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Research Article

Formulation of Diet Using Conventional and Non-Conventional Protein Sources

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ABSTRACT

A 60-day feeding experiment was conducted on Swordtail, *Xiphophorus helleri* to correlate the growth and the crude protein percentage of the feed. Nine experimental diets (Treatments) were formulated using different locally and cheaply available conventional and nonconventional protein sources keeping the basal ingredients same. These diets were tested on three replicate groups of 10 fishes (initial body weight: 0.7 ± 0.5 g) bred in circular fiber reinforced plastic tanks of 120 liter capacity with 100 liter of seasoned de-chlorinated tap water. Fishes were fed 3% of their body weight. The growth performance of Swordtail was studied in terms of Weight Gain, Feed Conversion Ratio (FCR), Specific Growth Rate (SGR%/day) and Protein Efficiency Ratio (PER). The results indicated that Weight Gain was more in the Treatment that contained Chicken Waste and least in the Treatment that contained Marine Fish Waste. Specific growth rate%/per day and Protein efficiency ratio showed similar results. The Feed conversion ratio was greater in Treatment that contained Marine Fish Waste and least in Treatment that contained Chicken Waste. The study suggests that Chicken Waste as a major source of Protein can be effectively considered in the formulation of practical diet for better growth of Swordtail, *Xiphophorus helleri*.

Keywords: Conventional; Non-conventional protein sources; Growth performance; Feed formulation; Utilization; *Xiphophorus helleri*

INTRODUCTION

In fish farming, the most important factor to be considered is probably nutrition [1]. It contributes to about 50% of the production cost. Feeding is the major limitation of aqua culturists. Fish meal is one of the important constituents in the production of fish feed even though it makes the production cost to rise exponentially [2]. It is used as the main dietary protein source because of its nutritional quality and palatability properties [3].

Fishes are unable to synthesize Essential Amino Acids and so these remain inadequate in them. But they are needed for their overall growth and tissue development [4,5]. It has been reported that fish meal contains complete EAAs profile that can meet the protein requirement of the fish. Fish meal is expensive as a feed ingredient and so use of nonconventional feed resources has been reported with good growth and better cost benefit values [6]. Nonconventional feed resources are not common in the market, are not traditional ingredients in the commercial feed formulation and are non-competitive for human consumption. But they are cheap and are either by products or waste products from agriculture or farm feeds or processing industries. These include feed stuff from animals (snails, tadpoles, earthworms, fish silage, blood etc.), plants (wheat bran, rice bran, groundnut oil cakes etc.) [7]. The nutrient quality of feed ingredients is one of the major prerequisite along with their availability for production of good quality feeds. The basic nutrient that cannot be compromised in fish feed formulation and preparation is protein. It is important to determine the optimum dietary protein level energy and/or their ratio for practically and economically successful production of fish. Supplementation of feed with lipid is generally a more effective method than that with proteins and carbohydrates to increase energy density because lipid is an energy dense nutrient and easily metabolized by fish [8].

Omnivores are one of the most successfully reared ornamental fishes that have adapted well to captive conditions and to available nutrition. One such omnivore is Swordtail, *Xiphophorus helleri* that was used in this experiment. Mostly, formulated fish diets tend to be high in protein. Protein levels in diets decreases as fish age and its growth rate decreases. Fish meal must be one of the protein sources in fish diets as it contains essential amino acids and fatty acids. Fish is capable of absorbing minerals from the water. Vitamin C is an essential vitamin for fish, and most species are not capable of synthesizing their own. Also vitamins A, D, E and B complex should be part of fish foods. Commercially produced fish foods

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are usually sold as dry or semi moist pellets or as flakes. Pellets are usually the most complete diets. They can be made to float or sink very slowly. In the ornamental fish industry flakes have been used for many years and have the advantage of being soft enough for very small fish to consume. Semi-moist diets are soft and compact [9].

