RESEARCH PAPER



Can Powdered Microencapsulated Whole Egg, Ulva lactuca and Grasshopper Improve Growth Performance and Economic Viability of African Catfish (*Clarias gariepinus*) Fry?

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Abstract

Clarias gariepinus fry requires feed that is rich in protein and essential fatty acid, but protein-rich feed is quite expensive especially when derived from animal origin. The present study aims to find less expensive feed for fry affordable for C. gariepinus farmers. About 3600 fries (Weight =1.49±0.03 mg) produced artificially, were stocked in twenty-four plastic containers (measuring 30×35×24cm). The containers were divided into eight treatments (T₀ to T₇) in triplicate. Seven different powdered feeds F₁ to F7 (prepared from Ulva lactuca, Grasshopper and chicken egg) were respectively fed to the fry in the seven treatments T_1 to T_7 against Artemia Shell free as a control (T_0) for two weeks. The results obtained revealed significant differences in terms of growth and economic response among the experimental treatments (P<0.05). The finding shows reduction in feeding costs from 54.90% in control to 19.98% and 19.90% while maintaining high growth performance and net profit in T₆ (1:1 mixture of grasshopper and microencapsulated whole egg) and T₅ (1:1 mixture of U. lactuca and Microencapsulated whole egg) respectively. Thus, for affordable feed and best profit, more should be explored on the benefit of using ingredients in T₅ and T₆ as possible feed for C. gariepinus.

Introduction

The main objective of any hatchery is to produce sufficient numbers of high-quality seeds, ranging from fry and fingerlings from available broodstocks (Marimuthu and Hanifa, 2009). One of the most important parts in fish hatchery is fry rearing or care, which involves the provision of the right quality feed at the right time. Failure to do that will result in high fry mortality, as the fry will have no food reserves of their own once the yolk sac has been absorbed (Gietma, 2003). Like many fish, *C. gariepinus* fry requires live feed and protein-rich diets like *Artemia* at the onset of exogenous feeding (Rumesy, 1980; Ovie and Ovie, 2002; Ibrahim, *et al.*, 2008). However, preparation of live feed is associated with harmful microorganisms while the protein-rich feed is quite expensive, especially if the protein is of animal origin (Rumesy 1980). *Artemia naupuli* is reported to be deficient in some essential fatty acid (Olurin *et al.*, 2014) and lack of essential fatty acids reduces growth and nutrient utilization of fish (Parker, 2012). Despite several studies to replace *Artemia* with different dry powdered feed, there still exists a high cost. Therefore, there is a need to investigate the alternative feed source for the progress of *C. gariepinus* production that will be produced at very low price, affordable for small-scale farmers.

Microencapsulated Whole egg is cheap and contains balance nutrients needed during the first ten days of life of most species of fish. However, watersoluble vitamins (especially ascorbic acid) and calcium are not sufficient in the "whole egg" and therefore needs to be supplemented (Chow, 1980). Also, microencapsulated whole eggs appear in the form of liquid that needs to be prepared every day or stored in the refrigerator to avoid deterioration. Hence, developing the dry powdered microencapsulated whole egg, together with some ingredients like *Ulva lactuca* and grasshopper, that are rich in vitamins and minerals to supplement their absence in the "microencapsulated whole egg" is necessary.

Ulva lactuca consist of Protein (9.91 - 27.9%), Lipid (0.11-15.75%), Ash (0.11-39.1%), rich vitamins (e.g. Ascorbic acid) and minerals (e.g. Calcium, Iron) (Ortiz *et al.*, 2006; Garci-Casel *et al.*, 2007; Ergun, 2008; El Tawil, 2010; Mustafa and Saeed, 2014). *U. lactuca* has all the essential amino acids which constitute 5% out of the 12% of the total amino acids (Kumar and Khaldar, 2007) and essential fatty acid (Rohani-Ghadikolaei *et al.*, 2012). Grasshopper is rich in nutrients (Blasques *et al.*, 2012) especially minerals, fat (Sani, *et al.*, 2014) with abundant essential fatty acid (Adeyeye, 2011), and essential amino acid of 351 mg/g crude protein (Adeyeye, 2005). Grasshopper could partially or completely be a substitute for conventional protein source in animal feed (Ojewola and Udom, 2005).

