

Reproduction performance of female Nile tilapia (*Oreochromis niloticus*) fed on diets made using caterpillar meal (*Imbrasia truncata*) as replacement of fish meal

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The study determined the effects of caterpillar meal as a replacement of fish meal on reproduction performance of Nile tilapia (*Oreochromis niloticus*) broodstock. Three diets containing different percentages of caterpillar meal were fed to *O. niloticus*. The first diet (T0) contained 0% caterpillar meal, the second diet (T1) contained 15% caterpillar meal and the third diet (T2) contained 30% caterpillar meal. The diets were fed to duplicate groups of brood fish (average weight of 78.3 ± 6.5 g for males and 39.8 ± 8.17 g for females). Each group consisted of six females and two males stocked into a hapa and fed twice a day at 3% of their body weight for 96 days. There were no significant differences ($p > 0.05$) among the experimental diets with respect to Specific Growth Rates (SGR) but the difference was significant ($p < 0.05$) between diets T0 and the two other diets on the Survival Rate (SR). No significant differences were found between diets T0 and T1 on total % of spawning per diet. Inter-spawning intervals (ISI) showed irregular patterns in relation to diet ($p < 0.05$) between diets T0 and T1, but with diet T0, the females tended to spawn at shorter intervals. However for diet T2, the period before first spawning was significantly longer than that of diets T0 and T1. Mean gonadosomatic index (GSI) was lower in fish fed with diet T2 than those fed with diets T0 and T1. Diets T0 and T1 recorded the highest GSI with no difference between diets. The body composition of broodstock was not significantly affected by the changes in diets. These results revealed that the replacement of fish meal by caterpillar meal at 15% can lead to better reproduction performance on Nile tilapia broodstock reared in hapas. It was therefore recommended that diet T1 be used in feeding broodstock of Nile tilapia *O. niloticus*.

Keywords: Reproduction performance, *Oreochromis niloticus*, caterpillar meal

Introduction

Nile tilapia (*Oreochromis niloticus*) is one of the most commonly cultured fish species in fish farms in Kisangani region, Democratic Republic of the Congo (Ngalya et al., 2019; Lokinda et al., 2018). It is considered the most promising fish species for fish farming. The attributes which make Nile

tilapia so suitable for fish farming are its general hardiness, ease of breeding, rapid growth rate, ability to efficiently convert organic and domestic wastes into high quality protein and good taste (Balarin and Haller, 1982; Pullin and Lowe-McConnell, 1982).

The global production of tilapia is expected to exceed three million tonnes in 2010 and estimated to increase to about 8.9 million tonnes by the year 2020 (Tacon and Metian, 2008). This rapid rise in the global production of tilapia is due in part to the increasing intensification of farming systems and this has led to a critical need for large quantities of fingerlings for stocking grow-out systems. Furthermore, it is increasingly important to produce high quality tilapia fry due to the low fecundity of brood fish. Tilapia of the *Oreochromis* genus, whose female are mouth brooders and exhibit high parental care with relatively low number of eggs produced in each clutch. Given that broodstock nutrition is recognized as a major factor that can influence fish reproduction and subsequent larval quality of many fish species (Izquierdo et al., 2001; El-Sayed and Kawanna, 2008; Hajizadeh, et al., 2008). However, the shortage in world production of fish meal, the main conventional protein source, coupled with increased demand for fish meal in feeds for livestock and poultry is likely to reduce the dependence on fish meal as a single protein source in aquafeeds (El -Sayed, 1999).

