

Dietary Effect of African wild lettuce leaf meal (*Lactuca taraxacifolia*) on the Growth performance and Reproductive Parameters of African Catfish (*Clarias gariepinus*).

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Abstract

The effects of African wild lettuce leaf meal (*Lactuca taraxacifolia*) were investigated on the sperm and egg quality of *C. gariepinus*, (mean body weight, 190.29g). Five diets with crude protein of 35% were formulated with different inclusion levels of wild lettuce leaf powder. T1 (control) has 0 g of WLM, while T2, T3, T4 and T5 has 0.02, 0.03, 0.04 and 0.05g/100g of the diets. A total of 70 juveniles of *C. gariepinus* were randomly distributed twice into 120 liter tank at stocking density of 7 fish per tank and constant water level was maintained in the experimental tanks. The tank contained pond water (PH \approx 7, oxygen \approx 4.3 mg/l, temperature \approx 26. 2 0C). The fish were fed to satiation twice a day between 8.00hrs and 17hrs for a period of 84 days. At the end of the feeding trials, the sperm and egg sacs were excised from the fish samples selected and the sperm and eggs were collected for analysis. Data collected on sperm weight, sperm volume, motility, egg weight and egg number were subjected to one way analysis of variance (ANOVA) using Completely Randomized Design (CRD).The result shows that in treatment 5, Egg weight, number and volume were higher (73.75g, 55072.27, and 723ml, respectively) compared to treatments 2, 3, 4, and the control. The control had a higher sperm weight than treatments 2, 3, 4, and 5. Treatment 4 exhibited the highest sperm motility (72.00), and no significant differences were observed among treatments 1, 2, 3, and 5. The result of these study found that including 0.03g of Wild Lettuce Leaf Meal (WLM) in catfish diets resulted in the best growth performance and as well as a pro fertility agent which could be a future prospect in producing quality seeds at fish seeds multiplication centers thereby reducing the dependence on synthetic drugs.

Keywords: sperm; oocytes

1.1. Introduction

The African catfish (*Clarias gariepinus*) is a species of catfish of the family Clariidae and is an important fish species in both aquaculture and capture fisheries. It contributes 22% of animal protein in sub-saharan Africa and 40% of animal protein for consumption in Nigeria. In Africa, this catfish has been reported to be the biggest in size in terms of length and weight and popularly cultivated species (Olaniyi *et al.*, 2020).. In Nigeria, Catfish culture started from inception with aquaculture and is majorly the only hope of fish supply sustainability. In catfish farming, the impact of micro nutrients on performance is crucial. However, their higher prices, mainly due to importation costs, increase overall production expenses. (Alemayehu *et al.*, 2018). Therefore, finding cost-effective alternatives locally is essential for feed production. Feed additives are edible substances that are supplemented to feeds in minute amounts (either alone or in combination) for particular purposes, which are to improve fish performance and quality, to preserve the physical and chemical quality of the feed as well as that of the aquatic environment (Alemayehu *et al.*, 2018). Many plant additives have several components which are very useful in solving the rising cost of feed ingredients, negative effect of chemical and mineral supplements. Some examples of such plants are wild lettuce (*Lactuca taraxacifolia*), bitterleaf (*Vernonia amygdalina*), fluted pumpkin (*Telferia occidentalis*), waterleaf (*Talinum triangulare*) etc (Olaniyi *et al.*, 2020). Wild Lettuce (*Lactuca taraxacifolia*) also known as bitter, opium or African Lettuce is a highly neglected indigenous leafy vegetable in Nigeria. It is commonly called *efo yanrin* and mostly consumed majorly among the Yoruba tribe of Nigeria. The leaves can be eaten fresh prepared as salad or cooked in soups and sauces (Sakpere *et al.*, 2011). The leaves of *L. taraxacifolia* are fed to lactating cows in northern part Nigeria to increase the milk yield and to sheep's and goats mixed with natron to produce multiple births. *L. taraxacifolia* is given to livestock to induce multiple births. The leaf extract can also be mixed with breast-milk of a nursing mother and administered medicinally to cure partial blindness resulting from snake spit. In Benin it is used as a febrifuge (Ayensu, 2008).

