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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

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ABSTRACT
Fish larval nutritional requirements a major concern for the expansion of larviculture of many fish species. Live foods such as rotifer, daphnia and artemia are mostly used in the hatchery production of fish hatchlings. This experiment was carried out to determine whether live feed(Artemia) and formulated imported feed (Coppens) or the combination of the two feeds at different proportions will efficiently enhanced the growth and survivability of hybrid (*H. bidorsalis* x *H. longifilis)* hatchlings. After endogenous feeding, average of 70-80 hatchlings initial mean weight (0.12±0.00) g were counted in each experimental tank (70 cm x 45cm x 40 cm) replicate twice to give five treatments in a complete randomized design. Each diet was fed adlibitum for 56 days. The result shows that live food (D_1) (100% Artemia) was best utilized for growth and feed utilization by the Hybrid (*H. bidorsalis* x *H. longifilis)* catfish fry. Fry fed imported formulated diet (D_2) (100% Coppens) shows significantly ($p<0.05$) lowest growth rate and survivability compared to fry fed D_3-D_5 (different ratios of live and formulated diets). The best growth performance recorded in fry fed live feed might be as a result of increased in food intake coupled with high nutrient digestibility.

Keywords: Live Feed, Formulated Diets, Hybrid, Artemia, Hatchlings and Fry.

1. Introduction

In the 1970s, the production of farmed fish relied almost exclusively on the capture of wild fry for subsequent stockings in ponds and tanks. The complete domestication of much freshwater aquaculture species was only achieved during the last two decades. However, since then, the hatchery production of fry has become a routine operation for most cultivated fish species [10].
The cultivation of fish larvae under controlled hatchery conditions requires specific culture techniques since they are usually small, extremely fragile, and are generally not physiologically fully developed. Their small mouth size, the uncompleted development of their perception organs (eyes, chemoreceptor) and digestive system are limiting factors in proper feed selection during the early first-feeding or start feeding period [8].
There is dearth of information on nutrition in early stage growth of hybrid catfish (*H.bidorsalis x H. longifilis*). Sub optimal nourishment of the larvae due to inadequate diets is a major cause of poor survival. Nutrition generally encompasses the ingestion, digestion and absorption of nutrients. It involves the transportation of these chemical elements for assimilation and use by the body cells [12]. Sound nutrition promotes healthy larval development [6]. In fish larvae, the yolk provides nutrition during embryonic development and early Ontogenesis [1]. The transition from endogenous to exogenous food supply at first feeding marks a critical phase during which high mortality may occur. Unfortunately, larval diets are difficult to evaluate and current aquaculture practices are not always optimal for larval rearing [12].
Live feeds are feeds which consist of living organism that can be active or inactive. They are palatable to fish and allows for better digestibility [9]. Formulated diets are feeds which are combined together from different nutrient sources so as to be richer in nutrients. Thus, larvae can thrive well on compound diets if well adapted. The importance of using combined live feed with formulated diets lies mainly in supplying a more suitable and well balanced diet to fish larvae in a digestive form. It appears that this aspect of larviculture is not been seriously tackled. Ironically, this aspect holds the key to sustainability and growth of the industry. Since fish seeds

are the raw materials of fish farming, its non-availability or insufficient supply may lead to stagnancy or death of the industry.

Since the hybrid between *Heterobranchus bidorsalis* and *Heterobranchus longifilis* (*H. bidorsalis* x *H. longifilis)* is yet to be fully cultured, it is imperative to determine the best feeds for its early stage growth, especially at the expiration of endogenous feeding. This study therefore examined whether formulated diet or live diet may efficiently enhanced larva growth and survivability of Hybrid (*H. bidorsalis* x *H. longifilis*), in comparison with larva fed on both diets at different proportions.

2. Materials and Methods
2.1. Experimental diets
Two (02) kilograms of imported formulated dry feed (Coppens) size 0.3-0.5 mm and two tins of shell-free Artemia were purchased from Success Fish Breeding and Poultry Farms Nig. Ltd. Akure, Ondo State, Nigeria for the experiment. Both the Artemia and Coppens were in solid forms which were factory packed and served as live and formulated diets respectively. The feeds were divided into five treatments (D_1-D_5) as indicated below. Feeds were packed in dry, airtight small containers (labeled) prior to use.

