PRINT ISSN 1119-8362
Electronic ISSN 2659-1502

Full-text Available Online at
https://www.ajol.info/index.php/jasem
http://www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 27 (4) 793-801 April 2023

Replacement of Dietary Crude Protein of Soybean Meal by Tobacco Leaf Protein Concentrate on Growth, Haematological and Biochemical Responses of Catfish, *Clarias gariepinus*

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ABSTRACT: This study assessed the effects of replacing dietary crude protein of soybean meal with tobacco leaf protein concentrate (TLPC) on growth, haematological and biochemical parameters of catfish, *Clarias gariepinus* juveniles. The catfish (n=126, mean weight = 31.77±0.07g) were arranged in a completely randomized design with 6 treatments and 3 replicates with 7fish per experimental unit and fed for 40days. The treatments were 0, 20, 40, 60, 80 and 100% replacement levels of soybean meal with Tobacco leaf protein concentrate respectively. Diets had negative effects ($p > 0.05$) on mean weight gain (MWG), specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) across treatments. Fish in the control group recorded highest values for MWG (51.20±0.25g), SGR (2.40±0.01) and PER (2.48±0.02). Similarly, the best value for FCR (1.03±0.01) was recorded amongst the control group of fish. With the exceptions of WBC, NEUT, LYPH and MCV, significant differences ($p<0.05$) were recorded in the values of Packed cell volume, red blood cells, Haemoglobin, PLT, MCHC and MCH across diets. The biochemical parameters recorded no significant differences ($p > 0.05$) across treatments with the exceptions of CHO, HDL and LDL. Also, significant differences ($p < 0.05$) were observed in the values of GSH, SOD, CAT and MDA across treatments. The poor growth performance coupled with reduced physiological activities of TLPC at different inclusion levels, confirmed that it could not favourably replace soybean meal however, TLPC enhanced the enzymatic antioxidant activities in the experimental fish, consequently improving the health status of *C. gariepinus.*

DOI: **https://dx.doi.org/10.4314/jasem.v27i4.22**

Cite this paper as: LAWAL, M. O; ALUTA, U. P; ADEROLU, A. Z; AKINADE, B. T; AJISAFE, A. A (2023). Replacement of Dietary Crude Protein of Soybean Meal by Tobacco Leaf Protein Concentrate on Growth, Haematological and Biochemical Responses of Catfish, *Clarias gariepinus. J. Appl. Sci. Environ. Manage.* 27 (4) 793-801

Dates: Received: 17 February 2023; Revised: 08 April 2023; Accepted: 16 April 2023
Published: 30 April 2023

Keywords: Dietary effects; *Clarias gariepinus*; Soybean meal; Tobacco leaf; Growth performance.

The faming of aquatic organisms which accounts for over 50 percent of global fish production, is the fastest-growing food-producing sector across the world. Globally, about 424 aquatic species are farmed, benefiting millions of people through the provision of food security, poverty reduction and sustainable livelihood (Galappaththi *et al*., 2020). Over the past two decades, aquaculture has developed tremendously to become an economically significant industry all over the world. Comparatively, the industry continues to grow at an average global annual growth level of 8.8% per year over all other animal food production industries (Onada and Ogunola, 2017). The sustainable expansion of aquaculture production basically depends on the availability of inexpensive quality feed, since fish feed constitutes approximately 60 -70% of the total production. Thus, further development of the aqua-feed industries requires a consistent supply of nutritive raw materials for feed formulation (Fagbenro and Adebayo, 2005).

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The conventional groundnut and soybean meals are over-competed by man and his animals making the cost of producing animal feed overwhelming. Hence, to lessen the cost of feed in fish production it is imperative to replace the aforementioned convectional sources of protein with suitable alternatives (Hung *et al*., 2007; Monebi and Ugwumba, 2013). Considerable interest has been shown on the use of conventional plant oilseed meals such as groundnut, cotton seed, soybean and rapeseed (Agbede, 2005; Toko *et al*., 2008; Liu *et al.,* 2011). In furtherance of the above search, the inclusion of plant-based ingredients in fish feed has increased markedly over the years (Krogdahl *et al*., 2010). This include the use of leaves from legumes as valuable feed source in aquaculture, because of their abundance and are also rich in protein and minerals (Edelman and Colt, 2016). In addition, oil-bearing seeds and oil cakes which are by-products of the vegetable oil industry are similarly sources of plant protein (Gatlin *et al.*, 2007). They are high in protein and low in carbohydrate. However, their high fibre and cellulose contents limit their nutritional value in the feed of monogastric animals (Ghaly *et al*., 2012). Accordingly, to address the aforesaid challenges, leaf protein concentrate (LPC) is being used as source of protein when compounding animal feed, because it is potentially the cheapest, and most abundant source of available protein (Sodamode *et al*., 2013; Adeyemi and Osubor, 2016). The LPC is made by mechanically separating indigestible fibre and soluble anti-nutrients from much of the protein, minerals and vitamins in some fresh green plant leaves (Adeyemi and Osubor, 2016). Tobacco (*Nicotiana tabacum* L.), an herbaceous plant grown widely for cigarette production, has been investigated extensively in the past decades as a source of high-quality leaf protein. Studies revealed that 1 g of dried tobacco leaf contained 94–146 mg protein before the flue-curing process (Fantozzi and Sensidoni, 1983; Salvucci and Anderson, 1987). Besides, the ability of tobaccos to grow in high density enabled them to yield fourfold more protein per acre compared with soybeans (Sheen and Sheen, 1985; Lo *et al.*, 2010). Also, studies have shown that tobacco leaves are rich in soluble plant protein, which has high nutritional and medicinal value (Vansuyt *et al*., 2003; Teng and Wang, 2012). The African mud catfish, *Clarias gariepinus* is a significant aquaculture fish across the world, and Nigeria remains the leading producer of this fish (Kaleem and Bio Singou Sabi, 2021). This species has been extensively cultured because of its easy breeding, rapid growth and considerable economic value (Aluta *et al.*, 2021). Hence, it is of utmost importance to identify appropriate alternative feed ingredient to replace soybean meal in the diet of catfish for its sustainable production.

