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TOXICITY OF GRASSCUTTER (*THRYONOMYS SWINDERIANUS* TEMMINCK) FAECES TO *CLARIAS GARIEPINUS* BROODSTOCK

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ABSTRACT

This study investigates the performance of *Clarias gariepinus* broodstock fed with grasscutter faecal droppings. The physiological effects and growth performance of the fish were assessed after 8 weeks experimental feeding followed by 4 weeks of feeding with normal formulated (control) diet for fish. Ninety test fish were maintained solely on grasscutter faecal organic manure, while 30 fish maintained on the control diet served as the controls. The test fish suffered significant ($p<0.05$) weight loss compared to initial (pre-treatment) values and those of the control fish, but exhibited increased ($p < 0.05$) growth within 4 weeks of being fed the control diet. They also developed normocytic, normochromic anaemia, and leucocytosis characterized by lymphocytosis and heterophilia after 8 weeks of feeding. Analysis of the plasma metabolites of the test fish revealed hyponatraemia, hypochloraemia, acidosis, hypocreatininaemia, hypoproteinaemia, decreased enzyme activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Elevated plasma alkaline phosphatase (ALP) levels were observed, while the levels of plasma calcium, phosphorus, urea, potassium cholesterol, triglyceride, albumin, globulin and albumin globulin ratio remain unchanged throughout the experimental period. The haematologic and plasma biochemical changes in the test fish may be attributed to hepatorenal dysfunction, decreased protein metabolism, deficient protein content of grasscutter droppings and possibly associated with toxic materials in grasscutter faeces. All the haematological and plasma biochemical changes in the test fish returned to normal 4 weeks after reversion to normal diet, showing that grasscutter droppings are nutritionally sub-optimal and probably toxic to *Clarias gariepinus* broodstock and hence not recommended for total conventional feed replacement in *C. gariepinus* fish farming.

INTRODUCTION

Fish production has been recognized as one of the ways by which animal protein can be sustainably made available to the populace of developing countries, and especially tropical countries where there is an abundance of underutilized manure and organic waste. Bardach *et al.* (1982) emphasized the development of high yielding fish culture systems based on feeding fish with organic manure. When applied to ponds, poultry faeces is a form of organic manure that gives high productivity by stimulating the growth of phytoplanktons which are natural and efficiently convertible food fish (Hickling, 1962). With the current increased awareness and interest in small and semi-large scale domestication and farming of grasscutter as an alternative source of animal protein in this country (Ogunsanmi *et al.*, 2002), the use of grasscutter manure in fish farms is on the increase. Agbede *et al.* (1999) highlighted the toxic effects on the pathophysiology and

weight gain of *Clarias gariepinus* broodstock fish maintained solely on poultry faeces as weight loss, anaemia, leucopenia and hepatorenal dysfunction.

Tacon (1992) emphasized that nutritional fish pathology in aquaculture still remains scantily studied. Fish fed excess plant legumes including *Leucaena leucocephala* with toxic amino acid - mimosine and *Canaralia ensiformes*, containing the toxic L-canavanine have suffered nutritional pathologies and even death (Jackson *et al.*, 1992). Some pathological conditions arising from dietary imbalances as well as the presence of some toxic anti-nutritional factors have been reported (Ukachuckwu *et al.*, 1997). These include anorexia, retarded growth and poor feed efficiency. Falaye *et al.* (1999) attributed the rise in specific liver enzyme activities and cholesterol level as an indication of hepatoxicity and lipid metabolic dysfunction on Indian Carp (*Cirrhinus mirgala)* placed on diets in which soybean milk residue replaced more than 50% groundnut cake as a source of protein.

Therefore, the expediency of promoting sustainable fish production, and the potential usefulness of information on the effects of grasscutter faeces dietary treatments in fish farming stimulated the current investigation of the growth performance and plasma biochemistry of *Clarias gariepinus* fed solely with grasscutter faecal organic manure.

MATERIALS AND METHODS

Experimental Fish

One hundred and twenty *Clarias gariepinus* broodstock were used for the experiment. They were purchased in two batches from a commercial fishpond in Ibadan, Nigeria. The weights of the fish ranged between 110gm and 140gm (mean 120gm). Ninety of them served as the test fish while 30 served as control. The test fish were divided into three groups of thirty, each placed in two tanks containing fifteen fish per tank for each group.

