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Research Application Summary

Growth performance of *Clarias gariepinus* hatchlings fed on enzyme pre-digested dry diets from first feeding

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Abstract

Lack of affordable larval diets is largely held responsible for the low survival (10-30%) and slow growth of catfish (*Clarias gariepinus)* fry, required for production of farmed human food fish and as bait for the Nile Perch fishery within the L.Victoria basin. Unlike the role of enzymes from live feed organisms that has been well documented, the role of microbial enzyme- incorporated dry diets is poorly understood in fish larval nutrition. Survival and growth of catfish larvae fed on phytase and protease pre-digested dry diets was evaluated as an alternative to expensive imported live feeds. Seven iso-nitrogenous (55% crude protein) dry diets were formulated and incorporated with phytase and protease enzymes at 750, 1000 and 1250 Units/Kg of feed. They were fed to *C. gariepinus* hatchlings after two days of hatching (after york absorption) as weaning diets along with hatchlings weaned onto Artemia for the first 24 hours and then onto an imported diet (Ranaan CS-starter feed/799 for catfish) in triplicates under hatchery conditions. There was significant differences in larval with the lowest in the control; zero enzyme (10.98%), highest in diet with 1250 units of protease followed by 1000 units of protease 94.23% and the imported diet with 41.66% survival). There was no significant difference in weight gain among diets (P= 0.40) but slight difference was observed in specific growth with the highest in larvae fed on the imported diet (0.21g) and least (0.06) in the control. These results indicated that enzyme pre-digested dry diets can nourish catfish larval as weaning diets.

Key words: Catfish larval growth, dry diets, protease, phytase

Résumé

Le manque d'aliments abordables pour les larves est largement responsable pour de faible taux de survie (10-30%) et de croissance lente de la fraie du poisson-chat (*Clarias gariepinus*). Ces aliments sont nécessaires à la production des poissons d'élevage pour l'alimentation humaine et comme appât pour la pêche de la perche du Nil dans le bassin du lac Victoria. Contrairement au rôle des enzymes extraites à partir d'organismes vivants alimentaires qui a été bien documenté, le rôle des enzymes microbiennes incorporées dans les aliments secs des poissons est mal compris dans la nutrition larvaire. La survie et la

croissance de la fraie des poissons-chats nourrie avec les aliments secs prédigérés avec la phytase et la protéase ont été évaluées comme une alternative aux aliments ayant les organismes vivants importés coûteux. Sept rations sèches iso-azotées (55% de protéines brutes) ont été formulées et incorporées avec la phytase et la protéase à 0, 750, 1000 et 1250 unités / kg d'aliment. Elles ont été nourries aux alevins de *C. gariepinus* après deux jours d'incubation (après l'absorption du sac vitellin) comme régimes de sevrage en même temps que d'autres alevins sevrés en utilisant l'Artemia pour les 24 premières heures, puis sur un aliment importé (Ranaan CS-démarreur / 799 pour le poisson-chat) en trois répétitions dans des conditions d'écloserie. Il y avait des différences significatives dans la survie larvaire avec la plus faible obtenue avec le contrôle; zéro enzyme (10,98%), et la plus élevée dans le régime alimentaire avec 1250 unités de protéase (52.75%) suivie par 1000 unités de protéase (94,23%) et Ranaan (41,66%). Il n'y avait pas de différence significative entre les régimes concernant le gain de poids (P = 0,40), mais une légère différence numérique a été observée dans la croissance spécifique (P = 0,58) avec la plus grande étant observée chez les larves nourries avec l'alimentation importée (0,21 g) et la moindre (0,06) chez les larves nourries avec le contrôle. Ces résultats indiquent que les aliments secs prédigérés avec les enzymes peuvent nourrir les larves des poissons-chats comme aliments de sevrage.

