

A Mixture of Chicken Viscera, Housefly Larvae and Spirulina Waste as Replacement of Fishmeal in Nile Tilapia (*Oreochromis niloticus*) Diets

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Abstract

This research investigated different blends of spirulina waste (SW), chicken viscera meal (CVM) and housefly maggot meal (HMM) as alternative protein sources on growth performance, feed utilization and body composition of *Oreochromis niloticus* fingerlings. Triplicate groups of male fish (initial mean weight, 6.09±0.5g) were fed a commercial feed Skretting (SK), fish meal (FM) based-diet D₀ (30%FM), diet D₁ (FM+SW+HMM), diet D₂ (FM+SW+CVM), diet D₃ (FM+HMM+CVM) and diet D₄ (SW+HMM+CVM) for 84 days. Diets were isonitrogenous (35% crude protein) and isoenergetic (19 KJ/g gross energy). No effects were found on survival rate and body protein content of fish fed experimental diets. Specific growth rate, weight gain, feed conversion ratio and protein efficiency ratio (PER) of fish fed D₀ and SK did not differ significantly from those fed diet D₃. These parameters were significantly lower in fish fed the other diets (P<0.05). Feed intake significantly decreased in fish fed diet D₁ and D₂ while PER obtained did not differ than those fed control diets. The higher profit index was recorded in the tested diets and the lower in the control diets. The results of this study indicate that fishmeal level for *O. niloticus* could be reduced to 5 % by inclusion of CVM and HMM in combination.

Introduction

Diets are one of the major cost variables for most aquaculture species, representing up to 70% of the total production cost (Tacon & Metian, 2015). Fish meal is used as a major protein and considered the most desirable animal protein ingredient in fish diets because of its high protein content, balanced amino acid profiles, essential fatty acids, high digestibility and palatability, attractants and many growth factors (Médale & Kaushik, 2009). Due to the high cost of fish meal, limited availability and other considerations, there is interest in the partial or total replacement of this ingredient with less expensive animal and plant protein meals, without adversely affecting growth and health of cultured species (Hardy, 1996). It is consequently essential to introduce alternative protein sources, to ensure the prospective growth of this sector. Effective substitution

of fishmeal by alternatives protein would minimize both the full reliance on fishmeal as raw material and its high cost. Several researchers reported the use of plant by-product as dietary protein source either alone or in combination with other feed ingredients in formulating the fish diets. Plant by-product sources have been extensively studied as partial or total fish meal replacements in fish feeds. A major drawback in the use of plant proteins is the presence of anti-nutritional factors in fish culture (Gatlin *et al.*, 2007). They are deficient in one or more essential amino acids (NRC, 1993). However, the use of some food processing techniques reduces the effect of anti-nutritive factors (Fapohunda, 2012). The essential amino acid compositions of alternative protein sources for fish are generally not comparable with that of fish meal. (Agbo *et al.*, 2015). Moreover, most of the single animal ingredient were inadequate to totally replace fishmeal

(Tacon & Jackson, 1985). Therefore, combining different alternative protein sources which possess varying amino acids that could supplement each other has been strongly recommended to surmount these drawbacks (Luo *et al.*, 2011).

One readily available and renewable ingredient is chicken viscera, which is a by-product of the poultry processing industry that is rich in protein (35.2 %) and contains a favourable profile of indispensable amino acids for fish production (Giri *et al.*, 2010; Alofa & Abou, 2020). Its lower price makes it an ideal candidate for replacing fish meal (FM) in aquafeeds (Thompson *et al.*, 2012). Another sustainable oncoming is to convert organic waste into high nutritive value biomass such as insect larvae. Insect meal contains approximately 44.0–49.0% crude protein and good digestibility; these make it a precious protein source (Henry *et al.*, 2015). The potential of using of chicken viscera and maggot meals as dietary protein sources have been tested singly in a wide range of fish species (Giri *et al.*, 2010; Tabinda *et al.*, 2013; Wang *et al.*, 2017; Alofa *et al.*, 2020; Alofa & Abou, 2020).

On the other hand, *Spirulina platensis* is a microalga rich in protein (60–70 % in dry matter), essential amino acids, vitamins, minerals, essential fatty acids (linoleic acid, α -linolenic acid, and palmitic acid), and antioxidant pigments, such as β -carotene, xanthophylls, zeaxanthin, echinenone and cryptoxanthin (Abdel-Tawwab & Ahmad, 2009; Teimouri *et al.*, 2013). Besides to its well nutrient content, it is involved in the modulation of the immune response (Takeuchi *et al.*, 2002), physiological activity, stress response and disease resistance (Güroy *et al.*, 2011). The effects of whole spirulina meal on growth performance, feed intake and nutrient utilization have been investigated for many fish species (Takeuchi *et al.*, 2002; Palmegiano *et al.*, 2005; Palmegiano *et al.*, 2008). Because the cost of spirulina is not competitive with other protein sources (Palmegiano *et al.*, 2005), its waste can be used with other ingredients in an aquafeed formulation. However, the use of spirulina waste in the diet of *O. niloticus* induces the poorest growth, due to the decrease in feed intake observed in this fish (Alofa *et al.*, 2020). Commercial aquafeed formulations use a mixture of alternative protein sources to substitute fishmeal with balanced amino acid profile, yet most of the studies assessing these ingredients singly to replace fishmeal (Gatlin *et al.*, 2007). Varying degrees of the hit has been reached when trying to replace fishmeal protein with plant or animal protein sources in Nile tilapia. When combined with alternative protein sources, poultry by-products meal has been shown to completely replace FM in juvenile tilapia diets without adverse effects on growth (El-Sayed, 1998). Using a mixture of several animal or plant protein ingredients, to substitute fishmeal in fish feed has been demonstrated successfully (Guo *et al.*, 2007; Adewolu *et al.*, 2010).

