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Development of Fry Diets for African Catfish (*C. gariepinus*) Larvae in Uganda

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ABSTRACT

The African catfish (*Clarias gariepinus*) is a popular cultured species in Uganda and the demand for fingerlings outstrips supply. The low supply of fingerlings is partly attributed to lack of affordable fry diets as the available feeds are imported and expensive. The study investigated the growth of *C. gariepinus* larvae fed on locally formulated diets. The dietary treatments had three protein levels, 56.1% (P56), 58.3% (P58) and 60.3% (P60) and a commercial diet (ranaan starter) from Israel as P-control. The diets were evaluated by proximate analyses and followed by a completely randomized block biological experiment. A total of 600, eight day old larvae were stocked in each tank (80×40×40 cm) in triplicates and were fed treatment diets at 8.00 am, 12.00 noon, 2.00 and 6.00 pm. After 96 days, the fingerlings for each group were compared for growth effects and survival. The body weight of fish differed by treatment ($p = 0.007$). Weight gain over the study period was greatest for the control diet. Specific contrasts between diets showed growth of fish larvae fed P60 was better than in P58 ($p = 0.04$) and P56 ($p = 0.0X$). Furthermore, P56 and P58 did not show a statistically significant difference in growth. Fisher's exact test performed on liver histology for lipid deposition did not show statistically significant differences among the diets (p -value = 0.0727). Fish gained best with imported feed but based on this research, local feed ingredients formulation in P60 may have potential to be used to culture *C. gariepinus* larvae.

Key words: *Clarias gariepinus*, fry diets, growth

INTRODUCTION

Production of *Clarias gariepinus* is increasing exponentially in Uganda under semi-intensive pond culture as the most predominant system. To ensure optimal survival and growth, catfish larvae are reared in hatcheries where they are fed artemia for the first five days at onset of exogenous feeding. Thereafter, fish can be weaned onto dry fry feed. However, lack of affordable and quality feed is a major constraint to local fish production systems. The challenge therefore is to avail farmers with affordable quality feeds for a profitable business and increased production of

fingerlings. The main aim of the study was to determine the most effective diet formulated from locally produced ingredients based on evaluation of growth, palatability, food conversion and profitability.

African catfish larvae like any other larvae fish require a high protein level (>50%) (FAO., 2010) before they reach 5 g in size. Protein sources vary in quality and are the most expensive components of the diet. The present study set out with the main objective to develop a nutritionally balanced cost-effective fry feed. The specific objectives of the study were (1) To determine the chemical composition of feed ingredients and formulate three combinations of dry fry feed for weaning larval catfish and (2) To determine the effect of the three fry diets on growth, survival of *C. gariepinus* larvae.

MATERIALS AND METHODS

Study species and culture: Larvae of *C. gariepinus* (initial weight 7.73 ± 0.02 mg) weaned at eight days, were obtained from Buloba fish hatchery, Uganda. Three treatments and a control, in three replicates each were maintained in twelve tanks covered with black polythene to minimize disturbance from light (Mukai and Lim, 2011).

Ingredients for the fry diets: The ingredients for experimental diets were; *Rastrineobola argentea* (a pelagic cyprinid fish locally known as Mukene), roasted soy, whole rice, whole wheat, Nile perch fish oil (extracted from belly flaps of Nile perch) and vitamin and mineral mix containing; vitamin A: 7,000,000 I.U; vitamin D3: 2,000,000 I.U; vitamin E: 10,000 mg; vitamin K3 STAB: 200 mg; vitamin B1: 300 mg; vitamin B2: 800 mg; vitamin B6: 400 mg; vitamin B12: 2 mg; niacin: 3,000 mg; pantoic acid: 1,000 mg; folic acid: 100 mg; biotin: 75 mg; choline: 35,000 mg; manganese: 6,000 mg; iron: 4,000 mg; zinc: 5,000 mg; copper: 800 mg; cobalt: 30 mg; iodine: 100 mg; selenium 1%: 20 mg; antioxidant: 20,000 mg; olaquinox 10%: 20,000 mg; salox 12%: 50,000 mg; ronozyme P: 5,000 mg; ronozyme G2: 12,000 mg; carophyll yellow: 2,500 mg; carophyll red: 500 mg. *R. argentea* and roasted soy were used as protein sources. All dry ingredients were first milled to powder and sieved to 0.02 mm particle size and stored in airtight plastic bags prior to mixing. The ingredients and nutrient composition of the experimental diets used in this study is shown in Table 1.

