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Bioavailability of artemia nutrients used as live feed in production of cultured catfish larvae

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Abstract
Larvae nutrition is considered to be the 'bottle neck' for larval fish production. As a way forward, live feeds known to be rich in macronutrients are used as a potential food source for fish larvae rearing. Fish larvae experiments with artemia investigating safety and bioavailability were carried out in an indoor hatchery with soybean diet as control. Here, 8 tanks experimental system was installed for nutrition experiments with commercial artemia feed and the control. The treatments had 4 replications and each tank was fed simultaneously twice a day to satiation for 14 days. In response to all the treatments diets, we observed a weight gain superior to that in response to the control diet. No substantial differences in organ weights nor gut length occurred. Protein bioavailability from the artemia diets did not differ from the control diet ranging from 56.2% to 76.2% apparent biological value *ABV*. Similarly, the soy bean control diet *ABV* ranged from 41.4% to 66.4%. This study indicated that absorption was lower for soy bean protein as control diets compared to artemia nutrition, albeit substantial percentage survival measured following feeding trial showed high rate. Serum analysis did not revealed any heart, kidney or liver toxicity induced by any of the diets. Artemia and soy bean-rich diets were well accepted, tolerated and suitable for the maintenance of body weight, normal organ function and indeed for larvae rearing. Therefore, the dietary inclusion with artemia meal appears to have a growth promoting effect on Catfish larvae, which may be associated with other trace minerals or other nutrients.

Keywords: Bioavailability, safety, fish larvae, artemia diet

Introduction
The African Catfish generally had been economically important fish species that contributes significantly to annual fish productions in many countries of the world, most especially in Africa [1]. Over the decades, aquaculture which describes artificial cultivation of fish species most especially the larval rearing phase faces many challenges notably development of appropriate feeds and feeding mechanism, hatchery and grow-out technology, as well as water-quality management, poor breeding methods and diseases outbreak. The high demand for fish fingerlings in the phenomenal growing aquaculture industry has necessitated the need for larval production of culturable marine and fresh water fish. Particularly, the inadequate availability of one of Africa catfish *Clarias gariepinus* larvae poses a great threat to its cultivation. This called for the need for the detailed knowledge and understanding of larval rearing technique for its artificial propagation [2].

Feeding is an essential part of larvae farming, and it is a factor to ensuring that the larvae attain a desired harvesting size and weight within a specified period of time. It is also part of the major cost incurred in fish farm business. More specifically, nutrition is considered to be one of the most important issues in larvae research [3, 4]. Considerable research has been carried out during the last few decades to identify nutrient requirements and bioavailability for fish larvae [5].

Bioavailability refers to the proportion of nutrient that is absorbed from the diet and used for normal body functions and is one of the main challenges in producing effective larval dietary products [6, 7]. It is asserted that the bioavailability of nutrients is higher in food eaten in its natural state [8]. Even among unprocessed foods, not all foods are broken down and digested effectively. Feed with poor absorption rate result in nutrients being disposed from the body without providing any nutritional or medicinal benefit. The quality of proteins and their bioavailability can be assessed using the apparent biological value (ABV), which measures the efficiency of nitrogen uptake, or using the digestibility, which measures the amount of protein

absorbed, or using the net protein utilization (NPU), which gives the combination of biological value and digestibility and hence reflects protein quality [9].

At the early stage of growth, fish larva are said to subsist on natural live feeds such as artemia, diatoms, microalgae etc. as such initiating build-up of organic materials for phytoplankton and zooplanktons production becomes important. As the larvae mature, supplementary feeding becomes necessary in view of their level of food consumption and depletion in natural food in their habitat. Common supplement used in larvae rearing include *artemia nauplii*, copepod, soy bean based feed, diatoms, rice bran, chopped worms etc. Artemia is described as an important source of proteins used in quality fish larval production [9]. This study aims to evaluate the quality of proteins and their bioavailability from artemia live feed and soybean based diet in vivo. The safety measurement was also done in order to identify the underlying factors for this phenomenal feat.

Materials and Methods

African Catfish larval production

Details of the matured brood stocks identification (male and female), spawning, fertilization, fish larval production in hatchery have been given separately [10].

Preparation of Feeds for feeding Trials

Commercial Artemia of food-grade quality was purchased from Aquaculture feed store in the capital city of Uyo, Nigeria. Artemia was protected from light and kept at - 4 ºC until they were used. Fresh dry soybeans were obtained from a supermarket in Uyo, the capital city of Akwa Ibom State Nigeria. The Soybean was shelled, sorted and dried for 24 h at room temperature. The Soybeans was further dried in an oven at 60 ºC, then grind using local grinder, sieved with a household sifter (1/16¢ wire mesh) and was stored in screw cap bottles at room temperature until use. Feeding was done twice daily to satiation for 14 days.

