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Complete replacement of fish meal by unconventional proteins sources in diet of *Oreochromis niloticus* (L., 1758) fingerlings: growth performance, feed utilization and body composition

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Abstract

For a period of 42 days, a total replacement of the fish meal by a mixture of unconventional sources of proteins (*Azolla filiculoides*, *Dialium guineense* leaf, brewers' yeast and maggot) was carried out for the of *Oreochromis niloticus* fingerlings. Five experimental diets isoproteic ($34.6 \pm 0.5\%$) reference diet and four test diets (A1 to A4) were used to feed *O. niloticus* fingerlings of average weight. At the end, the final mean weight (FW) and the daily weight gain (DWG) in the test diets (13.66 to 14.84g) were lower than those obtained by reference diet (18.45g) with $P < 0.05$. The same observation was made with the SGR ($1.17-1.33$ vs 1.83% day⁻¹) and PI ($4.24-4.85$ vs 5.80) in diets. The survival rate was not affected by the experimental diets, in the same way for the FCR and the PER, except the diet A1 ($P < 0.05$). Productive Protein Values (PPV) were between fin the reference diet A0 and 2 (0.39 vs 0.35 respectively) re are no significant difference with $P > 0.05$. Threonine appears with methionine like factors limiting in test diets.

Locally available unconventional proteins sources can totally replace fish meal without compromising growth performance, feed utilization and carcass traits but a close attention must be paid to methionine and threonine in order to satisfy nutrients requirements of *O. niloticus* fingerlings.

Keywords: Unconventional, isoproteic, limiting factor

1. Introduction

In the development and management of an aquaculture enterprise, fish feed plays a vital role in its growth and expansion [1] and constitutes 40–50% of the operational cost in the intensive and semi-intensive aquaculture system [2].

The fish meal, principal ingredient of aquafeed, is often used like principal source of proteins because of its high percentage of proteins and its composition in amino acids meeting the fish requirements [3]. Its use leads to a strong pressure of the man on the aquatic environments and thus of the production related to the captures. It is crucial to reduce or substitute totally the use of fish meal in fish diet by replacing it with alternative protein sources because total dependence can affect the whole operation of aquaculture system and consequently reduce the production.

Unconventional feed resources are credited for being noncompetitive in terms of human consumption, very cheap by-products or waste products from agriculture [4]. The sources of plant proteins, because of their very great availability, were the subject of several studies in the replacement partial or total of the fish meal in the food of several fish species [5-8]. Moreover, the sources of animal proteins like the termites, earthworms, the tadpoles, the snails, the maggots were used in the replacement of the fish meal with various conclusions [9-13].

Azolla filiculoides is natural aquatic fern, rich in proteins [14] with a good profile in amino acids [15, 16] and increasingly developed in the feed fish [17-19]. *Dialium guineense* is a plant of the family of the Fabaceae whose leaf is rich in proteins with good amino acid content [20]. Brewers' yeast and maggots rich in proteins in amino acids were tested in the feed fish [12, 21, 22]. Advantage of using these unconventional proteins sources products or collected is their easy availability and their low cost.

The present study aimed to evaluate the effect of total replacement of fish meal by mixing unconventional proteins sources such as *Azolla filiculoides*, *Dialium guineense* leaf, brewer's yeast and maggot as protein source in the diets of Nile tilapia (*Oreochromis niloticus*) in order to determine the growth performances and feed utilization.

2. Material Methods

2.1. Study Site and Experimental Fish and Design

The study was carried out in 42 days at the Laboratory of Research on Wet Lands, Faculty of Sciences and Technics (University of Abomey-Calavi, Benin).

Tilapia fingerlings (*O. niloticus*) were bought the Research, Innovations Centre, for Agriculture, Breeding and Fishing Development in Takon province. The fish were treated with a 2.5% NaCl solution for 20 min on arrival to eliminate ectoparasite infection. Fish were selected randomly from the

rearing tank, weighed and then transferred to the experimental tanks one week before the start of the experiment for acclimatization to experimental conditions. The experiment was undertaken with five different diets fed in triplicate. At the beginning and end of the experiment, each acclimatized fish was individually weighed using a digital scale. Fifty homogeneous fish with an average initial weight (IW) of 7.94 ± 0.02 g. fish⁻¹ were selected and distributed in triplicate into each tank for each treatment.