Material and Method

Study Site and Source of Fish Seed

The experiment was conducted at the Aquaculture unit of Marine Science Station, located in the south beach Gulf of Aqaba. Fry of *C. gariepinus* was produced artificially by inducing the fish (Female = Wt 0.7 kg, Male = 1 Kg) through hormone treatment followed by artificial fertilization and incubation of fertilized eggs (De Graaf and Janssen, 1996). Approximately 56 gm of eggs (equivalent to 41,941 egg pieces by considering 1 gm of eggs to contain 750 eggs) were obtained from female brooder.

Ingredients Collection and Preparation

Roasted grasshoppers were purchased from the local market of Nigeria. The Grasshoppers were ground

The table eggs were purchased and "Microencapsulated whole egg" was prepared by beating the egg until the yolk and the white were homogenized. Boiled water (150 ml/ egg) was poured over the eggs while stirring constantly (Chow 1980). The powdered microencapsulated whole egg was produced through freezed-drying followed by gentle oven drying at 40°C for two days. Dried Microencapsulated whole egg was then ground and sieved to pass through 300 μ m mesh thereby collecting the powdered egg.

Macroalgae (*Ulva lactuca*) were collected from the intertidal zone in front of the Marine Science Station Gulf of Aqaba. The collected *Ulva lactuca* were washed with freshwater to remove the salts and associated matters then dried at room temperature. Dried *Ulva lactuca* was ground and passed through 300 μ m mesh in powdered form (Figure 1).

Proximate Analysis of the Tested Ingredient

Proximate compositions of the experimental ingredients were analyzed following the standard method of the Association of the Official Analytical Chemist (AOAC 1990) (Table 3).

Experimental Design

About 3600 fries of 4 days old of almost equal size (weight and length of 1.49 mg and 6.56 mm, respectively) were stocked in 24 plastic baskets (measuring 35 cm in diameter and 24 cm height wrapped with fine cloth mesh). Baskets were divided into eight experimental treatments: T1, T2, T3, T4, T5, T6, T₇ and T₀ each in triplicate and suspended on eight tanks with constantan circulation of water (Fig.2). Seven different powdered feed (namely: F1 = Ulva lactuca; F2 = Grasshopper; F₃="Micro-encapsulating" whole egg; F₄ = $F_1 + F_2$; $F_5 = F_1 + F_3$; $F_6 = F_2 + F_3$; and $F_7 = F_1 + F_2 + F_3$) were prepared and fed the fry to the satiation in the respective experimental treatments against T₀ fed with Artemia (0.5 mm) as control. The mixed feed was prepared in an equal proportion of 1:1. The stage of



a. = *Ulva Lactuca*, b. = Grasshopper, c. Microencapsulated Whole Egg Figure 1. Powdered ingredients.

feeding the *C. gariepinus* fry with *artemia* in an intensive system lasts for 14 days (FAO 2015), therefore, this feeding trial lasts for a period of two weeks.

Monitoring Growth (Weight and Length) of Experimental Fish

The Growth of all experimental fry was monitored on a weekly basis. Weighing of fry was conducted using digital weighing balance while the total length of the fish was measured using a graph paper attached to Petri dish.

