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PERFORMANCES OF FS FEED, ARTEMIA NAUPLII AND COMMERCIAL DIET ON EARLY DEVELOPMENT OF *Clarias gariepinus* LARVAE

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Abstract: Live feed is often used as natural diets for fish larvae in aquaculture. Live feed contains various nutrients such as essential proteins, lipids, carbohydrates, vitamins, minerals, amino acids, and commonly known as living capsules of nutrition. However, live feed requires high cost in maintenance and the production of live feed, such as *Artemia* sp., is limited nowadays. This study investigates the growth performance, survival, and feed utilization of *C. gariepinus* larvae fed on two artificial diets and *Artemia* nauplii. The feeding trials had started at the onset of exogenous feeding for 21 days. A total 540, three-day old larvae were fed one of three feeding treatments, where each trial had triplicates of: (1) FS feed; (2) *Artemia* nauplii; and (3) commercial feed. The FS Feed was formulated by UPM researchers as an alternative to live feed. The study showed that no significant differences were noted in the specific growth rates (SGR) from the three feeding trials. FS feed showed the lowest feed conversion rate (FCR) at 0.61 as well as significantly the lowest feed consumption by the larvae, 4.92 ± 0.82 ($P<0.05$) compared to other feeds. Furthermore, larvae survival after 21 days was significantly higher (51.67 ± 3.85) ($P<0.05$) in *C. gariepinus* larvae fed on FS feed (51.67 ± 3.85) and *Artemia* nauplii (43.89 ± 4.55) than commercial feed. This study focuses on the potential of the FS feed as live feed replacement for improving the survival of fish larvae. Since it is a formulated larval diet, the FS feed could also reduce the chances to transmit diseases to larvae when compared to live feed.

Keywords: *Artemia* nauplii, artificial diets, larvae feeding, growth, survival, feed utilization.

Introduction

For many fish species, the larval stage is considered critical in their life-history since fish undergo rapid and dramatic morphological and physiological changes during ontogenesis. Successful species reared in aquaculture depend mainly on efficient larval rearing where the availability of a suitable diet plays an important factor for larval growth and survival (Sarkar *et al*., 2006). Fish larvae lack fully developed digestive systems, thus starter feeds need to be ready to be consumed, efficiently digestible and provide essential nutrients that are needed for their growth and support the full development of the larvae gut (Fernández-Díaz & Yúfera, 1997; Giri *et al*., 2002). Larval nutrition relies on feeding with live feed, such as rotifers (*Brachionus plicatilis*) and brine shrimps (*Artemia*) (Citarasu *et al*., 2002; Curnow *et al*., 2006; Dhont *et al*., 2013; Najdegerami *et al*., 2015) during early days of hatching, followed by a combination of live and dry feed until they are completely weaned (Chepkirui-Boit *et al*., 2011). The use of *Artemia* is well established because of its year-around availability as shelf cysts, and contains all the nutritional requirements and is high in unsaturated fats (Bengston *et al*., 1991; Sorgeloos *et al*., 2001). One of the major drawbacks of feeding *Artemia* nauplii to freshwater larvae is that the nauplii die after 30 to 60 minutes in fresh water; therefore, larvae must be fed intermittently every 2 to 3 hours. Moreover, in many developing countries, *Artemia* feeding for larvae is not cost-effective because it must be imported, increasing the production cost, and limiting the profitability of fry production. This incentivises the development of affordable alternative feeds to promote and sustain larval production of fish. Recently, extensive research has been done to formulate artificial larval diet either as partial or full replacements for live feed (Borlongan *et al*., 2000; Kovalenko *et al*., 2002; Akbary *et al*., 2010; Nik Sin & Shapawi, 2017; Kemigabo *et al*., 2019)

Artificial feed is attractive because it's easier to manage and has lower production costs, but most have not been successful in raissing the majority of the fish larvae (Koven *et al.*, 2001; Montchowui *et al.*, 2012) primarily due to difficulty in assimilating artificial feed in early larvae (People Le Ruyet *et al.*, 1993). To achieve long-term growth of larvae, an artificial diet must be attractive, ingestible, digestible, and nutritionally complete similar to the live foods (Hamre *et al*., 2013), and larval diet particle sizes must be appropriate to the mouth size of the larvae. For instances, small feed particles can lead to the problem of water-soluble nutrients leaching, caused by a very high ratio of surface-area-to-volume. This factor contributes to the technical complexities in developing larval diets, other limitations include lack of information on the optimal nutrient content and suitable feeding time of larvae.

African catfish (*Clarias gariepinus,* Butchell 1822*)* is one of the most widely cultured species in tropical and subtropical countries. Its tolerance to environmental stress and diseases, easy marketability with high fecundity (Ishiyagi, 2007; FAO, 2016; Kemigabo *et al*., 2018) makes it commercially important . The expansion of its industry needs extensive larvicidal production, where it predominantly depends on the constant production of live feed (Jonathan *et al*., 2013; Enyidi & Nduh-Nduh, 2016), this offers drawbacks to large-scale larval rearing. The *C. gariepinus* larvae may grow up to 100% per day (Conceição *et al.*, 1998) and absolute dependency on live feed presents considerable challenges, as it requires intensive cultivation techniques and high-cost live feed production. This study evaluates the effect of artificial diets, FS feed, and commercial Artemia nauplii feed on the growth performances and nutrient utilization in *C. gariepinus* larvae.