Feed formulation is the process of mixing various feed ingredients in proportions required to provide the fish with appropriate amounts of nutrients needed at a particular stage of production at a realistic cost. It requires knowledge about nutrients and feedstuffs in order to enhance nutrient requirements, palatability, acceptability, digestibility, toxicity, as well as costs [10]. Most fish farmers use complete diets containing required protein (18-50%), lipid (10-25%), carbohydrates (15-20%), ash (<8.5%), Phosphorus (<1.5%) water (<10%) and trace amounts of vitamins and minerals.

The current focus of nutrition work is to reduce protein cost by replacement or supplementation of fish meal with cheaper conventional and nonconventional protein sources. The aim of this research work is 1) to formulate fish feed using locally and cheaply available conventional and nonconventional protein sources so as to make available information that will help in integrating any of these conventional and nonconventional feed sources into fish feed ingredients during the feed formulation by fish nutritionist and fish aqua culturists who may want to use them as on-feed ingredients and 2) to correlate the specific growth rate%/day of the fish, weight gain, protein efficiency ratio and feed conversion ratio.

MATERIALS AND METHODS

Study site

The study was conducted in the Fisheries Field Laboratory of ICAR - Central Coastal Agricultural Research Institute, Goa - India (Ela Farm – Old Goa). The Institute is situated in Tiswadi taluka near Old Goa (Latitude: 15030'52" N; Longitude: 73055'01" E). The experiment was conducted from August to October for a period of 60days. 500 numbers of Swordtail, *Xiphophorus helleri* fingerlings of about 0.8 gm were brought with oxygen packing from Government Fresh Water Fish Seed Hatchery, Kerim, Anjunem, Sattari-Goa.

Acclimatization of the experimental fishes

The procured fingerlings were treated with mild $KMnO_4$ for a period of two minutes (dip treatment) so as to remove the external parasites and pathogens. They were then acclimatized for a period of 15 days before starting the experiment. During the period of acclimatization the fishes were fed with a conditioning diet other than that of the experimental diet.

Formulation of the experimental diets (Treatments)

All the necessary feed ingredients (groundnut oil cake, fish meal, wheat bran, vegetable oil, snails, fish waste, chicken waste, earthworms, squids, mussels, prawn meal, mineral-vitamin mixture) were obtained from the local market (proximate composition of each ingredient is given in Figure 1. They were then dried overnight at 105°C to make them moisture free. After this the individual ingredient was powdered by using a domestic kitchen grinder. The powdered ingredients were then sieved through a mesh screen of 0.5 mm diameter. The proximate composition of the ingredients is given in Table 1.

The required ingredients for each treatment as given in Table 2 were weighed accurately on a mono pan electric balance and mixed thoroughly. The total mixture was made to 300 gms. With sufficient amount of water the dough was prepared and it was put into a pelletizer to prepare pellets of 2 mm diameter. These were then oven dried overnight at 800°C and stored in air-tight containers for the experimentation purpose.

Nine experimental diets (Treatments) were formulated. All the basal feed ingredients (groundnut oil cake, fish meal, wheat bran, vitamins and minerals) were kept same for all the diets. The main protein source in each experimental diet was changed with varied composition. The diets were labeled as Treatments (T-1, T-2, T-3, T-4, T-5, T-6, T-7, T-8 and T-9). The proximate composition of the experimental diets is given in Table 3.

Three replicates were maintained for each treatment and completely randomized design was followed for the experiment. Circular fiber reinforced plastic tanks of 120 liter capacity with 100 liter of seasoned de-chlorinated tap water were used. Ten healthy fishes of 0.7 ± 0.5 gm of weight were stocked in each tank. The fishes were fed 3% of their body weight for a period of 60 days. Waste from the experimental tanks was removed after every five days. The tanks were washed clean and water changed three times during the experimental period, after every twenty days of interval.