Oreochromis niloticus has many attributes, these include fast growth, tolerance to a wide range of environmental conditions, resistance to stress and diseases, ability to reproduce in captivity and short generation time and feeding on low trophic levels and acceptance of artificial feeds immediately after yolk-sac absorption, that make them an ideal candidate for aquaculture, especially in developing countries (Bhujel, 2014). Several studies successfully determined the possibility of dietary fishmeal replacement by maggots meal, chicken viscera or spirulina separately in diets for Nile tilapia (Wang *et al.*, 2017; Alofa & Abou, 2020; Alofa *et al.*, 2020). However, the use of a combination of spirulina waste with other animal proteins as an alternative protein source in diets for this species has previously not been reported. Thus, the current experiment was carried out to evaluate the effect of replacing fishmeal by animal protein (housefly maggot and chicken viscera) mixture with spirulina waste on growth performance, feed utilization and whole-body composition of *O. niloticus* fingerlings.

Materials and Methods

Experimental Procedure and Fish Source

This experiment was conducted at the Experimental Fish farming Unit of Laboratory of Ecology and Aquatic Ecosystems Management at the University of Abomey-Calavi, Benin. Fish were reared in concrete tanks (diameter= 1.2 m ; volume = 1000 L) in an outdoor recirculation system.

All-male populations of tilapia were obtained from a fish rearing farm ("Dieu Exauce", Tori Avamè, Benin). They were acclimated for one week before being randomly distributed into eighteen concrete tanks (six treatments in triplicate) with 50 fish per tank. Each diet was randomly attributed to triplicate groups of monosex male *O. niloticus* (6.09±0.5 g). Fish were hand-fed thrice a day (09:00 h, 13:00 h and 17:00 h) to apparent satiation and the amount of feed consumed recorded for each tank. The uneaten feed was collected 1 h after feeding, dried and weighed, in order to evaluate the real feed intake. Water was pumped to each tank at a rate of 4 L per minute from biofilter with a capacity of 5 m³. The photoperiod was 12 hours dark and 12 hours light (7:30 - 19:30 h) during experiment and tanks were covered two-thirds of their surface by racks to prevent algal development. Dead fish, if any, were removed from each tank and recorded. Every two weeks, fish were counted and weighed, tanks were cleaned of faeces and the half of rearing water was exchanged.

Ingredients and Formulation of the Diets

Housefly *Musca domestica* larvae were produced from chicken viscera substrates as described by Alofa and Abou (2020) and refrigerated at -20 °C. Then, they were dried in an oven (Memmert UN160 Plus) at 60°C

before being ground to a homogeneous size and stored in a refrigerator at -4°C before proximate analysis. Fishmeal was made by sun-drying *Sardinella* sp during three days before being ground. *Sardinella* sp were purchased from Dantokpa market. Spirulina (*Spirulina platensis*) wastes were provided by Spirulina Production Unit of the Regional Institute for Development and Health (SPU/RIDH), located at Pahou (Ouidah, Benin). These wastes were generated from the production and packaging process of spirulina. Blood meal was obtained following the procedures described by Alofa *et al.* (2016). The rest of the ingredients such as corn bran, soybean meal, cottonseed meal, palm oil and salt were purchased from local market in Abomey-Calavi.

Five isonitrogenous (35% crude protein) and isocalorics (19 kJ.g^{-1}) diets were formulated using different combinations of spirulina waste (SW), housefly maggot meal (HMM) and chicken viscera meal (CVM) as protein sources for this experiment. Commercial diet Skretting was also used as a reference diet. Chemical composition of dietary protein sources used for diet formulation is shown in Table 1.

Fish meal was substituted with different combinations of SW, CVM and HMM at 83.33 % and 100% of total protein. Diets were denoted SK (commercial diet Skretting), D₀ (fishmeal based-diet), D₁ (5% FM + 10% SW + 25% HMM), D₂ (5% FM + 10% SW + 28% CVM), D₃ (5% FM + 20% HMM + 20% CVM) and D₄ (5% SW + 20% HMM + 20% CVM). Blood meal, soybean meal, cottonseed meal and corn bran levels were adjusted accordingly. Dietary protein, lipid and gross energy were adjusted to satisfy the nutritional requirement of Nile tilapia (Jobling, 2012). Formulation, proximate composition and amino acids contents of the experimental diets are presented in Tables 2 and 3 respectively. Amino acids were estimated based on the amino acid proximate composition of the ingredients used in the formulation of the experimental diets. Prices of different ingredients used in the formulation of experimental diets are shown in Table 2.

All dry ingredients were ground using a hammer mill. Salt (mineral) and oil were added to the dry ingredients and were thoroughly mixed with warm water before being pelleted into sizes of 3 mm diameter using a pelleting machine (Bosch MFW3640A). The pellets were then sun-dried for 48 h and later stored in the refrigerator (4°C) until use.