Experimental diets, feeding and management of fish

Two types of diets were used: grasscutter faeces were used as the test while the control fish were given formulated diet made up of appropriate proportions of various ingredients (Table 1). The proximate analyses of the test and control diets according to the methods of A.O.A.C. (1990) are shown in Table 2. Grasscutter faeces (test feed) were collected from a commercial rabbitry along Badagry Road, Lagos, Nigeria and sun-dried for 2 days. Non-faecal materials such as wood shavings and stones were removed, the dried faeces ground into powder and then pelleted. The control feed, purchased in pellet form from a feed mill in Ibadan, Nigeria and the test feed were stored in different polythene bags and kept in a dry place (28±1°C) before use. The acclimatization, feeding and management of both test and control fish were carried out following Agbede *et al.* (1999). The body weight, standard (SL) and total lengths (TL) of each test and control fish were measured weekly in order to adequately adjust the quantity of feed arising from changes in body weight. The flow-through system was used with water in the tanks adequately aerated. The water quality parameters were maintained at acceptable optimal levels for tropical fish (Boyd, 1981).

Blood Collection and Analysis

One and a half millilitres (ml) of blood were collected at the beginning (week 0), weeks 8 and 12 from the caudal peduncle of 10 each of control and test broodstock as described by Stoskopf (1993). The blood samples were dispensed into tubes containing lithium heparin anticoagulant. Haematological studies were carried out immediately. The blood samples were centrifuged at 1,200G for 5 minutes at 30°C, and the plasma stored at –25°C until used for biochemical analysis.

Packed cell volume (PCV), haemoglobin (Hb) concentration, red blood cell (RBC) count, total and differential white blood cell (WBC) count, mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were determined as described by Agbede *et al.* (1999). Plasma sodium and potassium concentrations were determined by flame photometry (Corning Model 400, Corning Scientific Limited, England). Plasma chloride, phosphorus, calcium and bicarbonate levels were determined as described by Toro and Ackermann (1975). The samples were analyzed for urea by the diacetyl monoxime method of Croker (1967); alkaline phosphatase (ALP) activity was determined using a modified method of Frajola *et al.* (1965); alanine and aspartate aminotransminase (ALP and AST) activities were determined using the method described by Reitman and Frankel (1957); while gammaglutamyl transferase (GGT) activity was determined by the method of Szas (1969). The plasma total proteins and albumin levels were determined using the methods described by Henry *et al.* (1957), while plasma globulin level was determined by subtracting the albumin values from total protein values. Plasma cholesterol level was determined by the method of Zlatkis *et al.* (1953) and triglyceride level was determined by the enzymatic method as described by Toro and Ackermann (1975).

Statistical Analysis

The data for the control and triplicate group of test fish were pooled and subjected to analysis of variance (SAS, 1987), and the means were compared for significant differences, if any, using the Duncan's multiple range test (Duncan, 1959).

RESULTS

The mean weights of the experimental fish fed grasscutter faeces and the control diet, as shown in Fig. 1, revealed that fish fed control diet steadily gained weight during the feeding trials reaching an average of 189% over the starting weight in week 12. On the contrary, the test fish lost weight from week 2 of feeding up till week 8, when their mean weight was 56.5% of their control counterparts or 87% of their starting weight. However, upon feeding normal diet from the 9th week upwards, the test fish gradually regained weight but could not match that of the control fish at the end of the feeding trial at week 12 (Fig. 1). No mortality was recorded in both control and test fish lots during the period of study.

The control fish had steady increases ($p<0.05$) in both SL and TL throughout the period of feeding trials (data not shown). There was no significant change ($p>0.05$) in SL of the test fish (25.5±0.4cm at week 0 and 25.2±0.1cm) during the 8 weeks of the experimental feeding with the test diet. However, there was a significant increase ($p<0.05$) in mean SL values to 27.3±0.2cm at week 12, 4 weeks after reversion to the control fish diet. No significant changes ($p>0.05$) were observed in the mean values of the TL of both groups of fish throughout the feeding trial.

