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Effect of Substitution of *Artemia salina* Protein by Soya Protein in *Clarias gariepinus* Larvae Compounded Diets: Growth, Feed Efficience and Survival

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***Abstract*— *Artemia salina, the main first-feeding protein source of the catfish Clarias gariepinus larvae is relatively scarce and very expensive in Côte d'Ivoire and it raises the cost of catfish fingerlings production. To reduce the feed cost, feeding trial was completed with five isonitrogenous (35%) diets formulated by substituting artemia protein in control diet by soya protein at 25% (SB_{25}), 50% (SB_{50}), 75% (SB_{75}) and 100% (SB_{100}) level. Clarias gariepinus larvae initial body weight 0.0064 ± 0.001 g were stocked at 1 larvae L^{-1} and fed with the experimental diets three times daily ad libitum for 49 days. At the end of the growth trial, diets SB_{25} and SB_{50} present similar growth with the control diet. The low growth recorded from fish fed SB_{75} and SB_{100} highly affected final biomass despite the best survival rate recorded. Best values of feed conversion ratio were recorded from larvae fed control diet followed by SB_{25}, SB_{50}. High levels of soya proteins in diets affect feed palatability and larvae growth, vigour, motility and reactivity. Compounded feeds SB_{25} and SB_{50} can be used us low cost Clarias gariepinus larvae diets without adverse effects on growth and survival compared of artemia control diet.*

***Keywords*— Clarias gariepinus, larvae, soybean meal, growth, survival.**

I. INTRODUCTION

Availability of quality feeds, feeding strategies and control of cannibalism are essential in *Clarias gariepinus* larvae growth and survival [1], [2], [3]. The lack of available low cost larvae feeds has continued to be a major constraint to the competitive catfish culture in Côte d'Ivoire [1], [4]. In fact, Artemia nauplii capsulated cysts which is currently used as protein source in catfish *Clarias gariepinus* larvae feed remains the major constraint in larvae feeding [5], [6], [7]. This protein source is hardly available and locally expensive (210.57 USD kg^{-1}) and it raises the cost of catfish fingerlings production [8]. To reduce the feed cost, the use of *Artemia* in catfish larvae diets must be reduced as suggested by Siddiqui and Ahmed [9]. This can be achieved by replacing *Artemia salina* proteins with alternative highly available soybean meal. Soybean meal has 45-50% protein content and is the better plant protein ingredient used as alternative protein sources in fish diets [10], [11], [12]. It is also the primary plant protein used in catfish diets in Africa due to the fact that soya is widely used for vegetable oil production which increases the locally available of soybean meal for animal nutrition [13]. Also imported soybean meal has good availability and locally and imported soybean meal are reasonable price (0.60-0.95 USD kg^{-1}) compared to *Artemia salina* nauplii. Results of lot of feeding trial have shown considerable success in partial or total inclusion of soy bean meal in catfish *Clarias gariepinus* larvae and the fingerlings diets [14], [2]. According Francis et al. [15], vegetable protein can substitute fish meal to supply required protein needed for good growth. However, inclusion levels of vegetal protein in diet and their effective utilization by fish depending to species and growth stage due to the presence of high crude fiber content and antinutritional factors [16], [15], [17], [18]. Consequently, high inclusion of vegetal ingredients in fish diets could cause slower growth rates, poor performance and high mortalities [13], [17], [19], [20]. For effective substitution of *Artemia salina* by soybean meal in *Clarias gariepinus* larvae diets it's essential to determine the optimal level of replacement which promotes growth and survival. This study assesses the effect of gradual replacement of Artemia proteins by soybean proteins in *Clarias gariepinus* larvae diets on growth performances, feeds efficiency and survival.

II. MATERIALS AND METHODS

2.1 Experimental diets

For feeding trail, control diet was formulated at 35% protein with *Artemia salina* as the main diet protein source without soybean meal. Then, four isonitrogenous diets were formulated at 35% crude protein by substituting *Artemia salina* in control diet by soybean meal based on crude proteins as follows: SB_{25} = 25% of soybean protein replaced *Artemia salina* proteins; SB_{50} = 50% of soybean protein replaced *Artemia salina* proteins; SB_{75} = 75% of soybean protein replaced *Artemia salina* proteins; SB_{100} = 100 % of soybean protein replaced *Artemia salina* proteins (Table 1). All diets were composed and produced with methodology described by Ossey et al. [19]. Nutritional compositions of diets were determined and all diets were stored at -20°C until use.