Chemical analysis of wild lettuce leaf showed that it contains small quantities of mineral elements like iron (Fe), calcium (Ca), magnesium (Mg), Phosphorus (P), that function in major metabolic process of the human cells (Fasuyi, 2006). It is also considered to be a good dietary source of mineral, carbohydrate and protein (Mosh, 2006)

1.2. Experimental site

This experiment was conducted at the fisheries and Aquaculture unit of Teaching and Research Farm, Ladoke Akintola University of (LAUTECH), Ogbomoso, Oyo state Nigeria.

1.3. Processing of Wild lettuce leaf (test ingredient)

Fresh wild lettuce leaves was obtained from a local market in Ogbomoso, oyo state. The leaves were sun dried to a constant weight, ground to fine powder and stored in an air tight container prior the use for the experiment.

1.4. Experimental diets

The ingredients such as maize, wheat offal, GNC, soybean, fish meal, oyster shell, bone meal, premix, lysine, salt and vegetable oil were procured from a reputable feed mill in Ogbomoso. Five isonitrogenous (35%CP) diets were formulated in which treatment T1 contained (0g WL), T2 (0.02 WL), T3 (0.02g), T4 (0.03g) and T5 (0.05g) per 100grams of feed. The ingredients were mixed thoroughly and then pelletized to reduce dustiness for proper and easy acceptance by the juveniles. The pellets were sundried to constant weight and packed into air tight sack and stored for use.

1.5. Experimental fish

Two hundred (200) juvenile African catfish were obtained from a reputable farm in ogbomoso and acclimatized for the period of two weeks after which, one hundred (105) juvenile African catfish (190.32 ± 0.02 g) were randomly selected and divided into five (5) dietary treatments. The fish were stocked at the rate of 7 juveniles (3male: 4females) per tank (120L) and replicated three times (due to the number of fish within the weight ranges selected for the study). The water used was exposed for three days to allow oxygen dissolution into the water. The waste and faeces in all the tanks were siphoned every day to prevent pollution.

1.6. Data collection

Data such as fish weight and feed intake were collected during the feeding trial and the following parameters - Mean weight gain (MWG), Percentage weight gain (PWG), Specific growth rate (SGR), Feed conversion rate (FCR), Protein intake (PI), Protein efficiency ratio (PER) were calculated

Mean Weight Gain (MWG) = Final weight (g) – Initial weight gain (g).

Average Daily Weight Gain (ADWG)g/day = Mean Weight Gain (g) / Length of feeding trial (days)

Percentage Weight Gain (PWG) % = Mean Weight Gain/ Initial mean weight (g) x100

Specific Growth Rate (SGR) = $\frac{\log W_2 - \log W_1}{T_2 - T_1} \times 100$ W1 = initial weight (g), W2 = final weight (g), Log = natural log to base T2 –T1 = time interval between initial and final weight (days)

Feed Conversion Ratio (FCR) = Feed intake/ Net weight gain

Protein Efficiency Ratio (PER) = Net weight gain/ Amount of protein fed

1.7. Milts and eggs collection

Three male and female fishes were randomly selected from all the treatments for milt and egg collection. The fishes were deoxidized and the testes excised and eggs were siphoned from the female ovum with the use of hose. After which the sperm and eggs were viewed under the computer microscope.

1.8. Milt and egg analysis

The milt was analyzed with the use of a computer aided system and the data were, mathematically elaborated to obtain numerical indices expressing the statue of the ejaculation and egg production. The eggs were weighed on a digital scale and counted by volumetric method. The Egg volume, weight and number of eggs were determined.

1.9. Chemical analysis

Proximate composition of test ingredient (wild lettuce) was determined according to the methods of Association of analytical chemist AOAC (2000).

2.0. Statistical analysis

All data collected during experimental period were subjected to a one-way analysis of variance (ANOVA) using completely randomized design in accordance with SPSS and Duncan's multiple range tests was employed to reveal significant differences among the means.