Calculations

At the end of the experiment, fish were weighed and their total body length was also recorded individually. Survival rate (SR), specific growth rate (SGR), percent weight gain (PWG), feed conversion ratio (FCR), protein efficiency ratio (PER), condition factor (CF), feed intake (FI), yield, production, incidence cost (IC) and profit index (PI) were evaluated through the following formulae :

$$\text{Survival rate (SR, \%)} = \frac{\text{final number of fish}}{\text{initial number of fish}} \times 100$$

$$\text{Percent weight gain (PWG, \%)} = \frac{\text{final weight} - \text{initial weight}}{\text{initial body weight}} \times 100$$

$$\text{Daily weight gain (DWG, g/day)} = \frac{\text{final weight} - \text{initial body weight}}{\text{initial body weight}}$$

$$\text{Specific Growth Rate (SGR, \%)} = \frac{\ln(\text{final weight}) - \ln(\text{initial weight})}{\text{rearing period in days}} \times 100$$

$$\text{Feed intake (FI, g/fish)} = \frac{\text{total amount of the dry feed consumed}}{\text{number of fish}}$$

$$\text{Feed Conversion Ratio (FCR)} = \frac{\text{total dry feed consumed (g)}}{\text{body weight gain (g)}}$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{body weight gain (g)}}{\text{protein intake (g)}}$$

$$\text{Fulton's Condition Factor (CF)} = \frac{\text{final body weight (g)}}{\text{final body length (cm)}^3}$$

$$\text{Yield (kg/m}^3\text{)} = \frac{\text{final biomass per tank (kg)} - \text{initial biomass per tank (kg)}}{\text{volume (m}^3\text{)}}$$

$$\text{Production (kg/m}^3\text{/year)} = \frac{\text{Yield} \times 365 \text{ days}}{\text{rearing period (days)}}$$

$$\text{Incidence of cost (IC)} = \frac{\text{Cost of feed}}{\text{kilogram of fish produced}}$$

$$\text{Profit Index (IP)} = \frac{\text{Price of fish produced}}{\text{Price of feed consumed}}$$

Monitoring of Water Quality Parameters

Parameters such as temperature ($^{\circ}\text{C}$), pH, dissolved oxygen (mg/L), salinity (psu), conductivity ($\mu\text{S/cm}$) and total dissolved solids (mg/L) were

Table 1. Analysed biochemical composition (% dry matter) of ingredients used for diet formulation

Protein sources	Chemical composition (% dry matter)			
	Dry matter	Crude protein	Crude fat	Ash
<i>Sardinella</i> sp Fishmeal	92.0	66.0	7.9	15.8
Chicken viscera meal	90.9	35.2	22.0	6.3
Spirulina wastes	91.5	46.3	6.7	10.0
Maggot meal	92.7	48.8	21.0	6.3
Soybean oilcake	94.8	30.0	13.2	3.7
Cottonseed oilcake	90.0	40.5	7.0	8.0
Blood meal	90.9	71.9	1.7	6.4

measured weekly at a depth of 10 cm with a multiparameter (Hanna HI 9829, v1.04 USA). These parameters were checked three times biweekly. Water samples were taken inside the tanks and nitrate-N, and nitrite-N were measured once weekly with a DR 6000 spectrophotometer (Hach, Loveland, Co, USA).

Sampling for Fish Whole Body Proximate

The initial sample of twenty fish was randomly selected and stored at -20°C for whole body composition analysis. At the end of the experiment, the total fish number from each tank were counted and individual body length and weight were measured to calculate Fulton' Condition factor (Nash *et al.*, 2006). Ten fish from each tank were randomly selected for analysis of the whole body composition. Fish were oven-dried, finely grounded, and homogenized prior to analysis.

Biochemical Analysis

Proximate composition of ingredients, experimental diets and fish samples were determined according to standard procedures (AOAC, 2005). Dry matter was measured by drying samples in an oven (Memmert UN160 Plus) at 105°C for 24 h. Ash was determined by incineration at 550°C in a furnace (Nabertherm LT/SW) for 16 h. Crude protein was determined by using the Kjeldahl method and estimated by multiplying nitrogen by 6.25. Crude lipid content in samples was determined by Soxhlet method.

Statistical Analysis

Data are presented as mean±standard deviation (SD). The analyses were performed using Statistical Package for Social Sciences (SPSS IBM version 21 for windows v8.1, Chicago, Illinois, USA). The growth,

Table 2. Formulation and proximate composition of the experimental diets (SK : commercial diet Skretting, D₀ : 30% FM -Control diet, D₁ : 5%FM + 10%SW+25%MM, D₂ : 5%FM+10% SW+28% CVM, D₃ : 5%FM+20% CVM+20% MM, D₄ : 10% SW+20% CVM+20% MM) for Nile tilapia *O. niloticus*