2.2 Biochemical Analysis

The proximate compositions of experimental diets were determined according to AOAC methods [21]. Dry matter (DM) was determined after drying 5g of sample in an oven at 105°C for 24 hours until constant weight; crude protein (N=6,25) was determined by Kjeldahl method; crude lipid of sample was obtained by Soxhlet extraction with hexane; Ash was measured by incineration at 550°C for 24 hours in a muffle furnace, crude fibre were measured by acid digestion following by ashing dry residue at 550°C muffle furnace for 4 hours, while nitrogen-free extract (NFE) was calculated by difference. The gross energy contents of the diets were calculated based on their crude protein, lipid and carbohydrate contents using the energy equivalents of 22.2, 38.9 and 17.15 kJ g^{-1} respectively [22]. Ingredients and chemical composition of the compounds diets are presented in Table 1.

2.3 Experimental Fish and Feeding Trial

The experimental was carried out at the hatchery of the Centre de Recherches Océanologiques (CRO), Abidjan, Côte d'Ivoire. Three days-age *Clarias gariepinus* larvae initial body weight 0.0064 ± 0.001 g were transferred in aquarium (39. 40 cm × 50. 20 cm × 27. 00 cm), capacity of 50 L and acclimated four (4) days prior to beginning of the growth trial. Fish were counted and stored at density of 1 larva L^{-1} in each aquarium. Three replicates were constituted by diet and the feeding trial was conducted in 15 aquariums. Fish were fed three times daily (07:00, 12:00 and 17:00 hours) *ad libitum* for 49 days [19]. Every day, dead fish of each aquarium were removed and counted. Once a week, 15 larvae were randomly sampled in each aquarium for total length and wet weight measured. Then, all larvae were weighed and feed ratio was adjusted to reflect the new fish biomass. At the end of experiment, all survival fish were collected, weighted, measured and counted. Missing fish were presumed to have succumbed to the cannibalism [23]. During growth trial, the average water temperature, measured twice daily was 29.32 ± 0.50°C, average dissolved oxygen content of water was 04.65 ± 0.60 mg/L and average pH was 07.18 ± 0.30.

2.4 Growth Feed Efficiency Parameters

The growth and nutrient utilization parameters were calculated for each treatment as follows: weight gain (WG) (g) = final body weight – initial body weight; daily weight gain (DWG) ($gday^{-1}$) = final body weight – initial body weight / number of feeding day; specific growth rate (SGR) (%/day) = [ln (final body weight) – ln (initial body weight)] × 100/ number of feeding day; biomass gain (BG) (g) = final biomass – initial biomass; feed conversion ratio (FCR) = total weight of feed consumed (g) / biomass gain (g); total weight of feed consumed is obtained by total feed distributed fewer uneaten food; survival rate (SR) (%) = (final number of larvae / initial number of larvae)×100; cannibalism rate (CR) (%) = (number of larvae missing/initial number of larvae)×100; mortality rate (MR) (%) = (number of dead larvae/initial number of larvae)×100.

2.5 Statistical Analysis

Data analysis was performed using Statistica 7.1 software. All data are presented as mean ± standard deviation (SD). Results were compared using ANOVA one-way analysis followed by the Tukey's multiple range test to compare differences among treatment means. Significant differences were considered at $p < 0.05$.

III. RESULTS

Growth and feed efficiency parameters, cannibalism, mortality and survival rate of *Clarias gariepinus* larvae fed control diet and diets SB_{25}, SB_{50}, SB_{75}, and SB_{100} are presented in Table 2.

3.1 Growth

At the end of the growth trial, final body weight, weight gain, daily weight gain and specific growth rate recorded were significantly ($p< 0.05$) influenced by the levels of soy bean meal inclusion in the control diet. These growth parameters were significantly highest from larvae fed control diet SB_{25}, and SB_{50} which did not differ significantly ($p>0.05$) followed by the group of the fish fed SB_{75}. Larvae fed SB_{100} recorded the significant lowest values of these growth parameters. The fish final biomass and biomass gains decreased with the soy bean meal inclusion level in control diet. The significant ($p< 0.05$) highest biomass gain value was recorded from fish fed control diet (82.21 ± 0.11g) followed by SB_{25} (69.44 ±

0.12g), SB_{50} (57.42 ± 0.14g), and SB_{75} (47.73 ± 0.13g) and the lowest value was obtained by fish fed SB_{100} (47.73 ± 0.13g).