Calculation and Statistical Analysis of the Data Economic Analysis

The cost of the tested ingredients and materials used in hatching the fry during the present research were the only considered source for investment (Table 1&2). Sale Price of fry was obtained by converting the sale price of fingerlings (Size/price = 0.2\$/5000 mg FAO, 2015) in relation to the sizes of fry in each treatment. The formulae for economic evaluations were implemented as follows:

Cost of feed consumed by the fry(\$) = Feed consumed by fry × Total Cost of feed prepared Total quantity of feed *prepared*

Cost of hatching a fry (\$/Egg) = Total Cost of hatching materials (\$) Total amount of eggs obtained after stripping female

Investment cost (\$) = Cost of feed consumed by fry + cost of hatching a fry

Sale price of Fry (\$) = $\frac{0.23 \times \text{Final weight of fry after feeding trial(mg)}}{5000 \text{ mg}}$

 $Profit index = \frac{Sale Price of fry after feeding trial($)}{Investment cost ($)}$

Net profit = sales price of fry - Investment cost

The Equations Used in Data Analysis

The following equations were applied to the data obtained after feeding trial:

Weight gain by the fry (mg) = Average final weight of fry – Average initial weight of fry.

Length gain by fry (mm) = Average final length of fry – Average initial length of fry.

Specific growth rate (%/day) = $\frac{\log Wf - \log Wi}{T1 - T2} \times 100$

Feed conversion ratio = $\frac{\text{Total feed intake (mg)}}{\text{Total weight gain (mg)}}$

 $\begin{array}{l} \mbox{Relative growth rate (\%) = } \frac{Wf\mbox{-}Wi}{Wi} \times 100 \\ \mbox{Feeding efficiency (\%) = } \frac{Total weight gain (mg)}{Total feed intake (mg)} \times 100 \end{array}$

Where W_f = Final body weight of fish at time T2 (day), Wi = initial body weight of fish at time T1 (day).

The results were analyzed using one-way analysis of variance with the aid of sigma stat software Version 3.5. The Fisher LSD test was used to further compare the mean differences.



Figure 2. Section view of experimental design.

Results

In this study, it was found that Powdered microencapsulated whole egg, grasshopper and *U. lactuca* interacted in a variety of ways to produce viably economic diets for *C. gariepinus* fry production. The powdered diets produced were from single or mixture of ingredients with either low-nutrient-low-cost, low-nutrients-high-cost, high-nutrients-low-cost or high-nutrient-high-cost (Table 2&3). A summary of variation in growth performance and economic viability of the early stages of *C. gareipinus* fry were presented in Table 4. The initial mean weight and length of the experimental fry were not significantly different (P>0.05).

Growth Performance of the Fry

During feeding trial with Shell free Artemia as a control, there were significant differences in terms of growth performance among the treatments (P<0.05, see Table 4). Growth performance of fry (weight gain = 63.41 ± 1.3) in T₆ (1:1 mixture of powdered grasshopper and Microencapsulated whole egg) is higher than fry in the control (T₀ = Artemia shell-free, weight gain =

49.61 \pm 1.0). However, the growth performance of fry in control is higher than the fry of T₅, T₄, T₃, T₂, and T₁.

Economic Response of the Fry

Investment cost was significantly higher in control T_0 (187.5±1.3 × 10⁻⁵\$) than all of the experimental treatments (P<0.05). T₆ recorded high sale price (260 ± 5.44×10^{-5} \$), followed by control (204 ± 4.25 × 10^{-5} \$) and T₅ (165 \pm 3.6 \times 10⁻⁵\$) while the low price was recorded in the remaining treatments (P<0.05). Net profit in T_6 (154.4±5.2 × 10⁻⁵\$) and T_5 (59.5 ± 3.4 × 10⁻⁵\$) were higher than control (16.5 \pm 5.6 \times 10⁻⁵\$). However, Net profit in control not differ significantly with T₂ (16.9 $\pm 2.5 \times 10^{-5}$ \$) and T₄ (26.6 $\pm 2.7 \times 10^{-5}$ \$). Failure in profit was observed in T_1 (-14.3 ± 2.5 × 10⁻⁵\$) and T_7 (-12.7 ± 1.9×10^{-5} \$) while low profit in T₃ (5.3 ± 3.5 × 10^{-5} \$) (P<0.05) (see Fig. 3 & Table 4). The best profit index observed in T_6 (2.5 ± 0.05) and T_5 (1.6 ± 0.03) were the indication of prospect in using mixture of powdered feed of these treatments. Though no profit loss was observed in T₃, T₄, and T₂, their profit index values indicated that the powdered ingredients of these treatments are not feasible as an alternative feed for C. gariepinus fry.