Materials and methods

Fish Maintenance and Feeding Trials

The experiment was conducted at Wet Lab, Department of Aquaculture in Universiti Putra Malaysia. The larvae of *C. gariepinus* were artificially produced at hatchery using inducing hormones. Prior to fish breeding, brood fish were separated according to sex and acclimatized for a week in a fiberglass tank. The brood fish were fed daily with pelleted feed to enhance gonad maturation before undergoing hypophysation technique. Ovaprim was used to stimulate breeding with the dosage of 0.5ml/kg for a female fish and 0.25ml/kg for male fish. The broodstocks were kept and maintained at 27±0.5ºC. After 12 hours, each female brood fish was stripped, and eggs were fertilized and incubated at 27ºC. The incubation was provided with moderate aeration and left to hatch for 24 hours. The development of fertilized eggs was monitored from time to time until hatching. Subsequently, larvae of *C. gariepinus* were kept in 5L glass tanks, with a stocking density of sixty larvae per tank. The total number of *C. gariepinus* larvae used in the feeding trials was 540. Round the clock aeration was provided to maintain the optimum dissolved oxygen. Exogenous feeding was started at 3 days after hatching (DAH), when observed yolk sac was completely exhausted. Feeding trials were divided into three sets, where each set was assigned in triplicates and the trials included feeding on FS feed, *Artemia* nauplii, and commercial feed. Fish larvae were fed with trial diets three times a day *ad libitum* (0800,1200 and 1700) for 21 days. To maintain good water quality, uneaten feed and faeces were removed from the entire tank bottom every morning by siphoning, and about 10% of the water was changed every day. Water quality such as pH, dissolved oxygen, temperature, ammonia, nitrite contents were recorded weekly (Table 1) and the experimental tanks were kept under similar conditions at a water temperature of 29°C and 12 h light/l2 h dark photoperiod.

Table 1: Physico-chemical parameters of water in three feeding tanks for 21 days (mean ± SE)

Parameters	Trial Diets		
	FS Feed	*Artemia* Nauplii	Commercial
Temperature	27.9 ± 0.13[a]	28.0 ± 0.09[a]	27.9 ± 0.7[a]
pH	7.7 ± 0.38[a]	7.5 ± 0.38[a]	7.6 ± 0.30[a]
Dissolved oxygen	6.53 ± 0.24[a]	6.64 ± 0.06[a]	6.55 ± 0.2[a]
Ammonia	0.16 ± 0.10[a]	0.16 ± 0.08[a]	0.74 ± 0.34[a]
Nitrate	0.19 ± 0.08[a]	0.16 ± 0.04[a]	0.20 ± 0.08[a]
Nitrite	0.04 ± 0.01[a]	0.05 ± 0.02[a]	0.11 ± 0.02[b]

Value of mean within the same row with different superscripts are significantly different ($P<0.05$, ANOVA).

Preparation and Formulation of Trial Feeds

Artemia cysts used in this study were s supplied from Aquatic Enterprise Co., Malaysia. Hatching of the cysts were carried out as suggested by Sorgeloss *et al.* (1986). Before hatching, cysts were washed for six minutes in an active bleach liquor followed by rinsing in chlorine-free water. The cysts were incubated in a glass jar at a density of $0.6gL^{-1}$ for 24 h using saline water (25 ppt). Vigorous aeration was provided throughout the hatching period for efficient hatching of *Artemia* cysts into nauplii. FS feed is registered as trade secret with Universiti Putra Malaysia (T2019041601). The main ingredient of the FS Feed are fermented soy protein and fish oil and the feed is high in protein (46.40%) and lipid (35.80%). The main usage of this feed is to replace *Artemia* nauplii for the aquaculture industry. While commercial feed (Cargill Starter Diet- Code: 6283_1) consist of 46% of protein and 8% lipid. Both artificial feeds were ground and filtered through a 250 micron sieve for size consistency.

Biochemical Composition and Proximate Analysis

Ten grams (10g) of the artificial feed samples were analyzed following the procedure outlined in AOAC (1990). Moisture was measured by drying the sample in an air convection oven at 105°C overnight. Crude protein was quantified by the Kjeldahl method after acid digestion (% crude protein = % nitrogen × 6.25), while crude lipid was determined by extraction with petroleum ether using the Soxhlet method. The ash content in the feeds was analyzed by the combustion of samples in a muffle furnace at 550°C for 12 h.