Several studies examining the effects of many ingredients on fish reproduction performance have obtained different results according to type of ingredients, composition of the diets and fish species (El -Sayed and Kawanna, 2008; Zakeri et al., 2011; Ng and Wang, 2011; Chen et al., 2013; Patterson and Green 2015; Ghaedi et al., 2016; Zimba et al., 2017). However, alternative protein sources of comparable value than fish meal are therefore urgently needed (Nugroho, 2018). Based on these criteria, attention could be directed towards the locally available and cheaper protein source, which may create flexibility in diet formulations and the potential of insect protein as partial or complete replacement for fishmeal which attracted much attention in aquaculture (Rumpold and Schluter 2013; Sanchez-Muros et al., 2014; Henry et al., 2015; Lock et al., 2015). Examples include Black soldier fly (St-Hilaire et al., 2007a; Sealey et al., 2011; Kroeckel et al., 2012, Adeniyi et al., 2015, Katya et al., 2017), housefly maggots (Sogbesan et al., 2006), silkworm pupae (Zhang et al., 2008), super-worm (Jabir et al., 2012), the variegated grasshopper (Alegbeleye et al., 2012), mopane worms (Rapatsa and Moyo, 2017), earthworms (Monebi and Ugwumba, 2013) and yellow mealworm (Ng et al., 2001) are some of the insect proteins that have been used for replacement of fishmeal in fish diets. It has been reported that percentages of fish meal replacement of over 25% reduced the growth of fish (St-Hilaire et al., 2007b; Alegbeleye et al., 2012).

In Kisangani region, Republic of Congo, there are no commercial feeds for fish farmers but caterpillars are available and are cheap during the harvest period. However, information on the effect of partial or total replacement of fish meal with caterpillar meal on the reproductive performance of tilapia is limited and not documented. The present study was conducted to comprehensively evaluate the effects of dietary substitution of fish meal by caterpillar meal on the reproductive performance of female Nile tilapia (brood fish). The reproductive performance was assessed using the following parameters: time to first spawning, Inter-spawning intervals, number of spawn and gonadal somatic index, including the proximate composition of fish.

Materials and methods

Experimental diets

Three calculated isonitrogenous (30%) and isoenergetic (16.5 kJ/g) diets were formulated for the *Oreochromis niloticus* brood stock as shown in table 1. The cost of each diet was also determined. The diet in which fishmeal was used as the sole source of protein was designated diet T0 (control). Caterpillar meal combined with fishmeal was designated diet T1, and the diet in which caterpillar meal was used as the sole source of protein was designated diet T2. Diets were prepared by mixing the dry milled feed ingredients with the addition of boiling water until a desirable paste-like

consistency was reached. This paste was manually divided in small sizes and sun-dried at about 27–33°C and preserved in a plastic bag until it was to be used.

Broodstock

Broodstock were obtained from Professor Kankonda's station located in the North Eastern part of Kisangani (R.D Congo) at 0 ° 56 '034' 'N, 25 ° 21'713' 'E. Water was pumped from a nearby stream to fish pond where hapas were installed and filled to a depth of 1 m. Males and females were distributed in 3 × 5 × 1 m hapas (mesh size = 5 mm) installed in a pond (587.2 m²) in a completely randomized design with 3 treatments and two replicates. The male:female sex ratio used was 1:3 as recommended by Little and Hulata (2000). Fish were fed twice a day using formulated diets containing about 26 - 30% crude protein and 18 - 22% fat (Table 1), at a feeding rate of 3% body weight daily. Each hapa was stocked with 6 females tilapia (*Oreochromis niloticus*) (mean initial weight, 39.8 ± 8.17 g) according to Siraj et al., (1983); De Silva and Radampola, (1990); El Sayed et al., (2003) and two males (mean weight 78.3 ± 6.5 g). Each female was identified by cutting the tip of a specific fin prior to the study. The adaptation period of females to males and to the environment was 14 days. During this period, animals received the mixing of three experimental diets (Table 1). After adaptation period, the respective experimental diets were offered for 96 days from June to September 2018.

