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Original article

Effect of dietary fluted pumpkin (*Telfairia occidentalis*) extract on growth performance, body composition and haematological parameters of Nile tilapia (*Oreochromis niloticus* Linnaeus)

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Abstract

The effect of dietary fluted pumpkin extract on growth, body composition and haematological profile was investigated in Nile tilapia *Oreochromis niloticus.* Fingerlings of about 5.23-5.44 g were fed diets supplemented with four concentrations ((2.5, 5.0, 7.5 and 10.0 gkg^{-1}) of fluted pumpkin extract powder for eight weeks. Fish fed supplemented diets showed significantly improved growth performance and feed utilization over the control (0 gkg^{-1} fluted pumpkin extract powder) treatment. The highest specific growth rate (0.79±0.10% per day) and best food conversion ratio (0.98±0.14) were obtained in fish fed 2.5 gkg^{-1} fluted pumpkin extract powder diet. No differences occurred in fish carcass moisture, protein or crude lipid content among the treatments (p>0.05). Similarly no differences occurred in white blood cells among the treatments (p>0.05) but there were greater improvement in the white blood cells of fish fed on dietary fluted pumpkin extract powder compared to the fish fed the control diet. The results suggest that dietary supplementation with fluted pumpkin extract powder improved growth rate, feed utilization, white blood cells and survival of Nile tilapia *O. niloticus* fingerlings.

Keywords: Nile tilapia, *Oreochromis niloticus*, growth, fluted pumpkin extract

INTRODUCTION

The cichlid Nile tilapia is the third most important fish species in aquaculture after carp and salmon because of their high protein content and palatability (FAO 2O12) but in spite of the remarkable aquaculture potentials of Nile tilapia, the size of this species obtain under culture is very poor. Hence, researchers are continually looking for commercial diets that will permit optimal growth of fish without health hazards (Baruah *et al*. 2008). One of the ways to improve the growth of this fish is through the use of dietary supplements that include plant-based additives. The use of plant-based additives in aquaculture is one of the methods used to improve weight gain, feed efficiency, and/or disease resistance in cultured fish.

Telfairia occidentalis (fluted pumpkin) is cultivated in various parts of southern Nigeria. The darkish green leafy vegetable is used as food and herbal medicine. The leaf is a rich source of protein, oil, vitamins and minerals but low in crude fibre and also rich source of folic acid, calcium, zinc, potassium, cobalt, copper, iron, vitamins A, C and K (Ajibade *et al*. 2006).

Fasuyi and Nonyerem (2007) reported that *T. occidentalis* leaf contains active ingredient which is a plant growth promoter that promotes growth in birds, and this may have similar effects in fish. The leafy

vegetables possess anti-microbial and antiviral properties (Nwozo *et al*. 2004, Olorunfemi *et al*. 2005). *T. occidentalis* extract is also reported to increase hematological parameters (Alada 2000). The objective of this study was to determine the dietary effects *T. occidentalis* leaf extract powder concentration on growth, body composition and haematological parameters of *O. niloticus* fingerlings.

METHODOLOGY

Formulation of experimental diets

The Pumpkin leaves were purchased from a market in Akure, Ondo State. The leaves were air dried for 7 days in order to remove the moisture content and the dried materials were milled into flour and later sieved to remove the chaff from the milled leaves. 500 g of the powder (pumpkin leaves) was soaked in distilled water and allowed to ferment naturally at room temperature for 24 hours, After which it was filtered with a muslin bag. The filtrate was evaporated to dryness in water bath to obtain a solid extract. Amounts of 0 (control), 2.5, 5.0, 7.5 and 10 g *T. occidentalis* extract per kg feed were taken and mixed with a basal feed (30% crude protein), containing fish meal, yellow maize, soybean meal, blood meal, fish oil, vegetable oil, vitamin premix and starch. All dietary ingredients were milled to a 3 mm particle size. The ingredients were thoroughly mixed in a Hobbart A-2007 pelleting and mixing machine (Hobart Ltd, London, UK) to obtain a homogeneous mass, and cassava starch was added as a binder. The resultant mash was pressed without steam through a mixer with a 0.9 mm die. The pellets were dried at ambient temperature (27-30 °C) and stored at −20 °C in a refrigerator. Table 1 shows the proximate composition of the diets.