Larvae Growth and Survival Sampling

Clarias gariepinus larvae were observed daily starting from 3 DAH to observe their morphological development. The length and weight of fifteen *C. gariepinus* larvae were sampled weekly for 21 days using vernier caliper and electronic balance, respectively. Larvae were mildly anesthetized using MS 222: sodium bicarbonate mixture (ratio at 1:2), dosage at 15ppm and sampled larvae were returned to the rearing tanks after the measurement. Also, the larval survival rate for each feeding trial was recorded daily. The growth performance parameters were determined by the following formulae:

$$Specific\ Growth\ Rate\ (SGR) = \frac{(ln\, W_f\ - ln\ W_i\,)}{t} \times 100$$

W_f= final weight
W_i= initial weight
t= time between $ln\, ln\, W_f$ and $ln\, ln\, W_i$

$$Average\ daily\ growth = \frac{(Final\ weight(g)\ -\ Initial\ weight\ (g))}{Days\ of\ culture}$$

$$Survival\ rate\ \% = \frac{Final\ number\ of\ larvae}{Initial\ number\ of\ larvae} \times 100$$

Nutrient Utilization Indices

Nutrient utilization is important to measure feed efficiency of the larvae. Hence, feed intake, feed conversion ratio (FCR) and protein efficiency ratio (PER) were calculated using the following equations:

Feed intake (g) = Total weight of food consumed by larvae within the experimental period

$$Feed\ conversion\ rate\ (FCR) = \frac{Feed\ intake\ (g)}{Weight\ gain\ (g)}$$

$$Protein\ efficiency\ ratio\ (PER) = \frac{Weight\ gain\ (g)}{Protein\ intake\ (g)}$$

Statistical Analysis

All results were presented as mean ± SE. Statistical comparisons between trial diets were made using one-way Analysis of Variance (ANOVA) implemented in IBM SPSS Statistics 25. Results were considered significant at a 5% level.

Results and Discussion

Proximate Analyses of Trial Feeds

The proximate composition of three feeding trial diets on *C. gariepinus* larvae are presented in Table 2. Both FS feed and *Artemia* nauplii showed high crude protein content compared to commercial feed. The protein content is an important component in the diets of larvae to support the rapid growth of larvae by increasing body muscle mass for growing larvae (Cahu *et al*., 2003). Interestingly, lipid content in FS feed was the highest among all trial diets and gave the highest total energy of (25.86 kJ) compared to the other diets; *Artemia* nauplii (12.47 kJ) and commercial feed (12.10 kJ). Several studies on the effect of lipid supplementation on fish reported high-lipid diets improved growth performance, intestinal enzyme activities, and reduce excessive lipid deposition (Kim *et al*., 2012, Li *et al*., 2016). Lipids are important to supply more energy on top of protein supplementation, as lipids are readily digestible by the fish, particularly larvae (Tocher, 2003; Conceição *et al*., 2010). The commercial feed shows the highest moisture, fibre, and ash contents compared to FS feed and *Artemia* nauplii. Fish feed formulation should be a balance between all these biochemical properties. An optimum level of moisture, fibre, and ash content is important to ensure no excessive amount could lead to water pollution (White, 2013).

Growth and Survival of Larvae

Recently significant attention has been paid to the need to rear some marine and freshwater species entirely on artificial diet (Wang *et al.,* 2005; Andrés *et al.,* 2011; Vandecan *et al.,* 2011; Israel *et al*., 2014; Kujawa *et al*., 2016;). However, a major problem in freshwater fish larviculture, such as the *C. gariepinus,* is that poor quality of feed is associated with low disease resistance, causing high mortality and stunted larval growth. Alternative feed other than live feed is important to explore, as live feed cultivation is costly and does not ensure consistent and safe nutritional quality (Drossou *et al*., 2006). A newly formulated diet, FS feed

Table 2: The proximate composition (%: on dry matter basis) of the trial diets

Proximate Composition (Dry Matter Basis)	Trial Diets		
	FS Feed	*Artemia* Nauplii	Commercial
Crude Protein (%)	46.40	48.55	45.21
Lipid (%)	35.80	2.95	5.25
Moisture (%)	4.43	10.25	10.47
Fibre (%)	2.90	6.42	12.12
Ash (%)	3.60	14.74	20.61
Energy (kJ)	25.86	12.47	12.10

intended for larval rearing was designed in this study to improve fish health and growth. The trial diets namely FS feed, *Artemia* nauplii, and commercial feed have been tested to evaluate the growth performance, survivability, and nutrient utilization of *C. gariepinus* larvae. The exogenous feeding with trial diets was given to 3DAH larvae of *C. gariepinus*, as yolk had completely absorbed, and they had readily accepted all trial feeds.

In this present study, the initial standard length and body weight of *C. gariepinus* larvae used were homogenous. Since exogenous feeding has not yet been introduced, the growth of larvae for 21 days could be attributed to the effects of trial diets. The growth performance of *C. gariepinus* larvae in terms of initial and final standard body length, initial and final body weight, initial and final standard length, SGR, and average daily growth were presented in Table 3. On day 21 of the experiment, the final standard length of larvae fed on FS feed (11.49 ± 1.16) and commercial feed (11.72 ± 0.91) showed a significant difference from larvae fed on *Artemia* nauplii (10.61 ± 1.05) ($p<0.05$). While the larvae fed on three trial diets were recorded to have no significant difference in the final body weight ($P>0.05$). After 3 weeks of rearing, the difference in SGR and the percentage of weight gain were also not significant in larvae raised using three trial diets ($P>0.05$). The result of average daily growth of *C. gariepinus* larvae shows no significant differences among larvae fed on FS Feed (0.39 ± 0.02) and *Artemia* nauplii (0.35 ± 0.07) diets ($p>0.05$), but significantly enhanced average daily growth (0.41 ± 0.04) was observed in larvae fed on the commercial diet ($p<0.05$). Based on the results, there was no significant improvement on growth parameters of larvae raised using artificial diets and *Artemia* nauplii.