Fifteen fish were sampled and the individual length and weight were recorded before stocking. Biomass of brood fish (male and female together) from each replicate hapa was monitored every 2 weeks and eggs from the mouths of incubating females were checked. Renewal of water, cleaning of hapas, weight and ration adjustment was carried out every 2 weeks. Fish were fed once on control day and twice daily (08:30 and 16:00 h) on other days. Seeds were collected in plastic bowls from the mouths of incubating females. The post-spawning time of eggs was determined by the examination of eggs as described by Ahmed et al., (2007). The percentages of spawning females during the experiment were determined at the end of the experiment. Fish were removed from hapas and the biomass of each fish was measured. After that, three females were collected from each hapa and the lengths and weight were individually measured. Fish were then euthanized by cervical dislocation and the ovaries were taken and weighed. The collected carcasses were stored in a deep freezer for later analysis of the proximate composition of the whole fish. Based on the above measurements, the following biological parameters were calculated:

(Effiong et al., 2009)

(Charo-Karisa et al., 2006)

Water quality monitoring and analysis

The water quality parameters like temperature, dissolved oxygen, pH, conductivity, turbidity and nitrates were measured at the point of supply and drainage of the pond, and as well as in each hapa. The measurements were done once a week with a multi-meter (HACH HQ40D and HACH SL 1000). The mean values of those parameters were 29.43°C for temperature, 6.48 mg/l for dissolved oxygen, 4.86 for pH, 14.98 µS/cm for conductivity, 14.9 NTU for turbidity and 0.37 mg/l for nitrates. All the parameters remained within acceptable range for the rearing of Nile tilapia (Abdel-Tawwab et al., 2015), which is one of the most important fresh-water fish because of its capabilities to tolerate a wide range of environmental factors and stress conditions (El-Sayed, 2006). Water quality parameters were not significantly different among the experimental hapa nets.

Reproductive performance

The following reproductive parameters were determined: The time from stocking until the first spawning (time to spawn, TS) and the total number of spawning were calculated per hapa. Time to first spawning (days) was calculated as the time elapsed from stocking date to the first spawning.

Inter-spawning intervals (ISI; days) was measured as the time elapsed from one spawning to the next of repeat spawning fish only (Coward and Bromage, 1999). Total numbers of spawning per tank were also measured. The relationship between gonad weight and fish weight was used to determine gonadosomatic index (GSI).

Determination of diet and fish body chemical compositions

Fish carcasses collected during the feeding trials were frozen for subsequent analysis. The tested diets and whole-fish body from each treatment were analyzed according to the standard methods of AOAC (2005) for moisture, protein, fat, fibre and ash content. Moisture content was determined by drying the samples to a constant weight at 105 °C in a drying oven and the nitrogen content was determined using a micro Kjeldahl apparatus. Crude protein was estimated by multiplying the nitrogen content by 6.25. Lipid content was determined by ether extraction in a multi-unit Soxhlet extraction apparatus for 6 h. Fibre was determined from soxhlet extracted samples. Ash was determined by combusting dry samples in a muffle furnace at 550 °C for 6 h. All the chemical analyses were done in triplicates, and the values were reported as % dry matter basis. Gross energy values were calculated based on 23.64, 39.54 and 17.57 (KJ g⁻¹) for protein, lipid, and carbohydrate, respectively (NRC, 2011).

Statistical analysis

The effect of replacing fish meal by caterpillar meal on female reproductive performance was analyzed using the Analysis of Variance (One-way ANOVA). Where the F-ratio was significant (i.e. $p < 0.05$), treatment means were separated using the Tukey's post-hoc HSD test. In all the above analysis, significance was accepted at $p < 0.05$.

Results

The present study demonstrated that there were no significant differences ($p > 0.05$) among the experimental diets with respect to final weight, condition factor (K) and SGR. However, there were significant differences ($p < 0.05$) on SR among the experimental diets. Females that received diet T0 displayed a higher SR (100%) than those who received diets T1 (87.5%) and T2 (87.5 ± 17.7) (Table 2).

Females that received diet T1 spawned within 45 days whilst those that received diet T0 spawned within 72 days. There was a significant difference ($p < 0.05$) between diet T1 and T0 with respect to the time before the first spawning was recorded. The second spawning was recorded within 89 days for fish fed with diet T0 and 91 days for fish fed with diet T1. However, there was no significant difference ($p > 0.05$) between diet T1 and T0 with respect to this parameter. The inter-spawning intervals (ISI) ranged from 17 days for T0 to 46 days for T1 and there was a significant difference ($p < 0.05$) between the experimental diets. Fish fed with diet T2 did not spawn during the experimental period.

