Original Research Article

Growth Response, Nutrient Utilization and Viscerosomatic and Hepatosomatic Indices of Nile Tilapia (*O. Niloticus*) Linnaeus 1758, Fed at Various Inclusion Levels of Raw and Soaked Sprouted Elephant Ear Plant (*E. Cyclocarpum*) Leaf in Substitute for Soybean Meal

Abstract-Aims: The objective of the study was to examine the effects of Raw and soaked Guanacaste (*Enterolobium cyclocarpum*) leaf meal on the growth performance, nutrient utilization, histological indices of juvenile Nile Tilapia (*Oreochromis niloticus*)

Study Design: Complete Randomized Design

Place and Duration of study: Department of Aquaculture and Fisheries, Federal University of Agriculture, Abeokuta. Nigeria between May 2022 to September 2022.

Methodology: A 12-week feeding experiment was conducted to assess the use of Raw and Soaked *E. cyclocarpum* leaf meal (SECLm) as a partial substitute for soybean meal. The parameters considered include growth response, nutrient utilization and hepatosomatic and Viscerosomatic indices of *O. niloticus*. 300 fingerlings stage (13.00±2.0g) were collected. The fingerlings were randomly distributed in 30 plastic experimental tanks ($50 \times 33.5 \times 33.5cm^3$) at the stocking rate of 10fish/tank. Four isonitrogenous (40%) diets and isocaloric () were formulated in which supplemented soybean meal with the Soaked *E. cyclocarpum* Leaf meal at three inclusion levels, (10%, 20% and 30%) while 0% inclusion served as Control diet (CD). The diets were fed to triplicate groups of the experimental fish and were fed twice daily at 3% body weight.

Results: Data obtained from the experiments were analyzed using One-way Analysis of Variance (ANOVA). At the end of the feeding trial, the group of fish fed at SEcLM 3 had the best growth performance in terms of Weight gain, FCR and SGR (1.52±0.01 and 1.43±0.06) respectively. The mean weight gain of all the treatments increased across the treatments. Fish fed SEcLM 1 had the highest overall growth performance and the highest weight gain. The survival rate was not significantly different (p> 0.05) across the treatments. The group of fish fed treatment diet SEcLM 1 had the highest viscerosomatic index 1.67±0.11 while for the highest hepatosomatic index was the group fed the Control diets (0.26±0.10) and the group of fish fed SEcLM 1 (19.82±1.52) had the highest body weight, the group that displayed the longest gut length is the group fed SEcLM 1 (11.25±0.25).

Conclusion: The conclusion drawn was that feeding *O. niloticus* with *Enterelobium cyclocarpum* leaf meal and Soaked *Enterelobium cyclocarpum* leaf meal up to 10% and Raw at 30% could replace Soybean meal without any physiological stress and posing any threat to the fish.

INTRODUCTION

The fast-growing forage leguminous plant commonly called Guanacaste with the scientific name *Enterolobium cyclocarpum* has the following common names: sprouted elephant ear plant, devil`s ear plant, Monkey ear tree, caro caro, earpod tree. *Enterolobium cyclocarpum* is a flowering tree that belongs to the family Mimosadeae (Janzen, 1981) from the tropical regions of the America such as: central Mexico South, Northern Brazil, Venezuela (Allen, P.H 1956). It has been recognized globally because of its large proportion, provision of shade from intense sun and its abundance over time.

Guanacaste trees are known for their attribute or ability to tolerate a wide range of rainfall level, soil and temperature conditions. It thrives well and grows to maturity within short period of time. Guanacaste flowers are usually pollinated by insects (Bees). The pods from this tree are neglected by fauna, they are therefore accumulated at the base of the parent tree. (JANZEN, D.H. and MARTIN, P.S. 1982)

It has been identified as one of the most promising plant species for the agro-forestry especially in areas with high humidity. (Ezenwa 1998). Guanacaste contains high amount of nutrient required for consumption (majorly for ruminant animals and also serve as food for Human) and it is moderately palatable. (Hughes *et*. *al*. 1990, Kalitho *et. al*. 1996)

Guanacaste serves various purposes for both Human and Animals. They serve as ornaments because of their aesthetic value. It serves as canopy (i.e shelter) for coffee plantation. It also serves as forages for ruminants (e.g cattles). It also plays a vital role in the fertilization of soil through the process of Nitrogen fixation. The Guanacaste wood is lightweight and water resistant and therefore used in the construction of furniture and also shipbuilding. The seeds can also be boiled eaten while the seed pod is still green. (Witsberger, D. Current *et*. al. 1982).