The gross composition of experimental diets is as shown in Table 1 and it revealed all the ingredients used for the five diets formulated for the experiment.

Table 1: Gross Composition of Experimental Diet.

Ingredients	D1(0g)	D2(0.02g)	D3(0.03g)	D4(0.04g)	D5(0.05g)
Maize	20.60	20.60	20.60	20.60	20.60
Wheat Offal	10.30	10.30	10.30	10.30	10.30
Groundnut Cake	22.20	22.20	22.20	22.20	22.20
Soybean Cake	33.30	33.30	33.30	33.30	33.30
Fish meal	11.10	11.10	11.10	11.10	11.10
Bone meal	0.50	0.50	0.50	0.50	0.50
Oyster shell	0.50	0.50	0.50	0.50	0.50
Lysine	0.50	0.50	0.50	0.50	0.50
Vit. premix	0.50	0.50	0.50	0.50	0.50
Honey	0.50	0.50	0.50	0.50	0.50
Wild lettuce	-	0.02	0.03	0.04	0.05
Total	100.00	100.00	100.00	100.00	100.00
ME Kcal/g	3156.23	3156.24	3156.25	3156.26	3156.27
%CP	35.00	35.00	35.00	35.00	35.00

CP – Crude protein ME- Metabolizable energy

RESULTS

The proximate composition of wild lettuce leaf (*Lactuca taraxacifolia*) is in Table 2. The crude protein, dry matter, crude fiber, ash content, moisture content and crude fat were 28.35%, 91.30%, 11.30%, 19.70%, 8.70% and 3.30% respectively.

Table 2: Proximate composition of wild lettuce

Parameters	Crude protein	Dry matter	Crude Fibre	Ash Content	Nitrogen Free Extract	Crude Fat
Values	28.35%	91.30%	11.30%	19.70%	28.65%	3.30%

GROWTH PERFORMANCE

The growth performance and nutrient utilization of juvenile African catfish fed wild lettuce leaf meal diets was revealed in Table 3.

The study evaluated the growth performance and feed utilization of African catfish fed different levels of wild lettuce meal as additives. The Initial mean weight and Specific Growth Rate (SGR) showed no significant differences. However, Final Mean Weight (FMW), Mean Weight Gain (MWG), Percentage Weight Gain (PWG), Average Feed Intake (AFI), Feed Conversion Ratio (FCR), Protein Intake (PI), and Protein Efficiency Ratio (PER) were significantly different among treatments. Treatments T2 (0.02g inclusion level) and T3 (0.03g inclusion level) recorded the highest FMW values (321.57g and 347.22g, respectively). The highest MWG and PWG values (156.83g and 82.48%) were found in treatment T3. AFI was highest in Treatment T3 (100.17g) and lowest in Treatment T2 (90.85g), with no significant differences among T1, T2, T4, and T5. FCR was highest in Treatment T4 and Treatment T5 (0.97 and 0.88, respectively) and lower in Treatments T1, T2, and T3 (0.75, 0.69, and 0.63, respectively). The highest PI value (35.05g) was recorded in Treatment T3, with no significant differences among Treatments T1, T2, T4, and T5. The highest PER values (3.61%, 4.11%, 4.47%, and 3.25%) were obtained in Treatments T1, T2, T3, and T5, respectively, while the lowest PER value (2.95%) was in Treatment T4.

Table 3: Results of growth performance and feed utilization of African catfish fed varying levels of wild lettuce leaf meal

