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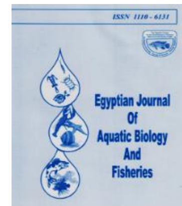

Using Shrimp Waste Protein Concentrate Prepared with Different Methods for Preparation of the Young Common Carp *Cyprinus carpio* L. Diets

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ABSTRACT

The current study dealt with preparing of shrimp waste protein concentrate with different methods and using it for feeding of common carp (*Cyprinus carpio*). Four diets were manufactured: the first, a control diet containing traditional feed materials (c); replacement diets were prepared by completely replacing the fish meal with shrimp waste powder (T2); using physically prepared shrimp waste protein concentrate (T1), and employing chemically prepared shrimp waste protein concentrate (T3). The fish were fed for 90 days. The results of the current study showed that the treatment (T2) was significantly superior in weight gain, daily growth rate, relative and specific growth rate, food conversion rate, and protein efficiency rate, reaching 91.40g, 1.01g, 94.61%, 0.73%/ day, 4.52, 0.67, respectively. The results also showed that the replacement process was successful according to the measured parameters. In conclusion, the study demonstrated the potential use of this prepared shrimp waste protein concentrate as an alternative to imported fishmeal in formulating diets for feeding young common carp.

INTRODUCTION

The economic aspect of fish nutrition is considered extremely important, as nutrition represents one of the largest costs of farming, sinve its cost exceeds 60% of the operational costs of the fish farms **(El-Sayed, 1998; Bolivar *et al*., 2011)**. Furthermore, the cost of feeding of fish diets is approximately more than 50% of the total cost of production **(Craig & Helfrich, 2017)**. In general, protein, especially animal protein, is considered one of the most expensive nutritional components, as it usually constitutes more than 50% of the diet cost **(El -Sayed, 1998)**.

Since animal protein is the most expensive component in fish diets, many researchers have directed their attention to finding alternative sources of protein, whether plant or animal, traditional or unconventional **(Ogunji *et al*., 2007)**. This attention is driven not only by cost consideration but also by the necessity of reducing or eliminating the use of high-cost nutrients in fish feed manufacturing **(Muzinic *et al*., 2006)**.

Feed costs constitute an important aspect of the cost of fish farming, and fishmeal is considered one of the preferred sources of animal protein in feeding farmed fish and shrimp, since it contains essential amino acids, vitamins, high palatability, and growth stimulants **(Majumdar *et al*., 2014)**.

Protein concentrates produced from animal waste, such as fish meal, shrimp waste meal, meat meal, slaughterhouse waste, and blood meal, contain a large amount of essential amino acids, in addition to the presence of some vitamins such as riboflavin and thiamine. During feed manufacturing, these sources must be mixed with vegetable proteins, and amino acids such as lysine and methionine must be added **(Orisasona, 2018)**.

Shrimp waste contains highly nutritious nutrients and can be used to produce products with high nutritional value. However, it causes many environmental problems and impacts **(Caruso, 2016)**. The urgent environmental problem of large unexploited quantities of waste availability must be taken into consideration **(Prasanthi *et al*., 2016)**. Although the presence and accumulation of large quantities of animal waste may cause environmental pollution, the optimal use of these wastes and their conversion into other valuable materials in many industrial, food and fodder applications is due to their high percentage of protein materials being of great benefit to humans and animals **(Vineis *et al*., 2019)**.

Al-Jader and Al-Khshali (2021) indicated the effects of partially replacing fish meal protein concentrate with shrimp waste powder at different percentages of 0, 16, 32, and 48%. They noted that the 32% replacement gave the best percentage in weight gain rate and specific growth rate. The feed conversion ratio, in addition to an increase in essential and non-essential amino acids, led to improved growth standards in *Cyprinus carpio*. **Abdel-Sada (2021)** used powdered waste from the oily shrimp Metapenaeus affinis as a partial substitute for fishmeal and soybean meal in *Cyprinus carpio* fingerling diets and compared growth standards, feed efficiency, level of saturation, body components, and the histological and chemical composition of the intestines. She showed that the use of powdered shrimp waste can contribute in enhancing fish health and enhancing growth rate and nutritional efficiency.