The commonly known and most cultured fish species called Tilapia belongs to the Cichlids family (i.e *Cichlidae*). It is endemic and widely distributed across Africa. This group of fish species consist majorly of three major or most cultured genera and they are: *Sarotherodon, Oreochromis and the Coptodon*. (Popma and Masser 1999.) Other genera of the Cichlid family include the*: Hemichromis*. These various genera of the Cichlid family possess different various attributes which makes them differ from one another. Amongst these three most commonly cultured genera, Oreochromis is known to be the major specie for commercial aquaculture around the globe. Tilapia has the following characteristics which makes them most preferred by fish farmers for commercial purpose. These characteristics are: prolific breeding (even in captivity), resistance to stress and disease, ability to feed on variety of feed (such as; plankton, periphyton, aquatic plants, benthic organisms) which helps to reduce cost of production for the farmer, ability to convert low quality feed to high quality protein required.

The Genera Oreochromis consist of various species like: *niloticus, aureaus, mossambicus* and so on. These species have varying features which make each of them distinct. *Oreochromis niloticus* commonly known as Nile Tilapia is a fish species native to the Northern half of Africa. (Froese *et.* al. 2015). Nile Tilapia can also be commercially called Mango fish, boulti e.t.c (Ibrahim, A.A.; El-Zanfaly, H.T. 1980*).* Nile Tilapia has been identified to be the second most farmed finfish in Africa giving a 10-12% growth per year. (Wallace 2015).

The most commonly recognized and used plant protein source included in fish feed is Soybean meal which is as a result of the vital and nourishing constituents such as: balanced amino acid profile, sufficient amount of vitamin and minerals, high amount of protein. Also, fish scientists have been able to identify that soybean meal is highly effective, palatable and easily digestible for the fish which helps in the adequate functioning, growth and development of the body system of the fish. (M.A Kilany and Dessouki 2014). Soybean meal has also been known to be edible and consumable by Human and for livestock. (Adeoye, 2016). The high rate of consumption and competitive use of soybean has resulted in hike in price of the commodity which has made the rate of production of fish feed more expensive. Therefore, it is economically advisable that other plant protein source that could be substituted for soybean meal (partially or wholly) should be considered for use in fish feed formulation. Some of these plant protein sources are: *E. cyclocarpum*, peas, oil seeds, sesame seed and other legumes. (Fish stat. *et*. al. 2002.)

However, since *O. niloticus* is an herbivore and could ingest and digest substantial amount of plant protein, it is therefore advisable to consider the use of other plant protein source like *E. cyclocarpum* which is also rich in required nourishing substances needed by the fish for proper survival of the fish. Although, this plant (*E. cyclocarpum*) has its own limitation as it contains some anti-nutritional factors which could mitigate against the utilization and absorption of the nutrients (Yasemin and Funda, 2010). However, these anti-nutrients can be removed or reduced by some processes, such as; fermentation, soaking, drying, heating and so on and also the inclusion of some feed enzymes could also help to aid the utilization of the feed nutrients. (Adeoye, Merrifield and Davies, 2013).

MATERIALS AND METHOD

The experiment on took place at Hatchery unit of the Fish farm, Department of Aquaculture and Fisheries Management, FUNAAB.

Collection of the Plant (Guanacaste)

Substantial amount of mature Guanacaste (E. cyclocarpum) leaflets was fetched from the roadside along the Fish farm and the lawn close to the Senate building (FUNAAB).

Procurement of Fingerlings

A total number of 500 all male Nile Tilapia (*O. niloticus*), fingerlings size (13.00 $\pm$ 20.0g) were procured from a reputable cage culture farm at Oyan Dam, Ogun state. Fish was transported in a 50 litres plastic bag filled with water to the hatchery section at the farm of FUNAAB.