The ability of certain fish larvae to digest the artificial diet is largely clarified by the morphological characteristics of the digestive tract and the secreted enzymes (Portella *et al*., 2008). The digestive system in *C. gariepinus* larvae exhibited pronounced development, and hormones from the pancreas and intestine started to secrete 48 hours after hatching (Verreth *et al*., 1992; Kolkovski, 2001b). At the beginning of exogenous feeding, larvae of *C. gariepinus* seem to be able to digest protein and lipid, so any reduced growth performance and larvae survival rate are not necessarily due to a lack of general digestive ability. As more information on the digestive activity of fish larvae becomes available (Kolkovski, 2001a; Kamarudin *et al*., 2011; Srichanun *et al*., 2012; Rønnestad *et al*., 2013), the more this presumption seems to be confirmed and it may be common for this species.

In our experiment, the survival rate was profoundly higher ($p<0.05$) in larvae fed on *Artemia* nauplii (43.89 ± 4.55) and FS feed, where FS feed yielded the highest survival rate (52.67 ± 3. 85). FS feed containing 46.40% of lipid showed better results in terms of larvae

Table 3: Growth performance parameters and survival rate of *C. gariepinus* larvae fed on three trial diets for 21 days

Parameters	FS Feed	*Artemia* Nauplii	Commercial
Initial standard length (mm)	5.86 ± 0.54[a]	5.7 ± 0.58[a]	5.78 ± 0.58[a]
Final standard length (mm)	11.49 ± 1.16[b]	10.61 ± 1.05[a]	11.72 ± 0.91[b]
Initial body weight (mg)	0.74 ± 0.1[a]	0.69 ± 0.11[a]	0.75 ± 0.06[a]
Final body weight (mg)	8.87 ± 0.49[a]	8.14 ± 1.57[a]	11.64 ± 5.27[a]
Specific Growth Rate, SGR	0.95 ± 0.02[a]	0.91 ± 0.08[a]	0.96 ± 0.13[a]
Average daily growth	0.39 ± 0.02[a]	0.35 ± 0.07[a]	0.41 ± 0.041[b]
Survival rate (%)	51.67 ± 3.85[b]	43.89 ± 4.55[b]	8.33 ± 4.2[a]

Value of mean within the same row with different superscripts are significantly different ($P<0.05$, ANOVA).

survival. A study by (Zheng *et al*., 2010) found that when darbarbel catfish, *Pelteobagrus vachelii* larvae were supplemented with dietary lipids, their survival improved by increased activity of lipoprotein lipase, hepatic lipase and pancreatic lipase, which helps in assimilating nutrients from the feed. Even though commercial feed gave the best average daily growth and final body length, is considered a poor diet since the mortality of larvae was significantly higher than larvae fed on *Artemia* nauplii and FS Feed. Survival has never been a major concern in the larval production of *C. gariepinus* due to its resistance to water quality and common diseases (Abraham *et al*., 2018), however, consumption of poor diet probably could contribute to mortality in the larvae. Alternatively, poor feeding can also contribute to starvation and poor diets acceptance to the larvae (Alegbeleye *et al*., 2012).

Based on the proximate analyses (Table 2), commercial feed and *Artemia* nauplii were lacking required lipid content, signifying the low survival of the *C. gariepinus* larvae. Lipids are in high demand in fast-growing fish larvae since they play important roles in the structural constituents of bio-membranes (Tocher *et al.*, 2008), involved in the early development of the digestive system (Cahu & Infante, 2001), metamorphosis and eye development (Bell *et al.*, 2003). Unenriched *Artemia* used in this study naturally have poor nutrients, particularly essential lipids that are required for good larval growth and development. During the early stage of development, fish larvae are unable to synthesize lipids at a rapid rate to meet their needs, Therefore, their diet needs to be supplemented through an exogenous diet. The *C. gariepinus* larvae probably could not digest the commercial feed which contains a high content of ash, due to incomplete digestive system development. This could also lead to high mortality during the feeding trial periods. Importantly, *C. gariepinus* larvae fed on the commercial diet did not exhibit a compromised survival rate when compared to larvae fed on *Artemia* nauplii and FS feed.