The haematological and plasma biochemical parameters of fish fed grasscutter faeces and control diet are presented in Tables 3 and 4, respectively. While there was no significant variation ($p>0.05$) in the haemogram of control fish during the course of the feeding trial, the test fish were severely anaemic ($p<0.01$) at week 8, with a return to normal haemogram in week 12 (Table 3). The anaemia was normocytic normochromic. Likewise, the test fish developed leucocytosis manifested as lymphocytosis and heterophilia ($p<0.05$). No significant change ($p>0.05$) was observed in the leucogram of the control fish through the feeding trial. The mean plasma levels of sodium, chloride, bicarbonate, creatinine, urea, total protein, albumin, cholesterol, AST and ALT of test fish were significantly lower ($p<0.05$) than those of the control fish at week 8 of the feeding trial. These plasma parameters returned to their initial levels following 4 weeks feeding of the fish with normal diet (Table 4). The activity of plasma ALP was however significantly increased ($p<0.05$) in the test fish by week 8 of feeding the fish with grasscutter faeces. This activity returned to the initial level after 4 weeks of reversion to normal diet (Table 4). There were no significant changes ($p>0.05$) in the mean plasma values of potassium, calcium, inorganic phosphorus, triglyceride and GGT of the test fish throughout the 12 weeks of experimental feeding (Table 4).

DISCUSSION

The results of the proximate analysis of the grasscutter faeces utilized in this study revealed that the crude protein content was 17.8% or about half of the optimum level of 35% required for Catfish (Dupree and Huner, 1984) and less than 50% of the control diet. Also, the fat, crude fibre, and moisture contents of grasscutter faeces were also lower than those of control diet. Though there was no mortality, the test fish lost weight, had no increase in standard length, developed normocytic normochromic anaemia, lymphopenia, heterophilia and moderate eosinophilia within the 8 weeks of being fed solely with grasscutter droppings. They also exhibited derangements in plasma biochemical parameters such as hyponatremia, hypochloraemia, metabolic acidosis, hypoproteinaemia, hypocholesterolaemia, and decreased plasma levels of creatinine, urea, AST and ALT. They however, exhibited increased plasma ALP activity. The above plasma derangements are usually associated with one form of tissue and organ damage as a result of toxicity (Agbede *et al.*, 1999). Progressive weight gain and reversion of haematological and plasma biochemical parameters to normal levels characterized reversion of the test fish to control diet, signalling improved nutrition and removal of exposure to toxic materials.

Nutrition, especially dietary protein intake is known to affect the live weight gain and haematological parameters of animals (Makinde *et al.*, 1991). Dupree and Huner (1984) reported linear relationship between fish weight gain and dietary protein. The implication of protein deficiency in the manifestation of several diseases has been fairly documented. Protein deficiency leads to susceptibility to disease and hence the rate of growth could be compromised (Umoh, 1979; Iyayi and Tewe, 1998; Agbede *et al.*, 1999). Apart from absolute protein insufficiency, the deficiency of essential amino acids in the diet has been reported to cause growth retardation of farmed fish (Roberts, 1978). In protein deficiency there is an indiscriminate handling of the amino acids (Eggum, 1970; Harper, 1971). Under this condition the metabolism of some serum metabolites could be altered. Since proteins form the basic unit of cells and other substances (especially enzymes and growth factors) that are necessary for body building, repairs, maintenance of homeostasis, regulation of vital body functions, energy source and defense against infectious agents (Kaneko, 1989), the lack or inadequate supply of proteins in feed usually would lead to increased net protein catabolism in order to maintain homeostasis and sustain life.

The weight loss in the test fish is a direct consequence of low protein intake and may be related to the poor palatability of the grasscutter faeces, which has been reported to impair digestibility (Dupree and Huner, 1984). The fact that the test fish steadily gained weight and had increased standard length following 4 weeks feeding with control (compounded) diet, further corroborates the significant disparity between protein sufficiency and deficiency as well as the crucial role of protein metabolism in body building, growth and the nutritional assessment of feeds.