Experimental diets		SK	D ₀	D ₁	D ₂	D ₃	D ₄
Ingrédients g 100 g ⁻¹	Prices						
<i>Sardinella sp</i> fishmeal	2.17	-	30	5	5	5	-
Spirulina waste	0.42	-	-	10	10	-	5
Housefly maggot meal	0.43	-	-	25	-	20	20
Chicken viscera meal	0.26	-	-	-	28	20	20
Blood meal	0.21	-	7	7	7	7	7
Corn bran	0.26	-	34	24	14	18	14
Soybean meal	0.65	-	15	15	18	15	17
Cottonseed meal	0.32	-	11	11	15	12	14
Palm oil	1.34	-	2	2	2	2	2
Salt (NaCl)	0.42	-	1	1	1	1	1
Proximate composition							
Dry matter (%)		-	90.18	90.11	88.95	89.98	89.92
Crude protein (% DM)		35	35.89	35.6	35.16	35.61	35.47
Crude lipid (% DM)		9	8.29	11.71	13.21	14.3	14.15
Carbohydrates (% DM)		-	47.77	46.16	42.18	41.87	42.21
Ash (% DM)		6.5	8.04	6.52	9.44	8.20	8.16
Gross Energy (kJ g ⁻¹) ^a		-	20.00	21.00	20.80	21.29	21.26
Diet cost (US\$.kg ⁻¹) ^b		1.84	0.98	0.56	0.53	0.54	0.46

a. Gross energy was calculated using the factors of 23.7 kJg⁻¹, 39.5 kJg⁻¹ and 17.2 kJg⁻¹ protein, lipids and carbohydrates respectively (Guillaume *et al.*, 1999).

b. Prices in US\$, 1 US\$= 599.05 FCFA at present. Including

Table 3. Calculated essential amino acid (EAA) contents of the experimental diets (% of dietary protein)

Essential amino acids	Experimental diets					Requirement (% of dietary protein)*
	D ₀	D ₁	D ₂	D ₃	D ₄	
Arginine	5.54	6.09	6.43	5.92	5.98	4.20
Histidine	2.67	2.53	2.51	2.56	2.58	1.72
Isoleucine	2.98	3.17	3.32	3.17	3.47	3.11
Leucine	7.16	6.70	6.49	6.50	6.87	3.39
Lysine	4.91	4.90	5.08	5.34	5.25	5.12
Methionine	1.94	2.04	2.15	2.11	2.13	2.68
Phenylalanine	5.08	5.49	5.91	5.66	5.53	3.75
Threonine	3.46	3.41	3.48	3.38	3.70	3.75
Tryptophan	1.19	1.13	0.97	1.11	0.98	1.00
Valine	4.52	4.41	4.40	4.33	4.64	2.80

* Essential amino acids requirements of Nile tilapia *O. niloticus* according

nutrient utilization and biochemical composition values were analyzed using one-way analysis of variance (ANOVA 1). The Tukey's multiple range test was used to compare differences among treatment means. Prior analysis, homogeneity of variance was determined using the Hartley statistical test. The differences were considered significant at $P < 0.05$.

Results

Water Quality

Water quality parameters values during the feeding experiment are presented in Table 4. The water temperature ranged from 29.88 ± 0.68 to 30.36 ± 1.49 °C, pH from 6.65 ± 0.30 to 6.82 ± 0.30 ; dissolved oxygen from 3.54 ± 0.67 to 3.74 ± 0.80 mg.L⁻¹; conductivity from 179.79 ± 89.08 to 185.65 ± 86.56 µS/cm; total dissolved solid from 92.50 ± 46.85 to 94.02 ± 45.27 ppm; salinity from 0.08 ± 0.039 to 0.08 ± 0.040 mg.L⁻¹; nitrate from 2.23 ± 0.38 to 2.73 ± 0.35 mg.L⁻¹ and nitrite from 0.03 ± 0.006 to 0.04 ± 0.004 mg.L⁻¹. No significant differences were observed in these parameters.

Survival Rate and Growth Performance

Culture performance of the fish are summarized on Table 5. The biweekly change in average fish body weight from beginning to the end of this trial is shown

in Figure 1. Over the experimental period, survival ranged from 89.29 % to 96.43 % among treatments (Table 5). This parameter was not affected by treatments. Fish fed SK and D₀ weighed 83.65 ± 2.55 and 80.96 ± 3.54 g at the end of the feeding experiment, and had a feed conversion ratio of 1.2 to 1.24 during feeding trial. Weight gain of Nile tilapia fed the animal blend diet D₃ (1224.5 %) at the 83 % replacement level was statistically similar to those of fish fed the control diets SK and D₀ (1273.5 and 1229.4 %, respectively), but final weight (66.68 ± 1.48 g), specific growth rate (2.85 ± 0.03 % per day) and daily weight gain (0.72 ± 0.02 g. days⁻¹) of fish fed the diet with 100% replacement fishmeal (Diet D₄) were significantly lower ($P < 0.05$). As shown in Figure 1, compared to control diet D₀ and Skretting, fish fed diet D₃ showed similar average weight growth pattern during feeding trial. However, the others experimental diets showed significant decrease in SGR and FBW. Mixture of animal protein sources (housefly larvae and chicken viscera) with spirulina waste-based diets did not affect fish condition factor (1.80 ± 0.04 - 1.97 ± 0.05).

Growth performance in fish fed the experimental diets D₁ and D₂ was significantly lower than those fed the control diet SK and D₀ in many responses ($P < 0.05$). Fish fed the SW and CVM combination diets (diet D₂) showed significantly lower daily weight gain and specific growth rate values than fish fed the control diets and not significantly differed from values of fish fed the SW and HMM blend (diet D₁) at the same dietary level.