Mots clés: croissance larvaire du poisson-chat, les aliments secs, la protease, la phytase

Background

Cat fish *(Clarias gariepinus)* is the most famous fish species in Sub Saharan Africa and comprises up to 70% of the total farmed fish products in Uganda (Isyagi, 2007; Ssebisubi, 2012; FAO, 2016). Its tolerance to high stocking densities, easy marketability, high fecundity and hatchability during artificial larval breeding gives it a competitive advantage over other fish such as tilapia (Hecht, 1996; Coppens, 2010; Oyedeji, 2016). However, lack of affordable dry diets to replace or supplement the expensive imported live feeds in use (Enyidi, 2015; Nabulime, 2015) restricts good larval nutrition resulting in low survival (10-30%) (Ayele, 2015), growth and inconsistent fry supply thus reducing its production (Chepkirui-Boit, 2010) especially around L.Victoria basin where it is in high demand as human food fish and as bait in the line fishing of Nile perch (Isyagi, 2007; Nabulime, 2015).

Literature summary

Clarias gariepinus larvae like many other fish do not possess a functional stomach at the on-set of external feeding (Dabrowski, 1984). This limits utilisation of dry diets for satisfactory growth when larvae is solely reared on dry diets as compared to when fed live feed organisms (Dabrowski, 1984). Live prey organisms contain endogenous enzymes that stimulate proteolytic activity in the fish larvae improving feed digestion at the on-set of exogenous feeding (Pillay, 1993; Kamarudin, 2011; Enyidi, 2015).This has promoted their use as larval diets especially the brine shrimp, Artemia (Ayele,

2015) mainly due to easy transportation and viability over a long time (up to five years) (Garcıa-Ortega, 1998; Wickins and O'CLee, 2008). Although other zooplankton and unicellular algae provide valuable diets (Leger, 1986; Pillay and Kutty, 1990) their absolute use is associated with cross contamination of larvae with pathogens, costly and time consuming production techniques (Leger, 1986; Pillay and Kutty, 1990; Ayele, 2015). This has increased demand and cost of Artemia making its use unattractive especially in regions like Sub Saharan Africa where it does not naturally exist. Evaluating the role played by exogenous enzymes in larval nutrition especially if incorporated into dry micro diets is still poorly under stood yet if perfected it could increase larval survival and growth thus shortening rearing periods to meet the growing fish demand. Hence the rationale for this study.

Study description

Seven diets were formulated to contain 55% crude protein as recommended by (NRC, 1993). Phytase and protease enzymes worth 750, 1000 and 1250 units /Kg of feed were added to six diets and thoroughly mixed. The seventh diet was left blank as the control and together with the other six diets were pelleted and reduced into dry crumbles. The crumbles were randomly assigned to 21 larval brown shrimp rearing tanks each stocked with 8000 catfish fry under hatchery conditions. Pre-decysted *Artemia nauplii* and a catfish starter feed (Raanan Cs 7994CO) crumbles from Israel (Aquafarm Consults Ltd, Kampala, Uganda) and fed alongside enzyme pre-digested diets as a check. Larvae were fed after every two hours over 24 hours daily and rearing tanks cleaned twice daily (at 7.00 am and 8:00 pm) for 4 weeks. Dissolved oxygen and temperature were maintained above 3.5mg/l and 26^{o}C as recommended by Boyd (1998) by aerating and warming rearing water, respectively. Levels of Nitrogen-ammonia, pH and phosphorus were measured weekly using test kits .The amount of feed fed was determined as the difference between the day's ration and the feed at the end of each day. Dead fish was recorded twice daily during cleaning and the total deaths used to estimate larval survival as;

Survival (%) = (No. of live larve / No. of initial stock) x 100

Fish weight was determined after putting a batch of larvae into a beaker with pre-weighed water and later counting the number of larvae in the batch to get the average individual weight as;

Average individual weight (g) = total batch weight (g) / total number of fish in the batch Weight gain (g)= final weight (g) - initial weight

Specific growth rate (SGR) over the rearing period was determined from the equation

SGR (% body weight gain / day) = [In (final weight - initial weight)/ production period] x 100

Where In = natural log (Fish Base 2016).

Feed utilisation was determined using the Feed conversion ratio (FCR) which was calculated as;

FCR =Total feed consummed (g) / Total weight gain (g)

Data were entered and summarized into Microsoft Excel and imported into PAST where ANOVA was performed and pair wise comparisons conducted with Turkey's test.

