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Nutritional Effect of African Wild Lettuce Leaf (Lactuca Taraxacifolia) On the Growth Performance and Liver Histology of African Catfish.

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ABSTRACT
The effects ofAfrican wild lettuce leaf meal (Lactuca taraxacifolia), were investigated on the growth performance and liver histology of Clarias gariepinus, (mean body weight, 190.29g). Five diets with crude protein of 35% were formulated with different inclusion levels of wild lettuce leaf meal. T1 (control) has 0 g of WLLM, while T2, T3, T4 and T5 has 0.02, 0.03, 0.04 and 0.05g/100g of the diets. A total of 105 juveniles of C. gariepinus were randomly distributed in triplicate 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 ≈ 7.2, oxygen≈ 4.3 mg/l, temperature≈ 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, livers were excised from random samples across the treatments.Data collected on weight changes and feed-intake were subjected to one way analysis of variance (ANOVA) using Completely Randomized Design (CRD). The research revealed that there is mild disseminated steatosis and congestion on all the liver of fish fed diets. There is moderate accumulation of fat on all the livers of the fishes fed African wild lettuce. The optimum requirement of African Wild Lettuce level in the formulation of practical diets for improved growth of Clarias gariepinus was Treatment 3. No significant visible lesion observed in the liver micrographs of the fish fed African Wild Lettuce based diets showed that a form of hepatoprotective effect in African Wild Lettuce meal was conferred on the liver of the fish fed with the test diets.
Keywords: Steatosis, Weight gain, Liver

I. INTRODUCTION

The African catfish (Clarias gariepnus) 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). Chemical analysis of wild lettuce leafshowed 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

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considered to be a good dietary source of mineral, carbohydrate and protein (Mosha, 2006). It is a wide known fact that the intensive use of synthetic drugs presents numerous disadvantages, for both the environment and fish health. Intensive use of antibiotics has resulted in its accumulation in the muscle of commercialized animals (Cabello et al., 2006; Romero-Ormazabal et al., 2012) and the development of resistant bacteria strains (Miranda and Zemelman, 2002; Seyfried et al., 2010). Also, the use of antiparasitic drugs like trichlorfon or praziquantel in bath treatments is hazardous for fish and aquatic environment. This also result in the development of resistance (Forwood et al., 2013; Umeda et al., 2006). Vaccines, considered to be the most effective method to prevent disease outbreaks in aquaculture, are too expensive for widespread use by fish producers and since it is extremely difficult to develop multiple strain vaccines, most vaccines are only effective against one type of pathogen (Pasnik et al., 2005; Sakai, 1999). Considering the numerous disadvantages of synthetic drugs, there is an increasing need for the development of alternative strategies in aquaculture disease management. Medicinal plants such as wild Lettuce (Lactuca taraxacifolia) can therefore provide a cheaper and more sustainable alternative in production of Catfish. Also, there has not been much documented research on the use of African wild lettuce as an additive in fish meal. Therefore, this study was carried out to investigate the dietary effect of wild lettuce on the growth performance and histology of African catfish.

II. MATERIALS AND METHODS.

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.

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.

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 0gbomoso. 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.

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±0.02g) 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.

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) = Log W2 -Log W1 /T2 –T1 x 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

Histology

At the end of the experiment, fish samples were randomly selected from all treatments, the livers were excised and fixed in 10% formalin before taken to the laboratory for analysis. The micrograph of the liver was developed by examining the cells under electronic microscope.

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Chemical analysis

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

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

III. 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

Parameters	Crude protein	Dry matter	Crude Fibre	Ash Content	Nitrogen Free Extract	Crude Fat
Value	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,

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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}	10.54
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}	635.42^{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

HISTOLOGY

Liver histology of African Catfish (Clariasgariepinus) Fed Varying Inclusion Levels Of African Wild Lettuce (Lactucataraxacifolia).

ThemicrographsLiverofAfricanCatfishfedlactucataraxacifoliawereasshowninplate AtoE.

1. Plate A revealed the liver with mild disseminated steatosis and mild disseminatedcongestion.
2. PlateBshowedtheliverwithmilddisseminatedcongestionandthelesionwereobvious.
3. Plate C presented the liver with mild disseminated steatosis and mild disseminatedcongestion.
4. Plate D presented the liver with mild disseminated steatosis and mild disseminatedcongestion.
5. PlateErevealedmoderatetomarkeddisseminated steatosisandfocalareacongestion.

Plate A: The micrograph Liver of African Catfish fed with control diet

Plate B: The micrograph Liver of African catfish with 0.02g(Diet2) of African wild lettuce Lactucataraxacifoliameal.

Plate C: The micrograph Liver of African Catfish fed with 0.03g(Diet3)of African wild lettucelactucataraxacifoliameal

Plate D: The micrograph Liver of African Catfish fed with 0.04g(Diet 4)of African wild lettuce lactucataraxacifoliameal

Plate 4.5:The micrograph Liver of African Catfishfed with 0.05g (Diet5) of African wild lettucelactucataraxacifoliameal

IV. DISCUSSION

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). Fakunle et al. (2013) reported that toxic components or anti-nutritional factors in mostagriculturalby-productsmayirritatethedigestivetractwhichiscapable ofdecreasingfeedintakeand growth.

The Histological observation in the present study is in agreement with the report of Olukunle et al. (2011) that liver histology of the control group contained a lot of lipids and had larger number of vacuolated hepatocytes than test diets fed groups linking it to a higher glycogen accumulation. Vacuolation of the liver is probably induced by the phobolesthers and other secondary metabolites in African Wild Lettuceca using oxidative stress and compromise the integrity of the membrane of the hepato-cytesorca using lipid peroxidation. The presence of numerous vacuolation in the liveras observed in this study seems to be pathognomonic lesion of fish exposed to high dietary lipid intake. Gatta et al. (2011) explained that high vacuolation of the hepatocytes is physiological response to dietary lipid intake, also, Olukunle (2011) made similar observation when evaluating the effect of different dietary oil sources on growth performance and nutrientutilization of Clariasgariepinus. Olukunle (2011) observed vacuolation in the liver offish fed diet containing Chrysophyllumalbidum seed meal. Adinortey et al., (2012) reported vacuolation in the liver of sharp snout sea bream (Diplodus puntazzo) when fed sunflower meal and the liver of rainbow trout (Oncorhyn chusmykiss) fed brassica-by-products respectively. The African Wild Lettuce meal could therefore be the constituent of hepato protection of the liver in fish fed African Wild Lettuce based diets. Antioxidant effects of bioactive components in plant sources could improve cell protection

V. CONCLUSION

The study revealed that African Wild Lettuce diet can be tolerated by African catfish. Treatment 3 performed better than the control and other treatments in terms of final mean weight, mean weight gain, percentage weight gain, average feed intake, protein intake and protein efficiency ratio thereby reducing the cost of production of Catfish in Nigeria. The results also revealed that the optimum requirement of African Wild Lettuce level in the formulation of practical diets for improved growth of C.gariepinuswere at0.03ginclusionlevel.

No significant visible lesion observed in the liver micrographs of the fishfed African Wild Lettuce based diets showed that a form of hepatoprotective effect in African Wild Lettuce meal was conferred on the liver of the fishfed with the test diets.

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