### **REGULAR ARTICLES**



# Utilization of marine fisheries wastes for the production of the freshwater fish *Cyprinus carpio*

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# Abstract

The present study was aimed to assess the effects of complete replacement of fish meal with fisheries waste meals on survival, growth performance, digestive enzyme activities, and muscle compositions of the freshwater fish *Cyprinus carpio*. The proximate composition and mineral contents of three different fisheries wastes, such as *Rastrelliger kanagurta*, *Sphyraena barracuda*, and *Fenneropenaeus indicus* were analyzed. Based on the nutrient content of these fisheries waste, one control fish meal diet and three different complete fish meal replacement diets (diet 1, diet 2, and diet 3 formulated with *R. kanagurta*, *S. barracuda*, and *F. indicus* waste meals, respectively) were formulated. Fingerlings *C. carpio* were fed with these diets for a period of 6 weeks. Results from feeding experiments showed insignificant (p > 0.05) differences in survival, growth, and feed intake of *C. carpio* fed with control and three different fisheries waste diets. However, the digestive enzyme activity and muscle biochemical compositions were significantly (p < 0.05) altered in *F. indicus* waste meal fed *C. carpio* compared to other fisheries waste meal and control diets fed fish groups. Therefore, the present study suggests that *R. kanagurta*, *S. barracuda*, and *F. indicus* waste meals can be considered as alternative feed ingredients for fish meal to formulate low-cost feeds for *C. carpio* culture.

Keywords Fish meal · Cyprinus carpio · Proximate composition · Feed · Fisheries waste · Digestive enzymes

# Abbreviations

FWMFisheries waste(s) meals(s)WMWaste(s) meal(s)WMDWaste(s) meal(s) diet (s)

# Introduction

Fish and fish products have presently emerged as the largest group in agricultural exports in the world. According to the FAO (2016), India has produced 4.9 million metric tons of aquaculture products during 2014. Among the different aquaculture practices, the culture of freshwater organisms

including grass carp (*Ctenopharyngodon idellus*), common carp (*Cyprinus carpio*), Nile tilapia (*Oreochromis niloticus*), catla (*Catla catla*), Scampi (*Macrobrachium rosenbergii*), and monsoon river prawn (*Macrobrachium malcolmsonii*) has been playing significant role in global food production. Among these freshwater organisms, *C. carpio* is considered as a very important cultivable species in Asian and European countries due to the presence of essential nutrients like protein, essential amino acids, fatty acids, lipid, vitamins, and minerals for mankind. Next to grass carp and silver carp, *C. carpio* ranked as the third position in world total finfish production which contributed about 9% of the global finfish production (FAO 2012).

Artificial feeds play a significant role in aquaculture operations which contains all essential nutrients like protein, amino acids, carbohydrate, lipid, fatty acids, vitamins, and minerals for farming species. However, the fish feed is one of the major cost components in aquaculture operations which reaches about 50–60% of total production cost (Adikari et al. 2017). Among the different types of feed ingredients, fish meal is a primary feed ingredient in commercial feed formulations which contains a chief source of protein, essential amino acids, lipids, vitamins, and minerals. However, the

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cost of fish meal is increasing continuously due to overexploitations and unpredictable availability in nature. Hence, it is a necessity to find out alternate feed ingredient for replacing fish meal with low-cost animal protein sources (Forster et al. 2003; Lunger et al. 2007; FAO 2010).

Waste from fish-processing industries is an important environmental contamination source. Fish heads, viscera (gut) gills, skin, and fins are discarded as fish waste. Dumping of this waste creates both disposal and pollution problems to the environment (Norziah et al. 2009). About 50% of waste materials from the total fish capture is not used as a food source and involves almost 32 million tonnes of waste per year in the world (Kristinsson and Rasco 2000). India alone generates greater than 2 million metric tonnes of fisheries by-products per year without recovery of any useful product (Nurdiyana and Mazlina 2009). This processing discards the major valuable components like nutrients, pigments, and enzymes (Sachindra et al. 2006). The utilization of fisheries wastes for the production of various value-added products, such as proteins, fatty acids, fish oils, amino acids, vitamins, minerals, enzymes, bioactive peptides, etc., is becoming a significant industry process which leads to reducing the waste and help to eliminate harmful effects on the environment (Fuchise et al. 2009; Gumisiriza et al. 2009; Esposito et al. 2010; Mathew 2010; Ghaly et al. 2013). Hence, the present study was aimed to assess the proximate composition and minerals including trace elements of marine fish waste meals, such as R. kanagurta, S. barracuda, and F. indicus, followed by evaluating the effect of complete replacement of fish meal with these fisheries waste meals on the survival, growth, digestive enzyme activities, such as protease, amylase and lipase, and muscle biochemical constituents, such as protein, amino acids, carbohydrate, and lipid of the freshwater fish C. carpio fingerlings as alternative feed ingredient for fish meal to formulate for low-cost fish feed.