2.2. Experimental Diets

The experimental diets consisted of one reference diet and four test diets, and were formulated to meet the nutrient requirements of *O. niloticus* [23]. The reference diet contained fish meal as the main crude protein (CP) source, whilst in the four test diets 100% of the fish meal CP was replaced with CP from mixing alternative local ingredients (Table 1).

Table 1: Proximate composition of the reference diet (A0) and test diets (g.100 g⁻¹ dry matter) for *O. niloticus* fingerlings.

Ingredients	A0	A1	A2	A3	A4
<i>Azolla filiculoides</i>	0.0	10.0	10.0	10.0	10.0
<i>Dialium guineense</i>	0.0	9.0	8.0	7.0	6.0
Fish meal	48.0	0.0	0.0	0.0	0.0
Soybean meal	20.0	20.0	20.0	20.0	20.0
Brewer's yeast	0.0	10.0	10.0	10.0	10.0
Cottonseed meal	20.0	20.0	20.0	20.0	20.0
Wheat meal	7.0	5.0	5.0	5.0	5.0
Maggot meal	0.0	19.0	20.0	21.0	22.0
Palm oil	3.0	3.0	3.0	3.0	3.0
Vitamin mix ^a	1.0	1.0	1.0	1.0	1.0
Mineral mix ^b	1.0	1.0	1.0	1.0	1.0
Starch	0.0	2.0	2.0	2.0	2.0
Total	100.0	100.0	100.0	100.0	100.0
CP	34.4	34.1	34.4	34.8	35.1

^a Vitamin premix contains (g 100 g⁻¹ of premix): ascorbic acid, 50.0; D-calcium pantothenate, 5.0; choline chloride, 100.0; inositol, 5.0; menadione, 2.0; niacin, 5.0; pyridoxine HCl, 1.0; riboflavin, 3.0; thiamin HCl, 0.5; DL-alpha-tocopherol acetate (250 IU g⁻¹), 8.0; vitamin A acetate (20,000 IU g⁻¹), 5.0; vitamin micro-mix, 10.0; cellulose, 805.5. Vitamin micro-mix contains (g kg⁻¹ of micro-mix): biotin, 0.5; cholecalciferol (1 µg = 40 IU), 0.02; folic acid, 1.8; vitamin B₁₂, 0.02; cellulose, 97.66.

^b Mineral premix contains (g kg⁻¹ of premix): calcium phosphate (monobasic) monohydrate, 136.0; calcium lactate pentahydrate, 348.49; ferrous sulfate heptahydrate, 5.0; magnesium sulfate heptahydrate, 132.0; potassium phosphate (dibasic), 240.0; sodium phosphate (monobasic) monohydrate, 88.0; sodium chloride, 45.0; aluminum chloride hexahydrate, 0.15; potassium iodide, 0.15; cupric sulfate pentahydrate, 0.50; manganese sulfate monohydrate, 0.70;

cobalt chloride hexahydrate, 1.0; zinc sulfate heptahydrate, 3.0; sodium selenite, 0.011.

2.3. Test Feed Ingredients

The chemical composition of the test feed ingredients is shown in Table 2. Cottonseed meal, wheat bran, soybean meal, palm oil and starch produced locally were purchased at the provender "La Qualité" of Abomey-Calavi. The fish meal used was obtained after milling fish (*Sardinella maderensis*) bought at Dantokpa market. Brewer yeast was collected as wasted production from private companies producing beer "SOBEBRA". The powder of *Azolla filiculoides* and *Dialium guineense* were respectively dried following the production method of [24]. Maggot produced according to [25] at the Research Station was washed, lyophilized (dried cold) before use.

Table 2: Proximate chemical composition (g.100 g⁻¹ dry matter) and essential amino acid (g.100 g⁻¹ dry matter) content of the test ingredients.