Experimental procedure

15 (fifteen) plastic tanks (33-litre), each filled with 20 liter of water were aerated continuously using an air compressor. 150 farm-raised *O. niloticus* fingerling siblings (with an average initial weight of 5.23-5.44 g) were acclimated to laboratory conditions for 14 days at the department of fisheries and aquaculture technology hatchery unit, Federal University of Technology, Akure, Ondo state, Nigeria before being distributed randomly into the fifteen tanks at a stocking density of 15 fish/tank representing five dietary treatments (0, 2.5, 5.0, 7.5 and 10.0 g *T. occidentalis* leaf extracts per kg feed) designated as FPE1 (control), FPE2, FPE3, FPE4 and FPE5). Fish were fed at 3% of their body weight (bw) per day in three equal meals, every five hours between 08:00 and 18:00. All fish were weighed and counted fortnightly and feeding rates were adjusted accordingly.

Table 1: Ingredients (g) and proximate composition (%) of feed ingredients

Ingredient	*Dietary treatments*				
	FPE1	*FPE2*	*FPE3*	*FPE4*	*FPE5*
Fishmeal	15	15	15	15	15
Yellow maize	25	25	25	25	25
Soybean meal	45	45	45	45	45
Fish oil	4	4	4	4	4
Vit premix	3	3	3	3	3
Binder (starch)	2	2	2	2	2
Vegetable oil	6	6	6	6	6
FPE	0	0.25	0.5	1.0	2.0
Proximate composition (%DM)					
Moisture	7.41	8.07	8.44	8.23	8.29
Ash	8.64	9.46	9.47	9.52	9.61
Fat	10.08	10.31	10.32	10.37	10.46
Crude fibre	5.57	6.13	6.18	6.23	6.27
Crude protein	31.66	31.00	31.84	32.53	33.19
NFE	38.64	35.03	33.75	33.12	32.18

Vitamin premix – A Pfizer livestock product containing the following per kg of feed: A = 4500 I. U, D = 11252 I.U, E = 71 I.U, $K_{3=}$ 2 mg, $B_{12=}$ 0.015 mg, panthothenic acid = 5 mg, nicotinic acid = 14 mg, folic acid = 0.4 mg, biotin = 0.04 mg, choline = 150 mg, cobalt = 0.2 mg, copper = 4.5 mg, iron = 21 mg, manganese = 20 mg, iodine = 0.6 mg, selenium= 2.2 mg, zinc = 20 mg, antioxidant = 2 mg. DM = dry matter

The experiment lasted for 56 days. At the end of the experimental period the following growth and feed utilization parameters were calculated: weight gain (WG), specific growth rate (SGR), food conversion ratio (FCR) and protein efficiency ratio (PER) using the following formulae as described by Brown (1957), Winberg (1956), Castell and Teiws (1980), and Miller and Bender (1955), respectively.

WG = final average weight (g)−initial average weight (g);

$SGR\ (\%\ d^{-1}) = 100 \times (\ln Wt - \ln W0)/t$

Where Wt and $W0$ represent final and initial body

weights of fish, respectively, and *t* represents the duration of the feeding trial;

FCR = dry weight of feed (g) / wet weight gain by fish (g); and PER = wet weight gain by fish (g) / protein intake (g)

Where protein intake (g) = protein (%) in feed × total weight (g) of diet consumed / 100.

Water temperature, pH and dissolved oxygen concentration were routinely monitored in all tanks. At the beginning and end of the feeding trial, pooled samples of 15 fingerlings were analyzed for carcass composition using AOAC (1997) procedures.

Twelve fish (four fish per replicate) were used for blood analysis and 5 ml blood samples from each treatment were collected by cardiac puncture using 5 ml disposable syringes, into treated Bijou bottles. The blood was stored at -40 °C prior to analysis. The blood analysis followed the methods described by Svobodova *et al*. (1991).

Statistical analyses

The effects of fluted pumpkin leaf extract as feed supplement on growth, haematological parameters were analyzed using one-way analysis (ANOVA) and significant differences among treatment means were compared using Turkey's multiple range test (Zar 1996).

RESULTS

Mean water quality parameters during the experiment were: dissolved oxygen 7.85±0.5 mgl^{-1}, pH 7.25 and temperature 27.5±0.40 °C. There were improvements in the growth responses of fish fed on *T. occidentalis* leaf extracts meal. The best growth responses were obtained in the fish fed on diet FPE2 (2.5 g *T. occidentalis* leaf extract powder per kg feed) while the slowest growth was obtained in the fish fed diet FPE5 (Table 2).

