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Preparation and utilization of fish waste protein concentrate in diets for young common carp, *Cyprinus carpio* L.

Sabreen M. Jaber, Salah M. Najim*

Department of Fisheries and Marine Resources, College of Agriculture, University of Basrah, Basrah, Iraq

** Corresponding author's E-mail: salah.mahdi@uobasrah.edu.iq*

ABSTRACT

This research was carried out to investigate the extraction of a protein concentrate from the viscera of the common carp, *Cyprinus carpio* L. to use as a partial substitute for fish meal in the diets of young common carp and to test its effect on growth indices and feeding efficiency. Two methods were adopted to extract the protein concentrate; physical method and chemical. Proximate composition of raw fish wastes, fish protein concentrates and different experimental feeds were analysed and several parameters of fish feeding and growth efficiency of cultured fish were studied. Fish were fed with a formulated diet for 60 days. The protein concentrate extracted by physical method was selected to prepare experimental diets for its higher yield. Four diets with substitution ratios of 0, 25%, 50% and 75% of fish meal were prepared. The results showed significant differences ($p < 0.05$) between the experimental feeds as to total weight gain rates, specific growth rate (SGR), food conversion ratio (FCR), protein efficiency ratio (PER) and protein productive value (PPV). The study concluded the possibility of partial replacement of fish meal up to 50% in the diets of young common carp without any significant deleterious influence on feeding and growth indices or noticeable adverse effect on cultured fish.

Keywords: Common carp, Fish waste, Protein concentrate, Fish meal, Carp nutrition.
Article type: Research Article.

INTRODUCTION

Fish waste is a good source of high-value proteins, amino acids, gelatin and enzymes. The massive yearly disposed quantities of wastes can produce large proportions of biologically active compounds and many pharmaceutical and biochemical products such as proteins, oils, amino acids, minerals, enzymes, biologically active peptides, collagen and gelatin (Najim *et al.* 2015). It could be used also for the production of fertilizers which are essential especially in organic agriculture in order to promote sustainable management of this emerging agriculture for healthy food production (Ahuja *et al.* 2020) due to the high contents of protein and other essential and valuable nutrients in fish wastes (up to 58%) with 19% of fats and minerals (Ghaly *et al.* 2013; Matrodzadeh *et al.* 2021). The elevation in fish production has led to an increase in fish waste from different fish processing activities and leaving it unexploited leads to major problems in terms of management and environmental impacts (Caruso 2016). In addition, the escalating demand for feed by aquaculture section given that feeding is the most expensive stage in aquaculture, an increased need appeared to improve the nutritional value of feed. Consequently, attention has turned to the use of low-cost alternative ingredients with balanced nutritional content in the manufacture of various feeds including plant and animal sources, among others, in order to reduce the pressure on fish meal though lowering the cost of feed manufacturing while maintaining feed quality and production sustainability (Sørensen 2012; Pour *et el*. 2023). Fish meal was always an excellent source of protein, essential amino acids, fats, vitamins and minerals and was continuously understood that it is not easily replaced in fish feeds (Muttharasi *et al.* 2019). Fish protein concentrate (FPC) has been studied extensively for being a high-quality protein powder with even higher protein concentration than the original fish. It is used to increase the nutritional value of foods with low

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protein content. It was indicated that it can be extracted by many technological approaches which share the same principals of removing water, fat, unpleasant fishy flavour and odors from the raw material and thus produce a sustainable and environmentally-friendly alternative protein component (Archer 2001; Ahmad *et al.* 2019). The present study was carried out to investigate the efficacy of fish waste protein concentrate prepared from the visceral wastes of common carp as a partial substitute for fish meal in diets of young common carp, *Cyprinus carpio*.

MATERIALS AND METHODS

Raw materials (Fish viscera sample)

The viscera of fresh common carp were obtained from the local markets of Zubair district, Basra City, Iraq. Upon arrival to the laboratory, it firstly washed with tap water, cut and minced in an electric grinder, placed in plastic bags and stored by freezing until performing chemical analyses and the preparation of the protein concentrate.