Table 4. Water quality parameters (\pm SE) in *O. niloticus* rearing tanks during the experimental period

Variables	Treatments					
	SK	D ₀	D ₁	D ₂	D ₃	D ₄
pH	6.69±0.25	6.65±0.30	6.66±0.33	6.78±0.47	6.82±0.30	6.75±0.50
Temperature (°C)	29.88±0.68	30.08±0.72	29.88±0.78	30.10±0.70	29.97±0.72	30.36±1.49
Dissolved oxygen (mg.L ⁻¹)	3.54±0.59	3.58±0.71	3.56±0.81	3.54±0.67	3.52±0.74	3.74±0.80
Conductivity (µS.cm ⁻¹)	180.77±83.58	185.65±86.56	183.03±87.20	181.38±86.90	179.64±89.08	183.02±82.46
TDS (mg/L)	93.69±45.15	94.69±45.19	94.02±45.27	93.60±45.96	92.50±46.85	93.31±42.29
Salinity (psu)	0.08±0.036	0.08±0.039	0.08±0.040	0.08±0.040	0.08±0.040	0.08±0.038
Nitrates NO ₃ -N (mg.L ⁻¹)	1.92±0.11	1.94±0.21	1.96±0.18	1.90±0.26	1.98±0.18	1.92±0.24
Nitrites NO ₂ -N (mg.L ⁻¹)	0.04±0.01	0.03±0.01	0.04±0.01	0.03±0.01	0.03±0.01	0.04±0.01

Table 5. Growth performance, survival rate and feed utilization of juvenile Nile tilapia fed with six experimental diets for 12 weeks

Parameters	Dietary treatments					
	SK	D ₀	D ₁	D ₂	D ₃	D ₄
Average final weight (g)	83.65±2.55 ^a	80.96±3.54 ^{ab}	60.43±1.98 ^d	71.13±2.43 ^{bc}	80.66±2.97 ^{ab}	66.68±1.48 ^{cd}
Survival rate (%)	92.86±3.37	95.24±3.37	95.24±3.37	89.29±5.05	96.43±1.68	91.67±1.68
Specific growth rate (%.day ⁻¹)	3.12±0.04 ^a	3.08±0.05 ^{ab}	2.73±0.04 ^d	2.93±0.04 ^{bc}	3.08±0.04 ^{ab}	2.85±0.03 ^{cd}
Daily weight gain (g.day ⁻¹)	0.92±0.03 ^a	0.89±0.04 ^{ab}	0.65±0.02 ^d	0.77±0.03 ^{bc}	0.89±0.04 ^{ab}	0.72±0.02 ^{cd}
Percent weigh gain (%)	1273.5±41.9 ^a	1229.4±58.1 ^{ab}	892.2±32.6 ^d	1068.0±39.8 ^{bc}	1224.5±48.7 ^{ab}	994.8±24.2 ^{cd}
Fulton's condition factor K	1.84±0.01	1.97±0.05	1.85±0.08	1.86±0.05	1.90±0.10	1.80±0.04
Yield (kg.m ⁻³)	3.01±0.22 ^a	2.99±0.26 ^a	2.16±0.16 ^b	2.41±0.24 ^{ab}	3.01±0.06 ^a	2.31±0.10 ^{ab}
Production (kg.m ⁻³ .year ⁻¹)	13.07±0.95 ^a	12.97±1.11 ^a	9.40±0.72 ^b	10.49±1.05 ^{ab}	13.08±0.28 ^a	10.05±0.45 ^{ab}
Feed intake (g.fish ⁻¹)	92.32±6.62 ^a	92.26±0.66 ^a	64.58±3.53 ^b	78.88±3.91 ^{ab}	92.40±6.50 ^a	88.02±4.46 ^a
Feed conversion ratio	1.20±0.04 ^a	1.24±0.05 ^a	1.20±0.01 ^a	1.23±0.01 ^a	1.24±0.04 ^a	1.47±0.04 ^b
Protein efficiency ratio	2.39±0.09 ^a	2.31±0.10 ^a	2.39±0.03 ^a	2.33±0.01 ^a	2.30±0.07 ^a	1.95±0.05 ^b

Values are represented as triplicate mean±SE, with 50 fish in each replicate. Different superscripts within a row indicate significant differences among means ($P < 0.05$). Initial weight=6,09±0,5 g

Feed Utilization

Results obtained for feed utilization are shown in Table 5. Feed intake differed between the groups and was significantly lower in fish fed diets D₁ and D₂ compared to those fed with other diets. FCR ranged between 1.20 and 1.47. Feed conversion ratio and protein efficiency ratio of fish fed D₄ (100% FM replacement) were also affected ($P < 0.05$), compared to fish fed the control diets. The significantly lowest protein efficiency ratio (1.95 ± 0.05) was observed in diet D₄. There were no significant differences in protein efficiency ratio between the other treatments ($2.30 \pm 0.07 - 2.39 \pm 0.09$). Fish fed diet D₁, D₂ and D₃ performed equally well in terms of FCR compared with fish fed the control diets (D₀ and SK). The higher FCR (1.47) and lower PER (1.95) were observed in the fish fed diet without fishmeal D₄.