The observed derangements in plasma biochemical parameters and enzyme profiles in the test fish could be directly or indirectly linked with toxic materials (high contents of ammonia, urates, nitrates and nitrites) in the faeces of grasscutter, as was the case with untreated poultry droppings (Agbede *et al.*, 1999). Hepatorenal impairment induced by the nitrates and nitrites present in grasscutter faeces could contribute to the hyponatraema, hypochloraemia and metabolic acidosis in the test fish (Kaneko, 1989; Ogunsanmi *et al.*, 1994; Agbede *et al.*, 1999). Zilva and Pannall (1984) reported that bone diseases with increased osteoblastic activity or hepatic dysfunction coupled with involvement of the biliary tract are the commonest causes of raised plasma alkaline phosphatase activity in the serum. Onifade *et al.* (1999) observed increased alkaline phosphatase levels in rabbits fed unsupplemented diet. The decreases in plasma ALT and AST activities in the test fish suggest an impaired production of these enzymes by the liver (Walmsley and White, 1994; Rosenthal, 1997) either as a result of hepatic dysfunction or low protein metabolism as a result of lowered intake or both.

In conclusion these results show that sole feeding *Clarias gariepinus* broodstock with grasscutter faeces had negative impact on the growth and could be toxic, hence not recommended for use in commercial fish farming unless fortified with supplementary high protein-based diets. Some form of processing is also advocated in order to remove or reduce the level of toxic materials that may be present in grasscutter faeces.

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Table 1: Composition of the formulated (control) diet

Feed ingredients	Composition (%)
Groundnut cake	27.80
Yellow maize	26.45
Fish meal	14.44
Blood meal	8.31
Brewer's waste	10.00
Bone meal	1.50
Oyster shell	2.00
Groundnut oil	5.00
Vitamin premix	4.00

Table 2: Proximate analysis (%) of experimental diets

	CP	Fat	CF	Ash	Moisture
Control diet	37.50	5.20	5.98	9.22	9.20
Test diet	17.80	3.68	1.03	7.59	8.44

CP = crude protein
CF = crude fibre

Table 3: Haematology of *Clarias gariepinus* broodstock fed formulated (control; n=10) diet and those on grasscutter faeces (test; n=10)

	Week 0		Week 8		Week 12	
Parameters	Control fish	Test fish	Control fish	Test fish	Control fish	Test fish
PCV (%)	36.5±0.9	37.1±0.6	36.8±1.0	28.2±0.4*	38.8±1.9	35.4±1.2
RBC ($x10^6$/µl)	2.4±0.1	2.5±0.2	2.4±0.2	1.8±0.2*	2.6±0.2	2.3±0.2
Hb conc (mg/dl)	11.0±1.3	10.5±0.5	10.8±0.5	8.1±0.3*	11.1±0.8	10.2±1.2
MCV (fl)	153.3±2.5	149.9±5.1	154.2±1.9	156.1±5.2	149.6±4.4	153.9±3.2
MCHC (%)	30.3±1.3	28.5±1.1	29.6±1.3	28.9±1.5	28.7±0.8	28.8±1.2
Total WBC ($x10^3$/µl)	41.1±3.6	42.3±3.1	42.3±2.3	48.2±0.5*	40.9±1.9	44.5±2.6
Lymphocytes ($x10^3$/µl)	27.8±0.6	29.6±1.5	28.8±2.1	32.2±1.1*	27.0±0.7	28.4±0.9
Heterophils ($x10^3$/µl)	10.9±0.3	10.5±0.1	10.9±0.3	14.1±0.3*	11.1±0.5	12.6±0.1
Eosinophils ($x10^3$/µl)	1.2±0.1	0.9±0.1	1.3±0.0	0.9±0.2	1.3±0.2	1.7±0.3
Monocytes ($x10^3$/µl)	1.2±0.0	1.3±0.2	1.3±0.1	1.0±0.3	1.5±0.3	1.8±0.1

Data presented as mean ± standard error of mean
*Indicates significant differences ($p < 0.05$) of test from control values

Table 4: Plasma biochemical values of *Clarias gariepinus* broodstock fed formulated (control; n=10) diet and those on grasscutter faeces (test; n=10)

