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Evaluation of using crayfish (*Procambarus clarkii*) as partial or complete replacement of fish meal protein in rearing the Nile tilapia (*Oreochromis Niloticus*) fry

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ABSTRACT

An evaluation of using crayfish as partial or complete replacement of fish meal protein in Nile tilapia fry was conducted .The obtained results showed that, the best feed conversion was noticed in control group, which fed on fish meal (FM) without any replacements, followed by group II which treated with crayfish meal (TCFM) 50%, then group IV, which received treated crayfish meal (100%), then group I where the worst value of feed conversion was noticed in diet containing crayfish by-product (CFBM) 50% replacement. Body composition showed no significant difference between different experimental diets and the control one, except with (TCFM) 100% diet.

Results of heavy metals analysis showed that fish of group II had the lowest concentrations of Cu, Cd, Pb, Fe and Zn than that in group I (CFM) and III (CFBM). On the other hand, it was noticed that the estimated levels of both cadmium and lead in fish of group II (TCFM) were lower than that of control group.

Key words: Crayfish, *Procambarus clarkii*, Nile tilapia, fish meal.

INTRODUCTION

As long as protein component represents 55-75% of the total diet cost , protein alternatives have the first priority in formulating diet of tilapia (Hanley, 2000) for the high cost of fish meal.

The freshwater crayfish (*Procambarus clarkii*), family cambariedae, has reached its greatest diversity allover the world (Holdich & Reeve, 1991) and could be considered the largest invertebrate in the freshwater systems (Cherkashina, 1995). It was accidentally introduced into Egypt in the River Nile during early eighties of the last century; since that time, it spread rapidly in the Egyptian freshwater ecosystems, i.e. streams, ponds, marshes, in polluted or clean waters (Ibrahim *et al*., 1995). However, the rapid growth of this species can be regarded as the main reason of dramatic environmental damage as it occurs in irrigation net works, digged in the ground and therefore damage the agricultural crops (Ibrahim *et al*., 1995). Hafez *et al*. (2003) as well as Agouz & Tonsy, (2003) tried to use crayfish as protein alternative .

Anderson *et al*. (1997) recorded that the highest concentration of lead and heavy metals was found in gills and hepatopancrease of the crayfish, while muscles and haemolymph had the lowest concentrations. Knowlton *et al*. (1983)

also found that lead clearance was significant in all evaluated tissues except the hepatopancrease , which is the organ of metal storage and detoxification of lead, therefore the removal of the digestive glands and gills was undertaken to decrease the amounts of heavy metals present in crayfish meal. In this study an evaluation of using crayfish meal as a source of protein in two concentrations (50 and 100 %) in rearing fry of the Nile tilapia was done.

MATERIALS AND METHODS

1-Experimental fish

A number of mono sex Nile tilapia fry (0.4-0.42 gm) were divided into five groups to evaluate the replacement of fish meal protein by different form and percents of crayfish .They were adapted for 2 weeks before starting the experiment, which lasted for 55 days. They were divided into 15 glass aquaria (30 fish / aquarium) of 30x30x30 cm (27L) filled with dechlorinated tap water and aerated to supply oxygen. The temperature was maintained in 27 C° and NH_3 concentration was maintained at 0.2 mg/L. The amount of food and the body weight was recorded weekly to calculate body gain, specific growth rate and feed conversion rate. At the end of the experimental period the nutritive value and the heavy metals were detected in samples of fish flesh.

2- Experimental diets

The red swamp crayfish were collected from markets and rinsed with tap water, then dried at 200 C°. The crayfish were treated by removing the digestive gland and gills from the whole body before grinding to be used as 50 % and 100% replacement of fish meal protein. Five isonitrogenious (30% CP) experimental diets were prepared according to the requirements of Tilapia fish (NCR, 1993). Diet protein contents (Table 3) were used as follow:

Diet 1: containing fish meal only as a source of animal protein for the control group.

