AQUATIC RESEARCH

E-ISSN 2618-6365

Aquatic Research 3(1), 24-35 (2020) • https://doi.org/10.3153/AR20003 *Research Article*

Evaluation of Spirulina (*Spirulina platensis*) wastes and live housefly (*Musca domestica*) larvae as dietary protein sources in diets of *Oreochromis niloticus* (Linnaeus 1758) fingerlings

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Cite this article as:

Alofa, C.S., Adite A., Abou, Y. (2020). Evaluation of Spirulina (*Spirulina platensis*) wastes and live housefly (*Musca domestica*) larvae as dietary protein sources in diets of *Oreochromis niloticus* (Linnaeus 1758) fingerlings. *Aquatic Research*, 3(1), 24-35. https://doi.org/10.3153/AR20003

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Submitted: 25.10.2019

Revision requested: 25.11.2019

Last revision received: 15.12.2019

Accepted: 15.12.2019

Published online: 18.12.2019

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ABSTRACT

This study was designed to evaluate spirulina wastes and live housefly maggot as partial replacement for fishmeal in the diets of Nile Tilapia. Four isonitrogenous (35%) and isocaloric (17-18 $KJ.g^{-1}$) diets were evaluated: commercial diet Skretting SK, control diet CD (30% fishmeal), and two others diets (SW and LM), corresponding to spirulina wastes and live housefly maggot inclusion respectively. Diets were hand-fed thrice daily to triplicate groups of fish to apparent satiation. After 12 weeks, the final weight and feed intake of fish fed diet CD and SK did not differ from those fed diets LM, but were higher than those fed SW diet ($p<0.05$). However, inclusion of SW had no effect on feed utilization. No significant differences were found in survival, feed conversion ratio, protein efficiency ratio and condition factor among the treatments ($p>0.05$). Whole-body protein contents were similar in all groups, whereas the lipid content was lower in SW group. It was concluded that a 62.56 % fishmeal protein could be replaced by live maggot in the diet of Nile Tilapia without negative effects on growth performances and quality of fish produced.

Keywords: Spirulina wastes, Live maggots, Fishmeal, Growth, Nile tilapia

Introduction

Nowadays, aquaculture industry provides half of all fish for human consumption (FAO, 2016; Esmaeili *et al.,* 2017). The expansion of this sector highly depends on industrially feeds (Tacon *et al.,* 2006). This feed rely on fishmeal as a major source of protein, highly digestible essential amino acids and fatty acids (Cho and Kim, 2011). The use of fishmeal as a major protein source in fish feed has heavily pooled to increased demand and prices for this raw material. Finding a suitable substitute for fishmeal is one means to reduce total operating costs in aquaculture industry (Webster *et al.,* 1997). Furthermore, plants protein do not constitute the utmost alternatives to fishmeal, and for this reason, the need to find new aquafeed ingredients presently remains a real challenge (Vizcaino *et al.,* 2014).

In plant protein, microalgae has received significant consideration in fish feed manufacturing because of its high protein content, vitamins, polysaccharides, polyunsaturated fatty acids, microelements and antioxidant pigments (Hemaiswarya *et al.,* 2011). Among the microalgae, Spirulina, which is a quite promising source of protein, is widely distributed and easily cultured in tank (Huo *et al.,* 2012). In recent years, Spirulina meal has been successfully used as a feed additive (Silva-Neto *et al.,* 2012) or alternative protein source (Teimouri *et al.,* 2013; Velasquez *et al.,* 2016) in aquafeeds to improve weight gain and carcass quality of fish. Because *Spirulina platensis* is one of the most habitually used dietary complements in human comsumption and many animal species, including fish. Its wastes, which is rich in protein, can be used as dietary protein source in Nile Tilapia diets. Abdelkhalek *et al.* (2015) indicated that *Spirulina platensis* supplementation in *O. niloticus* diets, could minimize deltamethrin (DLM) induced toxic effects by its mighty antioxidant activity. It is also a protective agent anti hepatotoxicity in freshwater catfish *Clarias batrachus* (Ahmad Dar *et al.,* 2014). Inclusion of *Spirulina maxima* in diets for juvenile common carp *Cyprinus carpio* results in increased growth rate (Ramakrishnan *et al.,* 2008).