Preparation of fry feeds: *Rastrineobola argentea* (Mukene), (80 g) milled to powder formed the biggest quantity of the ingredients. To this was added milled roasted soy, 3-4% whole wheat to improve pellet binding prior to crumbling while rice was added at 3-5% level since it has high fat and fiber levels. All diets were supplemented with vitamin and mineral mix according to NRC (1993) and mixed well. Water was added in ratio 30 parts water, 70 parts feed mash and passed through a vertical mixer (made by Tonnet Agro Engineering Co., Ltd) for 15 min. Fish oil was then added and dough mixed for more 5 min. The resulting dough was removed and manually transferred into a pelleting machine with a 2 mm die. The formed moist pellets were sun dried at 27 to 30°C to a moisture content of $7.04 \pm 0.65\%$ to store safely in airtight polythene bags.

Proximate composition analyses of the feeds was done following standard reference procedures (AOAC., 1999). Dry matter was determined by drying at 105°C to constant weight in the oven; while for ash (550°C for 5 h) in a muffle furnace; for crude fat (Soxhlet method) while crude protein was estimated by the Kjeldahl method ($N \times 6.25$). The study was conducted at Makerere University, College of Veterinary Medicine Animal Resources and Biosecurity (COVAB) and lasted 96 days.

Table 1: Formulations and proximate composition of experimental diets (DM basis)

Parameters	Dietary treatment			
	*Control (imported feed)	P58	P56	P60
Feed ingredients used				
Mukene (<i>Rastrineobola argentea</i>)		83.00	80.00	86.00
Whole milled wheat (<i>Triticum aestivum</i>)		3.00	3.00	3.00
Roasted soy		7.00	10.00	4.00
Whole milled rice		5.00	5.00	5.00
Vitamin and mineral mix		1.00	1.00	1.00
Fish oil (Nile perch)		1.00	1.00	1.00
Total		100.00	100.00	100.00
Proximate analyses of diets (%)				
Dry matter	93.01	92.04	93.20	93.56
Crude protein	55.58	58.30	56.00	60.00
Crude lipid	9.96	9.23	9.24	9.40
Ash	11.57	12.85	13.02	12.93
Moisture	6.99	7.96	6.80	6.44
NFE	15.90	11.66	14.94	11.23

*Ingredient composition of control diet (Ranaan CS-starter feed/799 for catfish) was unknown

Summary of experimental procedures: The stages followed in the study are as summarized in (Fig. 1).

Sampling

Growth rate measurements: Cross-sectional data was collected as described by Chambers and Miller (1995) to determine fish growth i.e., only the fish sizes at sampling (or harvest) that were available were used. Samplings was done on a weekly basis, a population of ten larvae fish was sampled per aquaria using scoop nets and then transferred in a beaker containing pre-weighed water to determine the weight. All fish were weighed and care was taken not to measure the same individual fish twice. After taking the weight measurements the fish were returned to the aquarium. Final sampling was done after 96 days of the feeding trail. The treatment effects were assessed by comparing the experimental diets with the control diet.

The specific growth rate was then calculated in terms of weight according to:

$$\frac{\ln \text{final weight} - \ln \text{initial weight}}{\text{No. of days}} \times 100$$

Histological examination of liver and kidney: At the end of the experiment, three fish from each diet were sacrificed by rapid chilling, immersed them in a bath with ice cubes at a temperature of 4°C for 20 min following American Veterinary Medical Association (AVMA). The fish were then dissected to obtain samples of liver and kidney for use as biomarkers to assess the effects of experimental and commercial feeds on the nutritional status of fish. Isolated samples were immediately fixed in 10% buffered formalin for 10 min and then histologically processed following procedures by Luna (1992) and Presnell *et al.* (1997). Thin slices of 5 µm sections were cut and stained with (Haematoxylin and Eosin) H and E for light microscopy examination.