Preparation of fish waste protein concentrate (FWPC)

Fish waste protein concentrate (FWPC) was prepared initiating with a weight of 2000 g of minced fish wastes in plastic containers which mixed thoroughly with 200 mL 6% glacial acetic acid. The mixtures were then placed in an autoclave for 40 minutes, afterward the product was cooled at room temperature. The cool mixture was filtered using a muslin cloth, the filter was neglected and the precipitate product was collected and dried by air drying. The final concentrated product was stored in refrigeration until tests were initiated.

Experimental diets

Four experimental diets were prepared (C, T_1, T_2, and T_3) with replacement ratios of 0, 25%, 50%, and 75% for fish meal, respectively (Table 1). Experimental diets were made after their components were ground, mixed and made into pellets by a manual meat mincer with a diameter of 2-3 mm. Feed pellets were left to dry by air drying at room temperature and stored into refrigeration to be used later in feeding trials.

Table 1. Dietary ingredients, proximate composition (%) and gross energy (Kcal/ 100 g) of experimental diets.

Ingredient (%)	Experimental diets			
	Control ©	T_1	T_2	T_3
Fishmeal	30	22.5	15	7.5
FPC	0	7.5	15	22.5
Soybean meal	21	21	19	17
Barley meal	5	5	8	10
Wheat meal	37	39	39	39
Corn oil	5	3	2	2
Vitamin-mineral premix	2	2	2	2
Proximate composition (%)				
Moisture	7.37	7.69	7.96	8.13
Protein	33.73	34.5	34.01	33.42
Lipid	3.47	3.98	4.35	4.71
Carbohydrate	47.04	46.11	46.89	47.9
Ash	8.39	7.71	6.79	5.84
Gross energy	409.94	415.07	418.92	423.06

Fish feeding experiments

Young common carp, *C. carpio* were obtained from the Aquaculture Unit, College of Agriculture, University of Basrah, Iraq. A total of 120 fish were randomly distributed into 12 experimental plastic tanks (25-L in volume)

each containing 10 fish. Four treatments were used with three replications for each treatment. Fish were fed with 5% of body weight, with two meals daily (08:00 AM and 01:00 PM) for 60 days. Fish were weighed biweekly where feed rations adjusted accordingly. About 50% of the water in the tanks was renewed daily and the accumulated waste was suctioned by siphoning upon the renewal of tank water in order to maintain water quality. Water quality parameters were within normal ranges for temperature (24.68 ± 0.70 ºC), dissolved oxygen (6.51 ± 0.98 mg L^{-1}), salinity (2.5 ± 0.38 ‰) and pH (7.28 ± 0.44) during whole experimental period.

Growth and Feeding Efficiency

At the end of the experiment, growth indices were calculated based on Jobling (1993) where the total weight gain, relative growth rate and relative growth rate were determined. Feeding efficiency was calculated following the equations mentioned by Hepher (1988) for feed conversion ratio, the efficiency of productive protein, the value of productive protein according to the following equations:

Mean weight gain MWG (g / fish) = fish final weight (g) – fish initial weight (g)
Relative growth rate RGR (%) = weight gain (g) / initial weight (g) × 100
Specific growth rate (% / day) = ln final weight (g) - ln initial weight (g) / period in days × 100
Food conversion ratio FCR = food intake (g) / wet weight (g)
Protein efficiency ratio PER (%) = weight gain (g) / apparent protein intake (g)
Protein productive value PPV (%) =protein gained (g) / protein consumed g × 100

Chemical composition

Experimental diets were analysed for moisture, protein, fat and ash contents using a Near Infrared Analyser (Model IL600), while the chemical composition of fish waste and protein concentrate was estimated based on AOAC (2003). Moisture content was determined by oven drying oven at 105 °C. Crude protein was estimated according to Micro Kjeldahl method, fat content by Soxhlet method with intermittent extraction using hexane as a solvent. Ash content was assessed using a Muffle furnace at 525 °C for 16 h. Carbohydrate contents were estimated by difference according to the following equation:

Carbohydrates % = 100 – (moisture % + protein % + lipid % + ash %)

The total caloric value was calculated from the calorie corresponding coefficients for proteins, lipids and carbohydrates, 5.5, 9.1 and 4.1 kcal/ g, respectively, according to the figures indicated by New (1987).