At the present time, some plant protein sources, such as Azolla meal (Abou *et al.,* 2007ab) ; cereal grain products and by-products (Guimarães *et al.,* 2008), corn co-products (Herath *et al.,* 2016), *Jatropha curcas* kernel meal (Krome *et al.,* 2016) ; soybean meal (Al-Feky *et al.,* 2016), have been used to partially or totally replacement fishmeal in diets of Nile Tilapia. However, greatest in amount plant-based feedstuffs have a large variety of anti-nutritional factors, which may decrease fish growth performance. To ensure high production and fast growth at least cost, a well-balanced formulated feed is necessary for profitable tilapia farming. In some countries, different by-products such as chicken viscera are frequently left to rot in environment. This by-product pose pollution and health problems to local communities. Moreover, the poultry production industry generates large amounts of by-products (Adler *et al.,* 2014). However, there is currently poorly used as a protein source in aquafeeds. We can used this by-products to produce enriched housefly maggot.

The housefly (*Musca domestica*) (Diptera : Muscidae) can feed on a wide variety of spoiled organic matter, such as distillers grains, fish offal, food and vegetable waste and animal manure (Salomone *et al.,* 2017). In addition, insect's larvae have the potential to convert the animal manure into precious biomass. For instance, blacks soldiers flies has been assessing as a prospective animal to use in bioconversion of manure to reduce waste remnant. They can reduce nitrogen waste by 75% and mitigate mass by 50% in poultry process (Newton *et al.,* 2005). As mentioned above, chicken viscera poses a potential feedstock for housefly larvae. Housefly maggots are rich in proteins and lipids, and research on their use as meals has given good results for several of the aquaculture species tested (Ogunji *et al.,* 2008; Lin and Mui, 2016). Although, several studies on maggot meal have been published (Ogunji *et al.,* 2008 ; Wang *et al.,* 2017), little reports have been performed about the use of live housefly ; this is case of African catfish (Emeka and Oscar, 2016) and no reports in Nile tilapia, the most important farmed tilapia species around the world. Thus, use of live housefly maggot for *O. niloticus* diets as fishmeal replacement is warranted. For this purpose, our study was aimed to assess a animal protein source (live housefly *Musca domestica* maggot) and single-cell protein spirulina *Spirulina platensis* wastes, tested separately, in practical diets for Nile tilapia substituting the fishmeal component in formulated experimental diets for this species.

Material and Methods

Fish and Experimental Procedures

Monosex male Nile Tilapia fingerling (*O. niloticus*) were obtained from Private fish farming ''Dieu Exauce'' located in Tori Avamey at Tori-Bossito (Benin). Tilapia were transported in oxygenated plastic bags to the Experimental Fish Farming Unit of Laboratory of Ecology of Aquatics Ecosystems of the University of Abomey Calavi, Benin, where the experiment was realized. Initially, 350 fish were stocked in $1m^3$ circular concrete tank and maintained during one week before start the feeding trial. During this time, they were fed with a mixture of experimental diets. A total of 600 fish with an average weight 8.65 ± 0.5 g were equally distributed into

four experimental triplicate groups and stocked into 12 circular concrete tanks (diameter : 120 cm with capacity of 1000 l). Before the beginning of the experiment, fish were starved for 24 hours. The fish were fed their assigned diets thrice a day (09 : 00 ; 13 :00 and 17 :00 h) to apparent satiation and the quantity of feed consumed recorded for each tank. A outdoor recirculation rearing system was used to conduct the experiment, with water flow set at 3 L min^{-1}. Fish were weighed collectively at the beginning and fortnightly for each tank to determine gain in weight.

Ingredients and Experimental Diet

Housefly *Musca domestica* larvae produced from chicken viscera and spirulina *Spirulina platensis* wastes were used as a partial protein replacement of fishmeal in fish diets. Chicken viscera were collected from the poultry processing industry "Agrisatch" (Abomey-calavi, Benin), and incubated in an rectangular areas (measuring 3m x 2m) as a substrate for housefly larvae development. The substrate was watered twice daily with water to prevent drying and exposed for two days to let houseflies to spawn eggs on it. The substrate was covered and left among 3 to 5 days to enable maggot to be grown before harvesting. The harvested houseflies maggots were washed and pre-cooked in warm water at 85°C during 15 minutes in order to prevent disease pathogens infection, before being incorporated in the practical diet.

Sardinella sp fishmeal was used in the formulation of experimental diets. This ingredient is purchased at the Dantokpa market and sun-dried for three days before being transformed into meal. Blood meal was obtained following the procedures described by Alofa *et al.* (2016). The rest of the ingredients for the diets such as soybean meal, cottonseed meal, palm oil and salt were obtained at local market. Dry matter, crude protein, ether extracts and ash of housefly maggot and spirulina wastes used in this experiment were analysed (Table 1) to assist in experimental diet formulation (Table 2). The costs of ingredients used in the formulation of practical diets are given in Table 3.