# Materials and methods

# Collection and processing of fisheries waste

The wastes including head, skin, viscera, fin, and tail of fish *Rastrelliger kanagurta* (Indian mackerel) and *Sphyraena barracuda* (Great barracuda), and head, legs and shells of shrimp, *Fenneropenaeus indicus* (Indian white shrimp) were collected from Ukkadam fish market in Coimbatore, Tamil Nadu, India. The waste materials of each species packed in separate polythene bags and transported to the laboratory. The collected fisheries wastes were sun-dried at  $28.64 \pm 1.77$  °C for 6 h per day for 2 weeks. During the drying process, fisheries wastes were covered with mosquito nets to protect from flies, insects, birds etc. Further, these dried waste materials were powered finely using mixer grinder and stored

individually in airtight plastic containers at -20 °C until further analysis.

## Analysis of proximate composition of fisheries waste

The proximate composition of each fisheries waste meal (FWM) including crude protein, lipid, fiber, nitrogen-free extracts (NFE), ash, moisture, and energy levels was estimated according to standard procedures (Table 1). The amounts of crude protein, lipid, fiber, moisture, and ash contents were analyzed according to standard procedures of AOAC (1995). The content of nitrogen-free extracts (NFE) was calculated using the formula [NFE (%) = 100 - (moisture + crude protein + crude lipid + total ash + crude fiber)] prescribed by Natarajan (2006). The level of gross energy was calculated by multiplying generalized physiological energy values of proteins (19 kJ), NFE (15 kJ), and lipids (36 kJ) with the energy levels contributed by the crude protein, NFE, and crude lipid fractions of FWM [energy contributed by crude protein (kJ/g) = protein content of feed  $(\%) \times 19 = x$ ; energy contributed by NFE (kJ/g) = NFE content of feed  $(\%) \times 15 =$ z; energy contributed by crude lipid (kJ/g) = lipid content of feed (%)  $\times$  36 = y; gross energy (GE kJ/g) = (x + y + z)/100] (Natarajan 2006).

# Analysis of minerals and trace elements

Content of trace elements (Zn, Cu, Cd, and Pb) and other mineral salts (Na, K, and Mg) of FWM was analyzed according to AOAC (1995) standard procedures (Table 2). Briefly, 1 g of each FWM was digested separately using 10-mL 9:2:1 ratio of HNO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub>, and HClO<sub>4</sub> in a hot plate at 80 °C. Further, the volume was made up to 25 mL, followed by filtered using Whatman filter paper No. 45. The trace elements and other minerals were analyzed using atomic absorption spectrometry (Perkin-Elmer; Model 2380) and results were expressed as mg/kg.

Table 1         Proximate composition	of collected fisheries waste
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Composition (%)	R. kanagurta	S. barracuda	F. indicus
Protein	$38.00 \pm 1.20^{b}$	$37.52 \pm 1.41^{b}$	$41.56 \pm 1.84^{a}$
Carbohydrate	$15.67\pm1.0^a$	$3.59\pm0.20^{\rm c}$	$9.02\pm1.05^{b}$
Fiber	$1.0\pm0.21^{b}$	$1.0\pm0.20^{b}$	$11.37\pm1.21^a$
Lipid	$8.17\pm0.84^{b}$	$18.86\pm1.42^a$	$4.55\pm0.71^{c}$
Ash	$31.29\pm1.17^b$	$33.83 \pm 1.71^a$	$25.19\pm1.54^c$
Moisture	$5.88\pm0.57^{b}$	$5.20\pm0.49^{b}$	$8.87\pm0.72^a$
Gross energy (kJ/g)	$12.69 \pm 1.40^{b}$	$16.81 \pm 1.42^{a}$	$12.27\pm1.51^{b}$

n = 3 (three samples from each treatment); mean  $\pm$  SD; mean values within the same row sharing the same superscript are not significantly different (p > 0.05)

 Table 2
 Contents of mineral salts and trace elements in the collected fisheries waste