Diet 2: containing 50% fish meal protein and 50% CFM (Crayfish meal) protein to be used in feeding group I

Diet 3: containing 50% fish meal protein and 50% TCFM (Treated Crayfish meal) protein to be used in feeding group II

Diet 4: containing 50% fish meal protein and 50% CFBM (Crayfish by-product meal) protein to be used in feeding group III

Diet 5: containing 100% TCFM (Treated Crayfish meal) protein to be used in feeding group IV.

Fish were fed twice a day (8 and 14 hr) at a level of 5% of body weight. At the end of the experimental period the nutritive value and the heavy metals were detected in some samples of fish flesh.

3- Analytical methods

Water quality (pH, NH_3, DO and salinity) was examined every week according to Boyd (1995). Protein was determined by Kjeldahl method, and fat was estimated using Sokselet apparatus according to A.O.A.C. (1984), while Ca and P concentrations were assayed by using spectrophotometer according to Klichling &Freiburg (1951) and Bett & Fraser (1959).

4-Heavy metals analysis

Tissue samples were dried in the oven at 100 C° and weighed then homogenized. One gram of dry sample (triplicated) was digested in concentrated nitric / perchloric acids mixture (7:3 v/v). Aunicom 969 Atomic Absorption Spectrometer with acytelen air flames was used, in accordance with the manufacturer's specification, for the determination of Cd, Pb, Cu, Zn and Fe in the digested samples and filtered water (Wood & Van Vleet, 1996).

5-Statistical analysis

The obtained data was statistically analyzed using analysis of variance (SAS program, 2000). Differences between means were tested by Duncan new multiple range test (Duncan, 1955).

Table (3). Diets composition of the experiment.

Matter	Diet (1) Control	Diet (2) Group I	Diet (3) Group II	Diet (4) Group III	Diet (5) Group IV
Y.C	46	35	37.5	26.5	22
S.B	36	37	35	39	40
F.M	15	7.5	7.5	7.5	-
C.F	-	15.5 CFM	15 TCFM	23 CFBM	30 TCFM
viT	2	2	2	2	2
Oil	1	3	3	2	6
Energy	4602	4493	4449	4425	4284

Y.C = Yellow corn | CFM = Crayfish meal(whole body)
S.B = Soya bean | TCFM = Treated Crayfish meal
F.M = Fish meal(hearing) | CFBM = Crayfish by-product meal

RESULTS AND DISSCUTION

Results of water analysis are shown in Table (1) indicated that the pH values were ranged between 7.10 and 7.6, which was the suitable range for tilapia growth (7-12) that reported by Huet (1972). The NH_3 and DO concentrations also were in the suitable range. Meanwhile, salinity being 0.1 ppt was so far from the toxic level (20.2 ppm) recorded by Boyd (1995) .The same results were recorded by Hafez *et al*. (2003). The obtained results indicated that replacing fish meal (FM) by crayfish meal (CFM), treated crayfish meal (TCFM) or crayfish by-product meal (CFBM) had no undesirable effect on the water quality.

Table (1). Effect of different dietary sources on water quality.

Treatment	pH	NH_3(mg/l)	DO(mg/l)	Salinity
Control	7.6	0.3	6.22	0.1
G I (CFM 50%)	7.3	0.2	5.01	0.1
G II (TcFM 50%)	7.4	0.2	5.2	0.1
G III (CFBM 50%)	7.42	0.2	5.35	0.1
G IV (TcFM 100%)	7.1	0.2	5.5	0.1

Chemical composition of fish meal in the whole crayfish, treated whole crayfish and crayfish by-product are presented in Table (2).The obtained results showed that (CP) contents of CFM was lower than that of fish meal being 28.87 % in CF, 35% in TCFM , 21.17 in CFBM and 72% in fish meal. Similar results were reported by Agoz &Tonsy (2003) and Hafez *et al.*(2003) for CFM .Results in Table (2) cleared that the highest CP content was detected in TCFM, while CFBM had the lowest CP contents. The obtained results also showed that the TCFM had the lowest level of E.E (ether extract). (4.26%) compared to their level in CFBM and CFM (8.05 and 9.6%). Regarding the nitrogen free extract it was found that TCFM contains the least amount of NFE (10.33%) compared to their amount in CFBM and CFM (15.07 and 18%) respectively, while the ash percent was 33, 39.4 and 43.7% in CFM, TCFM and CFBM respectively. Due to their physical structure, the crayfish and its by- products had higher CF content (10-12%) than that of FM (0.6%). These results agreed with that reported by Soliman (2000) and Hafez *et al.* (2003).