However, there were no significant differences in growth performance across the different *T. occidentalis* leaf extract powder concentrations. There were also improvements in the feed conversion ratio (FCR) of fish fed on diets FPE2 and FPE3 than the FPE4, FPE5 and FPE1. The average FCRs were 0.98, 1.08, 1.40, 1.47 and 1.19 for diets FPE2, FPE3, FPE4, FPE5 and FPE1 respectively. The PER was 0.06, 0.06, 0.04, 0.05 and 0.05 for the fish fed diets FPE2, FPE3, FPE4, FPE5 and FPE1. There were improvements in the protein content of fish fed on *T. occidentalis* leaf extract powder than fish fed the control diet.

Table 2: Mean growth performance and feed utilization of *O. niloticus* fingerlings fed experimental diets for 56 days

Parameters	*Dietary treatment*				
	FPE1	*FPE2*	*FPE3*	*FPE4*	*FPE5*
Initial mean weight (g)	5.44 (0.03)	5.24(0.10)	5.23 (0.02)	5.34(0.02)	5.40(0.05)
Final mean weight (g)	7.77(0.60)^{a}	8.15(0.30)^{a}	7.87(0.16)^{a}	7.33(0.14)^{a}	7.29(0.48)^{a}
Weight gain (g)	2.34(0.63)^{a}	2.92(0.12)^{a}	2.65(0.15)^{a}	1.99(0.12)^{a}	1.88(0.43)^{a}
PER	0.05(0.01)^{a}	0.06(0.01)^{a}	0.06(0.01)^{a}	0.04(0.00)^{a}	0.05(0.01)^{a}
SGR (% day^{-1})	0.64(0.15)^{a}	0.79(0.10)^{a}	0.73(0.03)^{a}	0.57(0.03)^{a}	0.53(0.10)^{a}
FCR	1.19(0.46)^{a}	0.98(0.14)^{a}	1.08(0.05)^{a}	1.40(0.04)^{a}	1.43(0.31)^{a}
FI (g/day)	2.79(0.06)^{a}	2.89(0.15)^{a}	2.86(0.09)^{a}	2.79(0.14)^{a}	2.68(0.29)^{a}

Values in parentheses are standard errors of means. Means in a given column with the same superscript letter were not significantly different at p<0.05. SGR= specific growth rate, PER = protein efficiency ratio, FCR = feed conversion ratio, FI = feed intake

However, there were no significant differences in protein contents across the different *T. occidentalis* leaf extract powder concentrations and the control diet. The body composition values are given in Table 3. The haematological parameters of *O. niloticus* (Table 4) showed no significant differences ($p \geq 0.05$) in red blood cell, haemoglobin or pack cell volume in all the treatments. However, there was a significant difference ($p<0.05$) in the white blood cell count of fish in the treatments. Fish fed on diets supplemented with *T. occidentalis* leaf extract powder had significantly higher ($p<0.05$) white blood cell counts.

Table 3: Chemical composition of whole body of *O. niloticus* fingerlings fed experimental diets (wet basis)

Composition (%)	*Dietary treatments*				
	FPE1	*FPE2*	*FPE3*	*FPE4*	*FPE5*
Moisture	4.71(0.15)^{a}	3.18(0.05)^{a}	3.11(0.04)^{a}	3.20 (0.03)^{a}	3.15(0.00)^{a}
Crude protein	57.37(0.21)^{a}	57.64(0.41)^{a}	57.89(0.07)^{a}	57.45(0.33)^{a}	58.23(0.89)^{a}
Crude lipid	12.44(0.33)^{a}	12.28(0.25)^{a}	11.74(0.28)^{a}	12.54 (0.42)^{a}	11.84(0.20)^{a}
Ash	15.64 (0.36)^{a}	18.58(0.07)^{ab}	17.21(0.48)^{b}	16.57(0.54)^{ab}	17.37(0.06)^{b}
NFE	9.85 (0.03)^{a}	10.33(0.64)^{a}	10.06(0.30)^{a}	10.25(1.28)^{a}	9.42(0.85)^{a}

Means in a given row with the same superscript letter were not significantly different at p<0.05. Values in parentheses are standard errors of means NFE = Nitrogen free extract

DISCUSSION

The results suggest that dietary *T. occidentalis* leaf extract promoted the growth of *O. niloticus* fingerlings at 2.5 and 5.0 g/kg feed concentrations; and when the concentration increased to 7.5 and 10.0 g/kg feed, there

was no additional growth. This was the first attempt to investigate potential of *T. occidentalis* leaf extract as a feed additive in aquaculture.