Minerals/elements (mg/kg)	R. kanagurta	S. barracuda	F. indicus
Na	$93.94 \pm 1.24^{\rm a}$	$76.17 \pm 1.58^{b}$	$76.74 \pm 1.45^{b}$
Κ	$17.50\pm1.18^a$	$16.88\pm1.50^b$	$16.08 \pm 1.47^{b}$
Mg	$10.08\pm1.20^a$	$10.04\pm1.10^{b}$	$10.04 \pm 1.01^{b}$
Zn	$50.0\pm1.0^{a}$	$49.62 \pm 0.64^{a}$	$43.66 \pm 0.95^{b}$
Cu	$4.64\pm0.1^{b}$	$5.00\pm0.18^{b}$	$27.99 \pm 1.0^a$
Cd	$0.97\pm0.01^{a}$	$0.28\pm0.01^{b}$	$0.25\pm0.01^{b}$
Pb	$1.39\pm0.09^a$	$1.28\pm0.47^a$	$1.45 \pm 0.70^{a}$

n=3 (three samples from each treatment); mean  $\pm$  SD; mean values within the same row sharing the same superscript are not significantly different (p > 0.05)

### Preparation of fishmeal replacement diets

Experimental feeds were prepared using commonly available feed ingredients (fish meal, soybean meal, groundnut oil cake, wheat bran, and tapioca flour) in the local markets. Briefly, fish meal, soybean meal, and groundnut oil cake were separately grounded using a mixer grinder and sieved through a 60-µm mesh. Each ingredient was weighed at desired concentrations (Table 3) to formulate 37% protein diets which is the optimum requirement for culturing of C. carpio. These weighed feed ingredients were thoroughly mixed at different ratios for preparing four different diets (one control fish meal diet and three 100% fish meal replacement diets (diet 1, diet 2, and diet 3 fish meal replaced with R. kanagurta, S. barracuda, and F. indicus waste meal, respectively). These blends were cooked in a closed aluminum container at 105 °C for 15 min, followed by cooling at room temperature. Further, Cod liver oil, vitamins, and egg albumin were added and thoroughly mixed for obtaining a stiff dough. The dough was pelletized by an indigenous hand pelletizer with the mesh size of 0.1 mm diameter (Pigeon manufactures, Kolkata, India) and was cut into  $3.0 \pm$ 0.18-mm-sized pieces. The pellets were dried at room temperature (27 °C) until constant weight was reached. The prepared feeds was stored individually in airtight plastic containers at -20 °C until to use the feeding trials. The proximate composition of the prepared feeds was analyzed according to the standard methods (AOAC 1995; Natarajan 2006) (Table 4).

#### Collection and acclimatization of experimental fishes

Fingerlings *C. carpio*  $(1.12 \pm 0.2 \text{ cm} \text{ and } 0.30 \pm 0.01 \text{ g length}$ and weight, respectively) were collected from the Bhavanisagar dam in Erode District, Tamil Nadu, India. Fingerlings were safely transported to the laboratory using plastic polythene bags half filled with oxygenated dam water and acclimatized to ambient laboratory conditions for 1 week in plastic tubs (50 L) with tap water. The acclimatized water was analyzed for temperature  $(27 \pm 1.04 \text{ °C})$ , dissolved oxygen  $(7.50 \pm 0.20 \text{ mg/L})$ , pH  $(7.43 \pm 0.21)$ , total dissolved solids  $(0.61 \pm 0.05 \text{ g/L})$ , biological oxygen demand  $(18.40 \pm 2.05 \text{ mg/L})$ , chemical oxygen demand  $(67.0 \pm 2.70 \text{ mg/L})$ , and NH<sub>3</sub>  $(0.017 \pm 0.001 \text{ mg/L})$  according to the standard methods of APHA (1995). During acclimatization period, adequate aeration was given to the fingerlings and they were fed with control feed prepared with basal feed ingredients twice (at 06:00 h and 18:00 h, respectively) per day, and about 60% of rearing tap water was renewed daily in order to maintain a healthy environment.