	Fish meal	<i>Azolla filiculoides</i>	<i>Dialium guineense</i>	Brewer's yeast	Maggot
Crude protein	66.2	27	22.05	50	54.6
Lipid	15.2	2.9	3.4	3.7	15.7
Ash	11.3	12.64	12.87	13.18	10.95
Essentials Amino Acids					
Threonine	2.31	0.67	0.71	2.40	2.09
Valine	2.77	0.53	0.79	2.80	1.91
Methionine	1.94	0.00	0.29	0.80	1.82
Isoleucine	2.45	0.33	0.50	2.30	3.05
Leucine	3.79	1.13	1.36	3.50	6.35
Phenylalanine	3.74	0.93	1.36	2.10	3.53

Histidine	1.75	0.47	0.64	1.20	3.01
Tryptophan	0.57	0.40	0.29	0.70	3.17
Lysine	4.22	0.73	1.14	3.80	4.23
Arginine	3.43	1.13	1.29	2.60	6.06
Total	26.97	6.32	8.37	22.2	35.22

2.4. Diet Preparation and Feeding

The feed was produced by careful mixing of the dry ingredients before adding palm oil and distilled water. Water was then added at 50% of dry matter to obtain a malleable paste. This paste is passed through a grinder (Moulinex HV8) and has given filaments of spaghetti with 1-2mm diameter. The manufactured foods are sun-dried for 24 h and fragmented to the desired size before being placed in storage boxes for packaging (temperature 4 °C) until the distribution. The fish were fed by hand with 5% of body weight per day at 8.00 h, 11.00 h, 14.00 h and 17.00 h.

2.5. Experiment Facilities

A device of 15 concrete circular tanks (3 per treatment) was used to perform the experiment. Each tank is half covered of its surface with a rack in order to avoid direct solar penetration that favors large variations in the temperature of the water but also the development of algal chlorophylls. The ponds are supplied with water from a drilling conducted on the station. Each tank is filled with a volume of 150 L of water and the water exchange rate is 3Lminute⁻¹. Aeration with one air-stone diffuser was provided to individual tanks via a low pressure electrical blower. In addition, the walls of tanks in the culture system were cleaned by manual scrubbing to remove slime from fish and microbes twice a week. Once per week, the total number of survivors in each tank was counted and fish biomass determined.

2.6. Chemical Analysis

The feed ingredients were analysed following [26] procedures: crude protein was assessed by the Kjeldahl method after an acid digestion using Kjeltac 2300 Auto Analyser, of mark Tecator Höganäs, Sweden. The amino acids of feed ingredients were analysed with a Waters HPLC system (Waters 474, Waters, Milford, MA, USA) including two pumps (Model 515, Waters), an auto sampler (Model 717, Waters), a fluorescence detector (Model 474, Waters) and a temperature control module. These amino acids analyses were done following the method previously described by [27]. Aminobutyric acid was added as an internal standard before hydrolyzation. Amino acids were derivatized with AQC (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate) and then separated with a C-18 reverse-phase column Waters Acc. Tag (150 mm × 3.9 mm). All of these analyses in duplicate were conducted in the Laboratory of Aquatic Animal Nutrition of Kagoshima University (Japan).

2.7. Water Quality Monitoring

Temperature (T °C) was recorded every three days with a thermometer at 8.00 h and 14. 00 h. Dissolved oxygen (DO mg L⁻¹) and pH were measured twice per week by oxymeter and pH meter respectively. Nitrite nitrogen (mg L⁻¹), nitrate nitrogen (mg L⁻¹) and total ammonia nitrogen (mg L⁻¹) were measured twice a month using the Hach Lange cuvette test method (DR 2800 visual spectrophotometer, Hach Lange GmbH, Germany).

2.8. Calculations

The following calculations were made:

Specific Growth Rate (SGR% day⁻¹) = [(ln FW – ln IW)/ T] × 100 and Daily Weight Gain (DWG) = (FW – IW) / T, where FW and IW refer to the mean final weight and the mean initial weight, respectively, and T is the feeding trial period in days.

Survival Rate (SR%) = [(Nf / Ni) × 100], where the Nf is final total number of fish and Ni is initial total number of fish.