Materials for Hatching	Quantity	Cost(\$)		
Broodstock	2	31.25		
Hormone	1ml	2.81		
Syringe	1(10ml)	0.13		
Net	1 yard	1.25		
Total cost of hatching materials		35.44		

Table 2. Quantity and cost of feed prepared in each treatment

Treatments	Quantity(mg)	Cost(\$)		
Artemia (T₀)	500000	28.13		
Ulva lactuca (T ₁)	50000	0.38		
Grasshopper (T ₂)	50000	0.31		
Table egg (T ₃)	50000	0.68		
$T_4 (T_1 + T_2)$	100000	0.69		
$T_5 (T_1 + T_3)$	100000	1.06		
$T_6 (T_2 + T_3)$	100000	0.99		
$T_7 (T_1 + T_2 + T_3)$	150000	1.37		

Table 3. Proximate composition of the experimental ingredients

Parameters (%)	Grasshopper	Microencapsulating whole egg	U. lactuca	Artemia shell-free	
Crude protein	13.5	16.4	1.4	54	
Crude lipid	10.5	39.4	3.3	9	
Ash	8.4	9	10.9	4	
Moisture	5.9	4.5	9.6	5	
Fibre				6	

Discussion

The differences in nutritional compositions and the cost of ingredients have affected the growth performance and economic responses of the fry in all the experimental treatments. As suggested by Uys (1985), *C. gariepinus* fry requires protein of around 55%, lipid of 9% and carbohydrate of 21%. Whether high or low nutrients, the success of fish growth depends largely on the level and quality of the nutrient of the ingredients used (Parker, 2012). The growth of early-stage *C. gariepinus* depends on the essential amino acids, fatty acids of the omega- 3 series (Bell *et al*, 1986; FAO, 2012), minerals (as calcium, iron) and vitamins especially ascorbic acid (FAO, 2012). However, level of these important nutrients above or below the optimum

requirements may cause poor growth performance hence affecting the economic response of the fry.

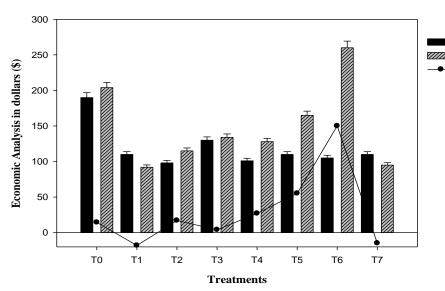
During this study, low protein and lipid contents to meet the requirement of *C. gariepinus* fry were associated with poor growth performance observed in T_1 (*Ulva lactuca*). While the excess of minerals and lipids is probably associated with poor growth in T_7 (1:1:1 Mixture of Grasshopper, *U. lactuca* and microencapsulated whole egg). This cause the observed profit lost in T_1 and T_7 .

Despite low growth performance, improvements were observed in T₃ (Microencapsulated whole eggs), T₄ (1:1 mixture of *Ulva lactuca* and Grasshopper) and T₂ (Grasshopper). This is because microencapsulated whole eggs and Grasshopper are rich in lipid which probably mates lipid requirement for *C. gariepinus* fry

Table 4. Growth performance and economic responses of the fry in different treatments