Feed Efficiency

Rearing *C. gariepinus* larvae with different trial diets showed significant variation in feed intake (Figure 1). Observed feed intake from lowest to highest was FS feed, commercial feed, and *Artemia* nauplii, respectively ($p<0.05$). FCR was from 0.61 – 1.16 and statistical analysis revealed there were no significant differences among FCR of *C. gariepinus* larvae fed on three trial diets ($p>0.05$) (Figure 2). Thus, it appears *C. gariepinus* larvae could use artificial feeds as it produced similar FCR as using *Artemia* nauplii alone, and also suggested that artificial diet could replace *Artemia* nauplii in the traditional feed regime. In aquaculture, a feed with FCR of 1:1 and 1:2 is considered very efficient (Huet, 1972). In our trial, *C. gariepinus* larvae fed on FS feed had yield away below average FCR (Figure 2) which might indicate that FS Feed has away better digestibility rate. The FS Feed also contains the lowest inorganic ash and fibre content compared to other feed, indicating that most of the feed is highly digestible and can easily be absorbed (Table 2). A similar finding was observed when comparing artificial diet and live feed by (Ruyet *et al.*, 2009). The high amount of energy in the FS Feed is more than double when compared to other feeds, thus it will reduce the need for the larvae to consume the feed, leading to lower FCR (Gibson & Gatlin, 2001; Craig *et al*., 2017). The protein efficiency ratio, PER (Figure 3) is the most significantly efficient in larvae with FS feed diet (0.20 ± 0.01) ($p<0.05$). This indicates the source of protein used in FS feed might be more easily digested by the *C. gariepinus* larvae compared to other feeds. Previous studies demonstrated limited utilization of protein in feed could be related to partial inhibition of protease and amylase levels by some dietary ingredients (Alarcon *et al.*, 1999), this suggests the potential of FS feed as larval diet, particularly for *C. gariepinus*.

From daily observation, some feeding tanks supplemented with commercial feed were missing individuals for 19 - 21 days (data not shown). This could indicate the occurrence of cannibalism, which occasionally happens when

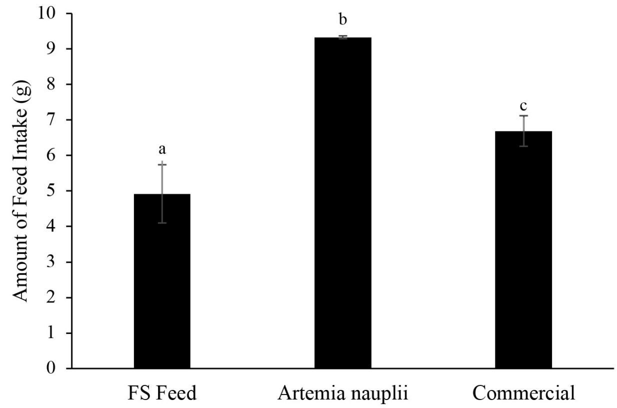

Figure 1: Amount of feed intakes of *C. gariepinus* larvae fed on different trial diets. Error bar show mean ± SE. Different lowercase letters (a-c) indicate statistically significant differences among trial diets ($p < 0.05$, ANOVA)

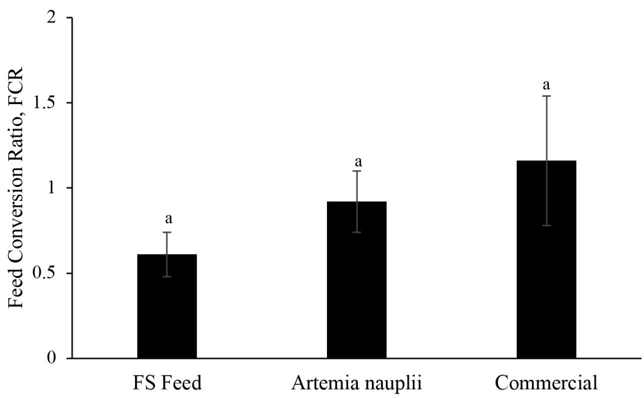

Figure 2: Feed conversion ratios, FCR of *C. gariepinus* larvae feed on three trial diets. Error bar show mean ± SE. Same lowercase letters indicate no statistically significant differences among trial diets ($p > 0.05$, ANOVA)

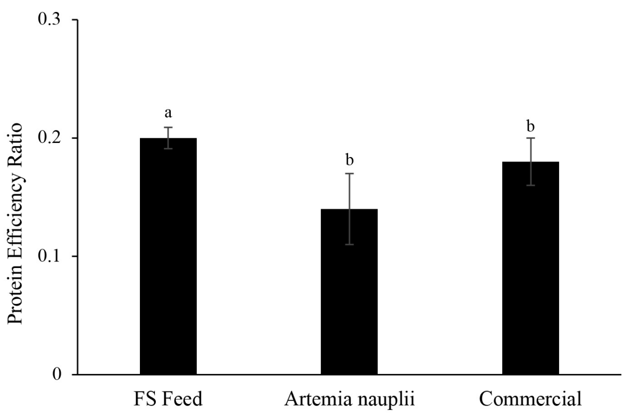

Figure 3: Protein efficiency ratios, PER of *C. gariepinus* larvae fed on three trial diets. Error bar show mean ± SE. Different lowercase letters (a-b) indicate statistically significant differences among trial diets ($p < 0.05$, ANOVA)

the feed lacks enough nutrient for the larvae. Larvae fed on exclusively commercial feed might present growth advantage due to some of the proteins that might be originated from cannibalism (Kestemont *et al.*, 2003; Al-Hafedh & Ali, 2004; Pereira *et al.*, 2017).