This aim of this study was to assess the effects of replacing dietary crude protein of soybean meal with tobacco leaf protein concentrate on nutrient utilization, growth, haematological and biochemical parameters of catfish, *Clarias gariepinus* juvenile.

MATERIALS AND METHODS

The experimental design: The experiment was a complete randomized design with six experimental diets in triplicates carried out for a period of 40 days, at the Fish Nutrition Unit of the Department of Marine Sciences, Faculty of Science, University of Lagos, Nigeria.

Preparation of Tobacco leaf protein concentrate (TLPC): 100g leaf from each of the freshly sampled weed was washed and pounded which ruptured the plant cell wall. The residue was squeezed to release leaf juice which contained protein. The separated leaf juice was heated at 80-90 °C for 10 minutes to coagulate the leaf protein. A rubber hose was used to siphon the coagulated protein from the whey, the protein coagulum was further filtered through muslin cloth and screw-pressed to remove the entire whey. The leaf protein was washed with water, repressed and sundried to get the leaf protein concentrate (Fellow, 1987; Agbede and Aletor, 2004; Agbede, 2005).

Experimental diet: Six iso-nitrogenous diets were formulated (Table 1). The *Nicotiana tabacum* leaf protein concentrate was used to substitute soybean meal at graded levels in the diets. The Control diet (0% TLPC), diet 1 (20% TLPC), diet 2 (40% TLPC), diet 3 (60% TLPC) diet 4 (80% TLPC), and diet 5 (100% TLPC). Soybean meal, fishmeal and groundnut cake were used as protein source, while maize, starch and oil were used as energy source. All feed stuffs were milled, weighed and mixed thoroughly for even distribution of the feed ingredients. Water was added to aid starch gelatinisation and the dough was passed pelletizer and passed through a 2 mm die. The pelleted feed was air-dried, sealed in zip lock bags and stored until it's needed. The proximate composition of formulated diets is shown in Table 2.

Experimental conditions and feeding regime: Three hundred *C. gariepinus* juveniles were obtained from Iceberg farm, Ikotun, Lagos State, and were transported in a 25L gallon to the fish nutrition unit, Department of Marine sciences, University of Lagos. The fish was acclimatized in an 800L capacity canvas tank fitted to a flow-through system and fed with 2 mm commercial feed (Blue crown) for a period of 14days. A total of 126 fish with an average initial weight of 31.77±0.067g each were randomly allocated in eighteen plastic tanks (52.5 ×33.5 ×21.0 cm^3) at 7 fish

per tank in triplicates per experimental group. The fish were fed thrice daily (8:0 0am, 12:0 0pm and 5:0 0pm) to apparent satiation with the experimental diets for 40 days. Water was changed every other day with de-chlorinated water from a borehole to maintain good water quality. The dissolved oxygen ranged from 4.5 to 6.0 mg/L and temperature ranged from 28.0 °C to 29.0 °C throughout the experimental period. The fish of each tank were bulk-weighed once every 10 days to determine the average weight gain while the quantity of the feed fed for each period was also recorded.

Table 1: Composition of experimental diets containing graded levels of *Nicotiana tabacum* leaf protein concentrate (TLPC)

Ingredient (g/kg)	**Control**	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**
Fish Meal (72%)	200	200	200	200	200	200
Groundnut Cake	320	320	320	320	320	320
Soybean Meal	250	200	150	100	50	0.0
TLPC	0.0	50	100	150	200	250
Maize	100	100	100	100	100	100
Starch	70	70	70	70	70	70
Oil	10	10	10	10	10	10
Di-calcium phosphate	20	20	20	20	20	20
Fish premix	25	25	25	25	25	25
Salt	5	5	5	5	5	5

Table 2: Proximate composition of formulated feed

Parameter (%)	**Control**	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 5**
Crude Protein	39.09	39.16	39.15	39.13	38.92	38.92
Moisture	11.10	10.30	9.35	9.70	9.55	10.00
Crude Fibre	4.39	4.49	3.75	3.99	3.37	3.24
Ether Extract	6.14	6.35	6.86	7.08	8.89	8.99
Total Ash	10.64	11.16	11.96	11.92	12.28	12.73
Carbohydrate	28.64	27.54	27.46	27.15	26.99	26.12