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There was significant difference in larvae survival among diets (P< 0.001). The diet with 1250 units had significantly higher survival rates than the control (P< 0.001) while survival among other diets did not differ significantly, i.e., the highest survival was 52.75 in 1250 protease > 42.30 in 1000 protease > 41.66 in imported diets> 39.87 in 750 protease> 37.49 in 1250 units of 37.08 in 1000 phytase, 37.02 in 750 phytase > 10.98 in the control phytase the control having significantly lower levels (10.9% (Fig. 1). Major mortalities that occurred in the control were as a result of starvation as evidenced by the negative food conversion ratio (FCR). Larvae appeared emaciated and weak due to poorly digested food in their gut with no cannibalism (either due to differential growth of fish or by lack of feeds) observed.

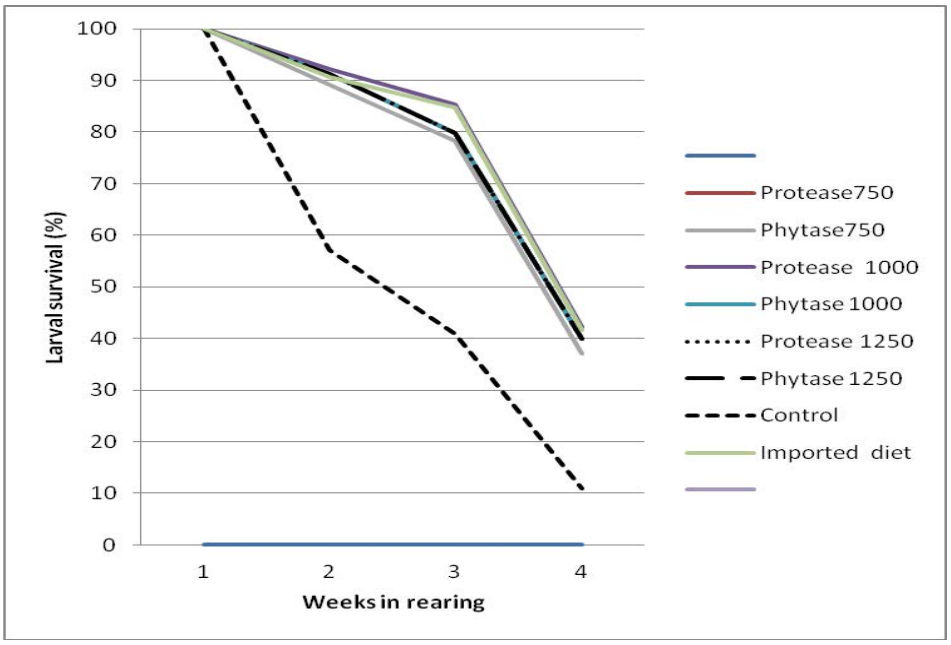

Figure 1: Larval survival in the different diets

No significant differences were observed in weight gain among diets (P = 0.40) but there was higher weight gained in larvae fed on the imported diet (0.21g) with the least (0.06) still in the control (Figure 2). There were however significant differences in specific growth rate among larvae fed on the imported diet and control only (P = 0.033), i.e., 30.08 mg/day and 21.58 mg/day, respectively (Figure 3).