### **Feeding experiments**

For this study, four groups of C. carpio fingerlings  $(1.88 \pm$ 0.27 cm length and  $0.34 \pm 0.11$  g weight) were assigned for this experiment in triplicate (three independent groups per diet) for a period of 6 weeks. One group was served as a control and fed the control feed (formulated with fish meal). The remaining three groups were fed with feeds formulated with respective FWM diets (diet 1, diet 2, and diet 3). The fish groups were separately maintained in a 20-L plastic aquarium with a stocking density of one fish/L. During the experimental period, about 60% of aquarium water was renewed every day by siphoning method with minimum disturbance to the fishes and the water quality parameters of each aquarium were maintained as same to acclimatization condition. Fish fingerlings were fed these experimental feeds at 5% of body weight (body weight was measured every 10-day interval for adjusting the feeding level) twice per day (at 06:00 h and 18:00 h). Fishes were maintained on a 12-h light/12 h dark photoperiod during the experimental period. The unfed feed and feces were removed by siphoning filtration during the feeding trial while renewing the rearing water.

#### Survival, growth, and food index analysis

The survival, growth performance, such as length gain (LG), weight gain (WG), and specific growth rate (SGR), and food index parameters, such as feed intake (FI) and feed conversion ratio (FCR), were calculated by the following equations: Survival (%) = no.of live fish/no.of fish introduced × 100.

- LG(cm) = final length(cm)-initial length(cm).
- $WG \ (g) = final \ weight \ (g)-initial \ weight \ (g).$

SGR (%/day) = log final weight (g)-log

initial weight (g)/total number of days  $\times$  100.

FI(g/day) = feed eaten (g)/total number of days.

FCR = feed intake (g)/weight gain (g).

# Estimation of digestive enzymes activity

Activities of digestive enzymes such as protease, amylase, and lipase were assayed in the fishes at the end of the experimental period. The whole intestine of fish from each feeding experiment was carefully dissected out and homogenized in ice-cold distilled water and centrifuged at 9300g under 4 °C for 20 min. The supernatant was used as a crude enzyme source. The activity of protease was analyzed by the casein-hydrolysis method of Furne et al. (2005). One unit of enzyme activity represents the amount of enzyme contributed to liberate 1 µg of tyrosine per min. Casein and L-tyrosine were used as substrate and standard, respectively. Amylase activity was determined by the starch-hydrolysis method (Bernfeld 1955). One unit of amylase activity was defined as the amount of enzyme that produced 1 µg of maltose per min. Starch and maltose were used as substrate and standard, respectively. Lipase activity was estimated by titration method (Furne et al. 2005). The activity of one unit of lipase was calculated as the volume of free fatty acids released from triacylglycerol per unit of time estimated by the amount of NaOH required for maintaining the constant pH and represented as milli equivalents of alkali consumed. Virgin Olive oil and lipase were used as substrate and standard, respectively. All these enzymes are expressed as

 Table 3
 Ingredients and proximate composition of experimental diets

Ingredients (g/100 g)	Control	Diet 1	Diet 2	Diet 3
Fish meal	25	0	0	0
Soybean meal	25	32	32	32
Groundnut oil cake	25	20	20	20
Wheat bran	10	8	8	8
Tapioca flour	5	5	5	5
Egg albumin	7	7	7	7
Sunflower oil	2	2	2	2
Vitamin mix <sup>a</sup>	1	1	1	1
Fisheries waste	0	25	25	25
Proximate composition	(%)			
Protein	44.43	38.64	38.54	39.54
Carbohydrate	20.92	27.58	24.21	24.87
Fiber	7.24	7.84	7.84	10.43
Lipid	5.57	5.52	8.20	4.62
Ash	12.22	11.42	12.01	11.14
Moisture	9.62	9.0	9.20	9.40
Gross energy (kJ/g)	13.58	13.46	13.90	12.90

Control: formulated with fish meal; diet 1: fish meal replaced by *R. kanagurta* waste meal; diet 2: fish meal replaced by *S. barracuda* waste meal; diet 3: fish meal replaced by *F. indicus* waste meal

<sup>a</sup> Becosules capsules (manufactured by Pfizer), each capsule contains Thiamine Mononitrate IP 10 mg; Riboflavin IP 10 mg; Pyridoxine Hydrochloride IP 3 mg; Vitamin B12 (as tablets 1:100) IP 15 mcg Niacinamide IP 100 mg; Calcium pantothenate IP 50 mg; Folic acid IP 1.5 mg; Biotin USP 100 mcg; Ascorbic acid IP 150 m specific activity (U/mg protein = enzyme activity/soluble protein (mg/mL). The protein content of crude enzyme extracts was analyzed according to the standard method (Lowry et al. 1951).