Profitability Analysis and Carcass Composition

As shown in Table 6, using of combination of SW, CVM and HMM in *O. niloticus* diets ensued in decrease of feed cost ($0.46 - 0.56 \text{ US}\$. \text{kg}^{-1}$) and incidence cost ($0.30 - 0.32 \text{ US}\$. \text{kg}^{-1}$) as well as increase profit index. The maximum reduction incidence cost was reached with fishmeal free diet. Fish fed with D₄ showed significantly higher profit index (3.28) than that of control groups SK and D₀.

Table 7 presents the whole-body composition of the fish. Dry matter (range : 92.26-94.77 %) and crude protein (range : 58.95-60.80 %) of fish fed all diets were not significantly different. Lipid deposition (range :

28.23-32.89%) in fish fed CVM diets is significantly higher, whereas ash content (11.67-30.41) decreased ($P < 0.05$). Increasing levels of animal protein sources resulted in higher lipid content in the fish carcass.

Discussion

Water quality parameters were maintained within the range recommended by Delong *et al.* (2009) for *O. niloticus*. There was no difference in survival and condition factor in the present study for any dietary treatments, which ranged from 89.29 to 96.43 % and 1.80 and 1.97 respectively among treatments. This is similar to what has been reported in same species (Suloma *et al.*, 2014; Khalifa *et al.*, 2018). The trend of survival rate recorded in this investigation is similar to that reported for *O. niloticus* (Obirikorang *et al.*, 2015; Kubiriza *et al.*, 2018). The high survival rate shows that *O. niloticus* fed each diet were in good health and mortality recorded during the trial could be attributed to stress due to physical handling.

Housefly maggot meal (HMM) and chicken viscera meal (CVM) used in the present work had a crude protein content of 48.8 % and 35.2 % respectively. These contents are similar to the values previously reported for these alternative protein sources (Giri *et al.*, 2010; Oké *et al.*, 2016). The analysis of the proximate composition of housefly larvae produced from chicken viscera substrate reveals a higher protein content than SW and CVM but lower than *Sardinella* fishmeal and higher in crude fat content than either SW or FM (Table 1). Here, monosex male population was used. Males are better than females due to their fast-growth rate, better

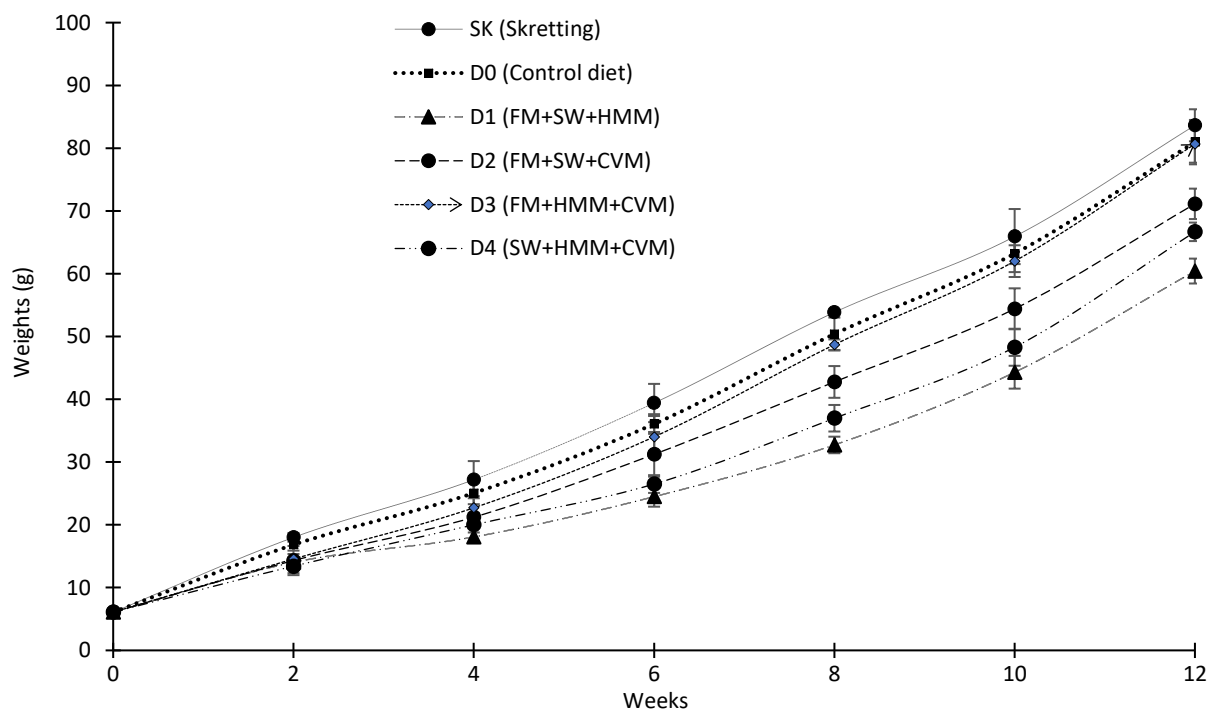


Figure 1. Changes in body weight (g) of Nile tilapia fingerlings fed the experimental diets for 12 weeks.

