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Effects of the Use of Plant-Based Food Additives on Growth, Body Composition, and Survival of Nile Tilapia (*Oreochromis niloticus* Linnaeus, 1758)

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Abstract

This study aimed to evaluate the impact of plant-based food additives on growth performance, body composition and survival of Nile tilapia. Four isonitrogenous (25% CP) and isolipidic (13% CL) experimental diets were prepared and designated as R1, R2, R3, and R4. R1 contains (3% *Cymbopogon citratus*), R2 contains (3% *Ocimum basilicum*), R3 contains (3% *Mentha spicata*) and R4 (control or 0% additive). 180 Nile tilapia fry with an initial average weight of 0.2g were distributed in 12 plastic tanks (50 L/tank) at the stocking density of 15 fish per tank in an isolated system. Fish were fed with experimental diets 3 times per day (8:00 am, 1:00 pm and 5 :00 pm) for 3 months. After 90 days of experiment, the results revealed that the final average weight ranged from 2.62g to 8.00g, the absolute average weight gain (AAWG) from 2.42 to 7.8g, the feed conversion ratio (FCR) from 1.31 to 2.67 and specific growth rate (SGR) from 3.06 to 4.36%/d. The survival rate (SR) varied between 64 and 100%. Of the parameters studied such as the absolute average weight gain (AAWG), the specific growth rate (SGR), the feed conversion ratio (FCR), and the survival rate, the best were obtained by the group of fish fed with the R2 diet containing 3% *Ocimum basilicum*. The results of the analysis of the flesh showed that the addition of 3% *O. basilicum* does not modify the contents of dry matter, crude protein, and ash but slightly increased the fat content. Based on the results, the inclusion of natural plant-based food additive (3% *Ocimum basilicum*) in the diet of Nile tilapia improved the parameters of growth, feed efficiency, and survival.

Keywords: additives, food, growth, fry, Nile tilapia

1. Introduction

The world total fish production has been stagnant since the 1990s (FAO, 2018), while population growth continues to increase and may even double by 2050 (Africa) according to Hounmanou *et al.* (2018). This situation will increase the demand for fish. Aquaculture is therefore expected to play an essential role in providing valuable animal protein-based foods to poor and food-insecure populations (Kobayashi *et al.*, 2015). The development of aquaculture is spectacular due to the contribution of the feed industry. However, some feed processing plants use additives to boost growth. These additives are chemicals, mainly hormones, and antibiotics that can cause side effects (Dada, 2015). The use of hormones and antibiotics is considered a major public health risk and measures to reduce and develop alternatives become a priority for international organizations (UN, FAO, WHO, OIE, EFSA). In this context, plants and herbs that possess antibacterial, antiviral, and antioxidant properties recognized by traditional medicines in many countries are investigated. For these reasons, the use of herbs and plants is encouraged by the World Health Organization (WHO) to reduce the use of chemicals.

Cymbopogon citratus, Stapf (Lemon grass) is a widely used herb in tropical countries. The compounds identified in *Cymbopogon citratus* are mainly terpenes, alcohols, ketones, aldehyde and esters. Some of the reported phytoconstituents are essential oils that contain Citral α, Citral β, Nerol Geraniol, Citronellal, Terpinolene, Geranyl acetate, Myrecene and Terpinol Methylheptenone (Shah *et al.*, 2011). These herb are tufted perennial C4 grasses with numerous stiff stems arising from a short, rhizomatous rootstock (Kumar *et al*., 2000) as with citrus flavor, and can be dried and powdered or used fresh. Common basil (*Ocimum basilicum* L.), a member of the Lamiaceae family is an annual herb, which grows in several regions around the world. Traditionally, basil has been extensively utilized in food as a flavoring agent, and in perfumery and medical industries (Telci *et al*., 2006). The leaves and flowering tops of the plant are perceived as carminative, galactogogue, stomachic and anti-spasmodic in folk medicine (Sajjadi, 2006). The mint species have a great importance, both medicinal and commercial. Indeed, leaves, flowers and stems of *Mentha spp*. are frequently used in herbal teas or as additives in commercial spice mixtures for many foods to offer aroma and flavor (Boukhebti *et al*., 2011)

In aquaculture practices many herbs and herbal products are included in the fish diet to cure diseases, promote growth, reduce stress, stimulate appetite, boost immunity and prevent infections in producing healthy fishes (Shalaby, 2004). The flavor imparted by herbs and herbal products added in fish diet changed the eating patterns, increased feed consumption and stimulated digestion by increasing the secretion of saliva, various digestive enzymes, bile, pancreatic enzymes activity and mucus in fishes (Lee and Gao, 2012). The objective of this study was to evaluate the impact of three plant-based food additives on growth performance, body composition and survival of the Nile Tilapia.