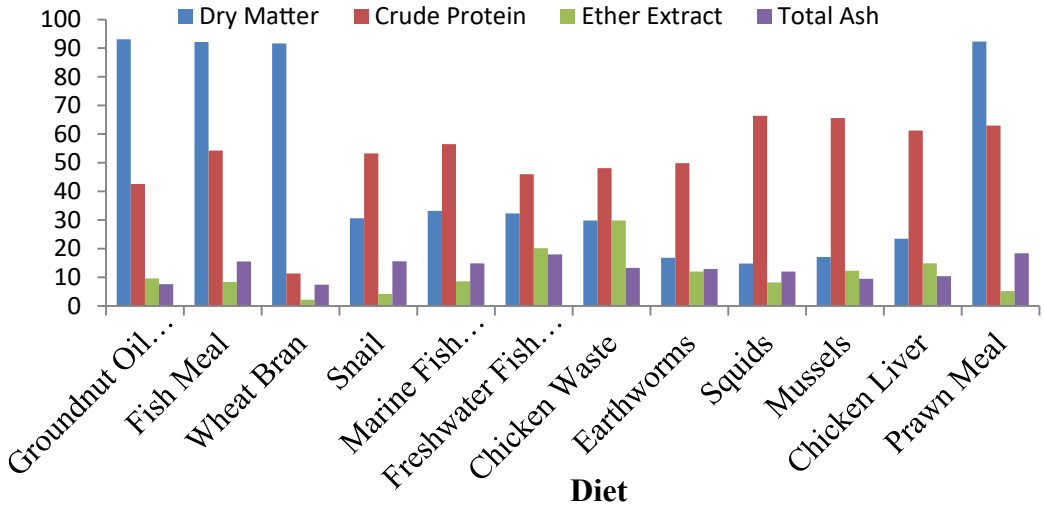

Figure 1: Proximate composition (% dry matter) of diet ingredients used for formulating the experimental diets (Treatments).

Table 1: Proximate composition (% dry matter) of Diet Ingredients used for formulating the experimental diets (Treatments).

Sr. No.	Feed Ingredients	Dry Matter	Crude Protein	Crude Fiber	Ether Extract	Total Ash
1	Groundnut Oil Cake	95.40	42.00	8.50	9.00	6.90
2	Fish Meal	93.80	42.00	6.00	6.00	31.50
3	Wheat Bran	91.30	12.25	10.60	1.80	6.90
4	Snail	30.26	52.50	0.80	4.00	16.00
5	Marine water Fish Waste	30.00	56.50	0.80	9.60	16.00
6	Freshwater Fish Waste	32.27	45.50	1.80	19.00	18.00
7	Chicken Waste	29.70	50.75	0.60	35.00	13.50
8	Earthworms	17.25	49.00	0.40	14.00	13.20
9	Squids	11.57	70.10	1.10	5.00	10.00
10	Mussels	13.71	57.20	1.00	13.40	9.40
11	Chicken Liver	15.20	66.10	2.00	23.50	10.40
12	Prawn Meal	26.70	64.30	1.20	5.00	14.10

Source: ICAR - Central Coastal Agriculture Research Institute (Accessed in May, 2021).

Table 2: Ingredients composition (% dry matter) of the practical diets.

Ingredients	Experimental Diets (Treatments)								
	T-1	T-2	T-3	T-4	T-5	T-6	T-7	T-8	T-9
Basal Feed Ingredients									
Groundnut Oil Cake	20.0	25.0	22.0	25.0	25.0	22.0	18.0	22.0	20.0
Fish Meal	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Wheat Bran	18.0	12.0	21.0	14.0	15.0	25.0	29.0	23.0	25.0
Vitamins & Minerals	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Oil	2.0	-	-	-	-	2.0	-	-	2.0
Experimental Diet Ingredient									
Snail	45.0	-	-	-	-	-	-	-	-
Freshwater Fish Waste	-	48.0	-	-	-	-	-	-	-
Marine water Fish Waste	-	-	42.0	-	-	-	-	-	-
Chicken Waste	-	-	-	46.0		-	-	-	-
Earthworm	-	-	-		45.0	-	-	-	-
Squid	-	-	-	-	-	36.0		-	-
Mussel	-	-	-	-	-	-	38.0	-	-
Chicken Liver	-	-	-	-	-	-	-	40.0	-
Prawn Meal	-	-		-	-	-	-	-	38.0

Table 3: Proximate composition (% dry matter) of the experimental diets.

