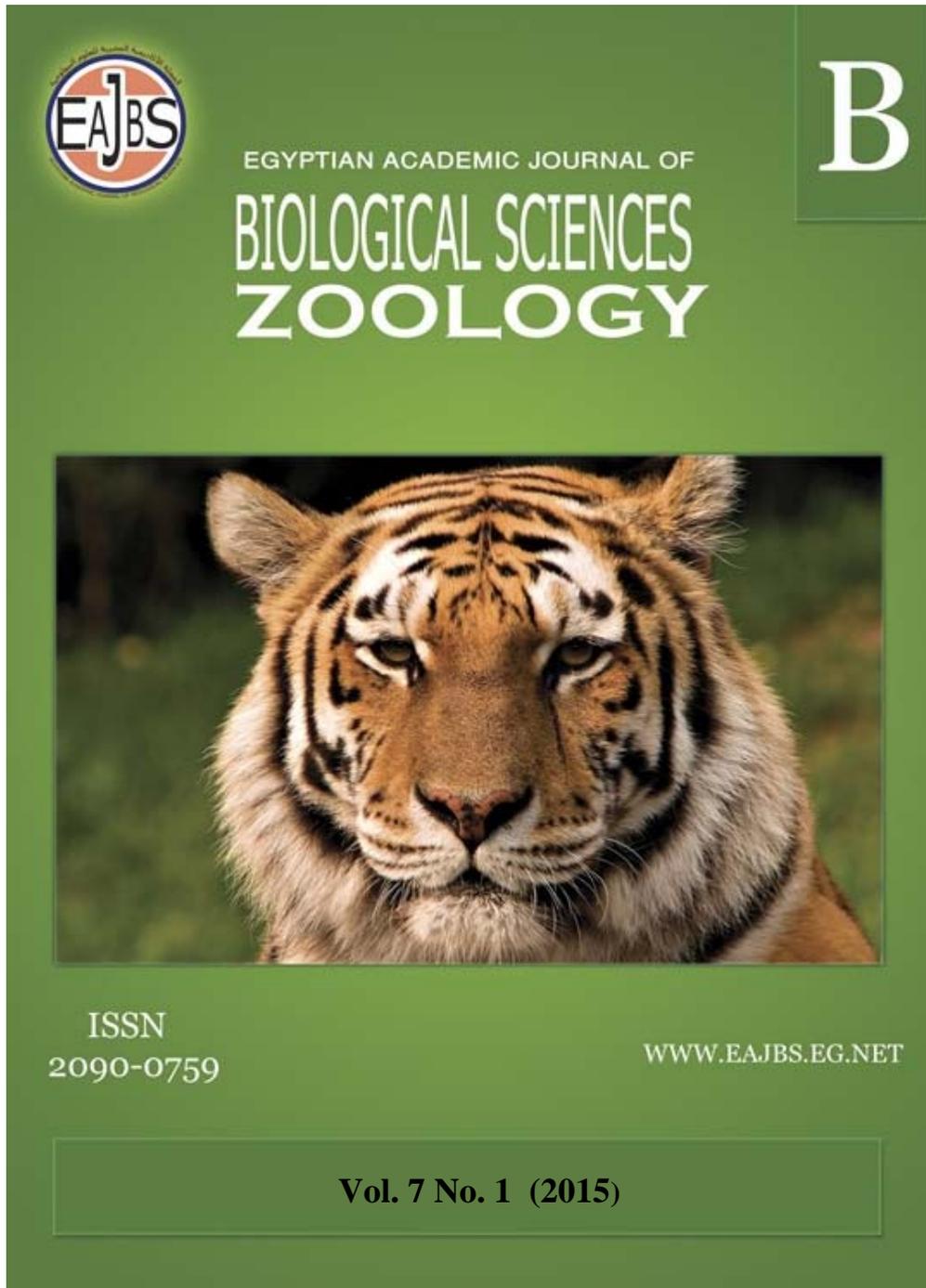


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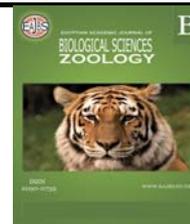


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Dietary fluted pumpkin (*Telfairia occidentalis*) improves reproductive indices in male African catfish (*Clarias gariepinus*) broodstock

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ABSTRACT

Background: Medicinal plants/herbs are used as natural additives in fish diets to enhance reproductive performance in fish. Many studies have shown that antioxidants can enhance fertility either directly or indirectly and that most plants rich in antioxidants have the tendency to increase sperm count, motility, and enhance sperm morphology

