: A review of potential water sources, species, and production systems. *Journal of World Aquaculture Society*, *39*, 291–310. https://doi.org/10.1111/j.1749-7345.2008.00169.x

Partridge, G. J., Sarre, G. A., Ginbey, B. M., Kay, G. D., & Jenkins, G. I. (2006). Finfish production in a static, inland saline water body using a Semi-Intensive Floating Tank System (SIFTS). *Aquacultural Engineering*, *35*, 109–121. https://doi.org/10.1016/j.aquaeng.2005.09.001

Pérez-Jiménez, A., Hidalgo, M. C., Morales, A. E., Arizcun, M., Abellán, E., & Cardenete, G. (2009). Antioxidant enzymatic defenses and oxidative damage in *Dentex dentex* fed on different dietary macronutrient levels. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *150*, 537–545. https://doi.org/10.1016/j.cbpc.2009.07.011

Pieper, A., & Pfeffer, E. (1980). Studies on the comparative efficiency of utilization of gross energy from some carbohydrates, proteins and fats by rainbow trout (*Salmo gairdneri*, R.). *Aquaculture*, *20*, 323–332. https://doi.org/10.1016/0044-8486(80)90093-9

Raizada, S., Purushothaman, C. S., Sharma, V. K., Harikrishna, V., Rahaman, M., Agrahari, R. K., & Kumar, A. (2015). Survival and growth of tiger shrimp (*Penaeus monodon*) in inland saline water supplemented with potassium. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, *85*, 491–497. https://doi.org/10.1016/10.1007/s40011-014-0372-1

Rick, W., & Stegbauer, H. P. (1974). α- Amylase measurement of reducing groups. In H. U. Bergmeyer (Ed.), *Methods of enzymatic analysis* (vol. *2*, 2nd ed., pp. 885–915). Weinheim, Germany: Verlag Chemie.

Robbins, K. R., Norton, H. W., & Baker, D. H. (1979). Estimation of nutrient requirements from growth data. *Journal of Nutrition*, *109*, 1710. https://doi.org/10.1093/jn/109.10.1710

Sá, R., Gavilán, M., Rioseco, M. J., Llancabure, A., Vargas-Chacodd, L., Augsburger, A., & Bas, F. (2014). Dietary protein requirement of Patagonian blennie (*Eleginops maclovinus*, Cuvier 1830) juveniles. *Aquaculture*, *428*, 125–134. https://doi.org/10.1016/j.aquaculture.2014.02.017

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*). *Animal Nutrition*, *3*, 156–163. https://doi.org/10.1016/j.aninu.2017.03.003

Saoud, I. P., Davis, D. A., & Rouse, D. B. (2003). Suitability studies of inland well waters for *Litopenaeus vannamei* culture. *Aquaculture*, *217*, 373–383. https://doi.org/10.1016/S0044-8486(02)00418-0

Schneider, W. C. (1957). Determination of nucleic acids in tissue by pantose analysis. In S. P. Calowick, & N. O. Kaplon (Eds.), *Methods of enzymology* (pp. 680). New York, NY: Academic Press.

Sealey, W. M., Gaylord, T. G., Toner, M., Ilgen, J., Fraser, W. C., Hooley, C. G., & Barrows, F. T. (2013). Growth and acute temperature tolerance of June Sucker juveniles fed varying dietary protein and lipid levels with and without supplemental dicalcium phosphate. *North American Journal of Aquaculture*, *75*, 124–132. https://doi.org/10.1080/15222055.2012.736449

Seong, M., Lee, S., Lee, S., Song, Y., Bae, J., Chang, K., & Bai, S. C. (2018). The effects of different levels of dietary fermented plant-based protein concentrate on growth, hematology and non-specific

immune responses in juvenile olive flounder, *Paralichthys olivaceus*. *Aquaculture*, *483*, 196–202. https://doi.org/10.1016/j.aquaculture.2017.10.023

Shaw, A. F. (1930). A direct method for counting the leukocytes, thrombocytes and erythrocytes in bird's blood. *Journal of Pathology and Bacteriology*, *33*, 833–835.

Shiau, S. Y., & Lan, C. W. (1996). Optimum dietary protein level and protein to energy ratio for growth of grouper (*Epinephelus malabaricus*). *Aquaculture*, *145*, 259–266. https://doi.org/10.1016/S0044-8486(96)01324-5

Shimeno, S., Shikata, T., Hosokawa, H., Masumoto, T., & Kheyyali, D. (1997). Metabolic response to feeding rates in common carp, *Cyprinus carpio*. *Aquaculture*, *151*, 371–377. https://doi.org/10.1016/S0044-8486(96)01492-5

Steinhart, M., & Eckman, R. (1992). Evaluating the nutritional condition of white fish (*Coregonous* sp.) larvae by the RNA/DNA ratio. *Journal of Fish Biology*, *40*, 791–799. https://doi.org/10.1111/j.1095-8649.1992.tb02625.x

Stone, D. A. (2003). Dietary carbohydrate utilization by fish. *Reviews in Fisheries Science*, *11*, 337–369. https://doi.org/10.1080/10641260390260884

Storey, K. B. (1996). Oxidative stress: Animal adaptations in nature. *Brazilian Journal of Medical and Biological Research*, *29*, 1715–1733.

Tacon, A. G. I. (1987). *The nutrition and feeding of farm fish and shrimp a training manual. 1. The essential nutrients*. FAO Brasilia Brazil, GCP/RLA/075/ITA Field Document 2/E, 117.