After 2 week of acclimatization, the fish were fed one of the four experimental diets (3 tanks per treatment) for 86 days : one commercial diet Skretting SK, one control diet CD (no housefly maggot and spirulina wastes), and diets to which 15 % and 25 % of spirulina wastes and live housefly maggot were added respectively. Diets were denoted LM (250 $g.Kg^{-1}$ live maggot ; 935 g.Kg basis live weight) ; SW (150$g.Kg^{-1}$ Spirulina wastes). Spirulina wastes was supplied by Spirulina Production Unit of the Regional Institute for Development and Health (SPU/RIDH), located in Pahou (Ouidah, Benin). These wastes were generated from the production and packaging process of spirulina.

Preparation

Diets were formulated to contain 35 % crude protein and 17-18 kJ.GE g^{-1} diet (Table1). Ingredients were grounded, weighed, and mixed. Mixtures were then pelleted using a meat grinder to form pellets. The pellets were sun-dried and stored in plastic bags at - 4 °C until use. For the preparation of diet containing live maggot, this by-product was pre-cooked ground with food grinder (Binatone BLG 450) and blended at least to make a paste before being to added to others ingredients.

Sampling and Water Quality Monitoring

Twenty fish were randomly selected to determine initial whole fish body nutrient composition and stored at -20°C until analysis. Biomass of each tank was recorded at the beginning and end of this trial. Ten fish per tank were randomly chosen (n = 30 per treatment). Fish weight, total length, were recorded to calculate condition factor (CF).

Water parameters such as hydrogen potential (pH), temperature (°C), dissolved oxygen (mg/L), salinity (*psu*), conductivity (µS/cm) and total dissolved solid (TDS mg/L) were measured weekly at a deep of 10 cm for each reared tank with a multiparameter probe (Hanna HI 9829 v1.04, Hanna Instruments Ltd., USA). Nitrite and ammonium were determined by cadmium reduction and phenate methods respectively using spectrophotometer Hach DR6000. These parameters were checked three times fortnightly.

Calculations

To show the effect of spirulina wastes and live housefly larvae inclusion on growth performance and nutritional indices, the next parameters were determined as average of the triplicates by the formulas given.

$$\textbf{Survival rate (SR, \%)} = \frac{\text{final amount of fish}}{\text{initial amount of fish}} \text{ X } 100$$

$$\textbf{Weight gain rate (WGR, \%)} = \frac{(\text{final body weight} - \text{initial body weight})}{\text{initial amount of fish}} \text{ X } 100$$

$$\textbf{Specific growth rate (SGR, \%)} = \frac{Ln(final\ weight\ gain) - \ln(\text{initial weight})}{\text{rearing period}} \text{ X } 100$$

Feed intake (FI, g/fish)
$$= \frac{\text{total amount of the dry feed consumed}}{\text{fish numbers X days}} \text{X } 100$$

Protein efficiency ratio (PER)
$$= \frac{\text{body weight gain}}{\text{total feed consumed protein content in diets}}$$

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

Condition factor (CF) $= \frac{\text{final body weight (g)}}{\text{body length (cm)3}} X\ 100$

Yield (Kg/m3)
$$= \frac{\text{final biomass per tank (g)} - \text{initial bimass per tank (g)}}{\text{water volume (1 m3)}}$$

Production (Kg/m3/year) $= \frac{\text{Yield x365}}{\text{rearing period}}$

Economic conversion ratio (ECR)

= Cost of diet x Feed Conversion Ratio (FCR)

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

Table 1. Formulation and proximate composition of experimental diets fed monosex Nile Tilapia fingerlings during 12 week

	Dietary treatments			
	SK[1]	**CD**	**SW**	**LM**
***Ingrédients* (g 100 g $^{-1}$)**				
***Sardinella sp.* fishmeal**		30	10	10
Spirulina wastes		–	15	–
Live housefly maggot		–	–	25
Blood meal		7	7	7
Corn bran		36	21	25
Soybean meal		14	25	22
Cottonseed meal		10	19	11
Palm oil		2	2	2
Salt (NaCl)		1	1	1
Proximate composition				
Dry matter (%)		90.16	90.31	90.24
Crude protein (% DM)		35.32	35.08	35.13
Crude lipid (% DM)		8.15	9.19	11.88
NFE[2]		36.42	34.15	31.95
Ash (% DM)		7.95	7.21	6.45
Gross energy[3] (kJ g^{-1})		17.85	17.82	18.58
Diet cost (US$. Kg^{-1})[4]	1.87	1.00	0.69	0.67