Diets/Treatments	Parameters			
	Dry Matter	Crude Proteins	Ether Extract	Total Ash
T – 1 (Snail)	95.72	39.99	6.95	15.14
T – 2 (Freshwater Fish Waste)	95.33	39.59	12.78	16.58
T – 3 (Marine water Fish Waste)	96.21	40.97	6.12	17.15
T – 4 (Chicken Waste)	98.43	39.95	16.78	14.52
T – 5 (Earthworm)	97.15	40.27	8.47	17.05
T – 6 (Squid)	99.7	41.58	8.01	15.5
T – 7 (Mussel)	96.61	41.36	7.51	17.4
T – 8 (Chicken Liver)	99.55	41.96	8.96	16.6
T – 9 (Prawn Meal)	99.29	40.77	6.99	15.7

Proximate analysis of feed ingredients and experimental diets were carried out by standard methods. Parameters analyzed were Crude Protein, Lipid, Total Ash, Dry Matter & Moisture (%) and Water Quality (Temperature, pH, Dissolved Oxygen (mg/litre), Hardness ($CaCO_3$/litre), Alkalinity ($CaCO_3$/litre), Nitrate (mg/litre) and Nitrite (mg/litre).

The parameters were calculated using the following formulae:

FCR = Dry matter feed intake (g)/Body mass gain (g)

SGR (%/day) = 100 ×ln (FBW) - ln (IBW)/Number of days

Where IBW and FBW are initial body weight and final body weight.

PER = Wet body mass gain (g)/Protein intake (g)

PWG = Final weight gain (g) – Initial weight gain (g)

RESULTS

The water quality parameters measured during the experiment are presented in Table 4. The analyzed values of water quality indicate that they are in the ideal range of fish rearing which may be responsible for no mortality or any adverse health condition in fish fed with different treatments.

The growth performance of Swordtail, *Xiphophorus helleri* fed with different experimental diets is presented in Figure 2 in terms of their Weight Gain, Feed Conversion Ratio (FCR), Specific Growth Rate (SGR%/day) and Protein Efficiency Ratio (PER).

The Final Weight of the experimental fish showed variations even though the Initial Weight of the fish was almost the same in all the nine treatments. The Final Weight recorded of the experimental fish was highest in Treatment – 4 (Chicken Waste) and Least in Treatment – 3 (Marine water fish waste). Weight Gain by the experimental fish was maximum in Treatment – 4 (Chicken Waste) and Least in Treatment – 3 (Marine water fish waste) (Table 5).

Feed Conversion Ratio (FCR) was greater in Treatment – 3 (Marine Fish Waste) and least in Treatment – 4 (Chicken Waste). The other Treatments showed results in between these two extremes.

Specific Growth Rate (SGR%/day) of the experimental fish was maximum in Treatment – 4 (Chicken Waste) and Least in Treatment – 3 (Marine water fish waste). The other Treatments showed results in between this range.

Protein Efficiency Ratio (PER) showed that Treatment – 4 (Chicken Waste) as the better diet followed by the other diets with Treatment – 3 (Marine water fish waste) as the least suitable for the growth and tissue development of the fish.

DISCUSSION

To meet the ever increasing demand of dietary protein in both international as well as domestic markets, fish culture has emerged as a promising animal husbandry practice. Success or failure of fish yielding venture is essentially determined by the nutritional status of the fish rearing and the availability of requisite diet at an appropriate cost. One of the major problems that the fish culturists face today is to find out an appropriate low cost supplementary feed for which a major budgeting input is being spent and their ever-soaring cost is the main deterrent to this industry.