Method: The effects of dietary aqueous extract of fluted pumpkin (*Telfairia occidentalis*) leaf powder on growth and reproductive indices of male catfish, *Clarias gariepinus* bloodstocks was investigated. Four isonitrogenous diets were formulated from practical ingredients where the control basal diet (FPE1) was without aqueous extract of *T. occidentalis* leaf powder and the other diets were supplemented by 50, 100, 150 and 200 g kg⁻¹ aqueous extract of *T. occidentalis* leaf powder respectively (designated as FPE2, FPE3, FPE4 and FPE5). The diets were manually fed to the broodstocks at a daily rate of 3% body weight (BW), twice a day (09:00 and 16:00 h) for 8 weeks. Fish were weighed collectively at biweekly intervals, their average weights were recorded and the daily amount of feed for each tank was readjusted accordingly.

Result: Fish fed experimental diets showed significantly improved reproductive indices over the control fish. Significantly higher ($P < 0.05$) gonadosomatic index was recorded for the fish fed diets of aqueous extract of *T. occidentalis* leaf powder compared to fish fed on control diet.

Conclusion: These results revealed that supplement diets with medicinal plant (*T. occidentalis*) improve gonadosomatic index, and reproductive indices of male *C. gariepinus* broodstocks and these plant has a potential pro-fertility property which can be exploited in fish fingerling production by hatchery operators.

INTRODUCTION

Medicinal plants have been used to enhance fertility and research has confirmed pro-fertility effects in some of the herbs tested in animals and fish. Fluted pumpkin (*Telfairia occidentalis*) is cultivated in various parts of southern Nigeria and contains Vitamin C, alkaloids and flavonoids which are phytoestrogens with antioxidant

properties. *T. occidentalis* belongs to the family Curcubitaceae and the powder and ethanolic extracts of the leaves has been credited with aphrodisiac or fertility in animal and men (Salman *et al.*, 2008), and may have similar effects in fish. This herb is a known vegetable which improves spermatogenesis in man (Nwangwa *et al.*, 2007). The darkish green leafy vegetable is used as food and herbal medicine. The leaf is also a rich source of protein, oil and minerals but low in crude fiber and also rich source of folic acid, calcium, zinc, potassium, cobalt, copper, iron, vitamins A and K (Ajibade *et al.*, 2006). The leafy vegetables possess anti-microbial and antiviral properties (Nwozo *et al.*, 2004; Olorunfemi *et al.*, 2005).

Many studies have shown that antioxidants can enhance fertility either directly or indirectly and that most plants rich in antioxidants have the tendency to increase sperm count, motility, and enhance sperm morphology (Oluyemi *et al.*, 2007; Adesanya *et al.*, 2007).

Clarias gariepinus are widely introduced around the world. They are found in the South and North Africa. They have also been introduced in Europe, Middle East, and in parts of Asia. *C. gariepinus* are found in slow flowing lowland streams, shallow lakes, swamps, ponds, ditches, rice paddies and pools left in low spots after rivers have been in flood.

C. gariepinus commands a very good commercial value in Nigerian markets (Ayinla *et al.*, 1994). It has been noted that farming is hardly imaginable without availability of fish seed (Chondar 1980). Based on a 1992 United Nations Development Project (UNDP) assisted base line study, the total annual fingerlings requirement for Nigeria was 250,000 million while the domestic production stood at 7.2 million (Nwokoye *et al.* 2007).

T. occidentalis has quite a number of antioxidants such as phenols. There is, therefore a high possibility that *T. occidentalis* can promote fertility. This study was therefore carried out to investigate the effects of the dietary supplementation of aqueous extract of *T. occidentalis* leaf powder on the reproductive indices in male *C. gariepinus* broodstocks.

MATERIAL AND METHODS

Fish, plant seeds and cultured facilities

The fresh leaves of *Telfairia occidentalis* were obtained from a local market in Ado – Ekiti, Ekiti State, Nigeria. The leaves were authenticated in the crop, soil and pest management department, Federal University of Technology, Akure, Nigeria using Odugbemi (2008).

The fresh leaves were air dried and milled to a fine powder using Maulinex electric blender. 500 g of the powdered leaves were soaked in 1 litre of distilled water for 48 hours, after which it was filtered with a muslin bag. The filtrate was evaporated to dryness in water bath to obtain a solid extract and mixed with a basal feed (40% crude protein), comprising standard amounts of fish meal, yellow maize, soy bean meal, blood meal, fish oil, vegetable oil, vitamin premix and starch.