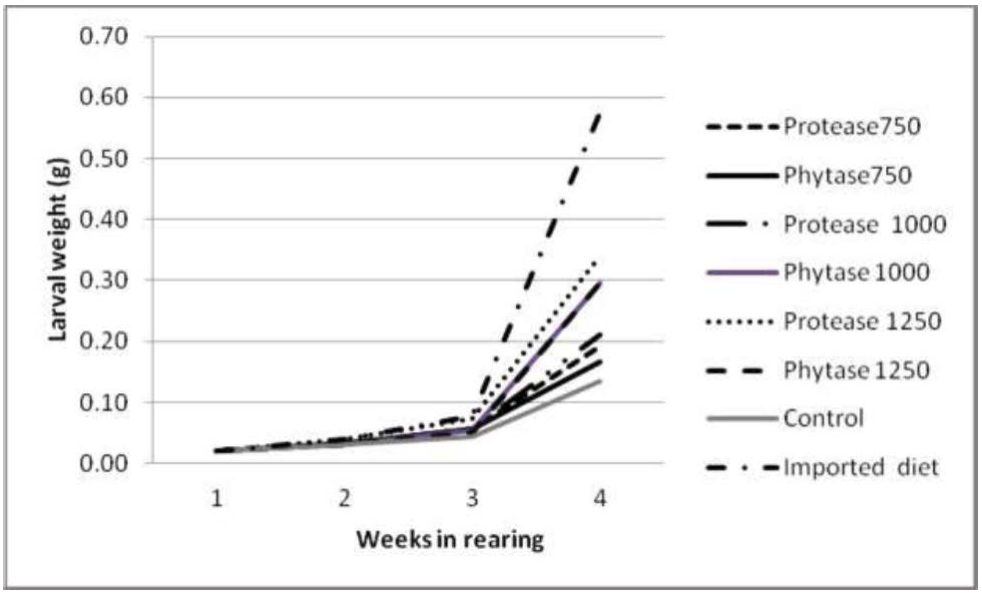

Figure 2: Average weight gain trends within treatment over the three weeks of hatchery rearing

The more the fish survived, the more they grew although this could only be informative if other factors such as environmental variables in the hatchery remained constant.

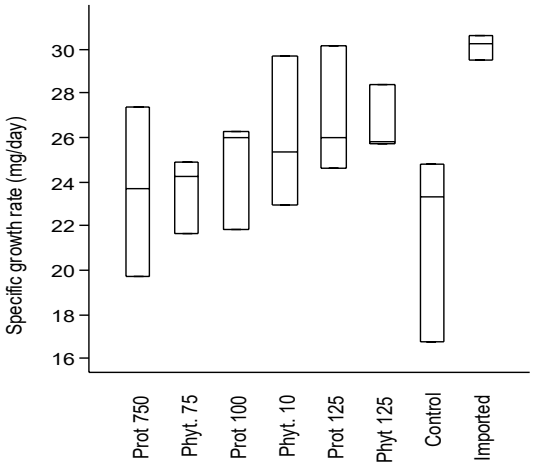

Figure 3 : Box plot of catfish larvae specific growth rate

Significant differences were also observed in the average Food Conversion Ration (FCR) among the diet treatments ($P<0.001$) being significantly negative in the larvae fed on the control diet ($P <0.01$) than in all other diets (Figure 4).

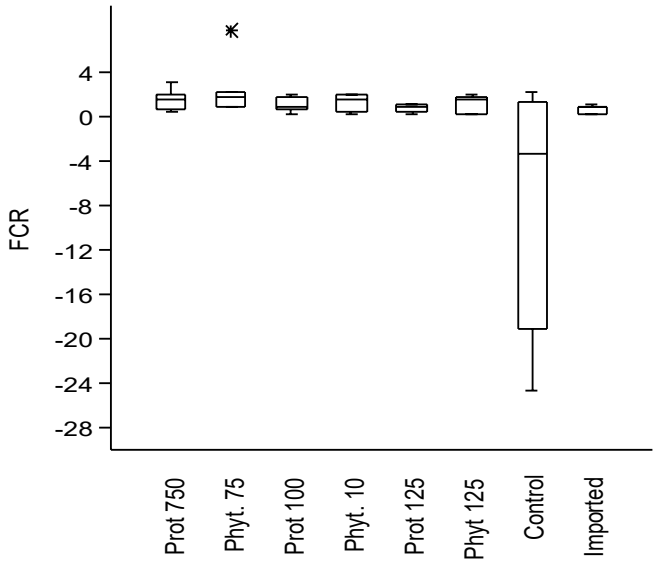

Figure 4: Average food conversion ration over the three weeks of larval feeding

Water quality parameters were also monitored. There were no significant effect of diet on temperature (P= 0.85), dissolved oxygen (P=0.98), ammonia (P = 0.023) and phosphorus (P =0.067). This was attributed to water heating, aeration and routine cleaning to maintain them within tolerable ranges. Slight differences were observed in pH values in 1250 and 750 Units of phytase (p = 0.051). Water temperature ranged from 26.5-27.6 ^{o}C, dissolved oxygen from 3.71-4.31, and pH ranged from 6.45 - 7. Ammonia and phosphorus ranged from 0.73-1.2 and 0.41-0.99 mg/l respectively. Pearson's correlation indicated no significant relationship (P = 0.10) between water quality parameters and the fish larvae survival, growth and FCR. However, larval survival and growth was influenced by the type of diet and not water quality.

The results of this study demonstrate that enzyme-pre digested dry diets can nourish *Clarias gariepinu*s larvae as first food under well managed hatchery conditions.

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