Table (2). Chemical composition of used animal proteins.

Matter	C.P	E.E	C.F	NFE	Ash
F,M	72	8.4	0.6	8.6	10.4
C,F,M	28.87	9.6	10.53	18	33
CFBM	21.17	8.05	12	15.07	43.7
TCFM	35	4.26	11	10.33	39.4

Results dealing with effect of food on growth rate and weight gain Table (4) revealed that fish of group (II) recorded the highest final weight (3.57 gm), while that of group (III) were the lowest (3.2 gm). Moreover, it can be noticed that there were no significant differences in weight gain between control and both CFM and TCFM, while fish of group III and IV recorded significant low weight gain compared to the control group. The specific growth rate showed the same pattern, like weight gain, where the highest rate was seen in group II, while fish of group III recorded significant low growth rate.

Regarding the total feed intake and feed conversion rate, the results in Table (4) indicated significant higher levels in all treated groups compared to the control one. These results agreed with those reported by Soliman (2000) , Agouz &Tonsy (2003) and Hafez *et al*. (2003).

Table (4). Feed conversion and growth rate of Tilapia fry fed on different levels of crayfish meals.

Criterion	Control	Group I	Group II	Group III	Group IV
Wo	0.41 ± 0.03	0.42 ± 0.03	0.40 ± 0.03	0.41 ± 0.03	0.42 ± 0.03
WF	3.68 ± 0.91	3.51 ± 0.88	3.57 ± 0.90	3.2 ± 0.7*	3.4 ± 0.9
Wg	3.27 ± 0.11	3.09 ± 0.15	3.17 ± 0.13	2.79 ± 0.09**	2.98 ± 0.1*
SGR	2.92 ± 0.28	2.83 ± 0.15	2.93 ± 0.28	2.78 ± 0.19*	2.8 ± 0.75
TFI	6.3 ± 0.47	7.4 ± 0.55*	6.83 ± 0.76*	7.19 ± 0.82*	6.86 ± 0.75*
FCR	1.93 ± 0.09	2.39 ± 0.1	2.15 ± 0.15*	2.5 ± 0.1*	2.3 ± 0.09
PER	1.69 ± 0.07	1.43 ± 0.04	1.66 ± 0.06*	1.3 ± 0.03*	1.44 ± 0.03

Wo = Initial Weight
WF = Final Weight
Wg = Weight gain
SGR = Specific Growth Rate
TFI = Total Feed Intake
FCR = Feed Conversion Ratio
PER = Protein Efficient Ratio

Chemical composition of tilapia fry fed on CFM, TCFM and CFBM are recorded in Table (5), which showed no clear alterations in body chemical composition of different groups, except that of group IV, which were given CFM protein 100% replacement, where the results revealed significant increase ($P<0.05$) in CP and EE content, while ash and calcium content were significantly decreased compared to the control group. The obtained results in Table (5) also cleared that EE % showed significant increase, while ash percentage was significantly decrease in fish of group I being 20.81% compared to the control one (17.15%). These results agreed with those recorded by Abd –El Fattah *et al* . (1998) and Agouz &Tonsy (2003).

Table (5). Chemical composition of tilapia fry fed on different levels of CFM replacing FM.