Plasma biochemical parameters	Week 0		Week 8		Week 12	
	Control	Test	Control	Test	Control	Test
Sodium (mmol/l)	130.3±1.7	128.0±2.4	131.8±1.9	118.4±3.2*	128.1±2.1	129.0±1.2
Potassium (mmol/l)	5.0±0.1	5.2±0.3	5.3±0.1	5.0±0.2	4.9±0.2	5.3±0.2
Chloride (mmol/l)	99.8±1.3	102.5±2.8	101.0±1.5	89.2±2.6*	100.1±2.1	101.7±1.6
Bicarbonates (mmol/l)	20.8±0.3	20.5±0.2	21.6±0.4	18.8±0.1*	19.9±1.2	20.1±0.3
Calcium (mg/dl)	8.4±0.3	8.5±0.2	8.3±0.2	8.4±0.2	8.5±0.1	8.4±0.3
Phosphorus (mg/dl)	4.9±0.2	4.9±0.1	5.0±0.3	5.1±0.4	4.8±0.2	4.8±0.3
Urea (mg/dl)	16.2±0.6	15.7±1.5	16.1±0.7	14.4±0.2*	16.5±0.4	15.9±1.1
Creatinine (mg/dl)	0.95±0.03	0.89±0.08	0.94±0.05	0.72±0.08*	1.05±0.02	0.99±0.06
ALP (IU/l)	167.3±4.3	163.0±6.2	169.3±5.3	185.2±3.9*	173±4.4	181.0±2.2
ALT (IU/l)	34.7±1.3	33.5±0.6	36.5±1.8	32.2±1.1*	34.1±1.2	34.2±0.4
AST (IU/l)	28.0±1.6	27.5±1.2	27.6±1.4	25.3±0.5*	28.2±1.1	27.9±2.1
GGT(IU/l)	7.0±0.4	7.2±0.4	7.3±0.2	7.1±0.2	7.2±0.3	7.4±0.2
Total protein (g/dl)	5.9±0.2	5.7±0.2	6.0±0.2	4.9±0.1*	5.8±0.1	5.7±0.3
Albumin (g/dl)	2.7±0.1	2.8±0.2	2.8±0.1	2.1±0.2*	2.6±0.3	2.2±0.2
Globulin (g/dl)	3.2±0.1	2.9±0.1	3.2±0.1	2.8±0.4	3.2±0.1	3.5±0.2
Cholesterol (mg/dl)	81.3±2.7	84.0±3.8	83.3±3.2	79.4±0.6*	81.9±1.9	82.2±2.7
Triglycerides(mg/dl)	63.0±3.6	64.0±3.4	65.0±2.4	63.2±4.2	63.2±2.3	64.5±1.2

Data presented as mean ± standard error of mean

*Indicates significant differences ($p < 0.05$) of test from control values

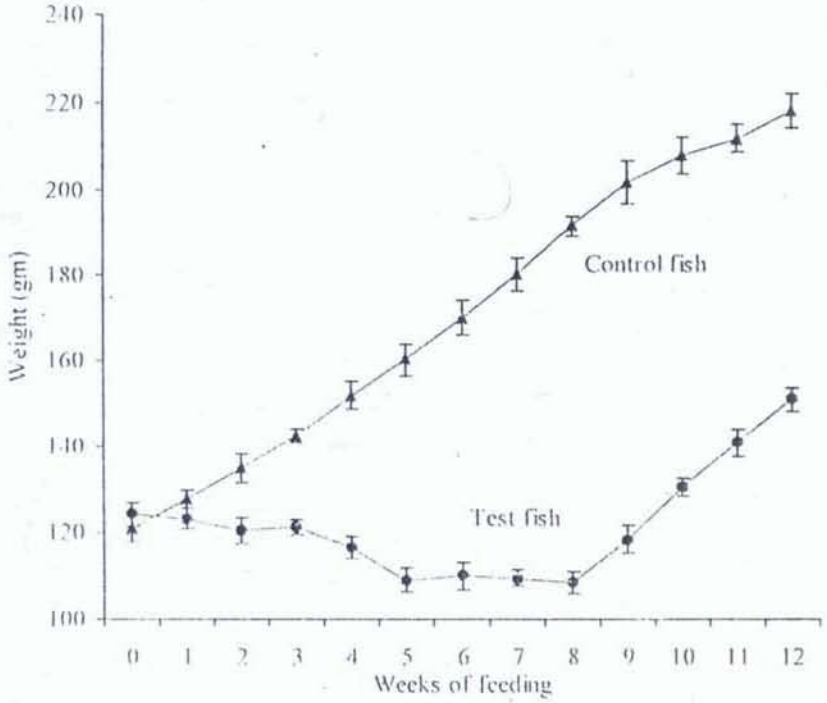

Fig. 1: Mean weights of *Clarias gariepinus* broodstock fed compounded diet (control) and those placed on grasscutter faecal droppings (test)