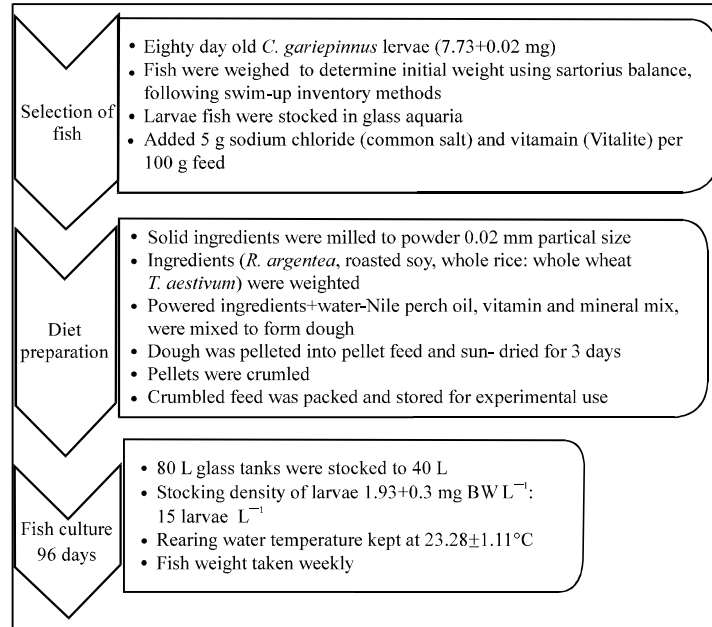


Fig. 1: Summary of stages involved in the experiment

Data analysis: Data was entered into a computer spreadsheet (Excel Microsoft Inc., Redmond, WA) and imported into a SAS file (SAS version 9.12, SAS Institute, Cary, NC) for data description and analysis. The effect of diet on the weight and length of the fish was tested using linear regression (PROC GLIMMIX). The effect of the diets on fat infiltration of the liver at 96 day was tested using Fisher's exact test. Significance was defined as $\alpha \leq 0.05$.

RESULTS

Feed attributes: Proximate analysis of experimental crumblled fry diets for *C. gariepinus* larvae gave values of crude protein (58.08±1.6) and crude lipid (9.46±0.34) (Mean values±standard deviations).

Ration palatability: We observed but did not measure, that eight day old larvae readily accepted the control commercial diet but feed acceptance for the formulated diet varied among diets and was poor in comparison to the control.

Fish attributes

Growth in weight: Growth responses of fish larvae in all the three treatments and the control fed both experimental diets and the control diet were influenced by diet type. The effect of dietary treatment on the body weight of fish interacted with time ($p = 0.0001$; Fig. 2). The weight of fish on day 96 differed significantly by dietary treatment ($p = 0.007$; Fig. 3). Fish fed the commercial control diet were significantly heavier than the fish fed the experimental diets. Compared to the control diet, fish fed P58, P56 and P60 were 853 ($p = 0.005$), 1,054 ($p = 0.001$) and 520 mg lighter ($p = 0.05$), respectively. Fish fed P60 were significantly heavier than fish fed P58 ($p = 0.01$) and P56 ($p = 0.002$). There was no significant difference in body weight of fish fed P58 and P56 ($p = 0.28$).