These wastes are characterized by their high digestibility and high protein content, which prompted scientists and researchers to focus on developing better methods for extracting proteins, especially non-traditional protein sources, using chemical, enzymatic and physical methods which aimed to improving their properties and producing modified protein concentrates that possess excellent functional properties that can be used in feed systems **(Tesfaye *et al*., 2018)**.

The current research aimed to optimize the exploitation of shrimp by-products, reducing disposal costs by treating them with various methods. These treated by-products are then used to feed common carp (*Cyprinus carpio*) fingerlings, contributing to the enhancement of sustainability in the fish farming sector.

MATERIALS AND METHODS

A total of 150 common carp fish were brought from the Aquaculture Unit which is located in Al-Haritha District, Basra Governorate, affiliated witht he College of Agriculture, University of Basra. The weight of the fish ranged between 95.40 to 99.95g, and the lengths ranged between 8 to 10cm. The average individual weight of the fish was 97.08± 1.67g. Subsequently, the fish were randomly distributed into 12 glass aquariums with dimensions (30 x 40 x 60cm) and a capacity of 50 liters.

The shrimp waste used in the experiment, represented by the head, tail and shells, was obtained from local markets in the city of Basrah as by-products of the shrimp cleaning and preparation process, in addition to using it as a raw material in the manufacturing of shrimp meal and protein concentrates. The shrimp waste was dried by spreading it in the open air over indirect sunlight with daily stirring to ensure complete drying. The dry waste products were grounded using an electric grinder to pass through a sieve with holes of 0.4mm to facilitate the process of mixing the meal with the rest of diet ingredients. The grounded quantities of shrimp waste were placed in plastic containers and stored until used in the manufacturing of the feed.

Protein concentrates

Shrimp waste protein concentrate was prepared physically according to the method described in **Dewita (2015)** by weighing 2000 grams of shrimp waste and adding 2000ml of distilled water to it. The mixture was placed in a pressure cooker for 45 minutes, and after completing the cooking period, the mixture was left to cool. Subsequently, the concentrate was placed in a transparent plastic container through which the formed layers can be seen. Afterward, the mixture was filtered with a centrifuge, and the sediment was collected and dried. The cooked waste was spread to dry in the open air for 24- 72 hours with continuous stirring to ensure drying. The products were grounded in an electric grinder for ease of use in the feed, then the product was stored in a refrigerator until use.

The shrimp waste protein concentrate was prepared by the chemical method according to the method mentioned by **Al-Kanaani (2014)**, which is the acid fermentation method, by using 2000 grams of shrimp waste with 200ml of glacial acetic acid (10% concentration), dissolving 5% citric acid in the mixture, and adding 200ml of distilled water. The mixture was mixed well, placed in a plastic container and closed tightly. It was left to ferment in the incubator at a temperature of 40- 45°C for 4- 7 days with continuous daily stirring in order to maintain a constant internal temperature. The resulting mixture was treated with a centrifuge, the precipitate was collected, and the supernatant was discarded.

Chemical analysis of the diets, including moisture, protein, fat, and ash contents, was conducted using an American-made near infrared analyzer. Four diets were then manufactured for feeding common carp fingerlings (Table 1). The required quantities of feed materials required to feed the fish during the experimental period were estimated, and the control diet (Treatment C) was prepared by using traditional feed materials. The replacement diets (T1, T2, and T3) were formulated as follows: T1 involved the complete replacement of fish meal with shrimp waste meal, T2 incorporated physically prepared shrimp waste protein concentrate in the feed, and T3 included chemically prepared shrimp waste protein concentrate. Growth parameters were calculated based on the method outlined by **Jobling (1993)**, as follows:

Weight gain (g) = Final weight rate (g) – Initial weight rate (g)

Relative growth rate (%) = [weight gain rate (g) ÷ initial weight rate (g)] x 100

Specific growth rate (%/d) = [(natural logarithm of final weight in grams - natural logarithm of initial weight in grams) ÷ period in days] × 100

Daily growth rate (g/d) = weight gain in grams ÷ period in days

Food conversion ratio = food intake ÷ increase in body weight

Protein efficiency ratio = increase in body weight ÷ protein intake

Table 1. Ingredients and calculated chemical composition of the diet used in the experiment

