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Effect of dietary protein sources on the growth performance, nutrient efficiency and biochemical body composition in *Heterotis niloticus* fingerlings

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Abstract
The present study was performed to investigate the effect of dietary protein sources on the growth performance, nutrient efficiency and biochemical body composition in *Heterotis niloticus* fingerlings. Five experimental diets were formulated to contain three sources of protein: diet D1 (based on fishmeal), diet D2 (based on earthworm meal), diet D3 (based on maggot meal). In diets D4 and D5, the proportion of fish meal was half replaced by earthworm and maggot meal, respectively. Fish with an initial body weight of 160.02 ± 18.60 g were fed with the experimental diets in triplicate groups per treatment. No significant difference was found on Final body weight (FBW), Specific growth rate (SGR), feed conversion ratio (FCR) and whole-body composition between fish fed with diet D4 and the control group that fed with a reference diet (D1) after 54 days. In view of growth performance, it appeared that earthworm meal could partly replace fishmeal in the diet of *H. niloticus* fingerlings.

Keywords: *Heterotis niloticus* fingerlings, protein sources, growth performances, body composition

1. Introduction

Reducing poverty and achieving food security is a challenge for all countries today. Meeting this challenge requires the participation of a large number of sectors including aquaculture. Aquaculture is a means of fish and aquatic species (shellfish, crustaceans, aquatic plants) breeding. It is a spreading sector for it now provides half of the fish for human consumption [1]. However, in developing and low-income food-deficit countries, levels of fish consumption remain relatively lower than in developed countries [2]. In 2010, sub-Saharan Africa contributed only 0.6% of the input in term of fish for human consumption, according to [3, 4]. This low rate of aquaculture production is mainly related to the high cost of these animals food. For [5], the high cost of food can be explained by the use of fishmeal as the main source of protein that represents more than 50% of cost production. The high purchase price of fishmeal is directed research towards alternative sources of protein, mainly to those that are not directly usable for human consumption [6, 7].

In Côte d'Ivoire, tilapia *Oreochromis niloticus* remains the main feeding specie in fish farm. The single or combination feeding food are mainly agro-food rest (71.10%). They are mainly from vegetal (soybean meal, maize meal, low rice meal and rice bran), and are used regularly to feed farmed fish directly [8]. The fact is that aquaculture production in Côte d'Ivoire remains low. If fishmeal cannot be excluded, it is important to reduce its use in food formulation to decrease the cost of fish production in order to increase fish species to raise in addition to *O. niloticus*. It is in this context that this study raises. It aims at evaluating the impact of food protein source on the growth, food utilization and body composition of *Heterotis niloticus* fingerlings. It is specifically a question of determining the effect of a partial or total substitution of fishmeal, respectively by earthworm meal and maggot meal in the feeding of this fingerlings specie.

2. Material and methods

2.1 Fish and experimental conditions

The study was carried out at the experimental fish station of the NGO "Association Fish Farming and Rural Development in Humid Tropical Africa" (APDRACI) located in Daloa city (Central-West of Cote d'Ivoire). Five rectangular ponds of 300 m^2 with a mean water height of 0.83 m have been used for the experiment. A total of 15 rectangular enclosure of 20 m^2 each (8m long and 2.5m wide) made of mosquito net have been installed in these ponds at the rate of 3 enclosures per pound. Enclosures have been referenced using numbers appropriated for food processing. The animals used for the experiment were fingerlings of *Heterotis niloticus* with a mean weight of 160.02 ± 18.60 g.

2.2 Experimental procedure

The ingredients used to formulate trial diets are: earthworm meal, maggot meal, fishmeal, maize meal, soybean meal, cotton flour and wheat bran flour. Palm oil, vitamin-amino acid premix and minerals have been used as a lipid supplement and to enhance the mineral and vitamin composition of foods. The composition of the raw materials used is summarized in Table 1.

Table 1: Proximate composition (% dry matter) of fish meal, earthworm and maggots meals

Ingredients	Composition				
	Moisture	Protein	Lipid	Ash	NFE
Fish meal	3.27	60.3	8.75	20.35	8.67
Earthworm meal	6.30	46.6	7.62	27.21	9.85
Maggot meal	6.61	42.35	12.07	18.88	19.46

NFE: Nitrogen free extract = 100% - (% protein + % lipid + % ash + % fiber)