1. Proximate composition : Crude protein : 35% ; Crude fat : 9% ; Fibre : 3.4% ; Ash : 6.5%, Calcium : 1% ; Phosphore : 1%, Lysine : 1.5% ; Methionine : 0.5% ; CuSO4 : 5mg/Kg

2. Nitrogen-free-extract (NFE) = 100 - (% moisture + % crude protein + % crude lipid + % ash + % crude fibre).

3. Gross energy (GE) 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).

4. Prices in US$, 1 US$= 586.69 FCA at present. Including labour and processing

Table 2. Analysed nutrient composition (as % dry matter) of feeds ingredients

Ingredients	Dry matter	Crude protein	Crude lipid	Ash	Crude fibre
Fish meal	92.0	66.0	7.88	15.77	1.0
Spirulina wastes	91.53	46.32	6.71	10	3.2
Maggot meal	91.7	48.8	20.1	6.25	6.1
Soybean oilcake	94.8	30	13.2	3.7	6.0
Cottonseed oilcake	90.0	40.5	7.0	8.0	14.0
Blood meal	90.9	71.9	1.7	6.4	1.6
Maize bran	91.4	6.2	3.1	1.4	12.3

Proximate Analysis

Dry matter, crude protein, crude lipid, and ash in feed ingredients and fish (Table 1) were determined according to standard procedures of Association of Official Analytical Chemists, AOAC (Horwitz and Latimer, 2005). Dry matter was determined by drying samples in an oven (Memmert UN160 Plus) at 105°C for 24 hr. Crude protein was calculated from the nitrogen content (N x 6.25) using the Kjeldahl method. Samples were first acid-digested. Crude lipid content in samples was determined by chloroform–methanol method (Folch *et al.,* 1957), while crude ash content was determined by incineration samples at 500°C for 12 h and weighing the residual ash. All analysis was performed in triplicate.

Statistical Analysis

Data were expressed as the mean ± SEM. of triplicate samples. All statistical analyses were conducted using Microsoft Excel and Statistical Package for Social Sciences (SPSS IBM version 20.0 for windows v8.1, Chicago, Illinois, USA). Prior analysis, homogeneity of variance was determined using the Hartley statistical test after log transforming (Dagnelie, 1975). Differences in the mean levels of the parameters between the dietary treatments were determined using one way analysis of variance ANOVA followed by Tukey's test of multiple comparison. The differences were considered significant when p-value were < 0.05.

Results and Discussion

The search for sustainable ingredients to replace fishmeal has been a real challenge for the Tilapia industry. At fishmeal substitution experiment, the quality of FM is of great importance on how tested products perform as FM substitutes (Biswas *et al.,* 2017). In this experiment, a high quality of FM produced from *Sardinella spp* with protein contents approximately 660 g kg^{-1} was used. Housefly larvae are converters of organic waste into expendable biomass of which the composition may attribute on the substrate. In this experiment, larvae were grown on chicken viscera.

The effects of SW and LM inclusion on tilapia performance, nutrient utilization and production are presented in Table 5. Although there was little variation in lipid contents as indicated in Table 1, all experimental diets were isocaloric and isonitrogeneous. Growth parameters were poor in fish fed SW diet and similar ($p < 0.05$) in those fed CD, SK and LM diets (Figure 1). There were no significant differences ($p > 0.05$) in final weight (80.96 – 88.54 g), DWG (0.86 – 0.95 g. $days^{-1}$), SGR (2.68 – 2.77 % $days^{-1}$) and annual production (13.88 - 16.03 Kg/m^3/ year) of Nile tilapia fed with control diets and LM diet (Table 5). These findings indicated that the growth performance and feed efficiency of *O. niloticus* juveniles fed live housefly larvae were not significantly affected by the replacement of fishmeal up to 66 %, showing that LM protein can be used to partially substitute FM in a practical diet of Nile tilapia. This is in agreement with the findings of Oyelese (2007) and Ogunji *et al.* (2008) that used it as partial FM substitute without affecting growth and feed utilization in Tilapia and catfish juveniles. The current study is in agreement with the earlier reported Tilapia studies and exemplifies the possible use of live housefly maggot as a partial substitute for FM in *O. niloticus* diets. Studies evaluating live housefly larvae in fish diets are highly few, but rising. Results of the current study in Nile Tilapia are similar to several studies in Teleost. In rainbow trout (*Oncohynchus mykiss*) for example, fish fed a diet with 18 up to 36% maggot meal (MM) produced from cow manures and fish offal had similar final average weight and weight gain as fish fed a control diet, whereas fish fed a diet containing 16 up to 33 % MM produced from cow manures only had significantly reduced growth parameters (Sealey *et al.,* 2011). This may be presumed that the nutrient content of fly larvae largely depends on their diet (Spranghers *et al.,* 2017). In the present experiment, specific growth rate recorded in all traitment were comparatively higher than those of the anterior study (Wang *et al.,* 2017) in which the SGR of Nile tilapia (initial weight : 68.89 g) fed housefly MM were ranged from 1.12 to 1.62 % per day. This difference might be due to the fish sizes or further rearing conditions.