Acclimatization of Nile Tilapia Fingerlings

The Nile Tilapia *(O. niloticus)* fingerlings was acclimatized under normal condition for 2 weeks and fed with 2 mm extruded (Blue Crown) feed during the period. After the acclimatization, 300fingerlings from the batch were randomly distributed 10each to 30 plastic aquaria tanks (2× 1.5×1m each) based on three treatments with each aquaria tank in the Hatchery complex. Feeding was stopped 48hrs before the commencement of the experiment. Unconsumed feed and feaces were siphoned and the water were changed using a flow through method.

Preparation of Guanacaste Leaf Meal

Mature *E. cyclocarpum* leaves were plucked around the Fish Farm area of the university, the leaves were air- dried in a room at standard room temperature (26^{0}C) in the absence of light for about 14days to reduce the moisture content of the leaves and to facilitate the leaf drop from the stalk. After two weeks, the dried leaves were milled to powder in a hammer mill and other feed ingredients were milled and sieved to acquire similar size configuration.

Aor the soaked, the air-dried leaves were soaked for 4-6 hours, then drained to dry and afterwards milled to incorporate with the formulated diet.

Proximate Composition Analysis

The air-dried leaf was subjected to various proximate composition analysis to qualitatively and quantitatively determine the moisture and chemical constituents (Moisture, Crude protein, crude fat, Fibre, Ash, CHO Saponins, Tanins phytic acid, Calcium, Potassium, Iron and Phosphorus) in the Biology Laboratory of University of Ibadan, Oyo State.

Statistical Analysis

Data were analyzed using a one-way analysis of variance (ANOVA) technique the means were separated by fisher's LSD test and compared using Duncan's multiple range test, as described by Snedecor and Cochran (1989). The significant differences level was defined at $P<0.05$.

RESULT

Proximate Composition of *E. Cyclocarpum* Leaves

Soaking the *E.cyclocarpum* leaves resulted in a slight increase in the crude protein content of *E. cyclocarpum* leaf meal, an increase in the moisture content compared to the raw state of the seeds. Also, there is a reduction in the percentage of antinutritional factors (phytic acid, Tannin and saponin). It influenced the proximate composition of *E. cyclocarpum* positively and also the Growth, survival and Nutrient utilization rate and Hepatosomatic and Viscersomatic Indices of the Fish was also observed. The tables below show the result of the soaking effect;

Table 1: Proximate composition of Soaked *E. cyclocarpum* leaf

Parameters	SecL
Moisture%	8.20
Crude protein %	21.25
Crude Fat %	7.27
Fiber	12.62

Ash	7.42
CHO	45.21
Phytase mg/100g	0.027
Tannins mg/100g	6436.29
Saponins mg/100g	194.85
Calcium mg/100g	1181.42
Potassium mg/100g	352.86
Iron g/100g	16.12
Phosphorus Mg/100g	79.60

SEcL: Soaked *E. cyclocarpum* Leaf meal

Parameters	REcL
Moisture%	6.80
Crude protein %	18.22
Crude Fat %	6.96
Fiber	9.80
Ash	8.37
CHO	49.85
Phytase mg/100g	0.032
Tannins mg/100g	6454.77
Saponins mg/100g	211.39
Calcium mg/100g	1641.15
Potassium mg/100g	604.28
Iron g/100g	21.50
Phosphorus Mg/100g	154.36

REcL: Raw *E. cyclocarpum* leaf meal

Growth, survival and Nutrient utilization Parameters

Feed Conversion Ratio (FCR)

Feed conversion ratio was highest in fish fed SEcLM 1 (1.54±0.05) that received the highest inclusion level of soaked leaf meal and there was no significantly different in fish treatment fed Raw diet 2, but there was a significantly different (P<0.05) from other treatment diets, Control diet has the lowest FCR of 1.42±0.02 which indicates an improved feed conversion ratio.

Specific Growth Rate (SGR)

Fish fed with diet SEcLM 2 had the highest SGR of 13.26±0.89 and was significantly different (P<0.05) from SEcLM 3 which had the lowest value (12.97±0.16) and Control diet.

Initial Body Weight (IBW)

The initial body weight of the fish fed with SEcLM 1 (8.36±0.04g) is the highest and was significantly different (P<0.05) from that of SEcLM 2 (8.24±0.02g) and control diet.