Ingredient	C	T1	T2	T3
Fish meal	25	0	0	0
shrimp waste meal	0	25	25	25
Soybean meal	20	20	20	20
Yellow corn meal	16	16	16	16
Wheat flour	15	15	15	15
Wheat bran	20	20	20	20
Vegetable oil	2	2	2	2
Vitamin & mineral premix	2	2	2	2
Calculated chemical composition of diet				
Moisture (%)	9.72	9.16	9.5	9.24
Crude protein (%)	26.96	26.24	27.82	28.51
Crude lipid (%)	5.89	7.18	6.18	6.28
Ash (%)	8.99	10.49	9.92	9.33
Carbohydrate (%)	48.44	46.93	46.59	46.63
Energy (Kcal/Kg)	4000	4020	4000	4050

The experiment was conducted from December 26, 2022 to March 26, 2023. Four treatments, each with three replicates, were applied in this experiment. The fish were fed 3% of their body weight, with two meals a day, at 09:00 in the morning and 01:00 in the afternoon during the experiment period. The aquariums were cleaned daily using a siphoning method before feeding to get rid of wastes, and an hour after serving the meal to get rid of uneaten food. Additionally, more than 50% of the water volume was replaced daily to maintain water quality. Fish weights were measured every two weeks, and the amount of food was adjusted accordingly. The environmental factors of aquariums water, including water temperature (°C), dissolved oxygen (mg/ L), pH, and salinity (PPT), were measured using a Chinese-made water quality meter, model AZ86031.

Statistical analysis

The growth experiment was designed using a completely randomized design (CRD) with four diets (treatments) and three replicates for each treatment. Mean differences were tested with analysis of variance (ANOVA), while the significant differences were tested by LSD test at 0.5% probability level using the statistical package for social sciences (IBM SPSS), version 26.0.

RESULTS AND DISCUSSION

The results of the current study showed that the environmental properties of pond water did not differ significantly during the experimental period, since the water temperature ranged between 24- 26°C, dissolved oxygen was between 5.9- 8 mg/ L, pH value was between 7.4- 8.2, and salinity was between 2.2- 3.0PPT. The results of the current study of the environmental factors used during the experiment period until the end of the experiment indicate that the level of temperature, pH, oxygen level, and salinity were relatively stable and within the appropriate ranges for cultivating common carp fish, since this species of fish have the ability to live within wide ranges of environmental conditions **(Goran *et al*., 2016)**.

Hepher (1988) identified optimal water conditions for common carp culture, recommending tempreatures between 25- 30°C, and a pH range of 6.5 – 9. He also noted the species' ability to tolerate salinity up to approximatley 11PPT. For suitable common carp farming, the concentration of dissolved oxygen should not be less than 3mg/ L. The observed results align with these recommendations, confirming their suitability for common carp farming.

Different letters in rows mean statistically significant difference between values at $P \leq 0.05$. Growth rates and weight gain are considered among the most important scientific and practical standards used in evaluating the quality of the feed and its protein content **(Hepher, 1988)**. The main factors affecting growth rate are the rate of food intake and the efficiency of its utilization **(Riche *et al.*, 2004)**. Furthermore, in their study **Hardy and Halver (2002)** stated that fish are among the most efficient farm animals in utilizing food. The results of the statistical analysis of weight gain rates, daily growth, relative growth, and specific growth indicated that there were significant differences ($P \leq 0.05$) between the replacement and control diets.

Table 2. Measures of live mass weights and feed efficiency measures of common carp fingerlings during the experimental period (Mean ±SD)

Parameter	Treatment			
	C	T1	T2	T3
Initial biomass (g)	96.94 ±1.91a	98 ±2.59a	96.62 ±0.88a	96.77 ±1.56a
Final biomass (g)	171.3 ±4.65a	178.29 ±6.58ab	188.03 ±7.87b	135.61 ± 5.05c
Weight gain (g)	74.35 ±2.78a	80.28 ±4.28a	91.4 ±8.10b	38.84 ±5.06c
Daily growth rate (g/d)	0.82 ±0.03a	0.88 ±0.04a	1.01 ±0.09b	0.43 ±0.05c
Relative growth rate (%)	76.68 ±1.45a	81.89 ±2.86a	94.61 ±8.69b	40.13 ±5.34c
Specific growth rate (%±/d)	0.62 ±0.00a	0.66 ±0.01a	0.73 ±0.03b	0.37 ±0.03c
FCR	5.14 ±0.09a	4.91 ±0.08a	4.52 ±0.23a	8.75 ±0.91b
PER	0.66 ±0.01a	0.66 ±0.01a	0.67 ±0.03a	0.36 ±0.04b

The rates of weight gain, daily growth, relative growth, and specific growth recorded the highest values (91.40g, 1.01g/ day, 94.61%, and 0.73%/ day, respectively) in the diet prepared from physical shrimp waste protein concentrate (T2), and the lowest values 38.84g, 0.43g/ day, 40.13% and 0.37%/ day, in the chemical preparation of shrimp waste protein concentrate (T3), respectively. Additionally, the results indicated that the preparation of protein concentrates had a significant effect on growth parameters compared to the control treatment, and this indicates the success of the replacement process.