Protein sources and its levels are the most important criteria in aquaculture fish formulations [8]. Rising costs and the uncertain availability have necessitated the identification of alternative protein sources for use in fish diets. Protein is the most and expensive component of fish diets. Protein is essential for the maintenance, growth, reproduction and may also be used for energy metabolism when need arises. Studies have shown that adequate level of non-protein energy such as lipids and digestible carbohydrate in the diet could minimize the use of protein as a source of energy [11].

The results showed that the diets, in which proteins from more than one source, that is both animal as well as plant were added, it proved to be better than the diet where only animal protein was used. The use of various protein sources in combination is more effective than a single source in replacing fish meal in carp diet.

Table 4: Water quality measured in the growth performance of Swordtail, *Xiphophorus helleri* fed with different experimental diets.

Sr. No	Parameters	Range
1	Temperature	25.2 – 27.4°C
2	pH	7.4 – 7.9
3	Dissolved Oxygen (mg/litre)	7
4	Hardness ($CaCO_3$/litre)	87.6 – 101.7
5	Alkalinity ($CaCO_3$/litre)	95.0 – 105.0
6	Nitrate (mg/litre)	12-19
7	Nitrite (mg/litre)	0.05 – 0.08

Table 5: Growth performance of Swordtail, *Xiphophorus helleri* fed with different experimental diets.

Parameters	T-1(Snail)	T-2(Freshwater fish waste)	T-3(Marine fish waste)	T-4 (Chicken Waste)	T-5 (Earthworm)	T-6(Squid)	T-7 (Mussel)	T-8 (Chicken Liver)	T-9(Prawn Meal)
Initial Weight (g)	$7.33^{a} \pm 0.138$	$7.39^{a} \pm 0.212$	$7.57^{a} \pm 0.075$	$7.58^{a} \pm 0.022$	$7.50^{a} \pm 0.022$	$7.56^{a} \pm 0.177$	$7.47^{a} \pm 0.229$	$7.56^{a} \pm 0.089$	$7.56^{a} \pm 0.096$
Final Weight (g)	$12.090^{ab} \pm 0.153$	$12.117^{ab} \pm 0.404$	$10.840^{c} \pm 0.159$	$12.770^{a} \pm 0.047$	$11.560^{bc} \pm 0.025$	$12.300^{a} \pm 0.280$	$12.470^{a} \pm 0.157$	$12.707^{a} \pm 0.455$	$12.353^{a} \pm 0.094$
Weight Gain (g)	$4.76^{ab} \pm 0.274$	$4.73^{ab} \pm 0.409$	$3.27^{c} \pm 0.095$	$5.19^{a} \pm 0.140$	$4.06^{bc} \pm 0.028$	$4.74^{ab} \pm 0.439$	$4.99^{a} \pm 0.260$	$5.12^{a} \pm 0.508$	$4.79^{ab} \pm 0.014$
FCR	$0.047^{bc} \pm 0.004$	$0.047^{bc} \pm 0.005$	$0.069^{a} \pm 0.002$	$0.044^{c} \pm 0.002$	$0.056^{b} \pm 0.002$	$0.049^{bc} \pm 0.006$	$0.046^{c} \pm 0.006$	$0.045^{c} \pm 0.004$	$0.047^{bc} \pm 0.001$
SGR (%/day)	$0.834^{ab} \pm 0.049$	$0.823^{ab} \pm 0.063$	$0.599^{c} \pm 0.011$	$0.869^{a} \pm 0.027$	$0.721^{bc} \pm 0.01$	$0.810^{ab} \pm 0.073$	$0.855^{ab} \pm 0.052$	$0.864^{a} \pm 0.071$	$0.818^{ab} \pm 0.01$
PER	$0.119^{ab} \pm 0.016$	$0.119^{ab} \pm 0.010$	$0.080^{c} \pm 0.002$	$0.130^{a} \pm 0.004$	$0.101^{bc} \pm 0.001$	$0.114^{ab} \pm 0.011$	$0.121^{ab} \pm 0.007$	$0.122^{ab} \pm 0.012$	$0.117^{ab} \pm 0.001$