2. Materials and Methods

2.1 Experimental Feeds

In order to determine the effectiveness of some medicinal herbs in fish feed, three practical diets were prepared, each containing a specific herb and a control diet without incorporating any herb. The diets were formulated to be isonitrogenous (25% CP) and isolipidic (13% CL). Diet 1 (R1) contains 3% *Cymbopogon citratus*, Diet 2 (R2) contains 3% *Ocimum basilicum*, Diet 3 (R3) contains 3% *Mentha spicata* and Diet 4 (R4 or control) contains 0% additive. Apart from the additives used, all the diets had the same ingredients (fishmeal, corn flour, rice bran, millet bran, *Sterculia* gum, yeast and premix vitamins and minerals) as shown in table 1. After pre-treatment of the ingredients (removing some impurities), the ingredients were ground to a fine powder. The powder of different ingredients was weighed accordingly and mixed. When ingredients were thoroughly mixed, oil and 30% water were added to obtain dough. The dough obtained from each treatment was passed through a Moulinex to produce spaghetti-like filaments that were dried in a traditional dryer for 30 minutes to 1 hour. These dried filaments were ground into a powder with a mortar before being packaged in glass jars for feeding the fry.

Table I. Formulations and proximate composition of experimental feeds

	Diets			
Ingredients (g)	**R1**	**R2**	**R3**	**R4**
C. citratus	3	0	0	0
O. basilicum	0	3	0	0
M. spicata	0	0	3	0
Fish meal	32	32	32	32
Millet bran	20	20	20	20
Rice bran	10	10	10	10
Fish oil	5	5	5	5
Corn flour	22	22	22	25
Yeast	4	4	4	4
Sterculia gum	2	2	2	2
Minerals premix[a]	1	1	1	1
Vitamins premix[b]	1	1	1	1
Total	100	100	100	100

a = phosphorus 7%; calcium 17%; sodium 1.5%; potassium 4.6%; 7.5% magnesium; manganese 738mg; 3000mg zinc; 4000mg iron; 750mg copper; iodine 5mg; cobalt 208mg; calcined and ground attapulgite qs 1000g; fluorine content of the complex 1.5% (approximately), dose 1kg per 100kg of food.

b = lives at 250000 IU; lives D3 250000UI; lives E 5000mg; lives B1 100mg; lives B2 400mg; lives B3 (pp) 1000mg; saw B5 pentode Ca2000mg; lives B6 300mg; lives K3 1000g; saw C 5000mg; H biotin 15mg; choline 100g; special expiant (anti oxidant (BHT), attapulgite crushed and calcined) qs 1000mg.

2.2 Growth Trial

Nile tilapia fry (*Oreochromis niloticus*) of three weeks old from the West Africa Agricultural

Productivity Program (WAPP) on the masculinization of tilapia were used. The fry were transported to the IUPA experimental unit at UCAD in 2019, where the experiment was conducted. They were acclimatized for two weeks. One hundred and eighty (180) Nile tilapia fry with an initial average weight of 0.2g were randomly distributed in 12 plastic tanks (50 L/tank) at the stocking density of 15 fish per tank in an isolated system. At the start of the trial, the weight and length of each fish were taken. Fish were fasted twenty-four hours before weighing. Each tank was equipped with an air stone for oxygenation of the water. The tanks were cleaned daily to remove feces and uneaten feed. Each experimental diet was administered by hand to a triplicate group of fish three times per day (8:00 am, 1:00 pm and 5:00 pm). The first month, fish were fed at 10% of their biomass, the second month, 8% of their biomass, and the third month 6% of their biomass. The amount of feed given was readjusted after each sampling according to the evolution of the biomass. The experiment lasted for 12 weeks.

2.3 Sampling

Sampling was done every 15 days by weighing individual fish in each tank. At the end of the experiment, all the fish were counted, the weight of each fish were taken for determination of average weight gain (AWG), specific growth rate (SGR), feed conversion ratio (FCR) and survival rate (SR).

2.4 Mathematical Formulae for Evaluating Growth Parameters

Growth performance parameters such as absolute average weight gain (AAWG), feed conversion ratio (FCR), specific growth rate (SGR), survival rate (SR), were determined according to the mathematical formulae below:

- AAWG (g) = final average weight - initial average weight

- FCR = total dry feed fed / weight gain

- SGR (%/d) = [(ln (final weight) - ln (initial weight)) / duration of the experiment] * 100

- SR (%) = (number of final fish / number of initial fish) * 100

2.5 Chemical Analysis of Feed and Muscle

Before the experiment, samples of the three herbs (additives) and four diets were sent to a local laboratory for proximate analysis in order to determine their composition (protein, fat, ash, fiber, and dry matter contents). After the experiment, the fish were sacrificed, muscles collected from each treatment and also sent to the same laboratory for analysis in order to determine the biochemical composition (Dry Matter (DM), Crude Protein (CP), Ash (C) and Fats (F)).