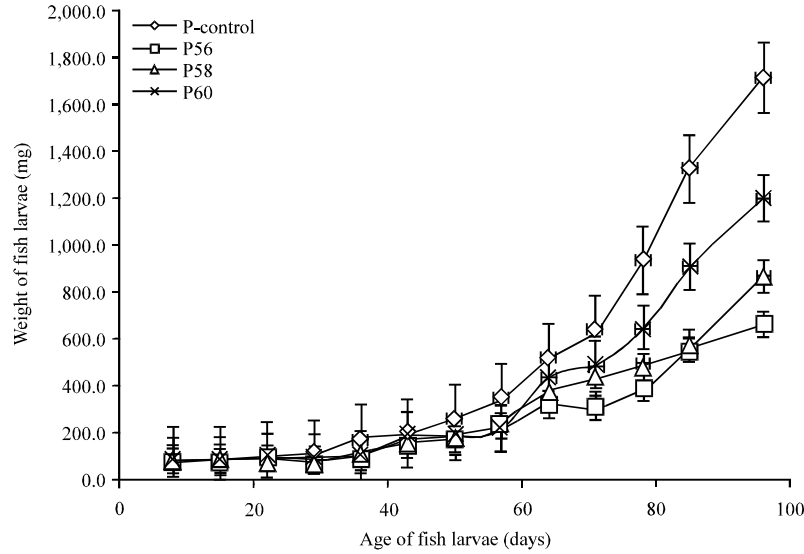


Fig. 2: Weekly recorded mean body weights of *C. gariepinus* larvae fed experimental and control diets over 96 days

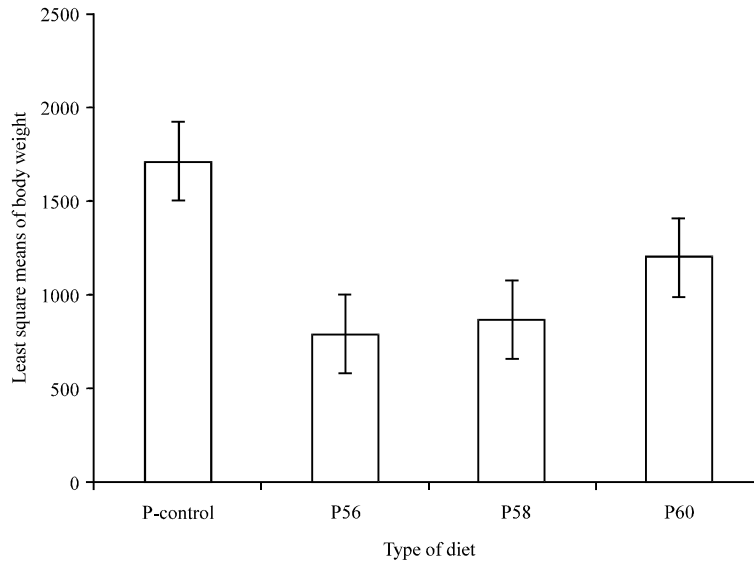


Fig. 3: Least squared means of the body weight of *C. gariepinus* larvae with three experimental diets and a commercial control diet at the end of the study (96 day). Means \pm SEM (df = 8) ($p < 0.05$)

Length of fish larvae: The length of the fish larvae on 96 day differed significantly by dietary treatment ($p = 0.004$). Least squares means for control, P58, P56 and P60 were 6.7, 6.7, 5.1 and 4.6 cm, respectively. Fish larvae fed P60 did not differ in length from the control diet ($p = 0.94$). However, fish larvae fed P58 and P58 had similar lengths ($p = 0.28$) and were significantly shorter in length than fish fed the control diet ($p = 0.01$ and 0.002 , respectively) or P60 ($p = 0.01$ and 0.002 , respectively).

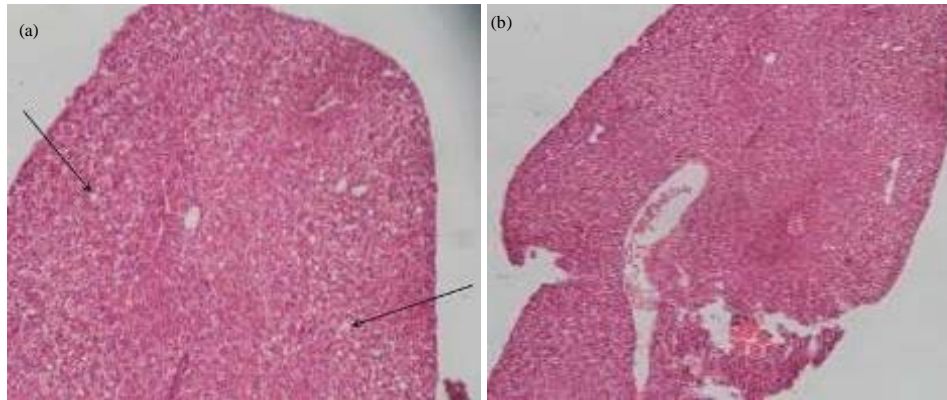


Fig. 4(a-b): Cross section of liver showing lipid infiltration after 96 days of feeding on experimental diets, (a) Liver X 20 fat infiltration and (b) Liver X 10 fat infiltration