Conclusion

The experiment demonstrated that *C. gariepinus* larvae can accept artificial feed on their first feeding. This could greatly reduce the cost of fish larvae culture since artificial feed does not require any pre-treatment or preculture. It is also shows that giving artificial feeds to *C. gariepinus* larvae yielded similar growth performance and survival rate as live feed. Although the microbial analysis was not conducted in this experiment, we believe that giving an artificial diet could reduce the chances of cross-contamination either from bacteria or viruses, which could enhance survival among larvae.

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References

Abraham, T. J., Mallick, P. K., & Paul, P. (2018). African catfish Clarias gariepinus farming practices in North and South 24 Parganas districts of West Bengal, India. *Journal of Fisheries*, *6*(1), 579.

Akbary, P., Hosseini, S. A., Imanpoor, M., Sudagar, M., & Makhdomi, N. M. (2010). Comparison between live food and artificial diet on survival rate, growth & body chemical composition of *Oncorhynchus mykiss* larvae. *Iranian Journal of Fisheries Sciences*, *9*(1), 19 - 32.

Al-Hafedh, Y. S., & Ali, S. A. (2004). Effects of feeding on survival, cannibalism, growth & feed conversion of African catfish, *Clarias gariepinus* (Burchell) in concrete tanks. *Journal of Applied Ichthyology*, *20*(3), 225-227.

Alarcon, F. J., Moyano, F. J., Diaz, M., Diaz, C. F., & Yufera, M. (1999). Optimization of the protein fraction of microcapsules used in feeding of marine larvae using in vitro digestibility techniques. *Aquaculture Nutrition*, *5*, 107-113.

Alegbeleye, W. O., Obasa, S. O., Olude, O. O., Moronkeji, T., & Abdulraheem, I. (2012). Growth performance & nutrient utilization of African mud catfish (*Clarias gariepinus*) fingerlings fed different levels of fermented pigeon pea (*Cajanus cajan*) meal. *Israeli Journal of Aquaculture - Bamidgeh*, *64*, 1-8.

Andrés, M., Rotllant, G., Sastre, M., & Estévez, A. (2011). Replacement of live prey by formulated diets in larval rearing of spider crab *Maja brachydactyla*. *Aquaculture*, *313*(1-4), 50-56.

AOAC. Association of Official Analytical Chemist. (1990). *Official methods of analyses of association of analytical chemist* (15th eds.). Washington DC: AOAC.

Bell, J. G., McEvoy, L. A., Estevez, A., Shields, R. J., & Sargent, J. R. (2003). Optimising lipid nutrition in first-feeding flatfish larvae. *Aquaculture*, *227*(1-4), 211-220.

Borlongan, I. G., Marte, C. L., & Nocillado, J. N. (2000). Development of larval diets for milkfish (*Chanos chanos*). *Journal of Applied Ichthyology*, *16*(2), 68-72.

Cahu, C., Infante, J. Z., & Takeuchi, T. (2003). Nutritional components affecting skeletal development in fish larvae. *Aquaculture*, *227*(1-4), 245-258.

Cahu, C., & Zambonino Infante, J. (2001). Substitution of live food by formulated diets in marine fish larvae. *Aquaculture*, *200*(1-2), 161-180.

Chepkirui-Boit, V., Ngugi, C. C., Bowman, J., Oyoo-Okoth, E., Rasowo, J., Mugo-Bundi, J., & Cherop, L. (2011). Growth performance, survival, feed utilization and nutrient utilization of African catfish

(*Clarias gariepinus*) larvae co-fed Artemia & a micro-diet containing freshwater atyid shrimp (*Caridina nilotica*) during weaning. *Aquaculture Nutrition*, *17*(2), e82 - e89.

Citarasu, T., Babu, M. M., Raja, R., Sekar, J., & Peter Marian, M. (2002). Developing Artemia enriched herbal diet for producing quality larvae in *Penaeus monodon*, Fabricius. *Asian Fisheries Science*, *15*, 21-32.

Conceição, L. E. C., Dersjant-Li, Y., & Verreth, J. A. J. (1998). Cost of growth in larval & juvenile African catfish (*Clarias gariepinus*) in relation to growth rate, food intake & oxygen consumption. *Aquaculture*, *161*(1-4), 95-106.

Conceição, L. E., Yúfera, M., Makridis, P., Morais, S., & Dinis, M. T. (2010). Live feeds for early stages of fish rearing. *Aquaculture Research*, *41*(5), 613-640.

Craig, S., Kuhn, D., & Schwarz, M. (2017). Understanding fish nutrition, feeds & feeding Steven. *Virginia Cooperative Extention*, 1-6.

Curnow, J., King, J., Bosmans, J., & Kolkovski, S. (2006). The effect of reduced *Artemia* & rotifer use facilitated by a new microdiet in the rearing of barramundi *Lates calcarifer* (BLOCH) larvae. *Aquaculture*, *257*(1-4), 204-213.

Dhont, J., Dierckens, K., Støttrup, J., Van Stappen, G., Wille, M., & Sorgeloos, P. (2013). Rotifers, Artemia & copepods as live feeds for fish larvae in aquaculture. In *Advances in Aquaculture Hatchery Technology* (pp. 157-202). Elsevier Ltd.