Protein Intake (PI) per fish = [total feed intake (g) × protein in the diet (%)] / Nf.

Feed Conversion Ratio (FCR) = [total feed intake (g) / total weight gain (g)].

Protein Efficiency Ratio (PER) = [total weight gain (g) / protein intake].

Protein Productive Value (PPV) = body protein gain / protein intake

2.9. Statistical Analysis

Data obtained from the experiment were subjected to one-way analysis of variance after verifying the normality and the homogeneity of variance using the STATVIEWS (version 5.01). Least-Significant-Difference test of Fisher was used to compare differences among individual means. Treatment effects were considered significant at $P < 0.05$

3. Results

The water quality parameters monitored during the experimental period showed that water temperature (°C) was 28.0 ± 0.5 (27.6–28.5) whereas pH and DO content (mg.L⁻¹) were 5.09 ± 0.27 (4.82–5.36) and 5.43 ± 0.44 (4.82–5.87) respectively. Total ammonia (TAN = $0.15 \text{ mg.L}^{-1} \pm 0.1$ (0.1–0.3), nitrite (NO₂⁻ = $0.05 \text{ mg.L}^{-1} \pm 0.02$ (0.03–0.1) and nitrate (NO₃⁻ = $0.8 \text{ mg.L}^{-1} \pm 0.01$ (0.4–1.2) concentrations were low during the experiment.

The CP content feed ingredients was highest in fish meal, followed in descending order by maggot meal, brewer's yeast, *Azolla filiculoides* meal and *Dialium guineense* leaf meal (Table 2). Lipid content was highest in animal sources of protein such as maggot meal followed fish meal, whilst the ash content showed different pattern amongst the feed ingredients. The lowest values was obtained with *A. filiculoides* meal, *D. guineense* leaf meal and brewer's yeast while in ash, there were fish meal and maggot meal. The essential amino acid (EAA) profiles varied amongst feed ingredients (Table 2). In general, most individual EAA were low in *A. filiculoides* meal and *D. guineense* leaf meal was high fish meal, brewer's yeast and maggot meal. The chemical composition and EAA profiles were different amongst diets, with a crude protein content of 30.3-31.8 g.100 g⁻¹, lipid content of 4.98-6.13 g.100 g⁻¹ and total EAA content of 22.2-49.5 g.100 g⁻¹. The test diets (A1 to A4) were only deficient methionine and threonine contrary the reference diet A0 but the diet A1 was deficient in several essential amino acids (Table 3).

Table 3: Chemical composition and amino acid (g.100 g⁻¹ dry matter) content of the reference diet (A0) and test diets.

	A0	A1	A2	A3	A4	<i>O. niloticus</i> requirements ^[23]
Crude protein (%)	31.8	30.3	30.9	30.5	30.5	
Lipid (%)	5.21	6.61	6.13	4.98	5.09	
Ash (%)	9.82	13.96	12.38	12.13	11.24	
<i>Essentials Amino Acids</i>						
Threonine	4.0	1.8	3.0	3.0	3.0	3.8
Valine	4.5	1.8	2.9	2.9	2.9	2.8
Methionine	3.0	0.6	1.6	1.6	1.7	2.1-2.8
Isoleucine	5.2	2.7	4.4	4.5	4.5	3.1
Leucine	7.0	3.4	7.0	7.0	7.1	3.4
Phenylalanine	6.8	2.8	4.8	4.8	4.8	3.8
Histidine	3.3	1.5	3.2	3.3	3.3	1.7
Tryptophan	1.3	0.9	2.7	2.7	2.8	1.0
Lysine	7.0	2.7	5.1	5.1	5.2	5.1-5.7
Arginine	7.4	4.0	7.5	7.5	7.6	4.0-4.2
Total	49.5	22.2	42.2	42.4	42.9	