Growth and Nutrient Utilization study: At the end of the feeding trial, the fish growth performance and Nutrient utilization indices were calculated following Bekhan *et al.* (2006):

1) Mean Weight Gain (MWG) (g) = Mean Final Weight (g) - Mean Initial Weight (g)

2) Specific Growth Rate (SGR)= $\frac{\text{Loge W2 (g)- Loge W1 (g)}}{\text{T2-T1 (day)}}$ x 100

3) Feed Conversion Ratio (FCR)

$$\text{FCR}=\frac{\text{Feed eaten in dry mass (g)}}{\text{Weight gain (g)}}$$

4) Protein Efficiency Ratio (PER)

$$\text{PER} = \frac{\text{Mean Weight Gain (g)}}{\text{Protein Intake (g)}}$$

Where Protein Intake (PI) = Total feed intake x Protein content of feed.

*Collection of blood samples for hematological and biochemical analysis***:** At the end of feeding trial, the fish were starved for 24hrs prior to sampling. Blood was collected from two fish per tank (n = 6 fish per experimental group) via the caudal vein using 2-ml syringe and 23-gauge needles. It was emptied into Heparin bottles for haematology and plain bottles for biochemical analyses.

Haematological analysis: The Neubaeur haemocytometer was used to determine erythrocyte (RBC) and leukocyte (WBC) counts. Haemoglobin level (Hb) was obtained by the cyanomethaemoglobin spectrophotometry method. Pack cell volume (PCV) was measured using the standard microhematocrit method. Also, the Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Neutrophils (NEUT), and Lymphocytes (LYPH) were determined using semi-automated haematology analyzer (Mindray).

Biochemical analysis: The protein (PRO), glucose (GLU), cholesterol (CHO), triglyceride (TG), high density lipoprotein cholesterol (HDL), and low-density lipoprotein (LDL) were analysed at the Department of Clinical chemistry laboratory, University of Lagos Teaching Hospital using an automatic biochemical analyser by the colorimetric method (Coz-Rakovac *et al*., 2008).

*Determination of Serum antioxidant parameters***:** The catalase (CAT), malondialdehyde (MDA), superoxide dismutase (SOD), and reduced glutathione (GSH) were carried out in the Department of Clinical Chemistry laboratory, Lagos University Teaching Hospital.

Data analysis: Results are presented with means ±SD of three replicates. All data were subjected to one-way analysis of variance (ANOVA) performed using SPSS version 16.0 and followed by Duncan's multiple range tests. *P* -value of < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The results of growth and nutrient utilization parameters of *C. gariepinus* juveniles fed graded levels of Tobacco leaf protein concentrate (TLPC) are shown in Table 3. Dietary inclusion of TLPC significantly decreased ($p > 0.05$) weight gain, SGR, PER and increased FCR of *C. gariepinus* when compared with those fed the basal diet. The highest (51.20±0.25g) and lowest (11.50±0.27g) mean weight gains were recorded in groups of fish fed control diet and diet 5 respectively. Similarly, the best (1.03±0.01g) and worst (2.65±0.03g) values for FCR were recorded in groups of fish fed control diet and diet 5 respectively. In the present study, we were able to replace up to 100% soybean meal with TLPC. Soybean meal (SBM) is an alternative protein ingredient currently of great interest to the aquaculture industry. However, the presence of antinutritional factors and its high-cost limits its use in crude and processed forms in aquafeeds (NRC, 1993; Ogbonna *et al*., 2014). The results from this study showed that the experimental diets were poorly utilised by *C. gariepinus* juveniles as reflected in the poor growth performance compared with the control diet. This finding supports the claim by Adewolu and Adamson (2011), who reported reduced fish growth rate after feeding *Amaranthus spinosus* leaf meal as potential dietary protein source in the practical diets for *C. gariepinus.* Similarly, Adebayo (2017) observed that despite the high crude protein level in corn gluten meal and other plant protein materials, the formulated diets were poorly utilised by the *Hetero-clarias* Juveniles, which was reflected in their growth performance. Furthermore, poor feed intake was observed among the groups of fish fed with the experimental diets compared with the control diet. This also contributed to the repressed growth observed among the experimental fish (Adebayo and Aladejare, 2015). Additionally, the reduced growth performance of fish fed diets with TLPC may be related to the limiting level of methionine and leucine, high antinutritional factors which depressed the feed intake and growth in fish at high levels of plant protein (Ngugia *et al.*, 2017). Contrary to the above findings, Fagbenro *et al.* (2017) observed, that the mean weight gain and percentage weight gain of *C. gariepinus* fed with test diets containing cassava leaf protein concentrate variety up to 40% substitution of Soybean meal were similar to those fed with the control diet. Similarly, Chavez *et al*. (2016) included water hyacinth leaf protein concentrate substituting up to 75% of soybean meal for white shrimp, *Litopenarus vannamei*, and recorded beneficial effects at 25% on growth compared to a control diet.