Five (5) isonitrogenous (30%) and isoenergetic (19 kJ/g energy) experimental diets have been formulated (Table 2) ; diet D1 based on fishmeal has been taken as reference; diet D2 based on earthworm meal, diet D3 based on maggot meal. In diets D4 and D5, the proportion of fish meal was half replaced by earthworm and maggot meal, respectively. For each experimental diet, the treatments were made in triplicate. The experiment was conducted in enclosures containing fish at a density of 0.3 fish/m^2. The daily ration has been determined according to the total fish weight in each enclosure. It was 5% of the total fish weight for the first month and 4% for the second month. The fish were fed daily at a frequency of three meals a day, the first feeding at 9 am, the second at 12 am and the third at 16. Only one control fishery occurred 27 days from the begining of feeding. During this fishery, the fish were measured, weighed separately before counting and returning in enclosure. Measurements of physicochemical parameters, ie temperature (Tº), pH, dissolved oxygen level (o2), were made from the first to the last day between 12 am and 13 for temperature and all the beginning of weeks before 9 am for pH and dissolved oxygen. The average values of these parameters through out the experiments were relatively stable and did not significantly vary from one pond to another. Temperature, dissolved oxygen, and pH varied with in the following limits: 29.40 ± 1.27 to 30.23 ± 1.28 ºC, 6.53 ± 1.04 to 6.83 ± 1.63 mg/L, and 6.51 ± 0.27 to 6.73 ± 0.28. Water transparency with a Secchi ranged between 0.61 ± 3.16 and 0.69 ± 1.02 m. The experiment lasted 54 days. At the beginning of the feeding trial, 5 fish was taken and kept frozen (-20°C) for subsequent whole-body proximate analysis. Also, at the end of the experiment, fish were weighted individually and three from the same enclosures were taken and kept frozen (-20°C) for further determination of whole-body composition.

Table 2: Experimental diets ingredients and proximate composition (% dry matter)

Ingrédients	Experimental diets				
	D1	D2	D3	D4	D5
Fishmeal	23	0	0	11.5	11.5
Earthworm meal	0	23	0	11,5	0
Maggot meal	0	0	23	0	11.5
Maize meal	23	7	19	15	25
Cotton flour	15	19	23	17	18
Soybean meal	14	19	20	17	18
Wheat bran flour	13	20	3	16	4
Palm oil	9	9	9	9	9
Vitamins mixture[1]	0.2	0.2	0.2	0.2	0.2
Minérals mixture[2]	2.8	2.8	2.8	2.8	2.8
Proximate composition					
Crude protein (%)	30.72	30.87	30.02	30.91	30.07
Crude lipid (%)	13.79	12.16	14.30	12.98	14.11
Ash(%)	7.72	18.77	7.66	13.25	7.51
NFE (%)	40.62	39.91	41.56	40.15	41.10
Gross energy (kJ/g)	19.17	18.44	19.37	18.81	19.23
Protein/Energy ratio (mg/kJ)	19.74	20.72	19.08	20.28	19.26

[1] Vitamins mixture: vit A: 2,500,000 IU; vit D3: 500,000 IU; vit E: 30,000 mg; vit K3: 2000 mg; vit B1: 2000 mg; vit B2: 5000 mg; panthotenic acid: 10,000 mg; niacin 5000 mg; vit B6: 4000 mg; folic acid: 2000 mg; vit B12: 80 mg; vit C: 20,000 mg; biotin: 200 mg and inositol: 80,000 mg [2]Minerals mixture: calcium = 23 g, Phosphore = 18 g, magnesium = 0.21 g, copper sulfate = 0.8 g, cobalt sulfate = 0.02 g, manganese sulfate = 0.6 g, zinc sulfate = 8.15 g, selenium sulfate = 0.04 g, ferrous sulfate = 0.9 g

2.3 Chemical analysis

Analyzes have been carried out in the nutrition laboratory of the Center for Research of Oceanology (CRO). Biochemical analyzes (moisture, crude proteins, crude lipids, fiber and ash) of diets and fish whole-body were performed in duplicate using [9] standard methods. The crude proteins has been determined by the Kjeldahl method (self-analyzer Kjel-foss), lipids by the hot method (Soxhlet type). The crude fiber has been analysed by the Weende method (acid and alkaline hydrolysis). The dry weight was determined by measuring the weight lost after drying for 24 h in an oven at 105 ° C. The ash have been determined after samples incineration of the muffle oven at 550 ° C for 24 hours.

2.4 Growth performance and nutrient efficiency parameters

To estimate fish growth and characterize tested food use efficiency, zoo technical parameters such as: Daily Weight Gain (DWG), Specific Growth Rate (SGR), condition coefficient (K), Voluntary Ingesting (VI), Feed Conversion Ratio (FCR), Protein Efficiency Ratio (PER), Survival Rate (SR) and Yield were calculated as follows:

DWG (g/day) = [Final body weight (g) – Initial body Weight (g)] / Number of experimental day; SGR (%/day) =[ln[Final body weight (g)] – ln[Initial body Weight (g)]] / Number of experimental day × 100; FCR = Feed intake (g) / Fish weight gain (g); PER = Fish weight gain(g) / protein intake (g); K = 100 × Final body weight (g) / Fish total length^3 (cm); VI (%/day) = 100 × Feed intake (g) / [(Initial body weight + Final body weight) / 2)] / number of experimental day; SR (%) = 100 × (Final fish number / Initial fish number); Yield (t/ha/year) = [Final total weight (g) × 365] / [Area (m^2)× number of experimental day].