Parameters	To	T ₁	T ₂	T ₃	T4	T ₅	T ₆	T 7
Initial weight (mg)	1.49±0.03ª	1.49±0.03 ^a	1.49±0.03ª	1.49±0.03ª	1.49±0.03ª	1.49±0.03ª	1.49±0.03ª	1.49±0.03 ^a
Initial length (mm)	6.56±0.26ª	6.56±0.26 ^a	6.56±0.26ª	6.56±0.26ª	6.56±0.26ª	6.56±0.26ª	6.56±0.26ª	6.56±0.26ª
Final weight (mg)	51.10±1.1	23.0±0.55	28.8±0.67	33.5±0.73	31.9±0.73	41.3±0.90	64.9±1.36	23.8±0.55
Final length (mm)	17.23±0.22	16.0±0.23	16.3±0.23	16.43±0.21	15.1±0.20	16.83±0.23	18.77±0.25	12.7±0.14
Weight gain (mg)	49.61±1.0 ^b	21.51±0.5 ^f	27.31±0.6 ^e	32.01±0.7 ^d	30.41±0.7 ^d	39.81±0.9°	63.41±1.3ª	22.31±0.5 ^f
Length gain (mm)	10.67±0.22	9.44±0.23	9.74±0.23	9.87±0.21	8.54±0.20	10.27±0.23	12.21±0.25	6.14±0.14
Relative growth rate (%)	3329.31±71	1443.6±37	1832.9±45	2148.3±49	2040.3±49	2671.8±60	4255.7±91	1497.3±37
Specific growth rate (%)	10.97±0.06	8.49±0.07	9.02±0.07	9.66±0.067	9.50±0.07	10.31±0.07	11.71±0.06	8.60±0.07
Total food consumed (mg)	18.29±0.4	20.48±0.5	21.89±0.5	32.52±0.7	35.1±0.8	19.51±0.4	21.18±0.4	25.41±0.6
Food conversion ratio	0.37±0.01ª	0.95±0.03 ^d	0.8±0.01 ^c	1.02±0.03 ^e	1.15±0.01 ^f	0.49±0.005 ^b	0.33±0.003ª	1.14±0.01 ^f
Feeding efficiency (%)	270.3±9.1 ^b	105.3±4.0 ^e	125±1.3 ^d	98±3.4 ^e	86.96±0.8 ^e	204.1±2.02 ^c	303.3±3.01ª	87.72±0.85 ^e
Hatching cost (×10⁻⁵\$)	84.5±0.0	84.5±0.0	84.5±0.0	84.5±0.0	84.5±0.0	84.5±0.0	84.5±0.0	84.5±0.0
Cost of food consumed (×10 ⁻⁵ \$)	103±1.3ª	21.8±0.31 ^c	13.6±0.18 ^e	44.2±0.55 ^b	16.9±0.22 ^d	21±0.25 ^c	21.1±0.23 ^c	23.2±0.32 ^c
Investments' cost (×10 ⁻⁵ \$)	187.5±1.3ª	106.3±0.31 ^c	98.1±0.18 ^e	128.7±0.55 ^b	101.4±0.22 ^d	105±0.25 ^c	105.1±0.23 ^c	107.7±0.32 ^c
Sale (× 10 ⁻⁵ \$)	204±4.25 ^b	92±2.22 ^f	115±2.67 ^e	134±2.9 ^d	128±2.92 ^d	165±3.6°	260±5.44 ^a	95±2.21 ^f
Profit index	1.1±0.03 ^d	0.87±0.02 ^e	1.17±0.03 ^d	1.04±0.03 ^d	1.3±0.03 ^c	1.6±0.03 ^b	2.5±0.05ª	0.9±0.02 ^e
Net Profit (× 10 ⁻⁵ \$)	16.5±5.6 ^c	-14.3±2.5 ^e	16.9±2.5°	5.3±3.5 ^d	26.6±2.7 ^c	59.5±3.4 ^b	154.4±5.2ª	-12.7±1.9 ^e
Feed cost (%)	54.9±0.3	20.51±0.2	13.86±0.2	34.34±0.3	16.67±0.2	19.90±0.2	19.98±0.2	21.54±0.2

* The same superscript on raw indicated no significant differences



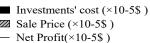


Figure 3. Economic response of the Fry.