feed conversion ratio and relatively higher survival rate (Angienda *et al.*, 2010; Omasaki *et al.*, 2017). According to the results obtained in this study, the use of chicken viscera meal and housefly maggot produced from this by-product can partially replace *Sardinella sp* FM in diets for monosex Nile tilapia without affecting growth performance, the feed conversion and protein efficiency ratios. The results of the current study show that fish fed diet D₃ containing chicken viscera and housefly maggot meal (ratio 1:1) had a growth performance similar to those fed control diets. Similar results have been reported by Burr *et al.* (2012) who observed that fishmeal levels could be reduced from 53 % to 10 % without a reduction in growth performance when mixtures of animal and plant protein sources were used in rainbow trout *Oncorhynchus mykiss* diets. Moreover, about 50% fishmeal was replaced by a mixture of rendered animal protein (feather, chicken offal and maggot meals, 4:3:2) in African catfish *Clarias gariepinus* diets without affecting growth (Adewolu *et al.*, 2010). Guo *et al.* (2007) reported that the combination of rendered animal protein ingredients can replace most of the fishmeal in practical diets for cuneate drum *Nibea miichthioides*. Previous study indicated dietary fish meal for Nile tilapia could be reduced to 10% by incorporating housefly maggot singly (Alofa *et al.*, 2020). Feed combination is mostly recommended as they induce better performance than when a single feed is used alone, so mixture accommodate a complementary blending of nutrients including amino acids (Santiago & Lovell, 1988). Growth is strongly related to feed intake and the ability of the fish to use ingested feed (Carter *et al.*, 2001). In this trial, the control groups showed feed intake levels higher than those of D₁ groups, and the effects of lower feed intake in the latter dietary treatments resulted in the lower specific growth rates.

O. niloticus is apt to use effectively cyanobacteria due to its omnivorous nature (Ibrahim *et al.*, 2013).

Curiously, when spirulina waste is used in combination with CVM (D₁) or HMM (D₂), it caused a negative effect on feed intake. Diets were not readily accepted by fish fed diets D₁ and D₂, showing that there were problems relating to the palatability of spirulina waste supplemented diets, except for total fishmeal replacement diet D₄, in which fish showed a relative acceptance. No publications on feeding spirulina waste to fish could be found. Nonetheless, the inclusion of microalgae such as the green algae *Desmochloris sp* derived from biofuel by-product, *Spirulina maxima* and *S. platensis* did not produce detrimental effects on fish growth in hybrid red tilapia *Oreochromis mossambicus* × *O. niloticus* (Ungsethaphand *et al.*, 2010; Garcia-Ortega *et al.*, 2015; Sarker *et al.*, 2016) and others species (Palmegiano *et al.*, 2005; Güroy *et al.*, 2012). On the contrary, the inclusion of whole spirulina meal in diets results in improved growth performance feed efficiency and digestibility for several species including yellowtail cichlid *Pseudotropheus acei* (Güroy *et al.*, 2012), Nile tilapia *O. niloticus* (Sarker *et al.*, 2016; Velasquez *et al.*, 2016) and rainbow trout (Teimouri *et al.*, 2013). Thus, the poor results obtained for the SW diet can be related to poor feed intake. In addition, feeds were offered *ad libitum*, palatability was adversely affected by spirulina wastes inclusion in diets, which might be because of the non-supplementation of amino acids. This difference can be explained by the low palatability of SW. However, reduced feed intake and low palatability were observed in Atlantic cod *Gadus morhua* fed diets containing algae mixture (Walker and Berlinsky, 2011).

Feeding the least fishmeal diets is most important in fish rearing industry (Wang *et al.*, 2008). Total replacement of fish meal by the mixture of animal and algae protein sources (diet D₄) reduced weight gain and feed utilization parameters. This observation is in line with that of Burr *et al.* (2012) who showed that there was an inverse relationship between growth

Table 6. Summary of cost benefit analysis of Nile tilapia fed the test diets

Diets	SK	D ₀	D ₁	D ₂	D ₃	D ₄
Parameters						
Feed cost (US\$.kg ⁻¹)	1.98	0.98	0.56	0.53	0.54	0.46
Cost of feed intake (US\$)	7.25±0.78 ^a	4.47±0.19 ^b	1.89±0.17 ^c	2.10±0.22 ^c	2.62±0.14 ^c	1.93±0.13 ^c
Cost of fish produced (US\$)	8.24±0.60 ^a	8.18±0.70 ^a	5.93±0.45 ^b	6.62±0.66 ^{ab}	8.25±0.17 ^a	6.33±0.29 ^{ab}
Incidence cost	0.88±0.031 ^a	0.55±0.024 ^b	0.32±0.004 ^c	0.32±0.002 ^c	0.32±0.010 ^c	0.30±0.007 ^c
Profit index	1.14±0.04 ^c	1.83±0.08 ^b	3.14±0.04 ^a	3.15±0.02 ^a	3.15±0.10 ^a	3.28±0.08 ^a

Different superscripts within a row indicate significant differences among means (P<0.05)

Table 7. Whole-body composition of *O. niloticus* fed the six experimental diets containing different combinations of chicken viscera, housefly maggot meal and spirulina waste (SK, commercial diet Skretting SK; D₀, control diet containing 30% FM; D₁, FM+SW+HMM; D₂, FM+SW+CVM; D₃, FM+HMM+CVM; D₄, SW+CVM+HMM)