Table 3: Growth and Nutrient utilization parameters of *Clarias gariepinus* fed with graded levels of Tobacco leaf protein concentrate

Parameter	Control	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
INW (g)	31.77±0.07	31.77±0.07	31.83±0.07	31.77±0.07	31.77±0.07	31.78±0.07
FNW (g)	82.97±0.23^{d}	50.63±0.48^{c}	45.77±0.19^{b}	45.03±0.15^{b}	44.63±0.17^{b}	43.27±0.20^{a}
MWG (g)	51.20±0.25^{d}	18.87±0.44^{c}	13.93±0.21^{b}	13.27±0.17^{b}	12.86±0.13^{b}	11.50±0.27^{a}
FI (g)	52.95±0.67^{d}	39.62±0.74^{c}	35.95±0.63^{b}	30.71±0.54^{a}	31.57±0.21^{a}	30.47±0.42^{a}
FCR	1.03±0.01^{a}	2.10±0.02^{b}	2.58±0.01^{e}	2.31±0.02^{c}	2.45±0.01^{d}	2.65±0.03^{e}
PI (g)	20.65±0.26^{c}	15.85±0.30^{b}	16.54±0.29^{b}	12.29±0.22^{a}	12.31±0.09^{a}	11.89±0.16^{a}
PER	2.48±0.02^{e}	1.19±0.01^{d}	0.84±0.01^{a}	1.08±0.00^{c}	1.04±0.00^{c}	0.97±0.01^{b}
SGR (%/day)	2.40±0.01^{d}	1.16±0.02^{c}	0.91±0.01^{b}	0.87±0.01^{b}	0.85±0.01^{b}	0.77±0.14^{a}

FNW - Final Weight Gain, INW - Initial Weight Gain, MWG - Body Weight Gain, FCR - Feed Conversion Ratio, PI – Protein Intake, PER - Protein Efficiency Ratio, SGR - Specific Growth Rate. Values along row with the same superscript were not significantly different (p > 0.05).

Table 4: Haematological indices of Clarias *gariepinus* fed with graded levels of Tobacco leaf protein concentrate

Parameter	Control	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
WBC (x 103/µl)	74000±3000.00	70600±1320.35	72000±577.35	73000±1652.27	73000±2081.67	72666.67±1452.97
PCV (%)	32.67±0.88^{c}	30.33±0.88^{ab}	31.67±0.88^{b}	29.33±0.88^{ab}	28.67±0.88^{a}	29.67±0.54^{ab}
HB (g/dl)	9.97±0.15^{c}	9.27±0.20^{ab}	10.23±0.20^{b}	9.73±0.13^{b}	8.5±0.29^{a}	9.40±0.21^{ab}
RBC (x 106/ µl)	4.43±0.09^{c}	4.27±0.12^{bc}	4.03±0.12^{abc}	4.03±0.09^{abc}	3.70±0.10^{ab}	3.87±0.12^{a}
NEUT (%)	1.33±0.33	2.33±0.33	1.33±0.33	1.33±0.33	1.00±0.00	2.33±0.33
LYPH (%)	98.67±0.33	98.00±0.52	98.67±0.33	98.67±0.33	98.33±0.33	98.00±0.58
MCHC (g/dl)	31.33±0.67^{a}	32.33±0.33^{ab}	33.00±0.58^{ab}	33.00±0.58^{b}	32.33±0.33^{ab}	32.67±0.33^{ab}
MCH (pg)	22.67±0.33^{a}	21.33±0.89^{a}	23.33±0.33^{ab}	24.33±0.33^{b}	23.00±0.58^{ab}	24.33±0.89^{c}
MCV (fl)	71.67±0.89	71.00±0.00	71.33±0.33	73.00±0.58	70.67±0.89	74.00±1.00

WBC- white blood cells, PCV- packed cell volume, Hb- haemoglobin, RBC- erythrocytes, NEUT- neutrophils, LYPH- lymphocytes, MCHC- mean corpuscular volume, MCH- mean haemoglobin concentration, MCV- mean corpuscular volume. Values along row with the same superscript were not significantly different (p > 0.05).

The results of WBC, PCV, Hb, RBC, neutrophil, lymphocyte, MCHC, MCH and MCV counts of experimental diets are shown in Table 4. WBC, neutrophil, lymphocyte and MCV counts showed no significant differences ($p > 0.05$) between the control and other experimental groups fed graded levels of TLPC. Also, significant differences ($p < 0.05$) were recorded in the values of PCV, Hb and RBC counts, with the fish group fed with control diets recorded the highest values across treatments. Similarly, MCHC and MCH counts differed significantly ($p < 0.05$) between the control and other experimental groups of fish fed graded levels of TLPC diets. The results of haematological variables in this study suggest that the test diets did not precipitate any severe effects on the health status of the experimental fish because all the hematological parameters measured in this study were within the recommended physiological ranges reported for *Clarias gariepinus* (Fagbenro *et. al*., 2013; Eyiwunmi *et al*., 2018). Because, red blood corpuscle, white blood corpuscle, neutrophils, and lymphocytes are indicators for fish health feed and anti-nutritional toxicity (Ozovehe, 2013). Moreover, differences in values of haemoglobin (Hb), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), blood platelets, and packed cell volume (PCV) between the fish of control group and others, indicated the healthy status of experimental fish (Abdul Kari *et al*., 2021).