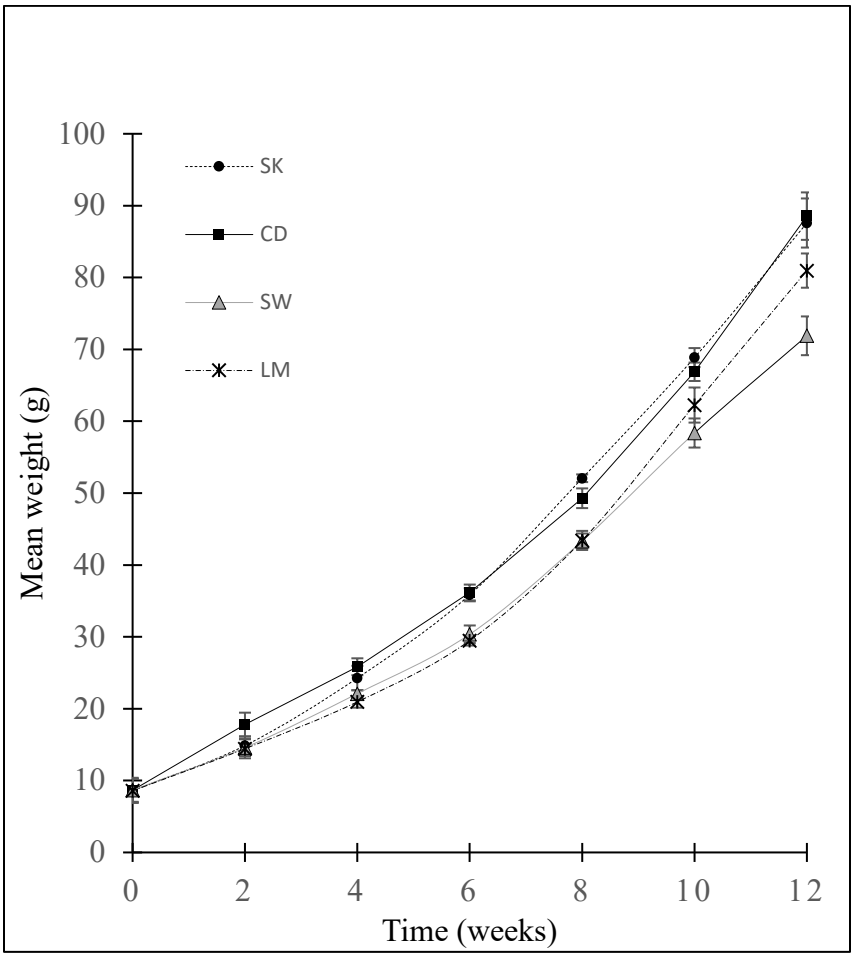

Figure 1. Mean weight (g) evolution of juvenile Nile Tilapia *Oreochromis niloticus* fed the commercial tilapia diet Skretting SK, the experimental diets containing *Sardinella sp.* fishmeal CD, Spirulina wastes SW and live housefly maggot LM during 12 weeks.

Table 3. Cost of ingredients used in formulating the diets

Ingredients	Price (US$.Kg^{-1})
***Sardinella sp* fishmeal**	2.24
Soybean meal	0.67
Cottonseed meal	0.33
Blood meal	0.22
Spirulina wastes meal	0.43
Housefly maggot meal	0.44
Corn bran	0.26
Palm oil	1.38
Salt (NaCl)	0.43

Table 4. Water quality parameters in *O. niloticus* rearing tanks during the experimental period