Treatment$_1$ (D_1) = 100% Artemia
Treatment$_2$ (D_2) = 100% Coppens
Treatment$_3$ (D_3) = 50% Artemia and 50% Coppens
Treatment$_4$ (D_4) = 60% Artemia and 40% Coppens
Treatment$_5$ (D_5) = 40%Artemia and 60% Coppens

2.2. Experimental fish
2.2.1. Hormone induction of broodstock and experimental set up
A hatchery raised gravid *Heterobranchus longifilis* broodstock weighed 2.5 kg and male *Heterobranchus bidorsalis* weighed 3.2 kg were procured from Success Fish Breeding and Poultry Farms Nig. Ltd. Akure Ondo State Nigeria, where the experiment was carried out. Prior to injection, the broodstocks were kept singly in aerated fibre tanks with 200 litres of water each. Broodstocks were re- weighed with weighing balance in order to determine the amount of hormone to be used. The milliliter (ml) of Ovaprim hormone injected to the female broodstock was calculated based on 0.5 ml/kg of fish. The injection was done intramuscularly and fish was returned into the tank. The male brood fish was not injected.

Stripping of eggs took place after the completion of latency period of 12 hours at 27 °C, while the male fish was sacrificed and milt prepared for fertilization of the stripped eggs. Using cleaned normal saline water of 0.9% cl, eggs were carefully stirred to complete the fertilization.

The experiment was conducted in 10 plastic tanks (70 cm x 45 cm x 40 cm) size each in two replicates for the five treatments. Average of 100 eggs were spread evenly on kakaban in each experimental tank and kept in controlled temperature. Hatchability commenced in 24 hrs and completed in all the tanks after 27 hrs. Kakaban were carefully removed with the unhatched eggs. And fry were left to feed endogenously for72 hours. At the expiration of endogenous feeding, healthy and strong hatchlings (average of 70-80 hatchlings) were left in each tank filled to 70% capacity with fresh water in a flow through system. The fish were fed alibitum every two hour for a period of 56 days.

2.2.2. Measuring of physico-chemical parameters
Physico-chemical parameters such as temperature, pH, and DO concentrations in each tank were monitored daily. Nitrate was monitored weekly during the experiment. Temperature was measured using a mercury glass thermometer, while pH was measured with a pH meter. DO was measured using dissolve Oxygen meter.

2.2.3. Proximate analysis
Proximate analyses (crude protein, lipid, fiber and ash composition) of Artemia, dry formulated imported feed and the combination of the two at different proportions were determined using [7] methods. Nitrogen Free Extract (NFE) was computed by taking the sum values for crude protein, crude lipid, crude fiber, total ash and subtracting this from 100

2.2.4. Growth parameters
Mean Weight Gain (MWG), Specific Growth Rate (SGR), Protein Efficiency Ratio (PER), Feed Conversion Ratio (FCR) and Protein Intake (PI) were calculated at the expiration of the experiment using the following equations:

Mean Weight Gain (MWG) = Mean final body weight – mean initial body weight

$$\text{Specific Growth Rate (SGR) per day} = \frac{\text{In}W_t - \text{In}W_i}{T} \times 100$$

Where T is the culture period in days

W_t and W_i were the mean final and initial weights (g) respectively.

$$\text{Feed Conversion Ratio (FGR)} = \frac{\text{weight of dry feed (g)}}{\text{Weight gain of fish (g)}}$$

Feed Intake (FI) = 5% body weight of fish per day.

Protein Intake (PI) = Feed Intake (FI) x % protein in diet

$$\text{Protein Efficiency Ratio (PER)} = \frac{\text{Gain in weight of fish (g)}}{\text{Protein Intake (PI) (g)}}$$

2.2.5. Statistical analysis
Data collected were analyzed using Statistical Package for Social Sciences (SPSS), Version 11, 2001 to determine significance between mean values. Duncan's multiple range test [5] was used to compare differences among means. Significant level was chosen at $p<0.05$.

3. Results
3.1. Proximate composition of experimental feeds
The proximate composition of experimental diets is presented in table 1. Diet (D_2) had the highest crude protein (56%), crude fat (15%), fibre (10%) and least in Ash content (0.40%).While diet (D_1) was least (6%) and highest (27%) in crude fibre and Nitrogen Free Extract respectively. The mixture of Artemia with Coppens (D_3 - D_5**)** at different proportions had intermediate values for the parameters analyzed.