The results of TLPC on serum protein (PRO), CHOL, TG, HDL, LDL, GLU, ALB and GLO of *C. gariepinus* are shown in Table 5. The values of serum protein, triglyceride, albumin, globulin and glucose recorded no significant difference ($p > 0.05$) between the fish of control group and others. However, slight differences were recorded in the values of CHO, HDL and LDL of fish fed with the control diet and diets 3 to 5. Albumin, globulins and total proteins ensure a healthy system and function as plasma carriers (Nya and Austin, 2009). In this study, the aforementioned parameters did not differ significantly across all groups, indicating healthy status of all the experimental fish. These results were in congruence with Kumar *et al.* (2010), who observed no significant effect in the protein and globulin levels in *C. carpio* fed 50 and 75% detoxified *Jatropha curcas* kernel meal. Lipids are biomolecules used by fish to stock energy and can be stored in many different organs (Guijarro *et al.*, 2003). The transport of lipids and other lipid-soluble components from the intestine to peripheral tissues is predominantly mediated by lipoproteins (Botham and Mayes, 2016). The ratio of HDL to LDL is vital because high levels of HDL and low levels of LDL as recorded in this study, are needed to reduce the chances of coronary heart disease (Ali *et al.*, 2012). Therefore, the values of CHO, TG, LDL and HDL recorded with the test diets, *N. tabacum* leaf protein concentrate showed no adverse effect on the state of health of experimental fish.

Table 5: Biochemical indices of *Clarias gariepinus* fed with graded levels of Tobacco leaf protein concentrate

Parameter	Control	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
CHO (mmol/L)	2.43±0.088^{ab}	2.57±0.145^{ab}	2.47±0.185^{ab}	2.20±0.100a^{a}	2.23±0.120^{a}	2.27±0.176^{a}
TG (mmol/L)	1.20±0.058	1.27±0.088	1.30±0.088	1.17±0.067	1.37±0.067	1.40±0.058
HDL (mmol/L)	1.23±0.033^{ab}	1.26±0.058^{ab}	1.24±0.088^{ab}	1.13±0.033^{a}	1.20±0.058^{ab}	1.43±0.031^{b}
LDL (mmol/L)	0.68±0.033^{ab}	0.70±0.058^{ab}	0.67±0.088^{ab}	0.53±0.088^{a}	0.40±0.058^{a}	0.93±0.088^{b}
PRO (g/L)	39.00±1.000	38.67±0.882	37.33±0.333	38.67±1.202	38.33±0.333	38.00±0.577
GLU (mg/dL)	42.67±0.882	39.67±0.882	41.67±1.202	42.0±01.155	41.00±0.577	40.67±1.202
ALB (g/L)	16.30±0.850	17.33±0.677	14.97±0.736	15.20±0.529	15.1±0.133	15.77±0.471
GLO (g/L)	22.70±0.315	21.33±0.240	22.37±0.664	23.47±0.677	23.20±0.416	22.23±0.317

CHO – cholesterol, TG – triglyceride, HDL – high density lipoprotein, LDL – low density lipoprotein, PRO - protein, ALB– albumin, GLO – globulin, GLU - glucose. Values along row with the same superscript were not significantly different (p > 0.05).

The results of antioxidant enzymes activities of the experimental fish fed tobacco leaf protein concentrate diets are reported in Table 6. Significant differences ($p < 0.05$) were observed in the values of GSH, SOD, CAT and MDA of fish fed with TLPC compared to the group of fish fed with control diet. The highest (1.11±0.04) and lowest (0.70±0.04) values for GSH were observed for fish fed diet 2 and control group respectively. Also, the lowest value (0.47±0.02) for MDA was recorded in fish fed with diet 3 and the highest value (0.70±0.03) was recorded in fish group fed with the control diet. Additionally, the highest (26.23±2.62) and lowest (15.73±0.62) values for CAT were observed for fish fed diet 4 and control group respectively. Similarly, the least (0.04±0.01) and highest (0.08±0.01) values for SOD were recorded for

the group of fish fed control diet and diet 4 respectively. In order to manage oxidative stress and associated tissue damage, organisms are equipped with multiple systems of antioxidant enzymes such as CAT, SOD, MDA and GSH to ensure optimum protection in the environment (Kurutas, 2016; Zhang *et al*., 2019). SOD is one of the major antioxidant defense enzymes produced in response to oxidative damage, which converts toxic superoxide anions into hydrogen peroxide (Wang *et al*., 2018), while Catalase is an enzyme found in nearly all living organisms exposed to oxygen, including bacteria, and animals which catalyzes the decomposition of hydrogen peroxide to water and oxygen (Chelikani *et al*.,2005). The elevation of CAT, GSH and SOD activities, and the reduction in the values of MDA in this study, suggest an adaptive response related to oxidative damage in order to counteract the impact of increased reactive oxygen species (ROS) generation. These results were in agreement with Zhang et al. (2022) who reported that dietary Flos populi extract fed to gibel carp (*Carassius auratus*) significantly increased serum superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) activities, while the content of malondialdehyde (MDA) in serum decreased significantly. Similarly, dietary supplementation of *Moringa oleifera* to sodium fluoride exposed fish (Nile Tilapia, *Oreochromis niloticus*) resulted in a significant reduction in MDA levels, and a significant elevation of SOD, CAT and GSH activities in a time-dependent manner (Ahmed et al., 2020). Additionally, Aluta *et al.* (2021) affirmed that Quantitative RT-PCR showed that SOD expression in hepatic tissues was upregulated as a result of feeding *C. gariepinus* with onion supplemented diet.