Criterion	CP %	EE %	Ash %	NFE %	Ca %	P %
Control	58.75 ± 2.7	17.15 ± 1.5	24.1 ± 3.8	0.0	7.55 ± 3.5	2.9 ± 0.7
Group I	58.88 ± 2.8	20.81 ± 2.3*	20.3 ± 2.9*	0.01	7.65 ± 3.7	3.6 ± 0.76
Group II	58.53 ± 3.1	19.66 ± 1.9	21.80 ± 2.8	0.01	7.41 ± 2.9	3.1 ± 0.88
Group III	50.58 ± 3.0	18.11 ± 1.1	22.31 ± 3.1	0.0	7.35 ± 3.1	3.0 ± 0.81
Group IV	60.75 ± 3.5*	20.91 ± 2.5*	18.33 ± 2.1**	0.01	6.30 ± 2.2*	3.0 ± 0.79

*Signefecantly different
**Highly signefecant

Results presented in Table (6) indicated that the highest concentrations of the heavy metals (lead, copper, cadmium, zinc and iron) were found in crayfish by-products followed by the whole body crayfish while the treated whole body crayfish had the lowest concentration. The obtained results shown in Table 6 cleared that the levels of heavy metals in the fish meal were nearly similar to that of the used CFM, except the levels of both Cd and Pb, which were very low. This was reflected on their occurrence in the fish fry muscle as shown in Table (7), where lead and cd levels in the fish fry muscles that fed on CFBM were significantly higher than those fed on FM, while it was significantly low in group II, which fed on 50% TCFM.

Table (6). Heavy metal analysis of crayfish groups.

H. metal (ppm)	CFM	TCFM	CFBM	FM
Cu	0.92 ± 0.07	1.42 ± 0.09	1.9 ± 0.08	1.6
Cd	0.53 ± 0.07	0.44 ± 0.02	0.72 ± 0.08	0.5
Pb	1.08 ± 078	1.076 ± 0.43	2.3 ± 0.23	0.8
Fe	0.71 ± 0.1	1.3 ± 0.3	1.41 ± 0.09	0.9
Zn	13.1 ± 0.92	15.84 ± 1.02	21.3 ± 0.12	18.4

Moreover, results in Table (7) showed that cadmium concentrations in fish fry muscles kept on TCFM was insignificantly higher than control group, being 0.167 and 0.141 ppm respectively, while its concentration was significantly elevated in groups III and IV compared with its level in control fish, being 0.185, 0.206 and 0.244 ppm respectively.

On the other hand the estimated levels of both zinc and iron as indicated in Table (7) were significantly high in groups III and IV, which fed on 50% CFBM and 100% TCFM, compared with their levels in the control group, while groups I and II showed no significant differences with the control group in zinc and iron concentrations . No available data dealing with heavy metals in tilapia fish fry fed on CFM and CFBM was found.

Table (7). Heavy metals and trace elements in fish fry muscles in the studied group.

H.m (ppm)	Control	Group I	Group II	Group III	Group IV
Cu	0.331 ± 0.09	0.34 ± 0.08	$0.263 \pm 0.05^{*}$	$0.442 \pm 0.11^{*}$	0.33 ± 0.09
Cd	0.141 ± 0.01	$0.185 \pm 0.03^{*}$	0.167 ± 0.03	$0.206 \pm 0.06^{**}$	$0.244 \pm 0.02^{**}$
Pb	0.40 ± 0.02	0.44 ± 0.02	0.43 ± 0.02	$1.6 \pm 0.05^{**}$	$0.65 \pm 0.06^{*}$
Fe	0.2 ± 0.01	0.21 ± 0.01	$0.176 \pm 0.001^{*}$	$0.36 \pm 0.05^{**}$	0.24 ± 0.01
Zn	0.98 ± 0.05	0.5 ± 0.09	1.2 ± 0.05	$2 \pm 0.1^{**}$	$1.9 \pm 0.1^{**}$

Finally, we can conclude that the best treatment was the second group which received (TCFM 50%) among the all treatments, and this could be attributed to the high CP content of FM, compared to CFM or its by-products.

The results indicated also that the use of (TCFM 50%) was more safe than non treated crayfish meal (CFM) and (CFBM). It is advisable to use treated crayfish meal to replace 50% of the fish meal in diets of Nile tilapia.

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