2.5 Statistical analysis
Data were expressed as mean ± SD (n = 3). The effects of diet were tested with one way analysis of variance (ANOVA), followed by Tukey's test. Differences were considered significant when P\0.05. Statistical analyses were performed using STATISTICA 7.1 software.

3. Results
3.1 Survival and growth performance
The fish survival rate in this test is between 88.88 ± 9.62 % and 100 %. The D4 diet provides the highest survival rate (100%). there is no significant difference between the survival rates of fingerlings fed with the different trial diets (p> 0.05). The general condition of the fish at the end of the experiment was quite satisfactory and no infection or pathology affected them during the experiment. Values mentioned in table 3 show that the weight gain (466.11 ± 23.65), specific Growth Rate (2.67 ± 0.24), yield (8.75 ± 0.79 t/ha/year), and condition factor (1.22 ± 0.05) are significantly higher (P <0.05) for D1. Nevertheless the weight gain of fish fed with D4 diet was similar to those fed with D1 almost all the breeding duration. In fact, D1 and D4 distinguished themselves from others by spectacular fish growth during the experiment. There is no significant difference between these two diets concerning fish growth performances (p > 0.05). The lowest growth was observed in D3 diet fish.

3.2 Nutrient efficiency
The feed utilization parameters are shown in Table 4. Feed conversion ratio (FCR) value was not significantly different among treatments (p > 0.05). Protein efficiency ratio (PER) was greater (2.75 ± 0.22) in fish fed with diet D1.

3.3 Biochemical body composition
The whole-body composition are given in Table 5. Body protein level ranged between 16.23 ± 0.03 % and 17.83 ± 0.08 %. Body lipid content ranged between 8.02 ± 1.50 % and 9.35 ± 0.39 %. Body ash content varied from 3.20 ±1.52 % to 4.37±0.28 %. There were no significant difference in the whole-body composition among treatment (P > 0.05). However, the body protein and lipid contents were affected by the experimental diets (P < 0.05).

Table 3: Growth performance parameters of *Heterotis niloticus* fingerlings fed trial diets

Diets	parameters					
	IBW (g)	**FBW (g)**	**WG (g)**	**DWG (g/day)**	**SGR (%/day)**	**Yield (t/ha/year)**
D1	144.44±19.24^a	610.55±15.03^a	466.11±23.65^a	8.63±0.44^a	2.67±0.24 ^{a}	8.75±0.79^a
D2	161.11±25.46^a	469.44±27.40^c	308.34±4.41^c	5.71±0.08^c	1.99±0.19^c	5.16±0.89^{bc}
D3	172.22±9.62^a	460±15.27^c	287.79±6.94^c	5.33±0.13^c	1.82±0.05^c	4.78±0.14^c
D4	172.33±9.81^a	592.78 ±11.70^a	420.45±5.06^a	7.78±0.09^a	2.29±0.07^b	8.53±1.08^a
D5	149.99±28.87^a	508.22±6.38^b	358.22±31.12^b	6.63±0.58^b	2.28±0.35^b	6.11±1.33^b
probability	ns	0.000001	0.0000001	0.000001	0.005624	0.00417

IBW: initial body weight, FBW: final body weight. Data are mean values ± SD (n = 3); means in the same row with the same superscript were not significantly different (P>0.05). ns = no significant difference.

Table 4: Nutrient efficiency parameters of *Heterotis niloticus* fingerlings fed trial diets

Diets	Parameters		
	FCR	**PER**	**VI (%/j)**
D1	1.26±0.07^a	2.75±0.22^a	3.35±0.03^a
D2	1.89±0.43^a	2.01±0.33^b	3.60±0.24^a
D3	2.02±0.03^a	1.85±0.04^b	3.61±0.09^a
D4	1.45±0.15^a	2.30±0.08^{ab}	3.55±0.12^a
D5	1.72±0.50^a	2.20±0.46^{ab}	3.68±0.29^a
Probability	*ns*	*0.0280*	*ns*

Data are mean values ± SD (n = 3); means in the same row with the same superscript were not significantly different (P>0.05). Ns = no significant difference.