The analysis was based on the procedure of the Association of Official Analytical Chemists (AOAC). Samples of feeds and fish muscles were dried to constant weight at 105 ^{o}C for 24 h to determine moisture, and consequently, dry matter values were determined. Crude protein (total Nitrogen x 6.25) was determined by using the micro-Kjeldahl method (Kjeltec System 1002 Distilling Unit, Tecator, Hoeganaes, Sweden). Crude fat was extracted by the Soxhlet method, and ash was determined by incineration of samples in a muffle furnace at 550 °C for 6h.

2.6 Statistical Analysis

Microsoft Excel was used to enter and calculate the data. The analysis of these data was carried out with the Statistical Analysis System software (SAS-PC) (Joyner, 1985) subjected to an analysis of variance (ANOVA). The results were presented as means of the three replicates. The Duncan test was used to compare significant differences between treatments. The difference was considered significant at *P*-values < 0.05.

3. Results

During the experiment, no pathological signs and symptoms were observed, and the fish accepted all the diets throughout the trial period. The survival rate varied between 60 – 100%. During the feeding trial, the culture environment was conducive to tilapia. Water temperature was monitored daily and ranged from 25.51 to 25.78˚C.

At the end of the experiment, parameters such as absolute average weight gain (AAWG), specific growth rate (SGR), feed conversion ratio (FCR), and survival rate (SR) were determined for each treatment. Concerning AAWG, ANOVA did not show a significant difference among the R1, R2, and R4 treatments, but these are statistically different from the R3 treatment. The same trends are apparent for all the others parameters studied (Table II).

Table II. Evolution of parameters of growth, survival and feed efficiency

	Diets			
Growth parameters	**R1**	**R2**	**R3**	**R4**
Wi (g)	0,2	0,2	0,2	0,2
Wf (g)	7,18	8,00	2,62	7,15
AAWG (g)	$6,98^{a}$	$7,80^{a}$	$2,42^{b}$	$6,95^{a}$
SGR (%/d)	$4,23^{a}$	$4,36^{a}$	$3,06^{b}$	$4,26^{a}$
FCR	$1,44^{a}$	$1,31^{a}$	$2,67^{b}$	$1,53^{a}$
SR (%)	84	100	64	91

^{ab}Different superscripts in each line indicate significantly different mean values ($p < 0.05$).

The muscle composition of *O. niloticus* fry at the end of the experiment are shown in Table III. Dry matter, crude protein, and ash contents of the flesh of the fish fed on R1 and R2 diets showed no significant differences compared to the muscle of the fish fed on the R4 diet (control diet). However, their lipid contents were slightly higher than the lipid content of the flesh of the fish fed on the control diet (R4). The analysis of the flesh also showed that the addition of 3% of *Mentha spicata* in the R3 diet did not cause any significant difference in the contents of dry matter and ash compared to the control diet (R4). The addition of 3% of *Mentha spicata* in the R3 diet showed a greater increase in lipid level and a slight decrease in the protein content of fish fed on the R3 diet compared to the control diet (R4).

Table III. Proximate composition of the tilapia with experimental diets

Fish	**DM (%)**	**CP (% DM)**	**Ash (%DM)**	**CL (% DM)**
R1	97,01	83,61	8,21	7,20
R2	97,5	84,32	8,22	7,42
R3	98,24	75,98	8,85	13,56
R4	97,99	85,56	8,15	5,97

4. Discussion

The values of the temperature recorded during this experiment ranged from 25.51 - 25.78°C. They were within the recommended values for Nile tilapia breeding water. According to El-Sherif *et al.* (2009), the optimum temperature for growth and survival of Nile tilapia fry breeding water is between 25 and 30 °C.

The results obtained from the experiment showed that the use of medicinal herbs has a positive effect on the growth and survival of Nile tilapia fry. Statistical analysis of the data revealed that the control treatment (R4) was not significantly different from diets R1 and R2 but was significantly different from diet R3. Of all the parameters studied, namely the absolute average weight gain (AAWG), the specific growth rate (SGR), the feed conversion ratio (FCR) and the survival rate (SR), in terms of absolute value, the best was recorded in the group of fish subjected to the R2 diet which had *Ocimum basilicum*. The enhanced growth in the *Ocinum basilicum*-supplemented diet may be because this herb enhanced the nutrient digestibility leading to improved nutrient utilization, which in turn could also explain the better growth. The flavor imparted by herbs and herbal products added in fish diet changed the eating patterns, increased feed consumption and stimulated digestion by increasing the secretion of saliva, various digestive enzymes, bile, pancreatic enzymes activity and mucus in fishes (Lee and Gao, 2012).