The addition of shrimp waste to common carp diets exhibited good growth rates compared to the control diet. Moreover, the protein concentrate diet prepared physically by the cooking method (T2) outperformed all other diets in terms of all growth parameters. This indication agreed with **Meyers (1986)** which confirmed the success of the replacement process and that one of the reasons for the good growth of fish fed with a diet containing shrimp waste meal is its high content of protein and amino acids that are similar to those found in fish meal.

The superiority of the T2 treatment over the rest of the treatments in terms of growth rates and feed conversion ratio may be due to the efficiency of the feed used. This feed provides the necessary requirements for growth and performance of the fish’s vital activities, with a focus on the essential amino acids. The diet components are integrated in terms of its balanced content of proteins and fats. **Al-Quoud (2003)** supports this, stating that the feed conversion ratio reflects the animal’s ability to convert food into weight gain while maintaining health.

The statistical analysis showed that there were significant differences ($P\leq 0.05$) between all treatments. The results of the current study did not agree with what was found by **Cavalheiro *et al.* (2007)**, who indicated that there were no significant differences in growth parameters between the Nile tilapia diets. Additionally, **El-Sayed (1998)** showed that there were no significant differences in growth when shrimp waste meal was added to the Nile tilapia diets as an animal protein source that may reduce the cost of production. While, the results of the current study were consistent with the findings of **Nwanna (2003)** when fermented shrimp head silage was used as a partial replacement for fishmeal in proportions of 5, 10, 20, 30 and 40% in the diets of African catfish Clarias gariepinus, since the results showed superiority to the treatment that contains 30% shrimp waste meal, which gave the best weight gain and feed conversion ratio. The results of the current study were consistent with what was found by **Al-Jader and Alkhshali (2021)**, since they showed that there were significant differences in the rates of weight gain, relative growth, and specific growth. In their study, the treatment in which 32% shrimp waste meal was added demonstrated a clear superiority in the rates of growth and weight gain compared to the other treatments. The World Health Organization (WHO) recommends the use of shrimp waste in feeding of small common carp due to its importance in improving growth and stimulating immunity in aquaculture **(Kandra *et al*., 2012)**.

The feed conversion ratio is considered one of the important economic parameters that indicate the efficiency of the food intake and converting it into actual weight gain, while maintaining its health condition **(Al-Quoud, 2003)**. The feed conversion ratio is one of the most important life standards for the fish, since it shows the relationship between the amount of feed consumed and weight gain **(Craig & Helfrich, 2017)**. While the protein efficiency ratio elucidates the relationship between the amount of protein consumed and weight gain **(Gerking, 1971)**.

The highest food conversion ratio was recorded in diet T3, which amounted to 8.75, and the lowest food conversion ratio was in diet T2, which amounted to 4.52, while protein efficiency ratio recorded similar rates in diets C, T1, and T2, and ranged between 0.66 and 0.67. The highest rate of protein efficiency was in diet T2, and the lowest percentage of protein efficiency ratio was in diet T3, which amounted to 0.36. The results of the current study showed that there was an increase in the food conversion ratio in the replacement diet compared to the control diet, since the best food conversion ratio recorded was in the T2 diet, which indicates the success of the replacement process and optimal absorption of nutrients by the body to increase growth. The results of the statistical analysis showed that there were significant differences ($P\leq 0.05$) between the T3 diet and the control diet, and there were no significant differences ($P> 0.05$) between the T1 and T2 diets compared to the control diet.