Statistical analysis

The results were expressed as the mean ± standard deviation. Analysis of variance using One-Way ANOVA was used. The Post Hoc test was used to compare treatment means at the 0.05 level. The IBM SPSS software package (version 26.0) was used for statistical analysis.

RESULTS AND DISCUSSION

Chemical composition of the raw fish viscera of common carp and fish protein concentrate Prepare

As indicated in Table 2, fish visceral wastes contained 71.39% moisture, 17.91% protein, 6.76% fat and 3.37% ash. Fish waste is an important source of value-added products such as proteins, amino acids, gelatin, oil and enzymes. It contains 58% proteins and 19% fat, 22% ash and 1% fibre in average and the variation in the chemical composition of fish waste may be ascribed to several factors including fish sex, age, food, time of the year and health (Murray & Burt 2001; Esteban *et al.* 2007; Ghaly *et al.* 2013). Moreover, other authors recorded recently moisture, protein, fat and ash contents of 63.69-68.74%, 12.03-18.30%, 7.11-22.98%, and 1.03-5.85%, respectively, for carp visceral wastes (Al-Hilphy *et al.* 2020; Salih *et al.* 2021). A large part of fish waste can be used in the production of high-value products. On the other hand, leaving it unexploited leads to major problems in terms of management and environmental impacts (Caruso 2016). The results in Table 2 indicated that the moisture, protein, fat and ash values of the protein concentrate reached 9.76%, 71.70%, 11.30%, and 6.02%, respectively, which is similar to what was reported previously by Mohammad & Al-Serajy (2013). They found in a protein concentrate prepared from fish waste where moisture, protein, fat and ash contents as 10.07%, 70.2%, 12.21%, and 6.4%, respectively. Abul-Fadel *et al.* (2018) reported that the values of moisture, protein, fat and ash

for a concentrate prepared from fish waste were 10.28%, 72.62%, 3.12%, and 13.98%, respectively. It was found that the chemical composition of the concentrate prepared from the residues of Indian carp (*C. catla*) was 5%, 68%, 1% and 9% for moisture, protein, fat and ash, respectively (Shanthi *et al.* 2021). Fish waste is characterized by high digestibility and high protein content. So, it is a good source for the production of protein concentrates that are used as protein source in fish feeds and various applications (Al-Noor *et al.* 2013; Najim *et al.* 2015).

Table 2. The chemical composition of carp visceral waste (FW) and the extracted Fish Protein Concentrate (FPC).

Composition	Content (%)	
	FW	**FPC**
Moisture	71.39	9.76
Protein	17.91	71.7
Lipid	6.76	11.3
Ash	3.37	6.02

Growth performance and feeding efficiency of young fish

Table 3 depicts the studied growth performance and feeding efficiency parameters of young common carp that were fed on the protein concentrate prepared from carp fish visceral waste. The results of the statistical analyses showed significant differences between all treatments and the second treatment T_2 which showed a significant superiority at the level of $p < 0.05$ for parameters such as weight gain, relative growth rate, specific growth rate, feed conversion ratio, protein efficiency ratio and value of productive protein (39.28 ± 0.46 g, %, /% 2.05 ± 0.02 days, 2.17 ± 0.01, 1.33 ± 0.01, 22.75 ± 0.04%), respectively. The continuous increase in growth rates in fish is mainly related to the continuous weight gain and for all treatments, especially T_2 it was elevated by the increased contribution of the good protein source in the diets. This positive effect is reflected clearly on the high rates of weight gain, relative growth rate and specific growth rate as a result of significant correlation with all vital functions in fish body, especially for its important roles in the metabolism, health and physiology of fish (Dabrowski & Ciereszko 2001).

Table 3. Growth and feed efficiency related parameters of young common carp *C. carpio* fed with experimental diets.