Indeed, for the absolute average weight gain, the R2 diet had 7.80g. This result is similar to the work of El-Dakar *et al.* (2015). With 2% *Ocimum basilicum* in the diet of the *Sparus aurata* at 1.88g initial average weight, they obtained a weight gain of 5.60g, which was higher compared to the control although no statistical difference was recorded. With 5% *O. basilicum* in the diet of *Oreochromis mossambicus*, the length of the fish increased

(Karpagam and Krishnaveni, 2014). Also, 2ml/kg of basil essential oil improved the growth of Nile tilapia (Elizângela *et al.*, 2019).

About the SGR value-wise, the R2 diet recorded the best (4.36%/d) followed by the R4 diet (4.26%/d) and then the R1 diet (4.2%/d), but there were no significant differences among them. The R3 diet had the lowest SGR (3.06%/d) and was significantly different from the rest. The inclusion of basil leaves in this study improved the SGR, and a similar result was recorded by El-Dakar *et al.* (2008) following the inclusion of 2% dried basil leaves in the diet of hybrid tilapia fry (*Oreochromis niloticus* x *Oreochromis aureus*). This result is in line with the work of Adewole *et al.* (2017), who showed that the use of *O. gratissimum* leaves as an additive in the diet of *Clarias gariepinus* improved to a certain level the SGR compare to control. Oluyemi and Funmi (2015) also reported that the addition of 40 mg/g of *O. gratissimum* leaves in the diet of *Clarias gariepinus* improved the SGR.

For feed conversion ratio (FCR), the two best results were observed with the diets that were included with basil (1.31) and citronella (1.44). These results are similar to those of Attalla (2009a), who reported that the use of forage as an additive significantly improved food intake, feed conversion ratio, and protein efficiency ratio. According to Amirkhani and Firouzbakhsh (2015), basil leaf extract at inclusion levels of 4% and 8% in fish feed improved growth and specific growth rate and the feed conversion ratio of common carp (*Cyprinus carpio*). The work of El-Dakar *et al.* (2015) also showed that the addition of *O. basilicum* in the diet of *Sparus aurata* improved the FCR of fish fed the diet containing basil compared to control.

The survival rate of fish subjected to 3% basil leaf powder in their diet was 100%. This result is similar to the data recorded by El-Dakar *et al.* (2015) with the use of 2% dried basil leaves, 2% dried basil seeds, and 2% basil seeds soaked and dried in the diet of *Sparus aurata*. This shows that survival is not affected by the inclusion of the basil products mentioned above in the diet of the fish species, as mentioned earlier. In addition, it has been proven that the addition of *O. basilicum* extract to the diet of *Cyprinus carpio* improved the resistance of fish compared to those subjected to the control regime which recorded a lower survival rate following a challenge test against *Aeromonas hydrophila* (Amirkhani and Firouzbaksh, 2015).

The biochemical composition showed that the dry matter, crude protein, and ash contents of the flesh of fish subjected to the R1, R2 and R4 diets had no significant difference. However, lipid levels in the flesh of fish fed R1 and R2 diets were higher than that of the R4 (control) diet. These results are different from those obtained by Oluyemi and Funmi (2015). They showed that the incorporation of 3% *Ocimum gratissimum* in the feed increased the dry matter, crude protein and ash contents and decreased the lipid content of the flesh. However, the fat content (7.42%) recorded with the R2 diet in this experiment is within the range of the fat content of the fish muscle, which is around 0.7 to 8.5% as supported by some researchers, including Visentainer (2005). R3-fed fish had increased dry matter, fat, and ash contents but decreased raw protein level compared to R4 (control) fed to fish. Qiu *et al*. (2003) fed formulated diets supplemented with or without TCM to Allogynogenetic crucian carp, and found that there were no significant differences in moisture and crude protein contents in the muscle; however, the muscle of fish fed the TCM supplemented diet has a significantly higher lipid content. In general, fat accumulation in a fish body results from the balance between dietary absorbed fat, de novo synthesis of fatty acids (lipogenesis) and fat catabolism via β-oxidation (lipolysis). These results differ from those of Adel *et al.* (2015) who showed that *Rutilus frisii kutum* fry fed 0%, 1%, 2% and 3% *Mentha piperita* in their diet for 8 weeks did not change the dry matter, lipid, protein, and ash contents of the flesh.

5. Conclusion

The results of this study indicate that the use of dry leaves of *Ocimum basilicum* and *Cymbopogon citratus* as food additives has a positive effect on growth and survival parameters. The study shows that supplementation of 3% *Ocimum basilium* and/or *Cymbopogon citratus* in the diet of tilapia fry boosts growth, improves the specific growth rate and the feed conversion ratio. However, the addition of 3% *Mentha spicata* in the feed has a negative effect on the studied growth parameters and survival. The study shows the possibilities of using plant leaves as growth factors for fish.

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