Parameters	SK	CD	SW	LM
pH	6.78 ± 0.29	6.81 ± 0.29	6.85 ± 0.33	6.83 ± 0.30
Temperature (°C)	29.88 ± 0.68	30.08 ± 0.72	29.88 ± 0.78	30.27 ± 1.47
Dissolved oxygen (mg. L^{-1})	3.12 ± 0.56	3.17 ± 0.57	3.15 ± 0.58	3.08 ± 0.31
Conductivity (µS/cm)	179.7 ± 84.1	185.1 ± 86.9	181.8 ± 88.0	183.0 ± 82.5
TDS (mg. L^{-1})	93.06 ± 45.56	94.63 ± 45.22	93.35 ± 45.67	93.31 ± 42.29
Salinity (psu)	0.07 ± 0.04	0.08 ± 0.04	0.08 ± 0.04	0.08 ± 0.04
Nitrate (mg. L^{-1})	2.23 ± 0.38	2.33 ± 0.25	2.27 ± 0.15	2.73 ± 0.35
Nitrite (mg. L^{-1})	0.04 ± 0.00	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01

Data are expressed as means ±SE (n = 3)
SK: Skretting, CD: control diet, SW: spirulina wastes diet, LM: live housefly maggot diet

Table 5. Growth performance, feed efficiency and annual production of *Oreochromis niloticus* fed the experimental diets for 12 weeks.

Parameters	SK	CD	SW	LM
Initial weight (g)	8.60 ± 0.10	8.66 ± 0.14	8.68 ± 0.14	8.53 ± 0.03
Final weight (g)	87.59 ± 3.42^{a}	88.54 ± 3.30^{a}	71.89 ± 2.70^{b}	80.96 ± 2.38^{a}
Feed intake (g $fish^{-1}$)	94.93 ± 3.20^{ab}	104.84 ± 3.33^{a}	83.73 ±3.17^{c}	94.81 ± 4.54^{bc}
Survival rate (%)	96.00 ± 2.00	94.00 ± 2.00	94.00 ± 2.00	91.33 ± 2.31
Weight gain (%)	918.8 ± 41.8^{a}	923.4 ± 54.7^{a}	728.8 ± 31.2^{b}	848.8 ± 30.8^{a}
Daily weight gain (g. $days^{-1}$)	0.94 ± 0.04^{a}	0.95 ± 0.04^{a}	0.75 ± 0.03^{b}	0.86 ± 0.03^{a}
Specific growth rate (% $days^{-1}$)	2.76 ± 0.05^{a}	2.77 ± 0.06^{a}	2.52 ± 0.04^{b}	2.68 ± 0.04^{a}
Feed conversion ratio	1.21 ± 0.09	1.32 ± 0.02	1.34 ± 0.02	1.32 ± 0.05
Protein efficiency ratio	2.37 ± 0.18	2.16 ± 0.04	2.14 ± 0.03	2.16 ± 0.09
Condition factor	1.92 ± 0.09	1.89 ± 0.12	1.80 ± 0.02	1.80 ± 0.07
Yield (Kg/m^3)	3.78 ± 0.25^{a}	3.73 ± 0.25^{a}	2.94 ± 0.07^{b}	3.27 ± 0.17^{ab}
Production (Kg/m^3/ year)	16.03 ± 1.06^{a}	15.84 ± 1.06^{a}	12.49 ± 0.30^{b}	13.88 ± 0.71^{ab}

Values in a row with different letters are significantly different ($p < 0.05$, Tukey's test).
SK: Skretting, CD: control diet, SW: spirulina wastes diet, LM: live housefly maggot diet

Water quality parameters values during the feeding trial were exposed in Table 4. The water temperature ranged from 29.88 to 30.27 °C, pH from 6.78 to 6.85, dissolved oxygren from 3.08 to 3.17 $mg.L^{-1}$, salinity from 0.07 to 0.08 $mg.L^{-1}$, nitrate from 2.23 to 2.73 $mg.L^{-1}$ and nitrite from 0.03 to 0.04 $mg.L^{-1}$. No significant differences were observed in these parameters ($p>0.05$). These parameters recorded were optimal for the monosex male Nile Tilapia used in the experiment, because the optimal temperature for this species must be in a range between 12 and 16ºC and the dissolved oxygen content should not lower than 3 mg. L^{-1} (Bhujel, 2000). Thereby, experimental diet did not affect significantly the pH of the dietary traitment, these remarks were in contrariness of the results noted from Promya and Chitmanat (2011) that recorded higher values of pH in tank with fish fed with a diet including algae. Survival rate of experimental fish were not affected by the presence of spirulina wastes in the diet, ranging from 91 to 96%. Similar data were observed for several fish fed spirulina meal diets (Sirakov *et al.,* 2012; Promya and Chitmanat, 2011).