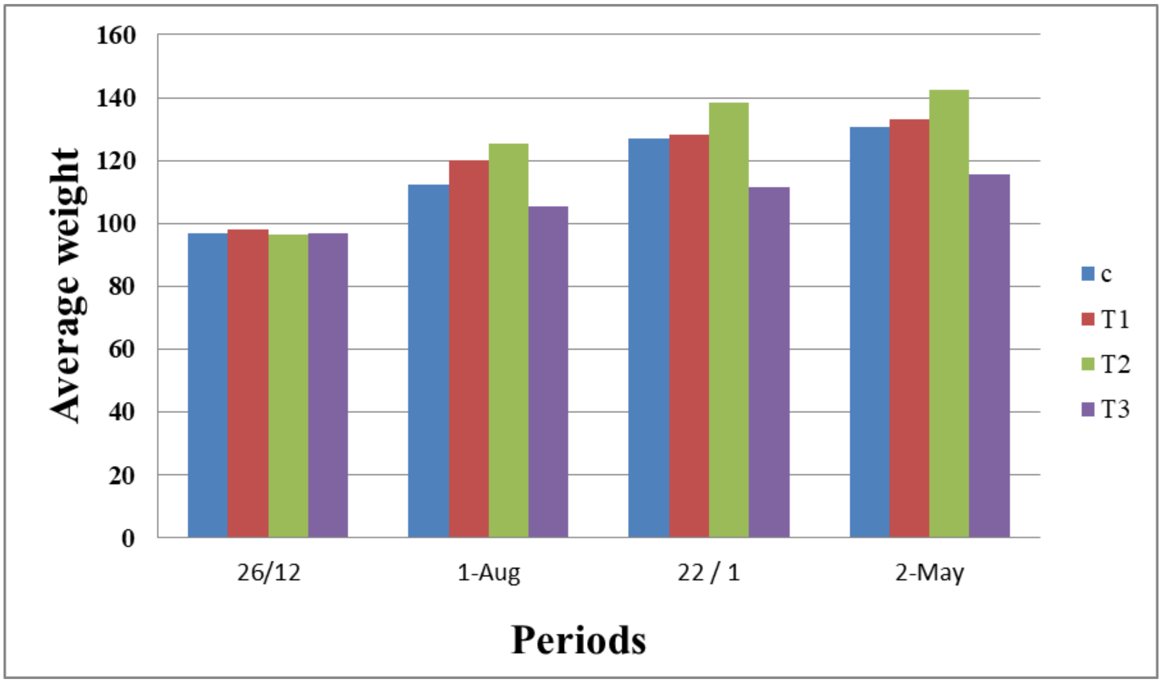

Fig. 1. Average weight of common carp fed shrimp waste protein concentrate during different experiment periods

Fig. (1) displays that T2 was superior than other treatments concerning the weight of common carp fed shrimp waste protein concentrate during different experiment periods after the first initial period. These results agreed with the findings of **Al-Jader and Alkhshali (2021)**, since they showed the effect of adding shrimp waste meal to common carp diets at rates of 0, 16, 32, and 48%. The study revealed that the diet in which shrimp waste meal was replaced by 32% outperformed the others, yielding the best results.

The results of the current study indicated that the protein concentrate diet of shrimp waste meal prepared physically by heating method (T2) recorded the best protein efficiency ratio. This is due to the high protein content of shrimp waste meal and amino acids that are similar to those found in fish meal. The results of the statistical analysis showed that there were significant differences ($P \leq 0.05$) between the protein concentrate diet prepared chemically by fermentation method (T3) compared to the control treatment, while there was no significant differences ($P > 0.05$) between the other two diets and the control diet.

The results of the current study agreed with the findings of **Raja Nandini (2014)**, since he demonstrated the effect of using different levels of shrimp waste meal, amounting to 0, 25, 50, 75, and 100% in Koi Carp diets. He found that the replacement percentage of 25 and 50% resulted the best conversion ratio and growth rate compared to other percentages. **Rachmansyah *et al.* (2004)** indicated that adding 10% shrimp heads meal to the diet of grouper Cromileptes altivelis had a negative impact on growth rates, feed conversion ratio, and protein efficiency ratio. The reason for this was the presence of chitin, which has limited digestibility in fish since Grouper is a carnivorous fish.

CONCLUSION

The current research highlights the feasibility of using shrimp wastes and its derivatives as fish meal alternative in diets intended for young common carp. This approach could play a significant role in eco-friendly use of aquaculture wastes, supporting fish farming and contributing to the sustainability of aquaculture sector, especially in developing countries.

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