#### Proximate composition analysis of fish

Concentrations of muscle total protein, total amino acid, and total carbohydrate contents of fish were analyzed according to the standard methods of Lowry et al. (1951), Moore and Stein (1948), and Roe (1955), respectively. The content of total lipid was extracted (Folch et al. 1957) and estimated according to the method of Barnes and Blackstock (1973). Ash and moisture contents of fish were determined by the standard procedures of AOAC (1995).

#### Statistical analysis

All the data were expressed as mean  $\pm$  SD. The data were analyzed by one-way analysis of variance (ANOVA), followed by Duncan's multiple range test (DMRT) using SPSS (16.0) software to compare the significant (p < 0.05) differences among treatments. The data of survival, specific growth rate, ash, and moisture were arcsine transformed prior to oneway analysis.

# Results

### Proximate composition of collected fisheries waste

In the present study, protein and fiber contents were significantly (p < 0.05) higher in *F. indicus* waste meal (WM) when compared to R. kanagurta and S. barracuda WM. Whereas the NFE content was significantly (p < 0.05) higher in the R. kanagurta waste meal, followed by F. indicus and S. barracuda WM. In this context, the lipid content was significantly (p < 0.05) higher in S. barracuda WM when compared to the other WM. The ash content was significantly (p < 0.05) higher in *R. kanagurta* and *S. barracuda* WM rather than in F. indicus WM, however, an insignificant (p > 0.05) difference was noted in ash content between R. kanagurta and S. barracuda WM. The moisture content was significantly (p < 0.05) higher in *F. indicus* WM than in R. kanagurta and S. barracuda. Meanwhile, the gross energy level was significantly (p < 0.05) higher in S. barracuda and F. indicus WM when compared to the R. kanagurta WM, however, there was no significant (p > 0.05) difference noted in the energy level in S. barracuda and F. indicus WM (Table 1).

Table 4 Survival, growth, and food index of C. carpio fed with fish meal replacement feeds

Parameters		Control	Diet 1	Diet 2	Diet 3
Survival (%)		$90.0 \pm 5.00^{a}$	$85.0\pm5.00^{\rm a}$	$86.0 \pm 5.70^{a}$	$88.0\pm2.88^{\rm a}$
Feed intake (g/day)		$0.36\pm0.010^a$	$0.35 \pm 0.011^{a}$	$0.33\pm0.012^a$	$0.34\pm0.014^{a}$
Feed conversion ratio		$1.84\pm0.31^{a}$	$1.48 \pm 0.10^{\rm b}$	$1.52 \pm 0.11^{\rm b}$	$1.45\pm0.10^{b}$
Length (cm) <sup>1</sup>	Initial	$1.88 \pm 0.27$	$1.88 \pm 0.27$	$1.88 \pm 0.27$	$1.88 \pm 0.27$
	Final	$3.56\pm0.14^{\rm a}$	$3.62 \pm 0.11^{a}$	$3.82 \pm 0.27^{a}$	$3.60 \pm 0.14^{a}$
Weight $(g)^1$	Initial	$0.34 \pm 0.11$	$0.34 \pm 0.11$	$0.34 \pm 0.11$	$0.34 \pm 0.11$
	Final	$0.80\pm0.065^{\rm a}$	$0.90 \pm 0.125^{a}$	$0.91 \pm 0.173^{\mathrm{a}}$	$0.90\pm0.036^a$
Length gain (cm) <sup>1</sup>		$1.68\pm0.16^{\rm a}$	$1.74 \pm 0.13^{\rm a}$	$1.94\pm0.30^{\rm a}$	$1.72 \pm 1.58^{a}$
Weight gain $(g)^1$		$0.46\pm0.07^{\rm a}$	$0.57\pm0.14^{\rm a}$	$0.53\pm0.19^{\rm a}$	$0.56\pm0.04^a$
Specific growth rate (% /day)		$0.57\pm0.03^a$	$0.68\pm0.04^a$	$0.64 \pm 0.02^{a}$	$0.68\pm0.03^a$

n = 3 (three samples from each treatment); mean  $\pm$  SD; mean values within the same row sharing the same superscript are not significantly different (p > 0.05)

 $^{1} n = 5$  (five samples from each treatment)

Control: formulated with fish meal; diet 1: fish meal replaced by *R. kanagurta* waste meal; diet 2: fish meal replaced by *S. barracuda* waste meal; diet 3: fish meal replaced by *F. indicus* waste meal

### Contents of mineral salts and trace elements

In the current study, the mineral salts, such as Na, K, and Mg, and trace elements, such as Zn and Cd contents, were significantly (p < 0.05) higher in *R. kanagurta* WM compared to *S. barracuda* and *F. indicus* WM, however, an insignificant (p > 0.05) difference was noted in these elements between *S. barracuda* and *F. indicus* WM. In this context, the content of Cu was significantly (p < 0.05) preeminent in *F. indicus* WM when compared to *R. kanagurta* and *S. barracuda* WM, whereas the difference between *R. kanagurta*, *S. barracuda*, and *F. indicus* WM was insignificant (p > 0.05) in the case of Pb content (Table 2).