Parameters	T ₁ (0g)	T ₂ (0.02g)	T ₃ (0.03g)	T ₄ (0.04g)	T ₅ (0.05g)	SEM
IMW (g)	190.82	190.78	190.39	190.14	190.20	90.4
FMW (g)	317.62 ^b	321.57 ^b	347.22 ^a	291.66 ^b	292.49 ^{ab}	10.5
MWG (g)	126.79 ^{ab}	130.79 ^{ab}	156.83 ^a	101.52 ^{bc}	102.29 ^{bc}	10.5
PWG (%)	66.44 ^{ab}	68.55 ^c	82.48 ^a	53.39 ^c	53.62 ^{cb}	5.47
SGR (g/day)	0.28	0.35	0.26	0.21	0.21	0.02
TFI (g)	667.13 ^a	585.79 ^c	500.85 ^{abc}	591.73 ^{ab}	635.43 ^{ab}	9.11
AFI (g)	95.25 ^b	90.58 ^b	100.17 ^a	98.21 ^b	90.77 ^b	3.33
FCR	0.75 ^c	0.69 ^c	0.63 ^c	0.97 ^a	0.88 ^a	0.49
PI (g)	33.67 ^b	31.76 ^b	35.05 ^a	34.33 ^b	31.39 ^b	1.11
PER (%)	3.61 ^a	4.11 ^a	4.47 ^a	2.95 ^b	3.25 ^a	0.23

^a, ^b and ^c means in the same row with the same superscript are not significantly different ($p < 0.005$).

IMW- initial mean weight, FMW- final mean weight, MWG- mean weight gain, PWG- percentage weight gain, SGR- specific growth rate, TFI- total feed intake, AFI- average feed intake, FCR- feed conversion ratio, PI- protein intake, PER- protein efficiency ratio and FI- feed intake

EGG QUALITY PARAMETERS

The Egg quality parameters of African catfish fed with different levels of wild lettuce leaf meal (additives) is as shown in Table 4.

All parameters were significantly different across the treatments ($p < 0.05$). Fish fed diet T5 had the highest value for egg weight (73.75g), those fed diet T4 had the least value while the fed diets T1 to T3 were comparable. This trend is similar for Egg number and egg volume.

Table 4: Eggs of African catfish fed varying levels of Wild lettuce leaf meal

Parameters	T ₁ (0g)	T ₂ (0.02g)	T ₃ (0.03g)	T ₄ (0.04g)	T ₅ (0.05g)	SEM
EGG W.	66.93 ^{ab}	56.32 ^{ab}	63.50 ^{ab}	40.21 ^b	73.75 ^a	4.32
EGG N.	48917.92 ^{ab}	43280.50 ^{ab}	46516.66 ^{ab}	29417.87 ^b	55072.27 ^a	3147.76
EGG V.	691.00 ^{ab}	506.50 ^{ab}	613.75 ^{ab}	450.25 ^b	723.00 ^a	38.68

^a and ^b means in the same row with the same superscript are not significantly different

($p < 0.005$)

EGG W- Egg weight, EGG N- Egg number, EGG V- Egg volume.

SPERM QUALITY

The sperm quality parameters of African catfish fed with different levels of Wild lettuce leaf meal is as in Table 5.

It was revealed in table 4.6 that fish fed the control diet (treatment T1) had the highest value (3.33g) of Sperm weight (SPW), while those offered T2, T3, T4 and T5 compare favourably. The highest value for Sperm volume (SPV) 3.50mls was obtained with Treatment 3, while Treatment T1, T2, T4 and T5 are similar and are quite comparable. The results of Sperm density and PH revealed no significant differences among all treatments. Treatment 3 and Treatment 5 recorded the highest Spermatocrit (SPMT) values of 38.00ml while Treatment 2 had the lowest value of 34.50ml. The highest value for Sperm motility (SPM) 72.00 was recorded with Treatment 4, while the lowest SPM value (62.50) was recorded with treatment 1. However, there were no significant differences among treatments 1, 2, 3 and 5.

Table 5: Sperm analysis of African catfish fed varying levels of Wild lettuce leaf meal

Parameters	T ₁ (0g)	T ₂ (0.02g)	T ₃ (0.03g)	T ₄ (0.04g)	T ₅ (0.05g)	SEM
SPM WT(g)	3.33 ^a	1.55 ^b	2.14 ^{ab}	0.96 ^b	1.31 ^b	0.25
SPM PH.	7.30	7.30	7.27	7.28	7.18	0.03
SPM.VL (ml)	2.00 ^b	2.50 ^{ab}	3.50 ^a	3.00 ^{ab}	3.00 ^{ab}	0.21
SPMCRT (%)	36.00 ^{ab}	34.50 ^b	38.00 ^a	36.00 ^{ab}	38.00 ^a	0.42
SPM DSTY.	233.00	237.50	233.50	245.50	227.75	3.01
SPMMTY (%)	62.50 ^b	64.50 ^b	65.00 ^b	72.00 ^a	65.00 ^b	1.12