Experimental procedures

The treatments had 4 replications and each tank was stock with 100 larvae of age 7 day old. Larvae were reared for 14 days, all in plastic tank (capacity 200 L) arranged in completely randomized design. They were fed to satiation twice a day. The initial length and weight of larvae was measured with a measuring scale and analytical balance respectively, prior to stocking. The 7 day old larvae were splits into treatment group I and Il and fed with *Artemia nauplii* at the rate of 0.05 g, 0.1 g, 0.5 g and 1.0 g and soybean laboratory prepared diet as control. The feeding experiment continued twice daily tills the end of the experiment. Temperature was maintained at 28 °C. The indoor rearing tanks were cleansed every other day and about one half of the water was replaced with fresh water every day to reduce the nitrogenous waste accumulated. At the end of the 14 days of the trial, 30 surviving larvae from each replicate were collected and analyzed. The initial and final weight and length parameters were recorded. The percentage survival and Specific Growth Rate *SGR* were calculated according to Srivastava *et al.*, [11] as:

1. Survival (%) = (Number of larvae stocked − Number of dead larvae) / Number of larvae stocked × 100.
2. Specific growth rate *SGR* = (*ln* final weight − *ln* initial weight) / Days of experiment × 100.
3. Condition factor (K): condition factor (K) was calculated according to Ayo-Olalusi, [12], with modification as shown below:

K = W x 100/ Lb

Where, W=weight of fish (mg), L=Length of fish (mm) and b is exponent of the length-weight relationship.

Bioavailability of Proteins

For analysis of the protein bioavailability, a method described by Neumann *et al.*, [13], with some modifications was adopted. Briefly, nitrogen content was measured in 24 h feces samples, collected on day 14. A protein conversion factor of 6.25 was used to determine the protein content in feces samples [14]. The apparent biological value (ABV), apparent digestibility (AD) and net protein utilization (NPU) were calculated using the following formulas:

ABV = NI – Ne (f) / Ne (f) x 100;
AD = NI – Ne (f) / NI x 100 and
NPU = ABV x AD / 100

Where NI = ingested protein; Ne (f) = protein excreted in feces. The ingested protein was calculated by multiplication of the consumed feed at day 14 by the protein conversion factor of 6.25.

Toxicity Markers

Song *et al.*, [15] methods previously described was used for the measurement of toxicity markers for kidney and liver in larvae. Aspartate aminotransferase (AST) was analyzed as a marker for liver health, Cystatin c for kidney health (Life Diagnostics Inc., West Chester, PA, USA). All measurements were done following the manufacturers protocols in plasma samples. AST was conducted by testing all samples in duplicate on one plate. Cystatin c measurement was performed by testing all samples in duplicate on two plates.

Statistical analysis

Data were analyzed through one-way ANOVA for completely randomized experiments. When significant differences were detected between the means, they were compared two-by-two with
Tukey's HSD test. The significant level of 5% was set in all statistical analyses. The statistical package SPSS 15.0 for Windows (SPSS Inc., Chicago, Illinois, USA) was used.

Results

Larvae of age 7 day old were fed for 14 days with artemia and soybeans protein at 0.05, 0.1, 0.5 and 1.0 g levels. During the feeding trials, no adverse effects like weight loss, inflammatory responses, adverse reaction, abstinence, bleeding could be seen. No significant differences between groups were observed for body weight from day 0 to day 14. However, Colon length, as well as organ weights of liver, lung and heart did not differ significantly between groups at the end of the study (Table 2). The spleen weight followed the same pattern with the artemia level 1.0 g group having a highest spleen weight. This is also true with the control diet (level 1.0 g group) (Table 2).

Figure 1 and 2 shows that the Toxicity markers for kidney (cystatin c) and liver (aspartate aminotransferase AST), as were analyzed in the larvae blood sample did not differ significantly in both treatment. For the analysis of the safety

of Artemia, toxicity markers of liver and kidney were assessed (Fig. 1& 2). The Artemia diets from the range of 0.05g to 1.0 g did not lead to significant difference in values of these parameters when compared to Control diet and hence reveal no toxic effects. Nonetheless, the treatment levels of Artemia diet showed an increased liver value (Fig. 2), when compared to the control diet.

Table 1: Body weight of larvae before and after 14 days diet consumption. Data are expressed as mean SD (n = 4).