C. gariepinus broodstocks used in the study were collected from Ondo State Agricultural Development Project fish farm, Akure, Ondo State, Nigeria. The broodstocks were transported to research farm of the department of fisheries and aquaculture technology, Federal University of Technology, Akure, Ondo State, Nigeria in plastic bowls. The fish were distributed into outdoor concrete tanks (1 × 1 × 0.6 m), filled with well water and acclimatized for 2 week, during which they were fed the basal diet. The concrete tanks were cleaned weekly, and about 50% of the

culture water was replaced with fresh, well water. Water quality parameters including O₂, pH and temperature were monitored daily. The average values of these parameters throughout the study were: O₂ = 6.35± 0.23

mg L⁻¹, pH 9.7± 0.20 and temperature = 26.20± 0.26 °C.

Experimental design

The broodstock (450 - 500 g) were stocked into concrete tanks (1 × 1 × 0.6 m) at a density of 6 (six) fish per tank with three replicates per treatment. Four isonitrogenous diets were formulated from practical ingredients (Table 1) where the control basal diet (FPE1) was without aqueous extract of *T. occidentalis* leaf powder and the other diets were supplemented by 50, 100, 150 and 200 g kg⁻¹ aqueous extract of *T. occidentalis* leaf powder respectively (designated as FPE2, FPE3, FPE4 and FPE5). The experimental diets were formulated to contain almost 40% crude protein. All dietary ingredients were weighed with a weighing top load balance (Metler Toledo, PB 8001 London). The ingredients were milled to a 3 mm particle size. Ingredients including vitamin premix and aqueous extract of *T. occidentalis* leaf powder were thoroughly mixed in a Hobart A- 2007 pelleting and mixing machine (Hobart Ltd,

London, UK) to obtain a homogenous mass, cassava starch was added as a binder. The resultant mash was then pressed without steam through a mixer with 0.9 mm diameter size. The pellets were dried at ambient temperature (27 – 30 °C) and stored at -20 °C in a refrigerator until the start of the experiment.

The diets were manually fed to the broodstocks at a daily rate of 3% body weight (BW), twice a day (09:00 and 16:00 h) for 8 weeks. Fish were weighed collectively at biweekly intervals, their average weights were recorded and the daily amount of feed for each tank was readjusted accordingly.

Table 1: Ingredients in and proximate composition (g) of experimental diets.

Ingredients	Experimental diets				
	FPE1	FPE2	FPE3	FPE4	FPE5
Fishmeal (65% Cp)	250	250	250	250	250
Soybean meal(45% Cp)	400	400	400	400	400
Yellow maize	150	150	150	150	150
Blood meal (85% Cp)	50	50	50	50	50
Fish oil	40	40	40	40	40
Vegetable oil	60	60	60	60	60
Vitamin premix**	30	30	30	30	30
Binder	20	20	20	20	20
<i>T. occidentalis</i> extract (mg/kg feed)	0	50	100	150	200
Proximate composition (% DM)					
Moisture	4.08	5.06	5.05	4.87	5.39
Crude protein	39.59	39.81	40.16	40.25	40.35
Crude lipid	14.47	13.16	12.16	14.54	11.85
Crude fiber	2.63	2.59	2.72	2.85	2.60
Ash	13.75	19.36	16.22	23.68	20.24
NFE	25.48	20.02	23.69	23.81	19.57

Vitamin premix** - An Animal Care^(R) Optimix Aqua product for catfish, containing the following per 5kg of premix: A = 20,000,000 I.U, D3 = 2,000,000 I.U, E = 200,000 mg, K3 = 10,000 mg, B2 = 12,000 mg, B12 = 9mg, B1 = 6,000 mg, B6 = 11,000 mg, C = 50,000mg, folic acid = 2,000 mg, Niacin = 80,000 mg, Calpan = 25,000mg, Biotin = 100 mg, x Zinc = 30,000mg, Copper = 5,000mg, Iron = 30,000 mg, Manganese = 50,000mg, Iodine = 1,000mg, Selenium = 100mg, antioxidant = 125,000mg.

NFE = Nitrogen free extract DM = dry matter

Evaluation of milt quality

Milt production and quality were determined at the end of the experiment. 3 fish from respective tanks of the same treatment were randomly collected, (9 fish from each treatment in total), were sacrificed and the testes removed. Milt volume was determined by making small incision into the lobes of the testes, the milt squeezed out into a Petri dish. This was measured with plastic syringe in ml.