The group fed with diet T2 had the lowest GSI (3.13 ± 0.73 %) when compared to those fed with diets T0 (4.00 ± 0.51) and T1 which recorded the highest GSI value of 5.02 ± 1.19 %. However, no significant ($p > 0.05$) difference was found between diets according to the GSI. During 96 days of the experiment, 50 % of females fed with diet T0 spawned while 40 % of those fed with T1 also spawned (Table 3). However, no significant ($p > 0.05$) difference was found between T1 and T0 on the spawning rates.

The body composition profile (Table 4), showed that the experimental diets did not significantly ($p > 0.05$) affect the following variables: crude protein, ether extract and body ashes. Except for ether extract content ($9.10 \pm 3.98\%$), all values of the variables decreased from the initial value. Fish fed with diet T2 had a higher crude protein content value ($49.5 \pm 1.56\%$) than fish fed with diet T0

(46.5 ± 1.68%) and T1 (46.0±1.05%), but there were no significant differences ($p > 0.05$) among the diets.

Discussion

The principal aim of the present study was to investigate the substitution of fish meal, commonly used by aquafeed industry, with caterpillar meal. Fish meal is produced from small marine pelagic fish and represents a finite resource. Because of several factors, including over fishing, resulting in dwindling catch and environmental changes which necessitate tight regulations, future demand for wild-caught fish will exceed supply (Sargent et al., 1999). Hence the need to evaluate potential substitutes for fish meal, an important ingredient in the formulation of aquafeeds. The results of the present study showed that SGR decreased with fish meal replacement, but no significant differences were recorded among the diet formulations. However, the findings of the present study are not consistent with those of previous authors, who found that the percentages of fish meal replacement with insects meal of over 25% reduced the growth of fish (St-Hilaire et al., 2007b; Alegbeleye et al., 2012). The lower SGR could be attributed to the composition of diets in terms of nutrient composition, the strain and age of fish used. According to the composition of diets, tilapia broodstock, if energy dietary reserves are not sufficient to support the reproductive functions, tissue protein is mobilized and catalyzed to be used as an energy source (Coward and Bromage, 1999; El-Sayed and Kawanna, 2008).

The results of the present study showed that fish fed with diet T1 spawned earlier than those fed with diet T0. This was most likely because diet T1 contained higher value of crude protein and lipid needed for egg yolk formation and other reproductive process. The findings of the present study were in agreement with studies by Grémare et al. (1988), Ng and Wang (2011) and Nesto et al., (2012), which suggested that, a high protein diet is helpful in speeding both gametogenesis and sexual maturity in fish because the nitrogen ration is the main factor which influences the reproductive output (Gunasekera et al., 1995). Nile tilapia brood stock requires about 40% dietary protein, 16.7 Mega Joules of Gross Energy /kg (energy) and 23.6 g/MJ (P/E ratio), for shorter spawning intervals and maximum reproductive output (El-Sayed and Kawanna, 2008), therefore, the reason for the low reproductive performance in the current experiment can be attributed to the lower values of protein, energy and P/E ratio of our diets.

The protein requirement of tilapia broodstock has been reported by many authors, with varying results. Values ranging from 30 to 40%, depending on species and size, dietary protein and energy sources, and rearing systems (Al Hafedh et al., 1999; Bhujel et al., 2001; El-Sayed et al., 2003). In the present study, spawning performance was poor in diet T2 (26% CP without fish meal), while fish fed with diet T2 did not spawn. This was in contradiction with the findings of El-Sayed et al. (2003) who reported that Nile tilapia fed with a diet containing 25% protein at 7‰ and 14‰ water salinity spawned early. The reason of the lack of spawning in that group of fish could be due to the imbalance of amino acid in the diets or to its fatty acids profile but this was not determined for this study. Eicosapentaenoic and arachidonic acids are precursors of eicosanoids, like prostaglandins (Bell et al., 1986; Tocher, 2003), which have a large variety of physiological actions in fish, including oocyte final maturation and ovulation (Sorbera et al., 2001; Planes and Swanson, 2008). Since amino acids and fatty acids profile of our diets were not done, research should be directed to establishing the composition in amino acids and fatty acids profile of diets and fish.