In table 4, the growth performances in test diets such as FW, SGR and DWG were similar but different for the reference diet ($P<0.05$). Moreover, the values of these parameters in diet A2 were numerically highest the other test diets except the diet A3. The feed conversion ratio (FCR) ranged from 1.71 to 2.59 with the best FCR in A0 (reference diet). Only the test diet A1 was significantly different with the reference diet ($P<0.05$). There were no differences ($P>0.05$) in survival rate between the reference diet and the test diets, but protein intake differed between diets. Protein efficiency ratio (PER) was not significantly different between diets except the test

diets A1 and A4 which were different with reference diet A0 ($P>0.05$). The values of PPVs were different among fish fed experimental diets except between the diet A0 and A2. The proximate composition of the whole fish body is given in Table 5. All fish displayed a change in the whole body composition (compared with that at the start of the experiment), which consisted mainly in an increase in all parameters of composition of fish. The protein content increased in all dietary treatments, but particularly in fish fed diet A2.

Table 4: Growth performance and feed utilization of *O. niloticus* fingerlings fed the reference diet (A0) and the test diets (A1 to A4).

Parameters	A0	A1	A2	A3	A4
IW	7.96±0.02	7.92±0.01	7.93±0.02	7.93±0.00	7.94±0.01
FW	18.45±0.89 ^a	14.28±0.07 ^b	14.33±1.40 ^b	14.84±1.07 ^b	13.66±0.26 ^b
FCR	1.71±0.02 ^a	2.59±0.293 ^b	2.16±0.45 ^{ab}	2.28±0.17 ^{ab}	2.30±0.17 ^{ab}
SGR	1.83±0.03 ^a	1.17±0.09 ^b	1.33±0.21 ^b	1.28±0.09 ^b	1.23±0.06 ^b
DWG	0.25±0.02 ^a	0.15±0.00 ^b	0.15±0.03 ^b	0.16±0.02 ^b	0.14±0.00 ^b
PER	1.13±0.03 ^a	0.76±0.11 ^b	1.16±0.04 ^a	0.84±0.07 ^b	0.88±0.08 ^b
SR	96±3.33	94±4.00	98±1.11	96±4.00	98±1.11
PI	5.80±0.27 ^a	4.85±0.23 ^b	4.24±0.36 ^b	4.86±0.37 ^b	4.43±0.07 ^b
PPV	0.39±0.03 ^a	0.23±0.01 ^b	0.35±0.05 ^{ac}	0.30±0.03 ^c	0.31±0.02 ^c

Mean values in the same line followed by the same superscript are not significantly different ($P>0.05$).

Table 5: Proximate composition (% fresh matter basis) of the carcass of *O. niloticus* fed experimental diets

Parameters	Initial	Experimental diets				
		A0	A1	A2	A3	A4
Protein	14.93	17.64±0.09 ^a	16.06±0.26 ^b	16.41±0.61 ^{ac}	16.33±0.48 ^c	16.28±0.32 ^c
Lipid	5.66	6.94±0.13 ^a	5.98±0.66 ^b	6.76±0.19 ^a	6.79±0.73 ^a	6.39±0.45 ^c
Ash	4.12	4.39±0.08	4.28±0.45	4.36±0.21	4.31±0.77	4.27±0.24

Mean values in the same line followed by the same superscript are not significantly different ($P>0.05$).

4. Discussion

Water quality parameters were not significantly different between treatments and were within the recommended range for the culture of *O. niloticus* [28]. The good water quality observed during the experimental period probably favored the higher survival rate of *O. niloticus* fed all diets.

The brewers' yeast, *Azolla filiculoides* and *Dialium guineense* leaf are very available in Benin. Moreover, the maggots can be produced from agro alimentary waste [25] and very good sources of proteins [21, 29] with a good essential amino acid contents. Moreover its very short cycle of production makes of him an excellent ingredient in the total replacement of the fish meal in the fish food.