Table 5: Proximate whole-body composition (% wet weight) of *Heterotis niloticus* fingerlings fed trial diets

	Initial	Diets				
		D1	**D2**	**D3**	**D4**	**D5**
Protein (%)	15.35±1.3	17.83±0.08^a	16.23±0.03^a	16.46±0.15^a	17.28±0.04^a	17.15±1.20^a
Lipid (%)	5.37±3.8	9.21±1.19^a	8.57±2.10^a	8.02±1.50^a	9.35±0.39^a	8.03±2.55^a
Moisture (%)	85.66±3.2	76.17±0.87^a	76.27±0.37^a	74.47±1.14^a	75.76±1.23^a	76.37±0.35^a
Ash (%)	5.31±0.61	4.37±0.28^a	3.37±1.12^a	3.27±1.28^a	3.18±0.28^a	3.20±1.52^a

Data are mean values ± SD (n = 3); means in the same row with the same superscript were not significantly different (P>0.05)

Discussion

At the end of this trial, the water physicochemical parameters remained relatively stable and have not been influenced by the diets tested. They were therefore compatible with the growth of *Heterotis niloticus* fingerlings. The survival rate at the end of trials, ranging from 88.88 ± 9.62 to 100 ± 0.00 %, did not significantly vary from one regimen to another. This makes it possible to attribute the registration of mortalities to the manipulations during the biometric checks instead of the composition of the trial diets since they occurred at the begining of the experiment and the day after the growth control. The lowest condition factor of around one (1) shows that the fish were all in better condition. It can be noted that, the overall, diets have registered profitable growth in fingerlings. The ingredients used to formulate experimental diets are found in the diet of the species in the wild. Fingerlings have therefore used the nutritional benefits of these foods to increase their growth performance. From all these diets, only fish fed the D4 diet achieved growth performance, food utilization performance and body biochemical composition very similar to those obtained by D1-fed fish that was better in all. The combination of fishmeal-earthworm meal in D4 diet formulation has certainly influenced the reasonable input of essential amino acids and other nutrients to fish. In fact, the raw protein (46.6%) and lipid (7.62%) contents of the earthworm meal used in this trial are closer to those reported by [10] who found crude protein levels between 60 and 70% and in lipids between 7 and 10% in the earthworm. [10] Asserts that the values found are comparable to those of fish and meat meal. Compared to these animal protein sources, earthworms are richer in essential amino acids, particularly lysine, methionine and cystine, in long-chain fatty acids, minerals and vitamins [10, 11, 12]. In *Heterotis niloticus*, the specific requirements for essential amino acids do not vary with the age of the fish. These requirements are closer to those of other omnivorous fish, with the exception of tryptophan and histidine, which are respectively lower and higher [13]. The D4 feed was therefore the only food a part from the control food D1, able to provide the fingerlings of *H. niloticus* with the essential nutrients (lipids, proteins, essential amino acids and minerals) for better zootechnical performances. The D2 diet enables to register low zootechnical performance in fish compared to that of D4. These results show that the nutritional value of earthworm meal can be improved by combining it with fishmeal in equal proportions. It is therefore possible to reduce or eliminate the amino acid deficiencies of *H. niloticus* fingerlings by a judicious combination of different by-products and a partial substitution of fishmeal by earthworm meal, rather than using this flour alone in place of fishmeal.

Compared to the D4 diet, the D5 diet has allowed fish to register lower growth and feed efficiently. The fish fed this diet voluntarily absorbed the feed with more envy, but the high FCR reveals that the conversion of this feed is relatively low. However, the difference in the formulation of the two diets is the maggot meal for D5 and the meal of earthworms for D4 which partially substituted the fishmeal. The protein (42.35%), lipid (12.7%) and ash (18.88%) contents of the maggot meal used are within the range of values proposed by [14, 15, 16]. These authors reported levels that vary between 37.2 and 55% for proteins, between 12.5 and 35.5% for lipids and between 7.15 and 11.65% for ashes. The above information shows that the lower growth performances registered in fish fed with D5 compared to D4 can come from its mineralogical composition which is itself related to the amount of maggot meal used. The diet D5 contains 11.5% of maggot meal. This amount of maggot meal in food could be too high and caused a disadvantage on the growth of *H. niloticus* fingerlings. Such a hypothesis has been justified by [17] in turkey poults with which he shows that the half substitution of fish meal by maggot meal in nutrition leads to a decrease of some essential nutrients except crude fiber. [18] Show that maggot meal can contribute up to 3% to the ration of poultry without compromising their performance and protein retention. The substitution of fishmeal for maggot meal rather than halfway should be at a lower level in the feed formulation for *H. niloticus* fingerlings. In the same trend, fish fed with Diet D3, containing 23% of maggot meal provided the lowest growth performances.

Conclusion

This study showed that expensive fishmeal can be replaced by earthworm meal in *H. niloticus* diet without negatively affecting growth performance. So, this protein source might be recommended in order to reduce fish production cost. Nevertheless, it would be interesting to analyze the effects of incorporating this protein in *H. niloticus* practical diets formulated with unconventional oil source.

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