Effect of dietary treatments on liver histology: Microscopic examination of the liver showed lipid infiltration in all the livers of the sampled fish (Fig. 4). Fat droplets (seen as round empty spaces) were seen in hepatic parenchyma and discrete vacuoles in fish fed diet P56 which was a sign of fat infiltration. Similarly, there was extensive infiltration (macrophages, neutrophils) in hepatic sinusoids for fish fed diet P58 and P60. Statistical analysis using Fischer's exact test showed that lipid infiltration in livers of fish fed experimental diets did not show significant differences ($p = 0.0061$, $n = 12$).

Fish survival: The fingerlings that survived longest were fed on the control treatment ($87.17 \pm 8.7\%$), followed by those fed P60 ($71.67 \pm 7.3\%$), P58 ($66.33 \pm 8.3\%$) and the lowest survival was noted for P56 ($56.83 \pm 5.9\%$).

Cost for local feed production: The cost of ingredients for production of 100 kg of local fry feed was used to estimate the economic effects of using the feeds.

DISCUSSION

The aim of this study was to assess whether there was a significant difference in the growth of *C. gariiepinus* fish larvae fed on experimental diets P56, P58 and P60 and P-control. Secondly, whether there were significant effects in lipid deposition and changes in liver morphology when diet treatments P56, P58 and P60 and P-control were fed to fish. Results from chemical analyses (Table 1) showed that the diets used in this study were within the range of dietary protein requirements 55 and 9% lipids (NRC., 1993). Although, Hoornycyk (2000) observed that heat treatment increased protein denaturation and decreased protein solubility, in the present study there was no heating of feed mash/dough. However, the quality of food protein ingredient used and method of feed processing employed improved protein availability, considering the theoretical protein supply (g kg^{-1}); P58 (47.62), P56 (47.2) and P60 (48).

During the first stages of experimental diet feeding there was slow growth of *C. gariiepinus* larvae and after 30 days onwards there was an exponential growth. Similar observations of low growth within the first 10 days were made by Segner and Verreth (1995) while feeding dry diet to *C. gariiepinus* larvae who noted that during the early phase of larvae fish, the glycolytic and

Table 2: Production costs of local fry feed compared with cost of imported feed

Local ingredients	Quantity (kg)	Unit cost (Shs)	Unit cost (USD)	Total cost (UgX)	Total cost (USD)
Mukene	86	7000	2.86	602,000	245,71.00
Whole rice	5	4000	1.63	20,000	8.16
Roasted soy	4	4000	1.63	16,000	6.53
Whole wheat	3	3500	1.43	10,500	4.29
Vitamin mix	2	5000	2.04	10,000	4.08
Fish oil	1	2000	0.82	1,640	0.67
Total cost/100 kg local feed				660,140	269.44