3.2 Feed Efficiency

Total quantity of feed used by aquarium and feed conversion ratio values recorded were affected by the level of *Artemia salina* replacement by soybean meal in control diet. Results showed that quantity of feed used decreased with the soybean meal inclusion level in diet conversely FCR significantly ($p < 0.05$) increased. The lowest value of FCR correlated with best feeds efficiency was recorded from fish fed control diet (01.88 ± 0.29) when the highest value of these parameters was obtained from fish fed diet SB_{100} (3.30 ± 0.18).

3.3 Cannibalism, Mortality and Survival

Cannibalism, mortality and survival rate values showed significant influence with the level of soy bean inclusion in control diet. Cannibalism rate values varied between 15.55 and 23.32%, mortality rate ranged between 1.25 and 2.21% while survival rate varied between 68.27 and 83.20%. The highest ($p < 0.05$) value of cannibalism rate was recorded from fish fed SB_{25} (29.75 ± 0.75 %) and SB_{50} (29.86 ± 0.66 %), followed by fish fed SB_{75} (25.60 ± 0.40 %) and control diet (23.32 ± 4.28 %) when the lowest cannibalism rate was observed from fish fed SB_{100} (15.55 ± 0.55 %).

Fish fed control diet recorded the highest mortality rate (02.21 ± 0.01) followed by those of fish fed SB_{25} (01.65 ± 0.14) and SB_{50} (01.87 ± 0.25), when the lowest mortality rate values were obtained from fish fed SB_{75} (01.30 ± 0.01) and SB_{100} (01.25 ± 0.12). The significant ($p < 0.05$) best value of survival rate was recorded from fish fed SB_{100} (83.20 ± 0.04), followed by control diet (74.47 ± 5.46) and SB_{75} (73.10 ± 0.15) and the lowest values of survival rate were observed from fish fed SB_{25} (68.60 ± 0.60) and SB_{50} (68.27 ± 0.03).

IV. DISCUSSION

At the end of the growth trial, feeds which artemia protein was substituted by 25% (SB_{25}) and 50% (SB_{50}) of the soya protein present similar growth with the control diet. Up to 75% of soya protein inclusion, values of final fish growth, weight gain, and daily weight gain recorded were decreased. These results show that artemia protein can be substituted by soya protein at 25 to 50% without adverse effects on *Clarias gariepinus* larvae growth. In fact, high levels of soybean meal increase anti-growth substances and indigestible carbohydrates levels in diets which lead to slow growth and poor feed performances [24], [25].

Consequently, low growth recorded from fish fed SB_{75} and SB_{100} highly affected final fish biomass by aquarium. Quantity of fish feed used also decreased with the levels of soybean meal inclusion. However, best values of feed conversion ratio were recorded from larvae fed control diet followed by diets SB_{25}, SB_{50} and SB_{75} when diets SB_{100} presents the lowest value of FCR. These results could show an increasing reduction of feed palatability, acceptability and digestibility when artemia proteins were gradually combined with soya protein in diet. Concerning cannibalism, several studies showed that it’s intensified by increasing size differences, suitable feeding practices, inter individual contacts, competition of food and stress [26], [27], [28], [29], [30], [31]. The low cannibalism value recorded with fish fed SB_{100} could confirm that soya protein diet SB_{100} was not accepted and not palatable for larvae which consequently reduces quantity of feed use, inhibits competition of food and stress, and entails slows growth for all the fish in aquarium. In these conditions, cannibalistic behaviour of larvae was reduced consequently in the groups of fish fed SB_{100} and these groups recorded the highest values of survival rate. Despite high survival rate recorded with SB_{100}, growth and feed efficiency values show that high levels of soya proteins in diets affect feeds palatability and larvae growth, vigour, motility and reactivity. In these conditions, 100% soya proteins diets are not recommended for *Clarias gariepinus* larvae growth. Conversely, survival rate (68%) obtained with feeds which artemia protein was substituted by 25% (SB_{25}) and 50% (SB_{50}) soya protein were similar to the survival rate (67-69 %) of the larvae *Clarias gariepinus* fed with commercial high proteins content (56-57%) diets reported by Yakubu et al. [3]. In addition, these two diets present similar growth results with control diets. In these conditions, artemia protein in 35% protein control diet can be replaced by 25 to 50% of soya protein for catfish *Clarias gariepinus* larvae growth.

V. CONCLUSION

Artemia protein in *Clarias gariepinus* larvae 35% protein diet can be replaced by soya protein to 25 and 50% for reduce the feed cost. Compounded feeds SB_{25} and SB_{50} can be used us low cost nutritive C*larias gariepinus* larvae diets without adverse effects on growth and survival compared of Artemia dietary control diet.