On the other hand, spirulina contains a large amount in proteins essential, vitamins, minerals, amino acids and fatty acids, antioxidant pigments and has been identified as a feed ingredient for cichlids; it seems to be a hopeful dietary protein source (Guroy *et al.,* 2012).

Feed intake had decreased ($p < 0.05$) in fish fed SW diet (83.73 ± 3.17 g. $fish^{-1}$). In contrast, fish fed LM diet (107.95 ± 3.31 g. $fish^{-1}$) had similar FI with those fed control diet SK

and CD (94.93 ± 3.20 and 104.84 ± 3.33 g fish^{-1} respectively). Our results were in contrast of the data received from Guroy *et al.* (2012), who showed that Spirulina meal has the potential to enhance the growth, reproductive performance and coloration on yellow tail cichlid *Pseudotropheus acei*. Several studies have shown that dietary Spirulina can affect the growth performance of diverse fish species. For example, it has previously been reported that 20 up to 40% of FM can be substitute with spirulina meal without negative effect on the growth performance of hybrid red tilapia (Ungsethaphand *et al.,* 2010). Moreover, Guroy *et al.* (2012) reported that spirulina meal could be replaced fishmeal up to 10% in yellow tail cichlid diets without any adverse effects on growth, reproductive performance or coloration. Likewise, it has been reported that that dietary inclusion of 8% Spirulina significantly enhanced growth performance of the ornamental red swordtail *Xiphophorus helleri* (James *et al.,*2006). Furthermore, according to Yeganeh *et al.* (2015), it's well known that the increase in HDL-cholesterol with spirulina inclusion suggests that Spirulina may improve the cardiovascular activity in rainbow trout (*Oncorhynchus mykiss*).

No significant differences in feed conversion ratio (1.21 - 1.34), protein efficiency ratio (2.14 - 2.37) and condition factor (1.80 - 1.92) were observed among these groups. However, fish fed SW diet showed significantly lower final mean body weight compared with other group being represented the lower value ($p < 0.05$, Table 5). The poor growth performance in fish fed SW diet might be due to the lower digestibility of microalgae, due to the presence of a cellular wall, as suggested Le Vay *et al.* (2001). It has been demonstrated in most studies that low growth rates of fish fed with plant protein–based diets were attributed with poor feed intake that was strongly influenced by the palatability of diets (Kader and Koshio, 2012). In the present study, FI was significantly decreased in fish fed SW diet which indicated that SW protein sources can negatively affect palatability. FI is highly influenced by the palatability of diets; it's one of the most important factor coupled with the efficiency on the utilization of protein sources in fish (Kader *et al.,* 2012). Plant protein are successfully used in feed formulations for rearing tilapia species because Tilapias have herbivorous or omnivorous feeding habits and lower level of the aquatic food chain. Likewise, growth performances obtained with the spirulina wastes protein in this study was lower than previously reported for others aquatic species of similar weight fed with spirulina such as the yellow tail cichlid *Pseudotropheus acei* (Guroy *et al.,* 2012), *Litopenaeus schmitti* larvae (Jaime-Ceballos *et al.,* 2006), the sturgeon *Acipenser baeri* (Palmegiano *et al.,* 2005) and *O. mossambicus* X *O. niloticus* (Ungsethaphand *et al.,* 2010). It has been demonstrated that high quality spirulina meal was an adequate and nutritious protein source that increased growth in several species such as Common carp *Cyprinus carpio* (Ramakrishnan *et al.,* 2008) ; sturgeon *Acipenser baeri* (Palmegiano *et al.,* 2005). However, Spirulina meal has high protein content (i.e. 66.9 %) compared with spirulina wastes (46.32 % crude protein) used in the present study. Therefore, the adverse effect following SW inclusion might be also due to the lower protein content observed in fish fed SW diet.

In our study, PER was more favorable in spirulina wastes based diet than in the control diet SK and CD. A similar observation was made using *Spirulina platensis* at different levels in sturgeon *(Acipenser baeri)* (Palmegiano *et al.,* 2005). However, The FCR and PER in *O. niloticus* fed Spirulina wastes based diets were similar than those of the control diets CD and SK. The results of this work are similar to those found by Teimouri *et al.* (2013) in which rainbow trout (*Oncorhynchus mykiss*) fingerlings fed with control diet, 7.5 and 10% *S. platensis* inclusion diets as feed supplement, showed comparable feed conversion ratio. Furthermore, the cellular structure (mucopolymer murein) of Spirulina alga is readily digestible and does not contain cellulose (Beresto, 2001). Wherefore, significant decrease on growth performance in fish fed SW diet may be associated by the lower feed intake observed in these fishes.