Final Body Weight (FBW)

The group of fish fed the control diet had the highest Final body weight gain (21.32±0.36g) and was significantly different (P<0.05) from all other dietary treatments. The Final body weight of the fish fed with SEcLM 1 (21.06±0.39g) is the highest and was significantly different (P<0.05) from that of SEcLM 3 (20.86±0.54g) and control diet.

Mean Weight Gain (MWG)

The group of fish fed the control diet had the highest Mean weight gain (13.08±0.33g) and was significantly different (P<0.05) from all other dietary treatments. Fish fed the SEcLM 1 has the highest Mean weight gain (12.70±0.41g) and was significantly different from those that were fed with SEcLM 3 (12.45±0.39g)

Mean Feed Intake (MFI)

The highest feed intake was recorded in the group of fish fed the SEcLM 1(19.56±0.07) and the least was observed in the group of fish fed SEcLM 2 (18.03±1.14) but was not significantly different (P>0.05)

Protein Intake (PI)

The group of fish that had the highest Protein intake was those that were fed with SEcLM 1 (7.82±0.03) and those with the least protein intake were those that were fed with SEcLM 2 (7.21±0.46), the difference is significant (P<0.05)

Protein Efficiency Ratio (PER)

The group of fish fed the control diet had the highest Protein efficiency ratio (1.76±0.02) and was significantly different (P<0.05) from all other dietary treatments. Fish fed the SEcLM 20% has the highest Mean weight gain (1.75±0.07) and was significantly different from those that were fed with SEcLM 10% (1.62±0.05)

Table 2: Growth, Nutrient Utilization and Survival

Treatments	Control Diet (0%)	SecL, REcL 10%	SecL, REcL 20%,	SecL, REcL 30%
Initial Body Weight	8.24+0.05[ab]	8.36±0.04, 8.24+0.15	8.24±0.02, 8.13+ 0.O1	8.42±0.17, 8.52+0.21
Final Body Weight	21.32±0.36[a]	21.06±0.39[a],19.49+0.77[b]	20.93±1.24[a], 21.25+1.38[b]	20.86±0.54[a], 13.88+6.9[a]
Mean Weight gain	13.08±0.33	12.70±0.41, 11.21+0.82[b]	12.69±1.25, 13.12+1.37[b]	12.45±0.39, 8.2+4.11[a]
Mean Feed Intake	18.60±0.48	19.56±0.07, 19.48+0.33[b]	18.03±1.14, 18.69+0.96[b]	18.87±0.52,12.91+0.46[a]
Feed Conversion Ratio	1.42±0.02	1.54±0.05, 1.75+0.1[b]	1.43±0.06, 1.44+0.88[b]	1.52±0.01, 1.05+0.53[b]
Survival Growth Rate	13.58±0.20	13.19±0.30, 12.67+0.67[b]	13.26±0.89, 13.66+0.9[b]	12.97±0.16, 8.54+4.29[a]
Protein Intake	7.44±0.19	7.82±0.03, 7.7+0.13[b]	7.21±0.46, 7.48+0.38[b]	7.55±0.1, 5.16+2.38[a]
Protein Efficiency Ratio	1.76±0.02[b]	1.62±0.05, 1.44+0.08[b]	1.75±0.07, 1.75+0.1[b]	1.65±0.01, 1.06+0.53[b]
APD	88.23+ 41.885[a]	89.11+26101[b], 884.95+55[a]	88.95+23580[b], 85.82+50[a]	88.80+16166[b], 85.40+12[b]

AFD	84.09±41.885[b]	87.31±26101[b], 83.77±83	87.08± 23580[b], 83.84 ±57	86.97±16166[b], 83.40±13
ACHOD	50.81 ±41.885[a]	62.98±26101[b], 79.19±1.00[b]	61.67±23580[c], 78.75±55[b]	63.97±16166[d], 78.90±12[b]

Means along the row having the same superscript are not significantly different at (P>0.05).
There is an inverse relationship between weight gain and inclusion level of SEcLM
APD: Apparent Protein Digestibility
AFD: Apparent Fat Digestibility
ACHOD: Apparent Carbohydrate Digestibility