Table 6: Serum antioxidant enzyme indices of *Clarias gariepinus* fed with graded levels of Tobacco leaf protein concentrate

Parameter	Control	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
GSH (µmol/ml/mg)	0.70 ± 0.04^{a}	0.95 ± 0.09^{ab}	1.11 ± 0.04^{b}	0.81 ± 0.05^{ab}	1.07 ± 0.05^{b}	1.03 ± 0.09^{b}
MDA (µmol/ml/mg)	0.70 ± 0.03^{c}	0.65 ± 0.04^{bc}	0.56 ± 0.03^{ab}	0.47 ± 0.02^{a}	0.48 ± 0.03^{a}	0.48 ± 0.03^{a}
CAT (µmol/ml/min/mg)	15.73 ± 0.62^{a}	17.60 ± 0.57^{a}	24.97 ± 0.97^{b}	20.50 ± 0.76^{ab}	26.23 ± 2.62^{b}	21.96 ± 1.42^{b}
SOD (µmol/ml/min/mg)	0.04 ± 0.01^{ab}	0.05 ± 0.01^{a}	0.06 ± 0.01^{abc}	0.06 ± 0.01^{abc}	0.08 ± 0.01^{c}	0.07 ± 0.01^{bc}

MDA: malondialdehyde; GSH: glutathione CAT: catalase, SOD - Superoxide Dismutase. Values along row with the same superscript were not significantly different (p > 0.05).

Conclusion: The poor growth performance recorded in this study indicated that tobacco leaf protein concentrate, could not favourably replace soybean meal however, the dietary TLPC enhanced the enzymatic antioxidant activities in the experimental fish, consequently improving the health status of *C. gariepinus.*

REFERENCES

Adewolu, MA; Adamson, AA (2011). *Amaranthus spinosus* leaf meal as potential dietary protein source in the practical diets for *Clarias gariepinus* (Burchell, 1822) fingerlings. *Int. J. Zool. Res.* 7: 128–37.

Abdul Kari, Z; Kabir, MA; Mat, K; Rusli, ND; Abdul Razab, MKA; Ariff, NSNA; Edinur, HA; Abdul Rahim, M; Pati, S; Dawood, MAO; Wei. LS (2021). The possibility of replacing fish meal with fermented soy pulp on the growth performance, blood biochemistry, liver, and intestinal morphology of African catfish (*Clarias gariepinus*). *Aquac. Rep.* 21: 100815.

Adebayo, IA (2017). Growth Responses of Hybrid Catfish (Heteroclarias) Juveniles Fed All Plant: Protein Diets Supplemented with L-lysine and L-methionine. *J Fisheries Livest. Prod.* 5: 219.

Adebayo, IA; Aladejare, T (2015). Utilization of fish offal meal in the diets of *Clarias gariepinus*. *J. Aquat. Sci*. 30:43-51.

Adeyemi, O; Osubor, CC (2016). Assessment of nutritional quality of water hyacinth leaf protein concentrate. *Egypt. J. Aquat. Res.* 42(3):269-272.

Agbede, JO (2005). Nutritional significance of leaf meals, protein concentrates and residues from some tropical leguminous plants. *J Food, Agric Environ. 3*(1): 25-30.

Agbede, JO; Aletor, VA (2004). Chemical characterization and protein quality evaluation of leaf protein concentrate from *Glyricidia sepium* and *Leucaena leucocephala*. *Int. J. Food Sci. Technol.* 39:253-261.

Ahmed, NF; Sadek, KM; Soliman, MKh; Khalil, RH; Khafaga, AF; Ajarem, JS; Maodaa, SN; Allam, AA (2020). Moringa Oleifera Leaf Extract Repairs the Oxidative Misbalance following Sub-Chronic Exposure to Sodium Fluoride in Nile Tilapia *Oreochromis niloticus*. *Anim. 10*(4): 26

Ali, KM; Wonnerth, A; Huber, K; Wojta, J (2012). Cardiovascular disease risk reduction by raising

HDL cholesterol – current therapies and future opportunities. *Br. J. Pharmacol.* 167: 1177-1194.

Aluta, UP; Aderolu, AZ; Lawal, MO; Olutola, AA (2021). Inclusion effect of onion peel powder in the diet of African catfish, *Clarias gariepinus*: Growth, blood chemistry, hepatic antioxidant enzymes activities and SOD mRNA responses. *Sci. Afr*. 12:1-8.

Bekhan, S; Dogankaya, L; Cakirogullari, GC (2006). Growth and body composition of European Catfish (*Silurus glanis*) fed diet containing different percentages of protein, *Israeli J. Aquac. – Bamidgeh*. 58:137–142.