Takahara, S., Hamilton, H. B., Neel, J. V., Kobara, T. Y., Ogura, Y., & Nishimura, E. T. (1960). Hypocatalasemia, a new genetic carrier state. *Journal of Clinical Investigation*, *39*, 610–619. https://doi.org/10.1172/JCI104075

Talukdar, A., Kumar, S., Varghese, T., Jain, K. K., Sahu, N. P., & Sahoo, S. (2019). Feeding gelatinized carbohydrate in the diets of magur, *Clarias batrachus* (Linnaeus, 1758): Effects on growth performance, enzyme activities and expression of muscle regulatory factors. *Aquaculture Research*, *50*, 765–777. https://doi.org/10.1111/are.13933

Tseng, Y. C., & Hwang, P. P. (2008). Some insights into energy metabolism for osmoregulation in fish. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology*, *148*, 419–429. https://doi.org/10.1016/j.cbpc.2008.04.009

Tucker, J. W. Jr. (1992). Marine fish nutrition. In G. L. Allan, & W. Dall (Eds.), *Proceeding of the aquaculture nutrition workshop* (pp. 25–40). Salamander Bay, NSW: NSW Fisheries Brackish Water Fish Culture Research Station.

Van Kampen, E. J., & Zijlstra, N. C. (1961). Determination of haemoglobin. *Clinica Chemica Acta*, *5*, 719–720.

Verma, A. K., Pal, A. K., Manush, S. M., Das, T., Dalvi, R. S., Chandrachoodan, P. P., … Apte, S. K. (2007). Persistent sub-lethal chlorine exposure elicits the temperature induced stress responses in *Cyprinus carpio* early fingerlings. *Pesticide Biochemistry and Physiology*, *87*, 229–237. https://doi.org/10.1016/j.pestbp.2006.08.001

Vieira, V. P., Inoue, L. A. K., & Moraes, G. (2005). Metabolic responses of matrinxa (*Brycon cephalus)* to dietary protein level. *Comparative Biochemistry and Physiology Part A*, *140*, 337–342. https://doi.org/10.1016/j.cbpb.2005.01.018

Wang, J., Jiang, Y., Li, X., Han, T., Yang, Y., Hu, S., & Yang, M. (2016). Dietary protein requirement of juvenile red spotted grouper (*Epinephelus akaara*). *Aquaculture*, *450*, 289–294. https://doi.org/10.1016/j.aquaculture.2015.08.007

Wassef, E. A., El Masry, M. H., & Mikhail, F. R. (2001). Growth enhancement and muscle structure of striped mullet, *Mugil cephalus* L., fingerlings by feeding algal meal-based diets. *Aquaculture Research*, *32*, 315–322. https://doi.org/10.1046/j.1355-557x.2001.00043.x

Whitfield, A. K., Panfili, J., & Durand, J. D. (2012). A global review of the cosmopolitan flathead mullet *Mugil cephalus* Linnaeus 1758 (Teleostei: Mugilidae), with emphasis on the biology, genetics, ecology and fisheries aspects of this apparent species complex. *Reviews in Fish Biology and Fisheries*, *22*, 641–681. https://doi.org/10.1007/s11160-012-9263-9

Winfree, R. A., & Stickney, R. R. (1981). Effects of dietary protein and energy on growth, feed conversion efficiency and body composition of *Tilapia aurea*. *Journal of Nutrition*, *111*, 1001–1012. https://doi.org/10.1093/jn/111.6.1001

Wooten, I. D. P. (1964). Microanalysis. In J. Churchill, & A. Churchill (Eds.), *Medical biochemistry* (4th ed., pp. 101–107). London, UK: London Churchill

Wootton, R. J. (1990). *Ecology of teleost fishes*. London, UK: Chapman and Hall.

Wrobleiuski, F., & Laude, J. S. (1955). LDH activity in blood. *Proceedings of the Society for Experimental Biology and Medicine*, *90*, 210–213.

Yang, S. D., Liou, C. H., & Liu, F. G. (2002). Effects of dietary protein level on growth performance, carcass composition and ammonia excretion in juvenile silver perch (*Bidyanus bidyanus*). *Aquaculture*, *213*, 363–372. https://doi.org/10.1016/S0044-8486(02)00120-5

Yengkokpam, S., Debnath, D., Pal, A. K., Sahu, N. P., Jain, K. K., Norouzitallab, P., & Baruah, K. (2013). Short-term periodic feed deprivation in *Labeo rohita* fingerlings: Effect on the activities of digestive, metabolic and anti-oxidative enzymes. *Aquaculture*, *412*, 186–192. https://doi.org/10.1016/j.aquaculture.2013.07.025

Yoshimatsu, T., Furuichi, M., & Kitajima, C. (1992). Optimum level of protein in purified experimental diets for redlip mullet (*L. haematocheila*). *Nippon Suisan Gakkaishi*, *58*, 2111–2117. https://doi.org/10.2331/suisan.58.2111

How to cite this article: Talukdar A, Deo AD, Sahu NP, et al. Effects of dietary protein on growth performance, nutrient utilization, digestive enzymes and physiological status of grey mullet, *Mugil cephalus* L. fingerlings reared in inland saline water. *Aquacult Nutr*. 2020;26:921–935. https://doi.org/10.1111/anu.13050