gluconeogenic capacities are low but are enforced during the later phase. The developmental pattern seen in growth rate of fish fed experimental and control diets was similar to other reports of similar catfish species (Rangsin *et al.*, 2012; Srichanun *et al.*, 2012; Khemis *et al.*, 2013). In all the four dietary treatments a relationship existed between weight of fish larvae and time but the daily increment in body material of fish larvae was not constant which was also noted by Chambers and Miller (1995) in larval Atlantic menhaden (*Brevortia tyrannus*) fish. Analysis of repeated measures using Glimmix, SAS showed there were no significant differences ($p > 0.05$) in the mean weight of *C. gariepinus* larvae fed varied proportion of protein diets (P56, P58 and P60) at the beginning of the study. But after 96 days, a pairwise test for mean weights showed significant differences in all the treatments compared to the control ($p = 0.0067$) but no significant differences were observed in growth rates of fish larvae fed P56 and P58 as well as between P60 and P58. However, the final weight for fish fed P60 was better than fish fed P58. Consequently, Specific Growth Rate (SGR) for fish fed P60 was higher (2.9) than that for fish fed P58 (2.5) diet. The observed growth rates in the present study where we used smaller fish (7.73 ± 0.02) were lower than growth rates of bigger fish larval catfish (150 mg) used in some previous studies (Adekunle and Joyce, 2014; Enyidi *et al.*, 2014). The observed growth rates of the catfish in this experiment suggest that all tested diets were suitable for rearing catfish larvae from start of exogenous feeding. But statistical analysis shows that the results are in agreement with many other studies, who also found that increase in dietary protein level are often associated with higher growth rates in many species (Jauncey, 1982; Kolkovski, 2001). Although at the start of exogenous feeding fish have all necessary enzymes for metabolism (Garcia-Ortega *et al.*, 2000), in the present experimental approach conducted on fish growth, slow growth was observed during the first days of feeding on locally formulated experimental diets. Although acid and alkaline proteases, amylase and lipase are present at a very early life stage (Rangsin *et al.*, 2012), there might have been a delay in progressive shift of alkaline proteases as the main digestive enzymes for protein digestion (Kolkovski, 2001; Pradhan *et al.*, 2013).

Low survival rates of fish larvae were observed during the experiment but as earlier observed by Sales (2011), larvae fed on compound diets have a 2.5 times higher chance to die than those fed on live feed. Larvae mortalities could be related to various factors like starvation, poor feed acceptance and infections of larvae (Abdulraheem *et al.*, 2012). The choice of good quality food which is acceptable to the fish species of interest is major problem in larviculture.

Although the results approached statistical significance, we were unable to conclude that the diets differed in lipid deposition of the liver. However, this study may have lacked sufficient sample size to detect meaningful differences in hepatic lipidosis. The finding of no difference therefore, is without certainty based on the small sample size and is subject to correction.

The cost for production of 100 kg locally formulated fry diet presented in Table 2 shows that, local diets can replace the imported feed with about 70% the saving for the farmers as opposed to when the imported feed is used.

CONCLUSION

Proper selection and incorporation of local feed ingredients may be useful in the formulation of dry fry experimental feeds for weaning larval catfish. Knowledge gathered from the present study towards larval fish acceptance, survival and growth when fed locally formulated dry fry feeds suggest that the chemical composition of ingredients and their combinations used in the study are suitable for dry fry feeds. Also, basing on the results, there is a potential for feed formulation P60 to be used in the commercial culture of *C. gariepinus* fish larvae. Also, improvements should be made when formulating fry diets to improve palatability. We recommend future studies using similar formulations also assess the effect of diets on physiological processes of digestion. It is anticipated that when this is done it would help to improve the diet so as to meet the specifications of digestive system of the target species. Furthermore, there is need to carry out preliminary starvation experiments using the study species in order to determine the age at which the dry formulated feed can be reliably given. Although unknown mortalities have been reported when dry feeds are used for feeding larvae fish, future studies should consider health and disease screening of egg to rule out the possibility of pathogen carry-over in the hatchlings.