Parameters	Treatments			
	Control	**T_1**	**T_2**	**T_3**
Initial weight (g)	16.17 ± 0.07	16.14 ± 0.10	16.15 ± 0.10	16.31 ± 0.07
Final weight (g)	29.54 ± 0.44	38.88 ± 0.29	55.43 ± 0.47	40.98 ± 1.05
Weight gain WG (g)	13.38 ± 0.48^{d}	22.74 ± 0.37^{c}	39.28 ± 0.46^{a}	24.67 ± 1.03^{b}
Relative Growth Rate RGR (%)	82.74 ± 3.22^{d}	140.92 ± 3.02^{c}	243.16 ± 3.19^{a}	151.20 ± 6.07^{b}
Specific growth rate SGR (% g-day)	1.01 ± 0.03^{d}	1.47 ± 0.02^{c}	2.05 ± 0.02^{a}	1.54 ± 0.04^{b}
Feed conversion rate FCR	3.78 ± 0.17^{a}	3.15 ± 0.11^{b}	2.17 ± 0.01^{c}	3.11 ± 0.11^{b}
Protein efficiency ratio PER	0.77 ± 0.03^{c}	0.92 ± 0.04^{b}	1.33 ± 0.01^{a}	0.92 ± 0.03^{b}
Protein productive value PPV (%)	12.15 ± 0.49^{c}	14.03 ± 0.46^{b}	22.75 ± 0.04^{a}	14.78 ± 0.49^{b}

Note: n = 3 (three samples from each treatment); mean ± SD; mean values within the same row with different superscript letters are significantly different ($p < 0.05$).

The improvement in feed conversion ratio as shown by Fig. 1. supports the cost-effective replacement of fish meal by fish waste protein concentrate as a protein source in fish diets as the same principal was indicated previously by Salih *et al.* (2021). The results agreed well with those of Hameed *et al.* (2021) in the effects of using fish waste mixed with 2% lactic acid in the diets of common carp, where the growth rates and feeding efficiency increased significantly compared to the control group. Additionally, when feeding common carp fingerlings on rations containing a protein concentrate prepared using local raw fish waste materials, Al-Hassoon (2017) obtained also better relative growth rates, growth, quality and feed conversion ratios. Najim (2012) reported that fish waste meal can be successfully used for replacing fish meal in fish diets without adverse effects on nutrition and growth

efficiency. On the other hand, Muttharasi *et al.* (2019) used marine fish waste meal and showed that it can be considered as an alternative feed ingredient for fish meal to produce effective and low cost feed for common carp.

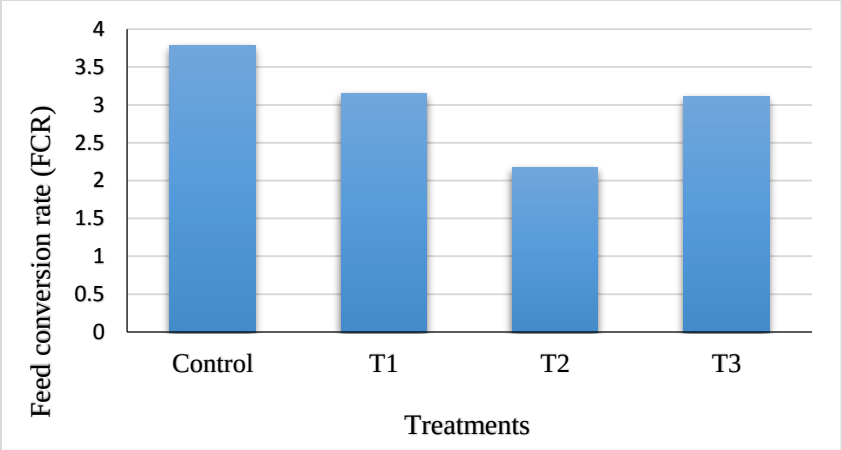

Fig. 1. Comparison of feed conversion ratio values for experimental diets.

CONCLUSION

It could be concluded from the results of the current study that it is possible to prepare a protein concentration from fish visceral wastes with reasonable proximate composition. This concentrate proved to be very suitable as a partial replacement for fish meal with substitution rates of up to 50% in the diets for young common carp without any noticeable adverse effect or significant deleterious alteration in growth and feeding efficiency of cultured fish.

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