Botham, KM; Mayes, PA (2016). Lipid transport & storage. Rodwell, VW; Bender, DA; Botham, KM; Kennelly, PJ; Weil, P(Eds.), *Harper's Illustrated Biochemistry, 30e*. McGraw Hill.
https://accessmedicine.mhmedical.com/content.aspx?bookid=1366&sectionid=73244168.

Chavez, NNG; Ragaza, JA; Corre Jr., VI; Serrano Jr., AE; Trafalgar, RFM (2016). Effects of water hyacinth (*Eichhornia crassipes*) leaf protein concentrate as a soybean protein replacement in white shrimp *Litopenarus vannamei* (Boone) postlarvae diet. *Aquac. Res.* 47: 2642-2649.

Chelikani, P; Ramana, T; Radhakrishnan, TM (2005). Catalase: A repertoire of unusual features. *Indian J. Clin. Biochem*. 20 (2): 131-135.

Coz-Rakovac, R; Smuc, T; Topic Popovic, N; Strunjak-Perovic, I; Hacmanjek, M; Jadan, M (2008). Novel methods for assessing fish blood biochemical data, *J. Appl. Ichthyol*. 24: 77–80.

Edelman, M; Colt, M (2016). Nutrient value of leaf vs. seed. *Front. Chem*. 4:32.

Eyiwunmi, FA; Augustine, O; Ovie, WK (2018). The Hematological parameters of Catfish (*Clarias gariepinus*) fed Fish Feeds with replaced Premix using Moringa Leaf Meal (MLM). *Madridge J. Aquac. Res. Dev.* 2(1): 35-39.

Fagbenro, OA; Adebayo, OT (2005). A review of the animal and aquafeed in Nigeria. In: Moehl, J; Halwart, M (eds.), *A synthesis of the formulated animal and aqua feed industry in Sub-Saharan Africa*, CIFA Occasional paper no. 26, FAO Rome, pp. 25–36.

Fagbenro, OA; Adeparusi, EO; Jimoh, WA (2013). Haematological Profile of Blood of African Catfish (*Clarias gariepinus*, Burchell, 1822) Fed Sunflower and Sesame Meal Based Diets. *J Fish Aquat Sci*. 8:80-86.

Fagbenro, OA; Oresegun, A; Nwanna, LC; Ilona, P; Fijabi, O; Adeleke, O; Agun, O (2017). Growth Response and Diet Utilization of *Clarias gariepinus* (Clariidae) Fed Diets Containing Cassava Leaf Protein Concentrate as Plant Protein Source. *Afr. J. Fish. Aquat. Resour. Manage*. 2:1-8.

Fantozzi, P; Sensidoni, A (1983). Protein extraction from tobacco leaves: technological, nutritional and agronomical aspects. *Qual Plant – Plant Foods Hum. Nutr*. 32: 351–368.

Fellows, P (1987). Village-scale leaf fractionation in Ghana. *Trop. Sci.* 27:77-84.

Galappaththi, EK; Aubrac, CJ; Ichien, ST; Hyman, AA; Ford, JD (2020). Climate change adaptation in aquaculture. *Rev Aquac*. 1–17.

Gatlin, DM; Barrows, FT; Brown, P; Dabrowski, K; Gaylord, TG; Hardy, RW; Herman, E; Hu, G S; Krogdahl, A; Nelson, R; Overturf, K; Rust, M; Sealey, W; Skonberg, D; Souza, EJ; Stone, D; Wilson, R; Wurtele, E (2007). Expanding the utilization of sustainable plant products in aquafeeds: a review. *Aquac. Res. 38*(6): 551-579.

Ghaly, AE; Maqhmond, N; Dare, D (2012). Nutrient composition of dandelious and its potential as human food. *Am J Biochem Biotechnol*. 8(2): 118-127.

Guijarro, AI; Lopez-Patino, MA; Pinillos, ML; Isorna, E; De Pedro, N (2003). Seasonal changes in haematology and metabolic resources in the tench. *J. Fish Biol*. 62: 803-815.

Hardy, RW; Tacon, AGJ (2002). Fish meal*:*Historical uses, production trends and future outlook for sustainable supplies. In: Stickney, RR; McVey, JP (Eds.), *Responsible Marine Aquaculture*). CAB international, pp.311-325.

Hung, LT; Truc, LTT; Huy, HPV (2007). Development of the aquafeed industry in India. In: Hasan, MR; Hecht, T; De Silva, SS; Tacon, AGJ (Eds.), *Study and analysis of feeds and Fertilizers for Sustainable Aquaculture Development*. FAO Fisheries Technical Paper. No. 497, pp. 363-377.

Kaleem, O; Bio Singou Sabi, A-F; (2021). Overview of aquaculture systems in Egypt and Nigeria, prospects, potentials, and constraints. *Aquac. Fish*. 6:535-547.

Krogdahl, A; Penn, M; Thorsen, J; Refstie, S; Bakke, AM (2010). Important antinutrients in plant feedstuffs for aquaculture: an update on recent findings regarding responses in salmonids. *Aquac. Res.* 41(3):333-344.

Kumar, V; Makkar, H; Becker, K (2010). Detoxified Jatropha curcas kernel meal as a dietary protein source: Growth performance, nutrient utilization and digestive enzymes in common carp (*Cyprinus carpio* L.) fingerlings. *Aquac. Nutr*. 17(3): 313-326.