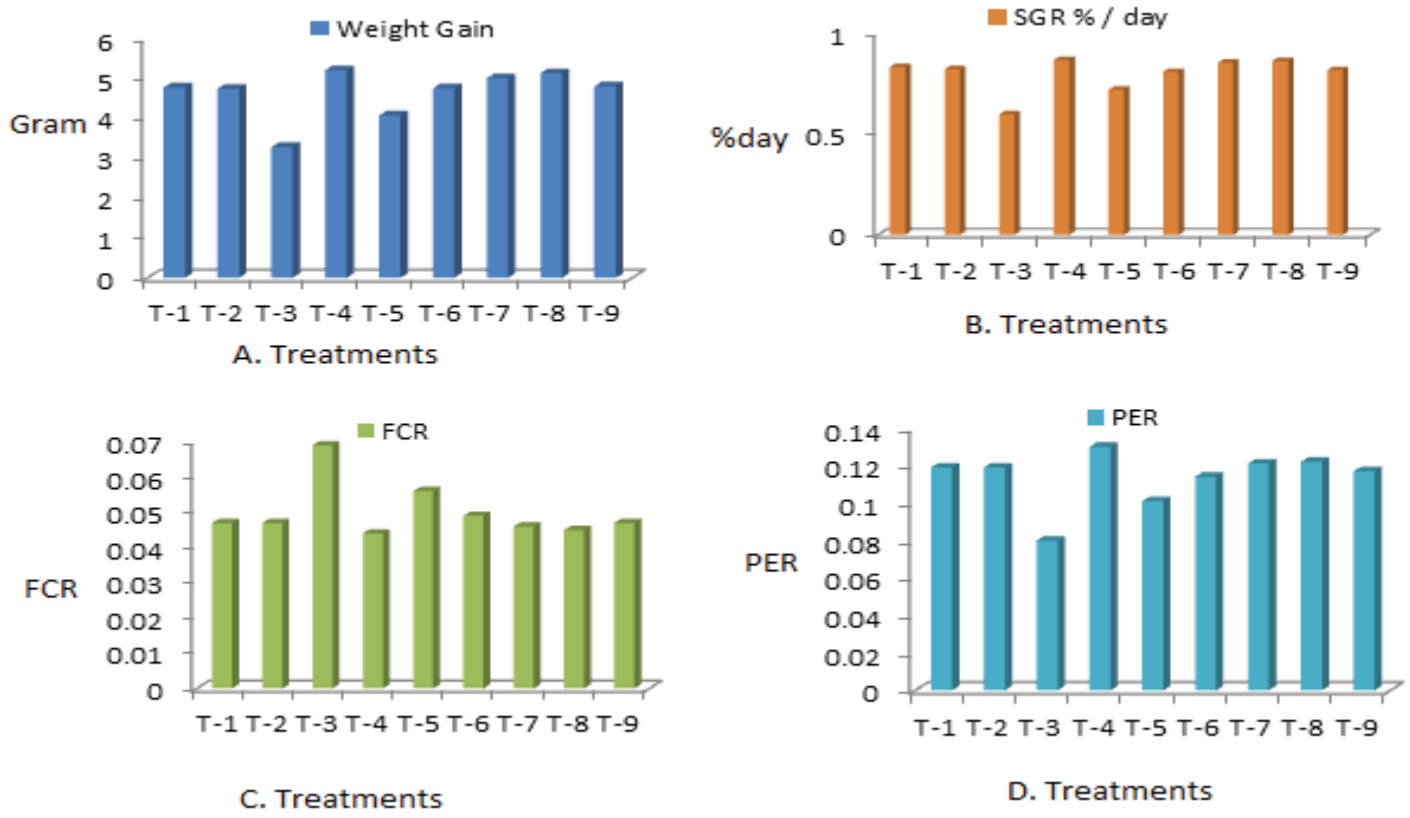

Figure 2: (A-D) Weight gain, SGR%/day, FCR and PER of fishes in different treatments.