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REFERENCES

- AOAC., 1999. Official Methods of Analysis. 16th Edn., Association of Official Analytical Chemists, Washington, DC., USA.
- Abdulraheem, I., S.O. Otubusin, O.T. Agbebi, O. Olowofeso and W.O. Alegbeleye *et al.*, 2012. The growth response of *Clarias gariepinus* hatchlings to different dry feeds. *J. Agric. Sci.*, 4: 75-80.
- Adekunle, A.I. and A.O.B. Joyce, 2014. Effect of partial and total replacement of live feed (artemia) with formulated diets in early stage growth of hybrid catfish (*Heterobranchus bidorsalis* x *Heterobranchus longifilis*) fry. *Int. J. Fish. Aquat. Stud.*, 1: 143-146.
- Chambers, R.C. and T.J. Miller, 1995. Evaluating Fish Growth by Means of Otolith Increment Analysis: Special Properties of Individual-Level Longitudinal Data. In: Recent Developments in Fish Otolith Research, Secor, D.H., J.M. Dean, S.E. Campana (Eds.). Belle W. Baruch Institute for Marine Biology and Coastal Research, South Carolina, ISBN: 9781570030116, pp: 155-175.
- Enyidi, U., J. Pirhonen and J. Vielma, 2014. Effects of substituting soybean (*Glycine max*) meal with bambaranut (*Voandzeia subterranea*) meal on growth performance and survival of african catfish (*Clarias gariepinus*) larvae. *Int. J. Fish. Aquat. Stud.*, 1: 152-157.
- FAO., 2010. FishStat statistics and information service. FAO Fisheries and Aquaculture Department.
- Garcia-Ortega, A., J. Verreth and H. Segner, 2000. Post-prandial protease activity in the digestive tract of African catfish *Clarias gariepinus* larvae fed decapsulated cysts of *Artemia*. *Fish Physiol. Biochem.*, 22: 237-244.

- Hoornyck, V., 2000. Heat treatment affects protein quality and protease activity in decapsulated cysts of *Artemia* when used as starter food for larvae of African catfish *Clarias gariepinus* (Burchell). *Aquacult. Nutr.*, 6: 25-31.
- Jauncey, K., 1982. Effects of varying dietary protein level on the growth food conversion, protein utilization and body composition of juvenile tilapia (*Sarotherodon mossambicus*). *Aquaculture*, 27: 43-54.
- Khemis, I.B., E. Gisbert, C. Alcaraz, D. Zouiten and R. Besbes *et al.*, 2013. Allometric growth patterns and development in larvae and juveniles of thick-lipped grey mullet *Chelon labrosus* reared in mesocosm conditions. *Aquacult. Res.*, 44: 1872-1888.
- Kolkovski, S., 2001. Digestive enzymes in fish larvae and juveniles-implications and application to formulated diets. *Aquaculture*, 200: 181-201.
- Luna, L.G., 1992. *Histopathologic Methods and Color Atlas of Special Stains and Tissue Artifacts*. American Histolabs, Downers Grove, Illinois Pages: 767.
- Mukai, Y. and L.S. Lim, 2011. Larval rearing and feeding behavior of African catfish, *Clarias gariepinus* under dark conditions. *J. Fish. Aquat. Sci.*, 6: 272-278.
- NRC., 1993. *Nutrient Requirements of Fish*. National Academy Press, Washington, DC., USA., ISBN-13: 9780309048910, Pages: 114.
- Pradhan, P.K., J. Jena, G. Mitra, N. Sood and E. Gisbert, 2013. Ontogeny of the digestive enzymes in butter catfish *Ompok bimaculatus* (Bloch) larvae. *Aquaculture*, 372: 62-69.
- Presnell, J.K., M.P. Schreiber and G.L. Humason, 1997. *Humason's Animal Tissue Techniques*. 5th Edn., Johns Hopkins University Press, Baltimore, MD., USA., ISBN-13: 9780801854019, Pages: 572.
- Rangsin, W., N. Areechon and R. Yoonpundh, 2012. Digestive enzyme activities during larval development of striped catfish, *Pangasianodon hypophthalmus* (Sauvage, 1878). *Kasetsart J. Nat. Sci.*, 46: 217-228.
- Sales, J., 2011. First feeding of freshwater fish larvae with live feed versus compound diets: A meta-analysis. *Aquacult. Int.*, 19: 1217-1228.
- Segner, H. and J. Verreth, 1995. Metabolic enzyme activities in larvae of the African catfish, *Clarias gariepinus*: Changes in relation to age and nutrition. *Fish Physiol. Biochem.*, 14: 385-398.
- Srichanun, M., C. Tantikitti, V. Vatanakul and P. Musikarune, 2012. Digestive enzyme activity during ontogenetic development and effect of live feed in green catfish larvae (*Mystus nemurus* Cuv. and Val.) Songklanakarin J. Sci. Technol., 34: 247-254.