Parameter	Initial	SK	D ₀	D ₁	D ₂	D ₃	D ₄
Dry matter	94.61±0.00	92.60±2.94	92.26±0.13	93.54±2.77	94.69±1.68	94.13±1.21	94.77±0.64
Crude protein	58.95±0.46	59.49±0.67	59.12±0.89	60.80±1.32	60.23±1.04	59.33±1.01	58.77±1.42
Crude fat	11.89±0.78	28.23±4.22 ^{bc}	29.95±0.08 ^{cd}	26.54±0.21 ^d	29.83±0.20 ^{bc}	32.89±0.53 ^a	33.54±1.12 ^{ab}
Ash	24.15±0.00	30.41±0.60 ^a	15.61±0.24 ^{cd}	14.77±0.64 ^{cd}	14.32±2.96 ^{cd}	11.67±3.24 ^d	19.80±0.07 ^{bc}

Different superscripts within a row indicate significant differences among means (P<0.05)

performance and total substitution of fish meal by soy protein concentrate combined with corn gluten and poultry by-products meal in rainbow trout diet. Total replacement fishmeal by soy protein concentrate in *Paralichthys olivaceus* diet decreased growth performance (Tushe *et al.*, 2012). In most investigations, *O. niloticus* fed diets containing a single alternative protein source or combination of protein sources shown decreased growth at high levels of replacement except when the practical diets contained 10-20 % fishmeal (Obirikorang *et al.*, 2015 ; Khalifa *et al.*, 2018 ; Kubiriza *et al.*, 2018). This could be associated with differences between amino acid profile and availability of alternative protein sources and fishmeal and the deficiency of other essential nutrients at some plant protein sources. Nevertheless, a combination of poultry meal, wheat flour, and corn protein concentrate proteins could completely replace fishmeal in diets for post-smolt Atlantic salmon *Salmo salar* (Davidson *et al.*, 2016). Moreover, soy concentrate could be completely replaced FM in *O. niloticus* fingerlings diet (Ribeiro *et al.*, 2016). Herath *et al.* (2016) indicated that distillers' dried grains with solubles were the best choice for fishmeal-free diet for *O. niloticus* juveniles (4.0–4.5 g).

The FCR observed in the current study ranged between 1.2 and 1.47. These values are similar to those observed in the experiment of Ogello *et al.* (2017) (1.22–2.17) and Hossain *et al.* (2002) (0.96–1.42) on tilapia in warm water recirculating system but for higher than those observed by Herath *et al.* (2016) under similar conditions. Mridha *et al.* (2017) obtained higher values of FCR for *O. niloticus* reared in a rain-fed rice–fish ecosystem for 120 days. The same trend was observed in juveniles of *O. niloticus* reared in tanks (1.37–2.34) during 86 days (Silva *et al.*, 2015; Ribeiro *et al.*, 2016). The FCR (1.22) values obtained with chicken viscera meal and maggot meal mixture D₃ is best than those reported by El-Sayed (1998) (1.86 - 2.48), which were obtained by using singly or combination of meat and bone meal, blood meal and poultry by-products meal. Except for fish fed diet D₄ (100% fishmeal replacement), protein efficiency ratio values in all experimental diets were higher than 2.00. This indicates efficient utilization of dietary protein.

The study shows that FM can be greatly reduced using a mixture of chicken viscera meal and maggot meal in diets for Nile tilapia with no significant effects on fish growth and body composition. These results contribute to the tilapia nutrition to enhance the sustainability of fish feeds by using a combination of animal protein sources ingredients. Fish fed the control diets showed lower crude lipid and ash contents in carcass than fish fed the experimental diets. This corroborates the results of previous studies on the cuneate drum (Guo *et al.*, 2007; Wang *et al.*, 2006) and grouper *Epinephelus coioides* (Milliamena, 2002). Considering that body lipid content did significantly differ between fish fed control diets and experimental diets, it is speculated that replacing fishmeal with a

mixture of CVM and HMM might negatively affect the digestive function of the gut of Nile tilapia. This could also be explained by the lipid content of these animal protein sources, used for diet formulation.

Economic analysis of using a mixture of SW, CVM and HMM as a fishmeal substitute was equally undertaken. The interest of using alternative protein sources in *O. niloticus* diets does not depend perforce in their nutrient profile but in their availability and low costs (Goda *et al.*, 2007). In this experiment, the economic analysis of these unconventional protein sources shows that these protein sources were more economical compared with fishmeal. Of all the experimental diets, the incidence cost (the price of feed per kg of fish produced) was highest in the commercial diet SK (0.88) followed to fishmeal based-diet D₀ (0.55). Using chicken viscera and housefly maggot meal in *O. niloticus* diets ensued in the decrease of feed cost as well as increase profit index (3.14–3.28 *versus* 1.14–1.83 with control diets). This is due to the low production cost of chicken viscera and maggot meals which is approximately 88 and 80 % of the cost of *Sardinella sp* fishmeal, respectively.

Finally, this study demonstrated that diet containing a combination of 20% chicken viscera meal and 20% housefly maggot meal with only 5 % fishmeal resulted in comparable Nile tilapia growth compared to a commercial feed and fishmeal-based diet. Thus, the inclusion of fishmeal in the diet of *O. niloticus* is reduced from 30 to 5 % using a combination of chicken viscera and housefly maggots. We suggest these ingredients as an appropriate alternative for marine fishmeal in the sustainable production of Nile tilapia, for ensuring the food security of the most vulnerable populations in Benin.

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