Table 4: Some haematological characteristics of *O. niloticus* fed the experimental diets

Parameter	Dietary treatments				
	FPE1	FPE2	FPE3	FPE4	FPE5
PCV (%)	26.00 (4.00)[a]	21.50(4.50)[a]	27.00(3.00)[a]	31.00 (2.00)[a]	31.00(3.00)[a]
Hb (g/100 ml)	8.70(1.40)[a]	7.15(1.55)[a]	9.05(0.95)[a]	10.35(0.65)[a]	10.30(1.00)[a]
WBC ($\times10^3$/µl)	5.95(1.50)[b]	7.95(1.85)[a]	7.30 (1.50)[a]	7.25(1.50)[a]	6.95(2.5)[a]
RBC ($\times10^6$/µl)	2.90(0.45)[a]	2.40(0.50)[a]	2.98 (0.33)[a]	3.40(0.20)[a]	3.40(0.30)[a]

Means in a given row with the same letter were not significantly different at $p < 0.05$. Values in parentheses are standard errors of means. PCV = packed cell volume, H b = haemoglobin estimation, WBC = white blood cell count, RBC = red blood cell count

Telfairia occidentalis extract have been reported to increase growth and haematological parameters in rat (Olorunfemi *et al.* 2005) and poultry birds (Fasuyi and Nonyerem 2007). However, no information was found on the effects of *T. occidentalis* leaf extract on tilapia survival and growth. The present study revealed that *T. occidentalis* leaf extract –based diet improve growth and increase the level of white blood cells in Nile tilapia.

These results showed that the *T. occidentalis* leaf extract in diet enhances nutrient utilization, which is reflected in improved weight gain, FCR and SGR. Generally, low feed conversion ratio were obtained in all treatments, but the poorest occurred in FPE5 (10 g/kg diet) (Table 2). Although better FCR values were obtained in diets FPE2 and FPE3 treatments compared to the control, differences in the treatment means were not significant (p>0.05). Similar results were reported by Turan and Akyurt (2005) who used the extract of red clover *Trifolium pratense* as a growth-promoting agent for catfish *Clarias gariepinus.* Immanuel *et al*. (2009) also reported that tilapia *O. mossambicus* fingerlings fed on diets supplemented by medicinal plants extracts exhibited faster growth than those fed with the control diet. Similar results were observed using medicinal plants extracts as growth-promoting agents for red sea bream *Pagrus major* (Ji *et al*. 2007), African catfish *C. gariepinus* (Soosean *et al*. 2010), carp *Cyprinus carpio* (Pakravan *et al*. 2012), black tiger shrimp larvae (Sankar *et al*. 2011) and narrow-clawed crayfish *Astacus leptodactylus* juveniles (Turan *et al*. 2012).

Kim *et al*. (1998) suggested that unknown factors in various medicinal herbs led to favorable results in fish growth trials. The present findings may indicate that availability of bioflavonoids in fluted pumpkin stimulate growth in fish. Therefore, the *T. occidentalis* leaf extract that promotes growth performance in the cichlid tilapia should be tested for its efficacy to induce efficient and economical propagation in other fish. The greater improvement in feed utilization observed with diets supplemented with 2.5-5.0 g fluted pumpkin leaf extract per kg diet suggested that the addition of *T. occidentalis* leaf extract powder improved feed utilization.

The whole body composition values obtained in this present study were similar to those reported by Pakravan *et al*. (2012) and Lee *et al*. (2012). White blood cell counts was significantly higher (p<0.05) in fish fed 2.5, 5.0 and 7.5 g *T. occidentalis* leaf extract powder per kg diets. The haematological values obtained in the present study are similar to those reported by Soosean *et al*. (2010) who used mangosteen (*Garcinia mangostana)* extract as a feed additive in African catfish *C. gariepinus* fingerlings. Differences in haematological indices of fish in this study could therefore be ascribed to differences in the dietary inclusions of *T. occidentalis* leaf extract powder in the diets.

CONCLUSION

It is evident from this study that aqueous extract of *T. occidentalis* leaf powder could enhance growth and haematological parameters of fish after incorporation in feed at dosages of 2.5-5.0 g/kg feed. It suggests that inclusion of fluted pumpkin in the diet would improve the feed utilization and non-specific immune infections in aquaculture. This study is a preliminary investigation conducted to provide an insight for the use of fluted pumpkin leaf extract as a dietary additive added to fish feed to enhance growth and disease resistance. Future research should focus on the purification of the active compounds and their evaluation in order to improve quality and their usage in culture system.

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