Unconventional proteins sources in diets did not affect the

survival of *O. niloticus* (survival rate 94%) indicating that the test diets did not have any major negative effects on fish health. Total replacement of fish meal by unconventional proteins sources (*A. filiculoides* meal, *D. guineense* leaf meal, brewer's yeast and maggot meal) has not been tested in *O. niloticus* fingerlings at our knowledge. By the end of the growth trial, groups fed test diets (A1 to A4) had a final mean weight and protein intake (PI) lower to the reference diet A0. These results can be explained by ingestion, the digestion and the presence of antinutritional factors. According to [30, 31], the plants contain substances of various natures which can disturb the appetite, digestion, the absorption of the nutrients and the metabolism of the animals, affecting their growth and sometimes their health. The digestibility of animal or

vegetable origin proteins is generally high in the fish (> 90%)^[23]. But the vegetable ingredients can reduce digestibility because of certain factors anti-nutritional like fibres and tannins^[31] and limit bioavailability of some amino acids^[32]. In general, the chemical composition and amino acid profile of test ingredient were in good agreement with published data^[23] except the diet A1. The crude protein content (34.1-35.1% CP) of this experimentation was within the range (25-35% CP) required for normal growth rate in *Oreochromis niloticus* fingerlings, but lower than the CP content required (40% CP) for maximum growth rate^[33]. The dietary content of arginine (4-7.6 g 100g⁻¹), histidine (3.2-3.3 g 100g⁻¹), leucine (3.4-7.1 g 100g⁻¹), isoleucine (4.4-5.2 g 100g⁻¹), tryptophan (1.3-2.8 g 100g⁻¹), lysine (5.1-7.0 g 100g⁻¹), phenylalanine (4.8-6.8 g 100g⁻¹) and valine (2.9-4.5 g 100g⁻¹) exceeded the requirements of *O. niloticus* except diet A1 whilst the dietary content of threonine (1.8-3.0 g 100g⁻¹) and methionine (0.6-1.7 g 100g⁻¹) did not meet the requirements of *O. niloticus* except a reference diet A0^[23]. According to^[34], when the food contribution in amino acids does not meet perfectly the needs for the animal, nitrogenized catabolism increases, the proteinic retention is reduced and thus the growth is slowed down and the increased rejections nitrogenized, which takes part in the pollution of the aquatic environment. The results showed that complete replacement of fish meal by unconventional protein source have differences in terms of growth performances and feed utilization between reference diet A0 and test diets (A1 to A4). These results are in agreement to earlier published studies in which *Heterobranchus longifilis* reduced growth when fish meal was completely replaced by crab meal^[35], *Clarias gariepinus* when an alternative animal protein mixture such as hydrolyzed feather meal, chicken offal meal and maggot meal was evaluated to replace fish meal totally^[36, 37]. Showed also that the growth performances and feed utilization was reduced when kikuyu grass and moringa leaves was used as protein sources in *Tilapia rendalli* diets to substitute fish meal at 100%. In contrast^[38], showed that fish meal protein in feed for striped catfish (*Pengasianodon hypophthalmus*) fingerlings can be replaced with protein from locally available plant and animal ingredients without compromising growth performance, feed utilization and carcass traits. Differences in protein intake account for a portion of the reduced fish weight gain, but differences in FCR and PPV except the diet A2 suggest that other essential nutrients may be limiting or that utilization of dietary protein and/or protein turnover may be altered in *O. niloticus* fed diets containing unconventional proteins sources. In addition, the results obtained with the test diets can be due to deficiency in threonine and methionine. Methionine is one of the first limiting amino acid in fish diets. According to^[39], an indispensable amino acid deficiency may cause reduced growth and poor diet conversion^[40]. Have asserted that after lysine and methionine, threonine is often the next limiting indispensable amino acid (IAA) in plant protein sources.

This work enables us to affirm that apart the profile in essential amino acids of the test diets, it is significant to take account of their digestibility in the formulation in order to obtain good growth performances and feed utilization. This study corroborate work of^[41] which affirmed that to formulate food with more precision and to predict the growth performance of the animal, it is suggested rather basing the formulations on digestibility than on the profile in essential amino acids.

The diets based maggot meal, brewer's yeast, *A. filiculoides* meal and *D. guineense* leaf meal as proteins sources enhance growth and thereby yield of *Oreochromis niloticus*. The results of this study showed that fish fed fish meal diet (A0) had the best growth than the fish fed diets based on unconventional protein sources as a complete fish protein replacement. The essential amino acids such as methionine and threonine appear as the limiting in the test diets. Also, it is important to determine the digestibility of unconventional proteins sources in order to improve the growth performances and feed utilization of fish.

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