Hepatosomatic and viscerosomatic indices

The hepatosomatic and viscerosomatic parameters of the experimental fish are shown in Table 7. The VSI (Viscerasomatic index) was highest in the fish fed SEcLM 10% with value (1.67±0.11) and there was a significant difference (p<0.05) from that of fish fed SEcLM 30% which had the least value of (1.07±0.02) The HSI (Hepatosomatic index) was highest in the fish fed SEcLM 20% with value (0.13±0.02) and there was no significant difference (p>0.05) from that of fish fed SEcLM 10% which had the least value of (0.10±0.05)

The Body weight was highest in the fish fed SEcLM 10% with value (19.82±1.52) and there was a significant difference (p<0.05) from that of fish fed SEcLM 30% which had the least value of (16.78±0.21)

The Length was highest in the fish fed soaked treatment diet 10% with value (11.25±0.25) and there was no significant difference (p>0.05) from that of fish fed SEcLM 30% which had the least value of (10.25±0.25).

The Condition factor (K) was highest in the fish fed SEcLM 30% with value (0.02±0.00), while the rest remains constant. Hence, there were no significant difference.

Table 3: Hepatosomatic and Viscerosomatic Indices

Parameters	Control	SEcLM 10%	SEcLM 20%	SEcLM 30%
Viscerosomatic indices	1.64±0.20 [a]	1.67±0.11 [a]	1.29±0.08 [ab]	1.07±0.02 [b]
Hepatosomatic indices	0.26±0.10	0.10±0.05	0.13±0.02	0.12±0.05
Body Weight	17.67±0.32	19.82±1.52	16.95±3.37	16.78±0.21
Length.	10.10±0.10 [b]	11.25±0.25 [a]	10.85±0.25 [ab]	10.25±0.25 [b]
K	0.02±0.00	0.01±0.00	0.01±0.00	0.02±0.00

Means with different superscripts along same rows are significantly different (p<0.05)

K: Condition factor

	Control (0%)	REcL10%	REcL (20%)	REcL (30%)
Villi height (nm)	1349.82±23.29^{C}	1373.91±9.00^{C}	1475.77±31.81^{b}	1543.41±3.38^{a}
Villi width (nm)	255.84±21.14^{a}	188.73±19.46^{b}	227.32±2.93^{ab}	118.45±0.44^{c}
Cryptal depth (nm)	371.89±26.25	359.46±6.01	354.49±3.50	353.11±1.77
Cryptal width (nm)	242.29±12.64^{a}	200.13±5.82^{b}	221.18±2.29^{ab}	119.12±1.40
Muscle thickness (nm)	244.03±22.11	266.51±6.14	257.27±3.68	239.00±3.48

Gut means with different superscript along same rows are significantly different (p<0.05)

DISCUSSION

The use of *E. cyclocarpum* leaf meal does not conflict with human food security and this study has demonstrated that the leaves have the potentials to partly replace soybean meal and considerably reduce expenditure on fish meal without compromising the growth performance of Juvenile Nile Tilapia. The water quality parameters that were monitored in the study showed that the experimental tanks were in good and favorable condition for the fish (*O. niloticus*) for adequate growth and physiological activities. Fish grow best at a temperature range of 25^{o}c -30^{o}c. Asfaw (2011). It was observed that optimum temperature for optimal growth performance with effective feed conversion fed to *O. niloticus* is 27.20^{o}c thus this value falls within the range of the present study. Also, optimum oxygen level required for optimal growth of all fish species ranges from 5-8mg/L Hussain (2009). Oxygen concentration is a very important factor that determines the major life processes of Fish and so should be properly checked from time to time for effective production and experimental work. Asfaw 2011 reported that the optimum pH range for fish growth and survival ranges between 6.0-8.0. The effects of higher pH are that high pH will result in stunted growth and induces the mortality rate. Therefore, from the experiment it can be deduced that the physico-chemical parameters of the water did not have negative impact on the fish performance during the course of the experimental work.

Soaking is considered the most commonly used techniques and can be stated as the simplest and cost-effective techniques to improve the nutritional status of food materials and also to minimize the concentration of anti-nutrients present. (Embaby, 2010). Several studies conducted have showed that soaking for 12-18hrs is the most effective method of reducing anti-nutrients level in food materials (grains, leaves, legumes) by partially or wholly solubilized in soaked water. (Emababy, 2010; Kajihausa *et.* al 2014). The use of this processing treatment is to promote the utilization and efficiency of the feed ingredient. The soaking process concentrated the other nutrients (crude fat, crude fibre) in the leaves. Proximate composition is important in that it allows the determination of the nutritive value of ingredient and the prediction of the quality of the product of several ingredients when mixed.