^a and ^b means in the same row with the same superscript are not significantly different (p<0.005)

SPM WT- Sperm weight, SPM PH- Sperm PH, SPM VL- Sperm volume, SPMCRT- Spermatocrit, SPM DSTY- Sperm density, SPM MTY- Sperm motility.

Discussion

Medicinal plants have been reported to be growth promoters and immune boosters in livestock and fish nutrition (Olaniyi *et al.*, 2020). The study's growth performance results are comparable, showing increased weight gain in treatments 1, 2, and 3, indicating effective conversion of feed protein into muscle. However, weight gain decreased above 0.03g inclusion of wild lettuce leaf meal. Treatment 3 (0.03g inclusion) exhibited the best performance in mean weight gain, percentage weight gain, and specific growth rate. Weight gain and specific growth rate are key indicators of diet productivity (Omitoyin and Faturoti, 2000).

Protein content in the diets enhanced fish growth and dietary energy supply. Specific growth rate (SGR) and feed conversion ratio (FCR) are vital factors for feed management and economic performance in aquaculture (Mokolensang *et al.*, 2003). These two parameters are closely associated with daily feeding rate or ration size and the rate at which they are converted to flesh (Inayat *et al.*, 2005). In this study, it can be observed that treatment T3 had the best conversion efficiency. Therefore, it can be deduced that treatment T3 was able to convert more of the feed administered into muscles, thereby increasing the weight gain of the fish. The hematological characteristics of fishes are essential for assessing their health, impacted by factors such as diet composition and fish activity (Keri *et al.*, 2012).

This study demonstrated that the inclusion of wild lettuce at 0.05g (treatment 5) led to improved egg volume, weight, and number (723ml, 73.75g, 55072.27) in fish compared to the control group. This aligns with findings by Olaniyi *et al.* (2020) regarding the relationship between egg weight and number. Adeparusi *et al.* (2010) and Dada (2012) also reported enhanced reproductive performance in catfish using medicinal herbs as

fertility-enhancing agents. Flavonoids, glycosides, and steroids present in wild lettuce leaves potentially contribute to increased egg production through estrogen production.

Sperm quality, particularly motility, is crucial for fertilization. This study observed increased sperm motility in all treatments compared to the control, likely attributed to the carbohydrate, lipid, and vitamin C content of wild lettuce leaves. The carbohydrate and mineral content of wild lettuce provides essential energy for sperm motility. The study also indicated that wild lettuce had a non-significant effect on semen pH and density, indicating potential safety for use as a feed supplement. While sperm weight decreased with wild lettuce supplementation, sperm volume increased, highlighting the importance of sperm motility for fertilization success.

These findings emphasize the potential of wild lettuce as a pro-fertility agent in fish breeding, contributing to enhanced egg and sperm quality, and ultimately, successful fertilization (Fauvel *et al.*, 2010; Ciereszko and Dabrowski, 2005; Ayoola, 2009).

CONCLUSION

In conclusion, the study suggests that wild lettuce leaf can be used as an additive at 0.03g inclusion level in diets to promote optimal growth without compromising fish health. It also demonstrates potential as a fertility enhancer, which could contribute to producing high-quality fish seeds in multiplication centers, reducing reliance on synthetic drugs in the future.

RECOMMENDATION

Further research should focus on the improvement of fish breeding technology for different species of fish using *L. taraxacifolia*, since the main aim of aquaculture is to maximize fish production and this plant has promising pro fertility property which can be exploited in

Aquaculture. Also, caution should be taken because higher concentration of wild lettuce leaf may not give the desired result, as seen in the result of the present research where inclusion level above 0.03g inclusion level may reduce the growth of the fishes.

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