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Table. 1: Formulation and proximate composition of experimental diets

Ingredients composition (%)	Soybean meal inclusion				
	Control diet (0%)	**SB_{25} (25%)**	**SB_{50} (50%)**	**SB_{75} (75%)**	**SB_{100} (100%)**
Artemia salina Meal	57.80	44.00	30.00	15.00	-
Soy bean meal	-	20.90	40.00	60.00	81.00
Maize flour	24.56	18.00	12.76	08.00	02.00
Maridav	10.00	10.00	10.00	10.00	10.00
Palm oil	02.00	02.00	02.00	02.00	02.00
Lysine	02.13	02.13	02.13	02.13	02.13
Methionine	01.61	01.61	01.61	01.61	01.61
VITAMYNOLYTE Super prémix	02.00	02.00	02.00	02.00	02.00
Total	100	100	100	100	100
Proximate analysis					
Moisture (%)	10.60	10.23	10.67	10.73	10.99
Crude protein (% DM)	35.13	35.63	35.44	35.16	35.15
Total fat (% DM)	04.76	06.43	07.95	09.58	11.25
Ash (% DM)	03.21	04.83	06.28	07.81	09.42
Crude fiber (% DM)	03.90	04.38	04.76	05.17	05.60
Nitrogen free extract (%)[3]	42.38	38.46	34.87	31.52	27.56
Gross Energy (kjg^{-1})[4]	16.93	17.03	16.96	16.95	16.92
P/E (g. kJ^{-1})[5]	20.74	20.93	20.90	20.74	20.77

Table. 2: Growth, feed efficiency and survival rate of larvae C. gariepinus fed the experimental diets

Parameters	Experimental diets				
	Control diet (0%)	**SB_{25} (25%)**	**SB_{50} (50%)**	**SB_{75} (75%)**	**SB_{100} (100%)**
Initial larvae number	50	50	50	50	50
Initial body weight (g)	0.0064±0.01	0.0064±0.01	0.0064±0.01	0.0064±0.01	0.0064±0.01
Final body weight (g)	$02.19±0.13^{c}$	$01.89±0.12^{c}$	$01.91±0.13^{c}$	$01.32±0.01^{b}$	$0.70±0.02^{a}$
Weight gain (g)	$02.18±0.16^{c}$	$01.88±0.31^{c}$	$01.90±0.13^{c}$	$01.31±0.01^{b}$	$0.69±0.02^{a}$
Daily weight gain ($gday^{-1}$)	$0.04±0.01^{b}$	$0.04±0.001^{b}$	$0.04±0.002^{b}$	$0.02±0.001^{a}$	$0.01±0.001^{a}$
Specific growth rate ($\%day^{-1}$)	$11.85±0.05^{d}$	$11.53±0.03^{c}$	$11.43±0.10^{c}$	$10.88±0.01^{b}$	$09.57±0.03^{a}$
Initial biomass of fish (g)	$0.32±0.001^{a}$	$0.32±0.001^{a}$	$0.32±0.001^{a}$	$0.32±0.001^{a}$	$0.32±0.001^{a}$
Final biomass of fish (g)	$82.53±0.10^{e}$	$69.76±0.20^{d}$	$57.74±0.22^{c}$	$48.05±0.15^{b}$	$28.95±0.30^{a}$
Biomass gain (g)	$82.21±0.11^{e}$	$69.44±0.12^{d}$	$57.42±0.14^{c}$	$47.73±0.13^{b}$	$28.63±0.15^{a}$
Quantity of feed used (g)	$155.16±0.11^{e}$	$147.89±0.10^{d}$	$128.18±0.16^{c}$	$110.90±0.17^{b}$	$95.53±0.21^{a}$
Feed conversion ratio	$01.88±0.29^{a}$	$02.12±0.06^{a}$	$02.22±0.07^{ab}$	$02.31±0.01^{b}$	$03.30±0.18^{c}$
Cannibalism rate (%)	$23.32±4.28^{b}$	$29.75±0.75^{c}$	$29.86±0.66^{c}$	$25.60±0.40^{b}$	$15.55±0.55^{a}$
Mortality rate (%)	$02.21±0.01^{c}$	$01.65±0.14^{b}$	$01.87±0.25^{bc}$	$01.30±0.01^{a}$	$01.25±0.12^{a}$
Survival rate (%)	$74.47±5.46^{b}$	$68.60±0.60^{a}$	$68.27±0.03^{a}$	$73.10±0.15^{b}$	$83.20±0.04^{c}$

Mean values ± SD in the same row sharing the different superscript are significantly different ($p<0.05$)

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