(Uys, 1987). However, Growth in T₃ (Microencapsulated whole eggs) is expected to be higher than the current performance of our study. The low growth performance observed might be due to the clumping of powdered microencapsulated whole egg particles exceeding the mouth size of the fry some minutes after we feed the fry. Microencapsulated Whole eggs lack some vitamins (e.g. Ascorbic acid) and sufficient minerals like calcium and iron (Chow, 1986; FAO, 2012). Also, Powdered microencapsulated whole egg of our study has excess dietary lipid and excess dietary lipid is reported to reduced nutrient utilization, resulting in low growth performance of Gilthead Seabream Sparus aurata (Emre et al., 2013). Therefore, we attributed the improved growth performance in T₆ (1:1 mixture of Grasshopper and Microencapsulated whole eggs), and T₅ (1:1 mixture of *U. lactuca* and Microencapsulated whole eggs) to be associated with high lipid content of Microencapsulated whole egg as well as the minerals, vitamins, essential amino acid and essential fatty acid content of grasshopper (Adeyeye, 2005; Adeyeye, 2011; Hui et al., 2013) and U. lactuca (Ortiz et al., 2006; Garci-Casel et al., 2007; Kumar and Khaldar, 2007; Rohani-Ghadikolaei et al., 2012) that possibly enriched the powdered microencapsulated whole eggs. Vitamin C (ascorbic acid) promotes lipid metabolism, thus reduced carcass lipid and increases protein levels (Ji et al., 2003). Minerals improve growth performance, electron transfer, regulation of acid-base equilibrium, and osmoregulation for fish normal life processes (Parker, 2012). However, growth performance in T₆ is higher than T₅ which could be due to the high protein, lipid and 1:1 (50: 50%) mixture of Mictoencapsulated whole egg and grasshopper of feed in T₆. Alegbeleye et al., (2011) shows that adding 25% Zonocerus variegates grasshopper meal enhanced growth performance of C. gariepinus. On the other hand, mixture of U. lactuca and "Microencapsulated whole egg" in the ratio of 1:1 (50: 50%) in T₅ probably slows the growth performance of fry in this group when compared with growth of T₆. Ergun et al. (2008) find that low-level inclusion (5%) of U. lactuca improved growth performance in Tilapia fed high-lipid diets. Eltawil, (2010) indicates that adding 15% level of U. lactuca in the diet of Red Tilapia resulted in increased weight and specific growth rate. It was further suggested that low dietary incorporation of Ulva in the diets of Nile Tilapia improved growth performance, feed utilization, physiological activity, disease resistance, carcass quality, and reduced stress response (Mustafa and Nakagawa 1995; Waseef et al., 2005; Valente et al., 2006). Therefore, C. gariepinus as omnivorous fish should have low level of inclusion of U. lactuca. Although only growth performance in T₆ is higher than control, improvement in growth performance and high net profit made us suggest the consideration of T₅ to be promising too.

Unlike control (T_0 = *Artemia* shell free), low investment cost and high profit were found in T_6 and T_5 .

However considering the high protein and lipid content of Artemia, control was expected to have high growth performance to compensate for the high investment cost as to have a high profit. Low growth in control could be due to the deficiency of some essential fatty acid in Artemia naupuli (Olurin et al., 2014) and lack of essential fatty acids reduces growth and nutrient utilization (Parker, 2012). Artemia naupuli enriched with cod liver oil improves growth and survival of *C. gariepinus* larvae (Olurin et al., 2014). Enriching Artemia with vitamin C and unsaturated fatty acid enhanced growth performance of Trout (Akbary et al., 2011), Acipenser persicus and Huso huso (Noori et al., 2011) and Sturgeon (Hafezieh et al., 2009) larvae.

Conclusion

In the aim of reducing the cost of feeding while maintaining the best growth and enhancing the profit in *Clarias gariepinus* seed production, the present study show that, the cost was reduced from 54.9% in the control (*Artemia* shell free) to 19.98% and 19.90% in T₆ (1:1 mixture of grasshopper and microencapsulated whole egg) and T₅ (1:1 mixture of *U. lactuca* and Microencapsulated whole egg) respectively. Our study indicated that the experimental ingredients of T₆ and T₅ can be utilized as alternative feed sources for *C. gariepinus* fry and can be affordable for fish farmers with great benefit. Therefore, more should be explored on benefit of combination of ingredients of feed in T₆ and T₅.

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