Motility durations were determined by placing 1µl of milt from each male on a Neubauer hemocytometer, a drop of distilled water was added and covered with a slip. The sperm activity was viewed under Olympus microscopic at 100 x magnification to see when all the sperm got stopped (Mims, 1991) while percentage motility from each sample was estimated using light microscope at 400x magnification immediately after addition of 20 µl distilled water as an activating solution. During spermatozoa activation, immotile sperm cell (ISC) was counted, and when the activation stopped, whole sperm cells (WSC) were counted (Canyurt *et al.*, 2008). The motile sperm cells (MC) were calculated as:

$$\begin{aligned} \text{MC} &= \text{WSC} - \text{ISC} \\ \% \text{MC} &= \text{MC}/\text{WSC} \times 100 \end{aligned}$$

Concentration of sperm was determined by counting the number of spermatozoa in sample dilute with distilled water (100 x) in a Burker haemocytometer, under 400x magnification (Rainis *et al.*, 2003). Gonado-somatic index (GSI) was computed according to Dahlgren (1979) as:

$$\text{wet weight of gonad/wet weight of fish} \times 100.$$

Water quality parameters such as temperature, pH and dissolved oxygen concentration were monitored daily throughout the study period using mercury-in-glass thermometer, pH meter (Hanna HI98106 model) and dissolved oxygen meter (JPP- 607 model).

A one-way analysis of variance (ANOVA) was conducted to test the effect of aqueous extract of *T. occidentalis* leaf powder gonadosomatic index and reproductive indices of male *C. gariepinus* broodstocks using SPSS Version 11.0. Least significant difference was used to compare means at $P < 0.05$ (Zar, 1996).

RESULTS

Data on reproductive performances of the male *C. gariepinus* broodstocks fed supplementary diet of aqueous extract of *T. occidentalis* leaf powder are presented in Table 2. Normal feed intake was observed for the experimental fish. There was significant difference ($P < 0.05$) in the weight of testes with fish fed diet FPE4 having the highest value while fish fed on diet (FPE2) had the lowest value. This reflects in a corresponding increase in milt volume. Similarly there was significant difference ($P > 0.05$) in gonado-somatic index and milt motility and this reflects in greater improvement in the motility duration of fish fed with diets supplemented with varying inclusion levels of aqueous extract of *T. occidentalis* leaf powder when compared with the control. However, there were significant differences ($p < 0.05$) in milt count across the fish fed different diets supplemented with aqueous extract of *T. occidentalis* leaf powder. The average milt volumes were 1.10 ± 0.05 , 1.10 ± 0.05 , 0.80 ± 0.05 , 0.58 ± 0.08 and 0.48 ± 0.03 , respectively for diets FPE5, FPE4, FPE3, FPE2 and FPE1.

The water quality parameters during the experimental period varied as follows: temperature, 22.18–24.50 °C; dissolved oxygen, 6.50–7.06 mg/l; pH, 6.94–7.63 recommended for catfishes.

Table 2: Parameters of *C. gariepinus* broodstocks as affected by the dietary supplementation of aqueous extract of *T. occidentalis* leaf powder for 56 days (mean \pm S.E).

Parameter	Dietary treatment (g kg ⁻¹ aqueous extract of <i>T. occidentalis</i> leaf powder)				
	0	50	100	150	200
Initial fish weight (g)	486.10 (0.90)	485.75(0.25)	487.15(1.95)	487.90(2.20)	486.40(1.10)
Final fish weight (g)	493.35(0.25) ^a	492.80(1.00) ^a	493.65(1.15) ^a	494.65(1.05) ^a	493.90(1.30) ^a
Weight gain (g)	7.45(0.65) ^a	7.05(1.25) ^a	6.50 (0.80) ^a	6.75(1.15) ^a	7.50(0.20) ^a
Weight of testes (g)	1.84(1.31) ^b	0.96(0.35) ^c	2.21(1.23) ^a	2.76(0.20) ^a	1.06(0.22) ^c
Milt volume (ml)	0.48(0.03) ^a	0.58(0.08) ^a	0.80(0.05) ^b	1.10(0.05) ^c	1.10(0.05) ^c
Milt count ($\times 10^4$ spz/ml)	123.75(22.25) ^a	195.00(7.50) ^a	199.25(10.75) ^{a,b}	277.50(18.50) ^b	246.75(40.25) ^b
Motility duration (sec)	19.50(6.50) ^a	35.00(7.00) ^a	33.00(1.00) ^a	37.50(4.50) ^a	32.50(4.50) ^a
% motility	49.53(2.43) ^a	52.03(3.33) ^{a,b}	58.88(0.38) ^{b,c}	68.00(3.55) ^{b,c}	60.80(0.25) ^c
GSI (%)	0.36(0.13) ^a	0.69(0.15) ^a	0.67(0.26) ^a	0.40(0.08) ^a	0.60(0.29) ^a

Mean in a given column with the same letter were not significantly different at $p < 0.05$. Values in parentheses are standard errors of means.