#### Survival, growth, and food index

In this study, survival, length, weight, length gain, weight gain, feed intake, and specific growth rate were insignificantly (p > 0.05) increased in *C. carpio* fed with different types fish meal replacement diets (diet 1, diet 2, and diet 3) when compared to control fish meal diet, whereas the feed conservation ratio was significantly (p < 0.05) higher in the control diet-fed fish group when compared to *S. barracuda* and *F. indicus* waste meal diets (WMD) fed fish groups. However, an insignificant (p > 0.05) difference was noted in feed conversion ratio between control fish meal diet and *R. kanagurta* WMD fed fish groups (Table 4).

## Activities of digestive enzymes

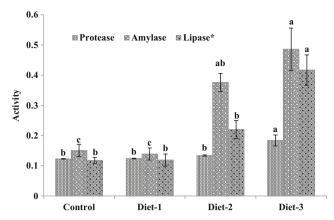
The activities of digestive enzymes, such as protease, amylase, and lipase, were significantly (p < 0.05) improved in fish fed with *F. indicus* WMD when compared to control and other fisheries WMD fed fishes, whereas an insignificant (p > 0.05) elevation was noted in protease and lipase activities among control, *R. kanagurta* and *S. barracuda* WMD fed fish groups. In context, the insignificant (p > 0.05) variation was seen in amylase activity between control and *R. kanagurta* WMD fed fish groups (Fig. 1).

# Proximate composition of C. carpio

In the present study, the protein content was significantly (p < 0.05) higher in *C. carpio* fed with *F. indicus* waste meal diet, followed by *S. barracuda* WMD, control fish meal diet, and *R. kanagurta* WMD, while the amino acid and lipid contents were significantly (p < 0.05) increased in *C. carpio* fed on *F. indicus* WMD when compared to control fish meal diet and other fish meal replacement diets fed fishes, however, level of these biochemical constituents between control and *S. barracuda* WMD fed fishes was statistically insignificantly (p < 0.05), while the carbohydrate content was significantly (p < 0.05) higher in *F. indicus* and *S. barracuda* WMD fed fishes when compared to control and *R. kanagurta* WMD fed fishes when compared to control and *R. kanagurta* WMD fed fishes when compared to control and *R. kanagurta* WMD fed fishes when compared to control and *R. kanagurta* WMD fed fishes when compared to control and *R. kanagurta* WMD fed fishes when compared to control and *R. kanagurta* WMD fed fishes when compared to control and *R. kanagurta* WMD fed fishes use insignificantly (p > 0.05) differed in control and fisheries WMD fed fish groups (Table 5).

# Discussion

Aquaculture has been identified as one of the fastest food production sectors which are responsible for the supply of fish protein for human consumption, and currently, 50% of world's



**Fig. 1** Digestive enzymes activity (U/ mg protein) of *C. carpio* fed with control and fish meal replacement feeds. n = 3 (three samplings from each treatment); mean  $\pm$  SD; Bars sharing the same alphabets are not significantly different (p > 0.05); \*, ×10<sup>2</sup>. Control: formulated with fish meal; Diet-1: fish meal replaced by *R. kanagurta* waste meal; Diet-2: fish meal replaced by *S. barracuda* waste meal; Diet-3: fish meal replaced by *F. indicus* waste meal

fishes products are used as food (Ryder 2018). However, aquaculture industries have been facing serious issue due to the high operation cost. Fish feed is one of the major components in aquaculture operation which contributes about 60% of the total production cost (Adikari et al. 2017). Among the feed ingredients, fish meal plays a major role due to the presence of highly digestible protein, essential amino acids, fatty acids, vitamin, and minerals (Tacon and Akiyama 1997; Gatlin et al. 2007; Tacon and Metian 2008; Navlor et al. 2009). However, fish meal is expensive due to low or unpredictable availability (Maliwat et al. 2017). According to Amaya et al. (2007), fish meal is the primary and most expensive ingredient in commercial feed formulations which contributes about 50% of total feed ingredients. Hence, it needs to search for alternative protein sources to replace fish meal in aquafeed formulations. In regard to this, several studies have been reported earlier with various alternative feed ingredients (Ali et al. 1994; Ai et al. 2006; Cavalheiro et al. 2007; Lazzarotto et al. 2015; Lazzarotto et al. 2018; Kriton et al. 2018).