Diet	Level [g]	Spleen w [mg/g BW]	Liver w [mg/g BW]	Lung w [mg/g BW]	Heart w [mg/g BW]	Colon l [mm/g BW
Control	0.05	2.4±0.6	1.6±1.0	1.3±0.7	2.3±1.0	2.2±0.5
	0.1	3.1±0.8	4.2±1.2	2.0±1.7	2.1±1.0	2.1±0.5
	0.5	3.1±0.4	4.1±2.0	3.1±0.6	2.3±1.2	2.0±0.3
	1.0	3.3±0.4	4.7±0.4	3.2±1.3	2.4±0.5	2.2±0.3
Artemia	0.05	3.0±0.4	4.2±0.2	1.2±0.6	2.0±0.8	2.3±0.5
	0.1	4.1±0.4	4.3±0.1	3.2±0.4	3.2±0.6	2.3±0.5
	0.5	4.0±0.6	4.4±0.8	3.4±0.1	3.3±1.1	1.9±0.3
	1.0	4.3±0.4	4.0±0.2	3.3±0.2	3.5±0.6	2.4±0.4

No significant differences as analyzed by ANOVA. Abbreviations: treatment level (trt level), initial body weight (BWd 0), final body weight (BWd 14), food consumption (FC), Specific growth rate SGR.

Table 2: Organ parameters of larvae before and rafter 14 days diet consumption. Data are expressed as mean SD (n = 4).

Diet	Level [g]	Spleen w [mg/g BW]	Liver w [mg/g BW]	Lung w [mg/g BW]	Heart w [mg/g BW]	Colon l [mm/g BW]
Control	0.05	2.4±0.6	1.6±1.0	1.3±0.7	2.3±1.0	2.2±0.5
	0.1	3.1±0.8	4.2±1.2	2.0±1.7	2.1±1.0	2.1±0.5
	0.5	3.1±0.4	4.1±2.0	3.1±0.6	2.3±1.2	2.0±0.3
	1.0	3.3±0.4	4.7±0.4	3.2±1.3	2.4±0.5	2.2±0.3
Artemia	0.05	3.0±0.4	4.2±0.2	1.2±0.6	2.0±0.8	2.3±0.5
	0.1	4.1±0.4	4.3±0.1	3.2±0.4	3.2±0.6	2.3±0.5
	0.5	4.0±0.6	4.4±0.8	3.4±0.1	3.3±1.1	1.9±0.3
	1.0	4.3±0.4	4.0±0.2	3.3±0.2	3.5±0.6	2.4±0.4

Means with different letters mark significant differences (ANOVA, $p< 0.05$); Abbreviations: Weight (w), length (l), Body weight (BW).

ABV, AD and NPU are values to express the protein bioavailability in vivo (Table 3). The Control Diet with Soy protein concentrate as protein source (ABV: 66.4%, AD: 72.4%, NPU: 54.3%) does not differ significantly from the artemia groups. However, differences between and among the artemia groups can be seen, showing the highest values for ABV: 76.2%, AD: 78.3% and NPU: 59.1%. Whereas, the lowest values for the ABV: 56.4%, AD: 57.3 and NPU: 66.8% are also obtained.

Table 3: Apparent biological value ABV, apparent digestibility coefficient ADC and net protein utilization NPU of proteins in larvae

Diet	Level [g]	ABV [%]	ADC [%]	NPU [%]
Control	0.05	41.4±1.6	48.2 ±0.9	52.3 ±0.2
	0.1	48.7±0.2	67.1 ±0.8	51.2 ±2.3
	0.5	58.7±1.2	72.3 ±0.3	49.4± 1.4
	1.0	66.4 ±2.8	72.4 ±2.5	54.3 ±0.3
Artemia	0.05	56.4 ±5.5	57.3 ±0.8	66.8 ±0.2
	0.1	65.2 ±2.9	65.6 ±0.6	55.8 ±2.4
	0.5	69.5 ±3.8	69.5 ±0.7	55.2 ±2.2
	1.0	76.2 ±5.4	78.3 ±3.1	59.1 ±1.2

Data are expressed as means (n = 4 for protein bioavailability).

Figure 1 and 2. Toxicity markers for kidney (cystatin c) and liver (aspartate aminotransferase AST) as were analyzed in the larvae serum. Data are expressed as means, no significant differences analyzed by ANOVA

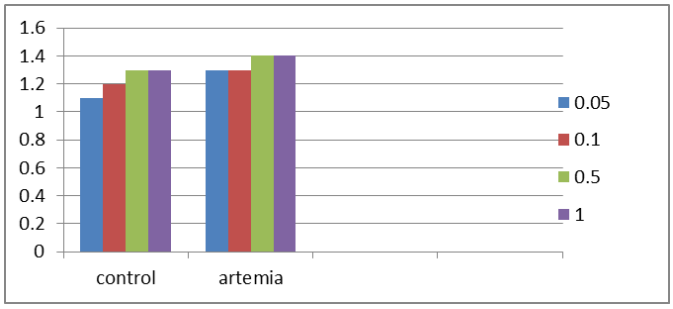

Fig 1: Toxicity markers for kidney (cystatin c.)