No significant effects of diets were observed regarding female GSI, because reproductive females allocate much of their energy reserves towards reproduction, not for growth (Patterson and Green, 2015). The result of the present study shows that the growth of females was lower but the GSI was higher for the diets T0 and T1. So, in order to reduce the use of fishmeal, the diet T1 is recommended for Nile tilapia female broodstock. The relationships between diets and the gonadosomatic index (GSI) of females in the present study were in accordance with the findings by Orlando et al. (2017) who worked on the reproductive performance of female Nile tilapia fed diets

with different digestible energy levels. Nile tilapia females have been reported to spawn at 20–30 g and up to 50 g (Popma and Masser, 1999) and within a period of 2 - 4 months under culture conditions (De Silva and Radampola, 1990; De Graaf et al., 1999; De Graaf, 2004). In nature, Nile tilapia have been reported to first sexually mature at 8 -16 cm and 10 - 12 months of age (Morales, 1991). Under culture conditions, tilapia maturation occurs sooner than in the wild, and more eggs are produced as a homeostatic response to the environment (Ahmed et al., 2007). The present findings indicated that, the spawning intervals of Nile tilapia were significantly affected by diets and tended to increase with the increase of caterpillar meal in the diet. During the first spawning, fish fed with diet T1 spawned earlier than those fed with diet T0, but on the second spawning it was the opposite. Fish fed with diet T2 did not spawn during the 96 days of the study.

The effect of dietary protein on spawning intervals of tilapia has been extensively studied elsewhere with varying results. Tilapia can spawn at intervals as short as 1 - 2 weeks, 15 - 20 days or 127 days (De Silva and Radampola, 1990; Tacon et al., 1996). Therefore, our findings on diets T0 and T1 are in conformity with the findings of El-Sayed et al., (2003) who found out that, the shortest spawning interval of 7 days was recorded in the group fed with a diet containing 40% protein, while the longest interval of 39 days was recorded in fish fed with 25% protein. Gunasekera et al. (1996), as well as Mashaii et al. (2016) observed also that the spawning intervals decreased with increasing dietary protein levels. However, spawning frequency in tilapias is seriously influenced by environmental factors and younger Nile tilapias often have shorter reproduction cycles. On the other hand, the percentage of spawning female Nile tilapia during the experiment was not affected under the diets T0 and T1.

In the present study females fed with diet T2 exhibited a numerical higher body protein deposition, while ether extract had the opposite response. However, there were no significant differences in the body composition of the fish between diets. In general, the protein body composition of broodstock fed with different diets for the present study did not agree with the findings of El-Sayed and Kawanna (2008), who suggested that the proximate composition of Nile tilapia broodstock was higher for fish fed with high protein diets. These variations may have been attributed to age and size of brood fish, the differences in diet composition, the P/E ratio, and experimental design such as the duration of study and environmental factors (Little and Hulata 2000; Mashaii et al., 2016).

The survival rate in our study was significantly different among the diets. The diet with only fish meal (T0) had 100% SR and the other two diets (T1 and T2) had 87.5% survival rates. This drop of SR can be attributed to handling during data collection because most of the deaths were recorded during data collection days. Further investigations are recommended to determine whether there was a causal factor related to the higher mortality in fish feed diets with caterpillar meal.

In conclusion, caterpillar meal had beneficial effects on the reproductive performance of female Nile tilapia, which includes a higher GSI, earlier first spawning activity, a low cost diet, an average inter-spawning interval, a high protein feed value when 15 % of fish meal is replaced by caterpillar meal. The replacement of expensive dietary fish meal with caterpillar meal will help in reducing the costs of broodstock diets as well as contributing to the environmental sustainability of wild fish stocks from which fish meal is derived. We suggest that the amino acids content and fatty acids profile be determined in those diets in future. The duration of the experiment needs also to be increased and parameters such as total egg production, egg hatching rates, and larval survival also need to be evaluated in future research.

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