Drossou, A., Ueberschär, B., Rosenthal, H., & Herzig, K. (2006). Ontogenetic development of the proteolytic digestion activities in larvae of *Oreochromis niloticus* fed with different diets. *Aquaculture*, *256*, 479-488.

Enyidi, U., & Nduh-Nduh, A. (2016). Application of phytogenics as first feed of larval African Catfish *Clarias gariepinus*. *Journal of Advances in Biology & Biotechnology*, *5*(3), 1-10.

Fernández-Díaz, C., & Yúfera, M. (1997). Detecting growth in gilthead seabream, *Sparus aurata* L., larvae fed microcapsules. *Aquaculture*, *153*(1-2), 93-102.

Gibson Gaylord, T., & Gatlin, D. M. (2001). Dietary protein & energy modifications to maximize compensatory growth of channel catfish (*Ictalurus punctatus*). *Aquaculture*, *194*(3), 337-348.

Giri, S. S., Sahoo, S. K., Sahu, B. B., Sahu, A. K., Mohanty, S. N., Mukhopadhyay, P. K., & Ayyappan, S. (2002). Larval survival & growth in *Wallago attu* (Bloch & Schneider): Effects of light, photoperiod and feeding regimes. *Aquaculture*, *213*(1-4), 151-161.

Hamre, K., Yúfera, M., Rønnestad, I., Boglione, C., Conceição, L. E. C., & Izquierdo, M. (2013). Fish larval nutrition & feed formulation: Knowledge gaps & bottlenecks for advances in larval rearing. *Reviews in Aquaculture*, *5* (Suppl 1). S26 - S58.

Huet, M., Timmermans, J. A., & Kahn, H. (1972). *Textbook of fish culture: Breeding & cultivation of fish*. London, UK: Fishing News Books.

Israel Adekunle, A., & Bola Joyce, A.-O. (2014). Effect of partial & total replacement of live feed (Artemia) with formulated diets in early stage growth of hybrid catfish (*Heterobranchus bidorsalis* x *Heterobranchus longifilis*) fry. *International Journal of Fisheries and Aquatic Studies*, *1*(3), 143-146.

Jonathan, B. Y., Maina, H. M., & Musa, Y. M. (2013). Fisheries and Aquatic Science. *Journal of Fisheries & Aquatic Science*, *8*(1), 287-290.

Kamarudin, M. S., Otoi, S., & Saad, C. R. (2011). Changes in growth, survival & digestive enzyme activities of asian redtail catfish, *Mystus nemurus*, larvae fed on different diets. *African Journal of Biotechnology*, *10*(21), 4484-4493.

Kemigabo, C., Jere, L. W., Sikawa, D., Masembe, C., Kang'ombe, J., & Abdel-Tawwab, M. (2019). Growth response of African catfish, *Clarias gariepinus* (B.), larvae & fingerlings fed protease-incorporated diets. *Journal of Applied Ichthyology*, *35*(2), 480-487.

Kestemont, P., Jourdan, S., Houbart, M., Mélard, C., Paspatis, M., Fontaine, P., Cuvier, A., Kentouri, M., & Baras, E. (2003). Size heterogeneity, cannibalism & competition in cultured predatory fish larvae: Biotic & abiotic influences. *Aquaculture*, *227*(1), 333-356.

Kim, K. D., Lim, S. G., Kang, Y. J., Kim, K. W., & Son, M. H. (2012). Effects of dietary protein & lipid levels on growth and body composition of juvenile far eastern catfish *Silurus asotus*. *Asian-Australasian Journal of Animal Sciences*, *25*(3), 369.

Kolkovski, S. (2001a). Digestive enzymes in fish larvae and juveniles - Implications & applications to formulated diets. *Aquaculture*, *200*(1-2), 181-201.

Kolkovski, S. (2001b). Digestive enzymes in fish larvae & juveniles - Implications & applications to formulated diets. *Aquaculture*, *200*(1-2), 181-201.

Kovalenko, E. E., D'Abramo, L. R., Ohs, C. L., & Buddington, R. K. (2002). A successful microbound diet for the larval culture of freshwater prawn *Macrobrachium rosenbergii*. *Aquaculture*, *210*(1-4), 385-395.

Koven, W., Kolkovski, S., Hadas, E., Gamsiz, K., & Tandler, A. (2001). Advances in the development of microdiets for gilthead seabream, *Sparus aurata*: A review. *Aquaculture*, *194*(1-2), 107-121.

Kujawa, R., Kucharczyk, D., Selezniow, G. F., Mamcarz, A., Ptaszkowski, M., & Biegaj, M. (2016). Substitution of natural food with artificial feed during rearing larvae of Sichel Pelecus cultratus (L.) under controlled conditions. *Turkish Journal of Fisheries & Aquatic Sciences*, *650*, 643-650.