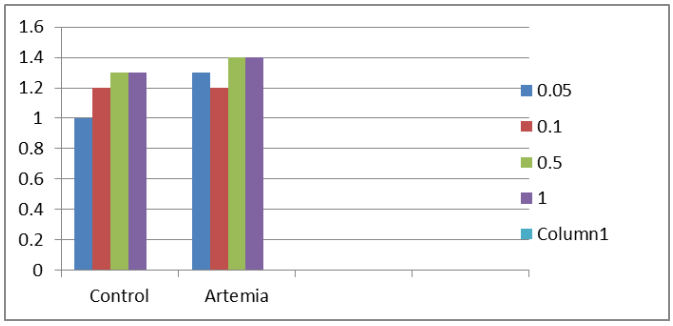

Fig 2: Toxicity markers for liver (aspartate aminotransferase (AST).

Discussion

The present study evaluates the bioavailability and safety of crude protein and Soy protein concentrate generated from two meal sources: Artemia and soybean respectively. It has been reported in many quarters that the quality of protein from vegetable sources is lower than the quality obtained from that of animal sources, simply because most vegetables lack one or more essential amino acids [16]. Soy bean are according to Saleh *et al.* [17] and Becker [18], deficient in the sulfur-containing amino acids methionine and cysteine, but otherwise possess a favourable amino acid composition such as Glutamic acid and aspartic acid (Becker, [18]. However, it has been shown by this study that ABV, AD and NPU, as parameters used in assessing the protein quality, did not differ between the different artemia diets and the Control Diets, even for the highest concentration of both diets. Soy protein concentrate, the protein fraction of milk, is a reference protein with BV, AD and NPU values ranging between 41.4% and 66.4%; 48.2% and 72.4% and 52.4% and 54.3 % respectively (Table 3). This study also showed that at least up to 76.2% of Artemia diet is rich in protein (Table 3) or at least 56.4 % of its protein fraction in the diet can be safely replaced by soybean proteins. This verifies the assumption that both artemia diet and soy bean protein concentrates do possess a good protein quality and that the proteins are sufficiently bioavailable. This is in consonant with the work of Neumann *et al.*, [13], in which they assessed the bioavailability and safety of nutrients from different types of microalgae diets in mice. To assess the bioavailability of protein from microalgae in their animal model, they discovered non significant differences in all the diets tested. Safety of artemia and soybean diets was assessed by measuring toxicity markers, organ weights and GI tract. Our study was able to show no adverse effects of the tested diets after 14 days of feeding. This is in contrast with the work of Erben *et al.*, [19]. It was shown in their study that inflammatory responses lead to significant changes in GI tracts of mice that were monitored during feeding trials. Furthermore, it can be assumed that no inflammatory response was initiated by the feeding of artemia and soy protein concentrates. Randers *et al.*, [20] and Johnson [21] reported separately that the toxicity markers, cystatin c and AST are also used as markers for organ health in humans. Our results showed no significant changes for the assessed markers, indicating no toxic effects of artemia on organ health. However, toxicity markers need to be monitored in further studies. Long term studies in vivo may be useful for animal husbandry and for evaluating toxicity in growth out fish trials. This could also be interpreted to mean that artemia and or soy protein concentrates can be fed to fish and used to replace up to certain percentage of fish meal without any adverse effects on growth and nutrient digestibility. This assertion is supported by Sørensen *et al.*, [22], in their study on the effect of microalgae on nutrient digestibility, growth and utilization of feed by Atlantic salmon *Salmo salar*. However, in contrast to our study, the microalgae cells were not disrupted in a form of processing, which leads to the observation that the decreasing bioavailability could be as a result of the inability of the digestive tract or system to digest the microalgae cells. This also means that a good downstream processing involving mechanical cell disruption will results in higher protein bioavailability than other downstream processing without mechanical cell disruption.

In summary, this study reveals the adequate bioavailability of crude protein from artemia and soy protein concentrate from soy bean meal. The study also reveals the safety of mechanical downstream processing involving cell disruption

in a short-term *in vivo* study. Furthermore, the safety of the artemia nutrient and soy bean meal was assessed showing no adverse effects for concentrations up to 1.0 g. This indicates that both diets could be used as an alternative source of proteins to replace fish meal.

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Conflicts of Interest: The authors declare no conflict of interest.

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