In the present study, a significant level of protein and fiber in the F. indicus WM indicates that the shrimp waste contains better source of protein and fiber rather than R. kanagurta and S. barracuda WM. The insignificant differences in the protein and fiber content between R. kanagurta and S. barracuda WM suggests that these two waste meals had about a similar level of protein and fiber contents. The NFE (carbohydrate) and lipid contents were significantly increased in R. kanagurta and S. barracuda WM, respectively; it indicates that these meals are good source of carbohydrate and lipids. The significant level of ash and energy contents in the selected WM indicated that these meals are the rich source of total mineral and energy levels. Similarly, significant amount of crude protein, lipid, carbohydrates, fiber, ash, and energy contents in the shrimp (Penaeus spp., Pandalus borealis, Trachypenaeus curvirostris, Penaeus notialis, Penaeus duorarum, Parapenaeus longirostris, and Penaeus kerathurus) WM and fish (Ethmalosa fimbriata, Sardinella sp., Upeneus sp., Rastrelliger brachisoma, Terapon jarbua, Liza macrolepis, Siganus javus, and Leiognathus sp.) WM have been reported (Ibrahim et al. 1999; Fanimo et al. 2000; Heu et al. 2003; Nwanna 2003; Sotolu 2009; Obasa et al. 2011; Esteban et al. 2007; Ramalingam et al. 2014). The significant decreases in the moisture content in R. kanagurta and S. barracuda suggest that these WM are rich source for nutrients.

In the present study, the significant elevations in minerals and trace elements, such as Na, K, Mg, and Zn in *R. kanagurta* WM, followed by *S. barracuda* and *F. indicus* WM indicate that these fisheries waste are good source essential minerals. Previously, the essential minerals, such as Ca, P, Na, K, Mg, Fe, Mn, Zn, and Cu contents in the shrimp *P. borealis* and *T. curvirostris* waste meal, and fish *E. fimbriata* and *Sardinella* sp., WM have been reported (Ibrahim et al. 1999; Esteban et al. 2007). The significant level of Ca and P contents has also been reported in shrimps (*P. notialis*, *P. duorarum*, *P. longirostris*, and *P. kerathurus*) head silage meal (Nwanna 2003). In this study, the detection of trace level of heavy

Parameters	Control	Diet 1	Diet 2	Diet 3
Protein (mg/g)	$164.14 \pm 0.86^{\circ}$	$108.08 \pm 2.88^{\rm d}$	$178.68 \pm 2.27^{b}$	$182.62 \pm 0.76^{a}$
Amino acid (mg/g)	$112.87 \pm 1.84^{b}$	$86.752 \pm 2.25^{\circ}$	$116.32 \pm 2.25^{b}$	$123.72 \pm 2.25^{a}$
Carbohydrate (mg/g)	$68.46 \pm 0.80^{\circ}$	$68.05 \pm 1.38^{\circ}$	$76.94 \pm 3.61^{b}$	$96.42 \pm 2.97^{a}$
Lipid (mg/g)	$35.15 \pm 1.03^{b}$	$22.47 \pm 0.90^{\circ}$	$35.34 \pm 0.51^{b}$	$37.43 \pm 0.76^{a}$
Ash (%)	$8.69 \pm 1.20^{a}$	$8.54 \pm 1.45^{a}$	$8.34\pm1.81^{a}$	$9.52 \pm 1.31^{a}$
Moisture (%)	$77.0 \pm 2.0^{a}$	$77.0 \pm 1.9^{a}$	$78.0 \pm 2.412^{a}$	$79.0 \pm 2.04^{a}$

 Table 5
 Proximate composition of C. carpio fed with fish meal replacement feeds

n = 3 (three samples from each treatment); mean  $\pm$  SD; mean values within the same row sharing the same superscript are not significantly different (p > 0.05)

Control: formulated with fish meal; diet 1: fish meal replaced by *R. kanagurta* waste meal, diet 2: fish meal replaced by *S. barracuda* waste meal; diet 3: fish meal replaced by *F. indicus* waste meal

metals, such as Cd and Pb, indicated that the selected FM are nontoxic in the heavy metal view.