Table 1: Proximate composition of experimental diets

Parameters (%)	Diets				
	(D_1) Artemia 100% (1kg)	(D_2) Coppens 100% 1kg)	(D_3) Artemia/Coppens 50% :50% (0.5kg : 0.5kg)	(D_4) Artemia/Coppens 60% : 40% (0.6kg : 0.4kg)	(D_5) Artemia/Coppens 40% : 60% (0.4kg :0.6kg)
Crude Protein	54.00	56.00	55.00	54.80	55.20
Crude fat	9.00	15.00	12.00	11.40	12.60
Ash	4.00	0.40	2.20	2.56	1.84
Fibre	6.00	10.90	8.45	7.96	8.94
NFE	27.00	17.70	22.35	23.28	21.42

3.2. Growth and survivability of experimental fish

The result of fish growth and survivability in the present study is presented in Table 2 below. Fry fed diet (D_1) had the highest Mean Weight Gain (MWG) value of 4.21±0.03 g, followed by fish fed diet (**D_4)** with a mean value of 4.01±0.06 g. The least growth performance was recorded in treatment two (D_2) with a mean value of 3.31±0.01 g. Fry survivability was significantly ($P<0.05$) highest (83.50±0.04)% in fish fed (D_1), while it was least (62.33±0.04) in treatment two (D_2).

Table 2: Survivability and growth performance of Hybrid (*H. bidorsalis* x *H. longifilis*
catfish fry fed with experimental diets for 56 days

	Experimental Fish				
Parameters	**D_1**	**D_2**	**D_3**	**D_4**	**D_5**
Initial Mean Weight (g)	$0.12^a \pm 0.00$	$0.10^a \pm 0.00$	$0.12^a \pm 0.00$	$0.11^a \pm 0.00$	$0.12^a \pm 0.00$
Final Mean weight (g)	$4.33^a \pm 0.021$	$3.41^d \pm 0.014$	$3.94^b \pm 0.03$	$4.12^b \pm 0.035$	$3.61^c \pm 0.07$
Mean weight gain (g)	$4.21^a \pm 0.03$	$3.31^c \pm 0.01$	$3..82^b \pm 0.01$	$4.01^b \pm 0.06$	$3.49^c \pm 0.06$
Specific growth rate (SGR)	$3.11^a \pm 0.02$	$3.00^b \pm 0.02$	$3.02^b \pm 0.03$	$3.05^b \pm 0.07$	$3.00^b \pm 0.03$
Protein intake (g)	$3.53^d \pm 0.03$	$2.43^a \pm 0.01$	$3.12^c \pm 0.02$	$3.38^d \pm 0.03$	$2.62^b \pm 0.01$
Feed conversion ratio (FCR)	$1.50^d \pm 0.01$	$1.97^a \pm 0.01$	$1.86^b \pm 0.01$	$1.65^c \pm 0.01$	$1.86^b \pm 0.01$
Mean Survival rate (%)	$83.50^a \pm 0.04$	62.33 ± 0.04	80.24 ± 0.04	75.46 ± 0.04	70.10 ± 0.04

Values in rows having the same letter are not significantly different ($p>0.05$)

3.3. Water Quality Analysis

The result of water quality analysis used for the experiment is presented in Table 3.Dissolve oxygen (DO) ranged between 6.37– 6.55 mg^{-l}; PH ranged between 6.84 -7.05 and temperature between 27.30 - 27.60. The nitrate values ranged between 0.20 - 0.22 mg/L in all the treatments throughout the rearing period. The values of water parameters measured were not significantly different ($p=0.05$) in all the treatments.

Table 3: Mean Values of water quality parameters in experimental tanks for Hybrid catfish (H. bidosalis x *H. longifilis*)

Treatments					
Parameters	**D_1**	**D_2**	**D_3**	**D_4**	D_5
DO (mg/L)	6.55± 0.01	6.43± 0.01	6.37 ± 0.02	6.42 ± 0.02	6.51 ± 0.02
PH	6.99 ± 0.01	6.86 ± 0.02	6.84 ± 0.02	6.86 ± 0.01	7.05 ± 0.03
Temp (^{0}C)	27.50± 0.10	27.60± 0.12	27. 50± 0.10	27.50± 0.10	27.30± 0.11
NO_3 (mg/L)	0.21 ± 0.002	0.22 ± 0.002	0.21 ± 0.001	0.21 ± 0.003	0.21 ± 0.001