The variation in the final whole-body proximate composition is reported in Table 6. Except dry matter and crude protein content, all whole body compositions were significantly affected by dietary protein source ($p < 0.05$). Crude lipid content in fish fed with SW diet were significantly lower than those in fish fed any other diets ($p < 0.05$). However, lipid contents was significantly higher in fish fed with LM diet, whereas ash content significantly decreased ($p<0.05$), reflecting the lipid and ash contents of this protein source. The results of the economic analysis are shown in Table 7. As it can be seen, profit index significantly increased with fish fed both SW and LM diets whereas economic conversion ratio decreased significantly. Economic analysis shows that inclusion of both SW and LM in the diet improves profitability.

Table 6. Proximate composition (%) of whole body of *Oreochromis niloticus* fed the experimental diets: CD, diet containing fish meal; LM, diet containing live housefly maggot and SW, diet with spirulina wastes meal.

Diets	Initial	SK	CD	SW	LM	p-values
Dry matter	89,82 ± 0,12	91,91 ± 0,04	90,50 ± 0,47	90,57 ± 0,06	91,45 ± 1,46	0,319
Crude protein	63,14 ± 0,70	61,40 ± 0,44	62,45 ± 0,08	60,26 ± 1,37	59,27 ± 1,13	0,090
Crude lipid	10,76 ± 0,59	32,59 ± 1,86^{a}	33,56 ± 1,66^{a}	26,15 ± 0,38^{b}	35,77 ± 0,59^{a}	0.007
Ash	16,52 ± 81,19	14,79 ± 0,68^{ab}	17,43 ± 2,37^{a}	15,29 ± 0,17^{ab}	11.13 ± 1.32^{b}	0,046

Values in the same column with different superscripted small letters mean significant difference ($p < 0.05$). Values show mean ± standard error, $n = 3$

Table 7. Summary of cost benefit analysis of Nile Tilapia fed the test diets

Diets	SK	CD	SW	LM	Anova p-values
Parameters					
Total feed used (Kg m^{-3})	4.56 ± 0.07^{ab}	4.93 ± 0.26^{a}	3.93 ± 0.12c	4.33 ± 0.32^{bc}	0.003
Cost of feeding (US$.m^{-3})	8.517 ± 0.12^{a}	4.93 ± 0.26^{b}	2.72 ± 0.08^{c}	2.90 ± 0.21^{c}	0.000
Price of fish produced (US$.m^{-3})	9.78 ± 0.65^{a}	9.66 ± 0.65^{a}	7.62 ± 0.18^{ab}	8.47 ± 0.43^{b}	0.003
Economic Conversion Ratio (US$. Kg^{-1})	2.26 ± 0.18^{a}	1.32 ± 0.02^{b}	0.92 ± 0.02^{c}	0.89 ± 0.04^{c}	0.000
Profit Index	1.15 ± 0.09^{c}	1.96 ± 0.03^{b}	2.81 ± 0.05^{a}	2.92 ± 0.12^{a}	0.000

SK: Skretting, CD: control diet, SW: spirulina wastes diet, LM: live housefly maggot diet

Conclusion

In conclusion, the results clearly indicate that *O. niloticus* fed housefly larvae performed better than those fed spirulina wastes diet in terms of growth performance and feed utilization. Thus, 20% of FM could be saved by including 25 % of live housefly larvae in the diet of Nile tilapia without any adverse effects on the growth performance and feed utilization. In this study there is no supplement aminoacids, feed stimulants or other marine fish products, which also have concern over their future availability like to FM. This ensures the plainness of diet formula for the successful production of this species in rural areas.

Compliance with Ethical Standard

Conflict of interests: The authors declare that for this article they have no actual, potential or perceived conflict of interests.

Ethics committee approval: This study was conducted in accordance with ethics committee procedures of animal experiments.

Financial disclosure: This research was funded by the Ministry of Higher Education and Scientific Research, Benin (Alofa C.S.'s Ph.D. thesis, University of Abomey-Calavi).

Acknowledgements: We are grateful to Mr. Appolinaire Effio, technician at the laboratory of zootechnics, Faculty of Agricultural Sciences, University of Abomey-Calavi for his assistance during the biochemical analysis. Mr Roger K. ADOUNKPE, Head of the Spirulina Production Unit of the Regional Center for Development and Health (Pahou, Benin) for the supply of spirulina by-products.

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