Kurutas, EB. (2016). The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. *Nutr. J.* 15(1): 71

Liu, X; Ye, J; Wang, K; Kong, J; Yang, W; Zhou, L (2011). Partial replacement of fish meal with peanut meat in practical diet for the pacific white shrimp, *Litopeaneus vannamei*. *Aquac. Res.* 43(5):745-755.

Lo, YM; Fu, H; Machado, PA; Hahm, TS; Kratochvil, RJ; Wei, CI (2010). Recovery of nicotine-free proteins from tobacco leaves using phosphate buffer system under controlled conditions. *Bioresour. Technol.* 101: 2034–2042.

Mekkawy, AA; Mahmoud, UM; Wassif, ET; Naguib, M (2011). Effects of cadmium on some haematological and biochemical characteristics of *Oreochromis niloticus* (Linnaeus, 1758) dietary supplemented with tomato paste and vitamin E. *Fish Physiol. Biochem*. 37:71–84.

Monebi, CO; Ugwumba, AAA (2013). Utilization of the earthworm, *Eudrilus eugeniae* in the diet of *Heteroclarias* fingerlings. *Int. J. Fish Aquac.* 5(2):19-25.

Ngugia, CC; Oyoo-Okothb, E; Manyalac, JO; Fitzsimmonsd, K; Kimotho, A (2017). Characterization of the nutritional quality of amaranth leaf proteinconcentrates and suitability of fish meal replacement in Nile tilapia feeds. *Aquac. Rep*. 5:62–69.

NRC (National Research Council), (1993). Nutrient Requirements of Fish. National Academy Press, Washington DC, 114 pp.

Nya, E; Austin, B (2009). Use of garlic, *Allium sativum*, to control *Aeromonas hydrophila* infection in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *J. Fish Dis*. 32: 963–970.

Ogbonna, SU; Uhuo, CA; Okereke, CN; Azi, SO (2014). Effects of soybean meal (*Glycine max*) on the growth performance and feeding habits of *Clarias gariepinus* fingerlings reared under laboratory condition. *Sky J. Biochem. Res*. 3(3): 024 – 027.

Olaniyi, CO; Salau, BR (2013). Utilization of maggot meal in the nutrition of African catfish. *Afr. J. Agric. Res*. *8*(37**)**: 4604-4607.

Onada, OA; Ogunola, OS (2017). Effects of catfish (*Clarias gariepinus*) brood-stocks egg combination on hatchability and survival of fish larvae. *J. Aquac. Res. Dev. 2*. https://doi.org/10.4172/2155-9546.s2-014.

Ozovehe, BN (2013). Growth performance, haematological indices and some biochemical enzymes of juveniles *Clarias gariepinus* (Burchell 1822) fed varying levels of *Moringa oleifera* leaf meal diet. *J. Aquac. Res. Dev*. 4:1–6.

Salvucci, ME; Anderson, JC (1987). Factors affecting the activation state and the level of total activity of ribulose bisphosphate carboxylase in tobacco protoplasts. *Plant Physiol.* 85: 66–71.

Sheen, SJ; Sheen, VL (1985). Functional properties of fraction-1 protein from tobacco leaf. *J Agric Food Chem.* 33:79–83.

Sodamode, A; Bolaji, OS; Adeboye, O (2013). Proximate analysis, mineral contents and functional properties of *Moringa oleifera* leaf protein concentrate. *J. Appl. Chem.* 4:47-51.

Teng, Z; Wang, Q (2012). Extraction, identification and characterization of the water-insoluble proteins from tobacco biomass. *J Sci Food Agr*, 92:1368 –1374.

Toko, II; Fiogbe, ED; Kestemont, P (2008). Mineral status of African catfish (*Clarias gariepinus*) fed diets containing graded levels of soybean or cottonseed meals. *Aquac.* 275:298-305.

Vansuyt, G; Souche, G; Straczek, A; Briat, J-F; Jaillard, B (2003). Flux of protons released by wild type and ferritin over-expressor tobacco plants: effect of phosphorus and iron nutrition. *Plant Physiol. Biochem*. 41(1): 27–33.

Wang, CY; Li, ZB; Sun, YZ; Chen, Q; Li, WJ; Huang, YC; Lu, J (2018). Effects of Chinese herbal medicines mixture on growth performance digestive enzyme activity immune response of juvenile Japanese seabass, *Lateolabrax japonicus. Aquacult. Nutr*. 24(2): 683–693.

Zhang, B; Li, C; Wang, X; Zhou, H; Mai, K; He, G (2019). The effects of dietary *Eucommia ulmoides* Oliver on growth, feed utilization, antioxidant activity and immune responses of turbot (*Scophthalmus maximus* L.). *Aquacult. Nutr*. 25 (2):367–376.

Zhang, X; Sun, Z; Wang, Y; Cao, Y; Wang, G; Cao, F (2022). Enhancement of growth, antioxidative status, nonspecific immunity, and disease resistance in gibel carp (*Carassius auratus*) in response to dietary Flos populi extract. *Fish Physiol. Biochem.* 48**:** 67–83