3.4. Proximate Composition of Experimental Fish (*H. bidorsalis* x *H. longifilis*)

The result of the proximate analysis of experimental fish after the experiment is presented in Table 4 below. Due to its initial small size, proximate composition of the newly hatched fish could not be ascertained before the experiment. After the experiment, the highest (68.76%) crude protein was in fish fed D_1**,** followed by D_4 (66.51%) and least in D_2 (60.98%).Also the ash content in fish was highest inD_1**(**16.17%) and least in fish fed D_2**(**12.86%. The same trend was observed in Crude fat level in fish, but the Nitrogen Free Extract was highest (21.95%) in fish fed $D_2$and least in fish fed D_1 (9.65%).

Table 4: Proximate Composition of the experimental fish (*H. bidorsalis* x H. longifilis) after the experiment

	Treatments				
Parameters (%)	D_1	D_2	D_3	D_4	D_5
Ash	16.17	12.86	14.02	15.85	12.95
Crude Protein	68.76	60.98	64.32	66.51	61.10
Crude Fat	5.42	4.21	4.80	5.26	4.20
Crude Fibre	Nd	Nd	Nd	Nd	Nd
NFE	9.65	21.95	16.86	12.38	21.75

Nd = Not detected

4. Discussion

A major problem in intensive larviculture of freshwater fish is inadequate food quality which leads to mortality, reduced growth and deformities. In the present study, fry fed purely on dry formulated diet recorded the lowest growth performance and survivability. While the best performance was exhibited by fry fed purely on artemia (live food). A week after the commencement of the experiment, some behavioral observations were noticed in the experimental tanks. Fry fed only formulated diet were visually smaller, less active and remained dispersed at the bottom of the aquaria in comparison with those fed live diet. It was also observed that live food seemed to be more attractable to the Larvae than other diets. Larvae fed the combination of the diets (live and formulated) at different proportions had an intermediate behavior, swimming either near the bottom or the walls. The general observation was that larvae seemed to wean from live food to formulated diet gradually and spontaneously during later stage of the experiment.

The above observations were in line with report made by [4] that source of food play an important role in delivering nutrients to fish larvae, which invariably influences their behavioral tendencies and survivability. Larviculture of Hybrid catfish (*H. bidorsalis x H. longifilis)*, as reported in this study improved markedly when live diet was provided [3] reported that co-feeding strategy in some aquaculture species may improve growth and survival rate in early stage growth. But small larval size coupled with difficulties in acceptance of formulated feed slows down successful larviculture of many promising aquaculture species. Diet selection by fish larvae has been studied [11, 9]. Feeding only formulated diet may result in both physiological and morphological immaturity as observed in this study. In addition, the result of the growth indices indicated that larvae fed live diet had increase food intake, nutrient digestibility and assimilation.

Development of suitable formulated larval diets can also alleviate immediate problem of transition from endogenous to exogenous feeding in larviculture [2]. The process produces particles which are readily accepted and contain high amounts of protein and fat. The balance of nutrients should support growth of the small larvae since adequate digestible protein and fat for energy are available in the preparation. The advantages of this type of diet formulation according to[9], are its versatility for utilization which can be adjusted to give a desired formulation and the flexibility with which diet particles are prepared with good water stability which present a soft flesh-like gel to the fish larvae.It has often been considered that formulated / dry feeds fed are inadequate to nourish small fry during the first stages of feeding and that such diet could be used only successfully after the fish had been fed on live food for some time . Though formulated diet may be consumed efficiently by the Hybrid (*H. bidorsalis* x *H. longifilis)* fry at the start of exogenous feeding, it might be suitable and even more adequate than live diet in a later hatchery stage

5. Conclusion

Live food plays an important role in delivering nutrients to freshwater fish larvae.In most cases, live feeds have to be cultured, therefore under hatchery management enough provision should be made leading to increase fingerling production. For the early stage growth of the Hybrid catfish (*H. bidorsalis* x *H. longifilis)* fry, feeding live organisms for a start before introducing formulated diet has proved to increase digestive activity as reflected in growth performance and survivability. However species- specific requirements for live feed are lacking. Therefore, it is hereby suggested, especially for a specific new fish species to have a product of known nutritional profile.

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