In aquaculture industries, assessment of survival, growth, and quality of feeds are considered as an important parameter which affects the economy of the cultivable organisms like fish and crustaceans. In the current study, the insignificant elevations in survival, length, length gain, weight, weight gain, feed intake, and specific growth rate of C. carpio fed with control (fish meal diet) and all three FWM (R. kanagurta, S. barracuda, and F. indicus) suggested that these selected FW performed on par to fish meal diet. The significant decreases in feed conversion ratio in S. barracuda and F. indicus WM fed fish group suggest the better quality of these feeds. The insignificant elevation in survival, weight gain, feed intake, specific growth rate, and feed conversion ratio has been reported in the catfish, Clarias gariepinus fed to fish meal and FWM (E. fimbriata and Sardinella sp.) diets (Sotolu 2009; Obasa et al. 2011). Lu and Ku (2013) reported that partial replacement of fish meal by shrimp WM showed an insignificant difference in survival and significant elevation in weight gain, feed efficiency, and protein efficiency ratio in juvenile cobia, Rachycentron canadum. Effects of replacement of fish meal by fish silage meal on the growth and nutrient efficiency of red tilapia Oreochromis mossambicus, Oreochromis niloticus, and Oreochromis aureus have also been reported earlier (Madage et al. 2015).

In this present investigation, significant elevations in the activity of digestive enzymes, such as protease, amylase, and lipase of C. carpio fed with F. indicus WMD, indicate that this shrimp meal has the ability to promote digestive enzyme secretion in the experimental fishes which led to better feed intake, survival, and growth performance. In context, an insignificant difference in the digestive enzymes activity among control (fish meal) diet, R. kanagurta and S. barracuda WMD fed fish groups indicated that these selected FWM performed on par to each other. Previously, the impacts of dietary fish silage on digestive enzymes (protease, amylase, and lipase) activity of Labeo rohita has been reported (Haider et al. 2017). The digestive tracts of L. rohita fingerlings produced a significant level of digestive enzyme activities, such as protease, amylase, lipase, cellulase, maltase, and invertase when fed on prawn head meal diet has also been reported (Sethuramalingam and Haniffa 2002).

Body chemical composition is a good indicator of the physiological status of an organism. The nutrients in edible organisms depend upon their proximate composition, such as protein, amino acid, carbohydrate, lipid, ash, and moisture levels (Vijayavel and Balasubramanian 2006). In this study, the significant improvement in muscle protein, amino acids, and carbohydrate contents of *C. carpio* fed with *F. indicus* and *S. barracuda* WMD showed that these waste meals had potent to improve the protein content in the experimental fishes due to the presence of considerable level of crude protein and carbohydrate in their by-products. Also, the insignificant variations in the muscle lipid content of C. carpio fed with the control diet, F. indicus and S. barracuda suggested that these FW influenced the lipid utilization in experimental fishes due to the presence of adequate amount of crude lipid in their meal. Further, in this study, the insignificant elevations of ash and moisture content in the fishes fed with control fish meal diet and all three fisheries WMD indicated that all these diets had better level of total minerals and other nutrients content. Similarly, C. gariepinus fed with FWM (E. fimbriata and Sardinella sp.) diets showed a significant elevation in protein, lipid, and ash content have been reported (Sotolu 2009; Obasa et al. 2011). The insignificant difference in protein, lipid, and ash contents of the fish, C. carpio, R. canadum, and Heterobranchus longifilis fed on fish meal, shrimp and crab WMD has also been reported earlier (Lu and Ku 2013; Keremah 2013; Ramasubburayan et al. 2013).

Results from the present study indicated that the FWM, such as *R. kanagurta*, *S. barracuda*, and *F. indicus*, are the good source of proximate composition and mineral contents. Feeding experiment revealed insignificant differences in survival, growth, feed intake, specific growth rate biochemical constituents and activity of digestive enzymes of *C. carpio* fed with control fish meal diet and fisheries WMD. Therefore, the present study suggests that the *R. kanagurta*, *S. barracuda*, and *F. indicus* WM can be considered as alternative feed ingredients for fish meal to produce cost-effective feeds for the culture of freshwater fish *C. carpio*.

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# Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

Ethical standards The manuscript does not contain clinical studies or patient data.

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