Li, J. Y., Li, X. F., Xu, W. N., Zhang, C. N., & Liu, W. B. (2016). Effects of dietary choline supplementation on growth performance, lipid deposition & intestinal enzyme activities of blunt snout bream *Megalobrama amblycephal* fed high-lipid diet. *Aquaculture Nutrition*, *22*(1), 181-190.

Montchowui, E., Lalèyè, P., N'tcha, E., Philippart, J. C., & Poncin, P. (2012). Larval rearing of African carp, *Labeo parvus*Boulenger, 1902 (Pisces: Cyprinidae), using live food & artificial diet under controlled conditions. *Aquaculture Research*, *43*(8), 1243-1250.

Najdegerami, E. H., Baruah, K., Shiri, A., Rekecki, A., Van den Broeck, W., Sorgeloos, P., Boon, N., Bossier, P., & De Schryver, P. (2015). Siberian sturgeon (*Acipenser baerii*) larvae fed *Artemia* nauplii enriched with poly-β-hydroxybutyrate (PHB): Effect on growth performance, body composition, digestive enzymes, gut microbial community, gut histology & stress tests. *Aquaculture Research*, *46*(4), 801-812.

Nik Sin, N. N., & Shapawi, R. (2017). Innovative egg custard formulation reduced rearing period & improved survival of Giant Freshwater Prawn, *Macrobrachium rosenbergii*, larvae. *Journal of the World Aquaculture Society*, *48*(5), 751-759.

People Le Ruyet, J., Alexandre, J. C., Thébaud, L., & Mugnier, C. (1993). Marine fish larvae feeding: Formulated diets or live prey? *Journal of the World Aquaculture Society*, *24*(2), 211-224.

Pereira, L. S., Agostinho, A. A., & Winemiller, K. O. (2017). Revisiting cannibalism in fishes. *Reviews in Fish Biology and Fisheries*, *27*(3), 499-513.

Portella, M. C., & Dabrowski, K. (2008). Diets, physiology, biochemistry & digestive tract development of freshwater fish larvae. *Feeding and digestive functions of fishes*. 227-279. Enfield: Science Publishers.

Rønnestad, I., Yúfera, M., Ueberschär, B., Ribeiro, L., Sæle, Ø., & Boglione, C. (2013). Feeding behaviour & digestive physiology in larval fish: Current knowledge & gaps & bottlenecks in research. *Reviews in Aquaculture*, *5*(1), S59-S98.

Ruyet, J. P.-L., Menu, B., Cadena-Roa, M., & Métailler, R. (2009). Use of expanded pellets supplemented with attractive chemical substances for the weaning of turbot (*Scophthalmus maximus*). *Journal of the World Mariculture Society*, *14*(1-4), 676-678.

Sarkar, U. K., Lakra, W. S., Deepak, P. K., Negi, R. S., Paul, S. K., & Srivastava, A. (2006). Performance of different types of diets on experimental larval rearing of endangered *Chitala chitala* (Hamilton) in recirculatory system. *Aquaculture*, *261*, 141-150.

Sorgeloos, P., Dhert, P., & Candreva, P. (2001). Use of the brine shrimp, *Artemia* spp., in marine fish larviculture. *Aquaculture*, *200*(1-2), 147-159.

Srichanun, M., Tantikitti, C., Vatanakul, V., & Musikarune, P. (2012). Digestive enzyme activity during ontogenetic development & effect of live feed in green catfish larvae (*Mystus nemurus* Cuv. & Val.). *Songklanakarin Journal of Science & Technology*, *34*(3), 247-254.

Tocher, D. R. (2003). Metabolism & functions of lipids & fatty acids in teleost fish. *Reviews in Fisheries Science*, *11*(2), 107-184.

Tocher, D. R., Bendiksen, E. Å., Campbell, P. J., & Bell, J. G. (2008). The role of phospholipids in nutrition & metabolism of teleost fish. *Aquaculture*, *280*(1-4), 21-34.

Vandecan, M., Diallo, A., & Mélard, C. (2011). Effect of feeding regimes on growth & survival of *Clarias gariepinus* larvae: Replacement of Artemia by a commercial feed. *Aquaculture Research*, *42*(5), 733-736.

Verreth, J. A. J., Torreele, E., Spazier, E., Sluiszen, A. Van Der, Rambout, J. H. W. M., Booms, R., & Helmut, S. (1992). The development of a functional digestive system in the African catfish *Clarias gariepinus* (Burchell). *Journal of World Aquaculture Society*, *23*(4), 286-298.

Wang, C., Xie, S., Zheng, K., Zhu, X., Lei, W., Yang, Y., & Liu, J. (2005). Effects of live food & formulated diets on survival, growth and protein content of first-feeding larvae of *Plelteobagrus fulvidraco*. *Journal of Applied Ichthyology*, *21*(3), 210-214.

White, P. (2013). Environmental consequences of poor feed quality & feed management. *FAO Fisheries & Aquaculture Technical Paper*, *583*, 553-564.

Zheng, K., Zhu, X., Han, D., Yang, Y., Lei, W., & Xie, S. (2010). Effects of dietary lipid levels on growth, survival & lipid metabolism during early ontogeny of *Pelteobagrus vachelli* larvae. *Aquaculture*, *299*(1-4), 121-127.