GSI = Gonadosomatic index = Gonads weight (g) \times 100 / fish weight

DISCUSSION

The results suggest that dietary aqueous extract of *T. occidentalis* leaf powder at all concentrations improved the reproductive indices and GSI of cultured male African catfish, *C. gariepinus*. These results showed that the aqueous extract of *T. occidentalis* leaf powder treatment enhances reproductive performance, which is reflected in improved milt volume, milt count, motility duration, % motility and GSI.

High motility durations were obtained in aqueous extract of *T. occidentalis* leaf powder treatments, but the lowest was obtained in control. Better milt count values were obtained in the aqueous extract of *T. occidentalis* leaf powder dietary treatments compared to the control and there were significant ($P > 0.05$) differences among the fish fed the supplementation. Aqueous extract of *T. occidentalis* leaf powder in diets also promoted reproductive performance in male rat (Salman *et al.*, 2008). Similar results were reported by Sharma *et al.* (2008) who used the aqueous extract of *Anacyclus pyrethrum* as a fertility enhancing agent for male rat. Dada (2013) reported that catfish *C. gariepinus* broodstocks fed on diets supplemented by medicinal plants exhibited improved reproductive performance than those fed with the control diet. Similar results were reported for using medicinal plants as fertility-promoting agents for catfish *C. gariepinus* (Dada and Ajilore, 2009; Adeparusiet *al.*, 2010; Dada and Ogunduyile, 2011).

The improvement in the quality of milt observed in this study could be attributed to the actions of the phytochemical composition of the leaves of *T. occidentalis* such as flavonoids, steroid. Fasuyi (2006) reported that the chemical composition of the leaves of *T. occidentalis* include proteins, fat, vitamin A, thiamine, riboflavin, nicotinamide, vitamin C and minerals such as zinc, iron, calcium and magnesium. Adeyeye and Omolayo (2011) also reported that amino acid composition of *T. occidentalis* includes lysine, histidine, arginine, cysteine methionine and leusine. Some of these active ingredients in *T. occidentalis* such as flavonoids, steroid have well documented spermatogenic activities. The arginine is a biochemical precursor in the synthesis of putrescine, spermidine and spermine which are essential for sperm motility (Steven, 2000).

It could therefore be concluded that the inclusion of *T. occidentalis* in the diets provided a steady supply of additional nutrients to the fish over the control fish. Zinc promotes growth, sexual maturation and reproduction and there has been evidence associating serum and semen zinc levels with male infertility (Mohan *et al.*, 1997). Carbohydrate-rich *T. occidentalis* could have increased sperm motility and volume by increasing glucose metabolism leading to the production of pyruvate and energy. Pyruvate is known for its preference as substrate essential for the activity and survival of sperm cells (Egbunike *et al.*, 1986). As antioxidants, the flavonoids and vitamins in *T. occidentalis* extract could maintain sperm morphology, sperm survival and sperm function (Nwanna and Oboh, 2007). The improvement in sperm count could be attributed to antioxidative properties of tannins and vitamin A presence in the leaves (Nkanget *et al.*, 2003). According to Sato *et al.* (2004), vitamin A protects the testis against lipid peroxidation, hence, promotes spermatogenesis and improves structural differentiation of epithelial cells of the epididymis. This explains the increase in milt quality in fish treated with *T. occidentalis*. These investigations showed that aqueous extract of *T. occidentalis* leaf powder possess promising profertility property which can be exploited in fish fingerlings production. Results of the studies provide baseline information and established safe limits of using aqueous extract of *T. occidentalis* leaf powder as profertility agent to increase the fingerlings production in *C. gariepinus* under hatchery conditions.

CONCLUSION

In conclusion, these investigations showed that aqueous extract of *T. occidentalis* leaf powder possess promising profertility property which can be exploited as feed additive to enhance milt quality of the male, *C. gariepinus*. Results of the studies provide baseline information of using aqueous extract of *T. occidentalis* leaf powder as profertility agent to increase the fingerlings production in *C. gariepinus* under hatchery conditions.

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