Anti-nutritional factors present in *E.cyclocarpum* leaves include: tannin, phytate and saponin had reduced or denatured which could have been due to the removal and hydrolysis of water soluble components in the anti-nutrients. There were residual anti-nutrients in the leaf meal even after the soaking process. Phytate reduced by 15.63%. The phytate is known to influence the utilization of nutrients by forming complexes with protein and limiting their utilization, also phytate decreased the availability of Ca, Mg, Zn, K and P. Phytate is known to have a negative effect on the growth performance of the fish. (V Kumar *et.* al. 2012) Tannin is mildly reduced by 0.29% through soaking and could also be responsible for the relative decline in growth with increase in inclusion level of SEcLM. Presence of Tannin in fish feed gives the feed an unpleasant taste and results to less consumption due to reduced palatability. (Becker and Makkar, 1999) Saponins are polar compounds, thus they are soluble in water. Saponin are attracting considerable interest as a result of their diverse properties, both deleterious and beneficial. A high saponin diet can be used to inhibit the dental caries and platelet aggregation and can also be used as an antidote for acute lead poisoning in human. (John Shi, *et*. al 2004) It is assumed in this study that saponin known for reduction in feed intake and growth performance could also be responsible for the observed poor performance of groups fed different inclusion level of SEcLM. The reduction could have facilitated a better digestion and improved nutrient utilization.

The growth and nutrient utilization of *O. niloticus* was influenced by inclusion levels of the soaked E. *cyclocarpum* leaf meal in the diet. Growth parameters served as indicators of fish's ability to utilize and retain nutrients in a given diet. In this study it was indicated in their mean weight gain (MWG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER). In the first few weeks of the feeding trial, the fish fed enthusiastically on all the experimental diets and thus resulted in rapid increase in the growth rate within the period aforementioned. There is inverse relationship between the weight gain and inclusion level which could attributed to the fact that the fibre content of the soaked leaf meal was higher than the fibre content of soybean meal, protein value of the soaked meal was lower than the soybean meal protein. This inverse relationship is in line with the findings of Azaza *et.* al 2008 who observed a decline in growth and weight gain as green ulva meal is increased in the diet of *O. niloticus* as a replacement for soybean meal. The rapid response to feeding could be as a result of minimal feeding during the acclimation period before the commencement of the experimental feeding trial in addition to initial starvation of the fish which increased their metabolic rate. Similar observation was reported in a study, Alegbeleye *et al.,* (2005) where *O. niloticus* fingerlings fed toasted Lima Bean meal recorded a rapid response to feeding in the first week of the experimental trial. Some researchers have reported either reduced growth or low feed intake in the fish as a result of presence of anti-nutritional factors in the plant protein hence similar growth may not be obtained (Bake *et al.,* 2015).

CONCLUSION

Observations after the 12 weeks experimental period showed that the diet containing Soaked *E. cyclocarpum* leaf meal is acceptable to *O. niloticus* fingerlings at all the dietary inclusion levels and that it was at the 10% Soaked *E. cyclocarpum* and 30% of raw E.cyclocarpum inclusion level that the fingerlings had improved and also best performance than all other treatments. Also, the hematological parameters of the experimental fish were within the normal range at all inclusion levels, showing that there was no abnormalities in the use of Soaked *E. cyclocarpum* leaf meal as a dietary energy replacement for soya bean meal in the diet of *O. niloticus* fingerlings, and this is present in great amount at the experimental area, which have little or no competition compare to other plant protein source, farmers are encourage to go into *E. cyclocarpium* seed planting for commercial use in aquafeed production.

This study shows that Soaked *E. cyclocarpum* leaf meal could be used by fish farmers to replace soya bean meal in the diet of *O. niloticus* fingerlings as this would help to reduce the cost of feed production and at the same time increasing the farmer's income. It is recommended that proper processing should be done to remove the nutritional stress factors present in the *E. cyclocarpum* seed. Also, further research studies should be carried out to check if Soaked *E. cyclocarpum* leaf meal can be used beyond 10% soaked and 30% raw inclusion level without imposing any threat or abnormalities on the fish and the final consumer.

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