In this study, various conventional and nonconventional protein sources were used as ingredients of fish feed. The experimental fish was fed with nine experimental diets which were prepared from the protein sources like snails, freshwater fish waste, marine water fish waste, chicken waste, chicken liver, earthworms, squids, mussels and prawn meal. The feed was in pelleted form.

Among the treatments, significant ($p<0.05$) higher weight gain was observed in the fishes fed with the experimental diet containing chicken waste as the protein source. The least weight gain was observed in fish fed with marine fish waste as the protein source. The Specific growth rate (SGR%/day) was significantly higher in fish fed with chicken waste and the least was observed in fish fed with marine fish waste. Chicken waste is a continuous source of protein throughout the year as well as it is readily and cheaply available. Poultry Byproduct Meal (PBM) has high potential to be incorporated in the diet of carnivorous fish species due to its high protein content and lower price compared to fish meal [12].

On the other hand, opposite results were observed where the feed conversion ratio was concerned. The feed conversion ratio was high in fish fed with marine fish waste and least in fish fed with chicken waste. The protein efficiency ratio was significantly ($p<0.05$) higher in fish fed with chicken waste and it was least in the fish fed with marine fish waste.

Several nutrients in feed are indispensable for growth and maintenance of fish. Protein is probably one of the most important factors largely affecting growth of fish and determining feed cost because of high proportion and price [13,14]. In addition, studies on the apparent digestibility PBM revealed that this product is well digested by several fish species [12].

It was also observed that the crude protein percentage in chicken waste as an ingredient is more but the crude protein percentage in the diet containing chicken waste is low. On the other hand, the crude protein percentage in marine fish waste as an ingredient is more and in the diet containing marine fish waste is less.

The results showed that the fish fed with chicken waste as a major source of protein can be effectively considered in the formulation of fish diet for better their growth performances and feed utilization of swordtail fish, *Xiphophorus helleri*.

Fish survival was not affected by experimental diets in the study. The high survival rates recorded indicate that feeding Swordtail with conventional and nonconventional feed diets could enhance their survivorship. This is made possible because *Xiphophorus helleri* is an omnivorous fish species which used efficiently animal protein source to cover energy requirements [15]. Moreover, these results indicate that the protein quality of feed formulated with unconventional animal protein source ingredient was well accepted by the fish.

An optimal EAA profile is a requisite for fish growth and nitrogen retention as reported by Peres and Oliva-Teles [16,17]. All the experimental diets in the current trial are of high protein quality [18], because each of them contains all the EAA with values higher than the requirement

CONCLUSION

The concept of healthy and wholesome aquaculture is an integral component in improving and sustaining aquaculture production to provide fish protein and other beneficial nutrients. However, more efforts in nutrition and fish health management involving various sectors of the aquaculture industry need to continue to ensure a steady, sustainable, and reliable supply of safe and high quality fish beneficial to public health while preserving the environment. Fish feed, fish feed ingredients, common conventional feedstuff, animal

and plant sources of unconventional feed, fish feed formulation and feeding methods are important for the effective management of fish farming.

As aquaculture production of fish becomes more intensified, practical diets need to be formulated to be cost effective and environment-friendly. Appropriate ingredients should be included to satisfy the nutrient requirements of the fish, in order to promote optimal growth of fish and boost the income of the small-scale farmers and commercial producers. Feed formulation for sustainable aquaculture should aim at increasing aquaculture system performance and profitability, enhancing disease resistance, increasing acceptability, palatability, and digestibility of practical diets, and maintaining environmental quality through sound feeding management and good aquaculture practices. The use of unconventional sources of proteins such as earthworm reduces the diets cost and improves the production cultured fishes.

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CONFLICT OF INTEREST

The author has no conflict of interest to declare.

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