

Full Length Research Paper

Substitution of soyabean meal with bioactive yeast in the diet of *Clarias gariepinus*: Effect on growth rate, haematological and biochemical profile

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The effects of substituting soyabean meal with yeast (*Sacharomyces cerevisiae*) meal in diets fed to *Clarias gariepinus* was studied for 60 days. Growth response, haematological and biochemical parameters were evaluated. 105 juvenile three-weeks old *C. gariepinus* composed of seven treatment groups replicated thrice were used for the study. Each replicate had five fishes. The Groups (A to G) were: Group A, 0% yeast meal (YM) (15 juveniles); Group B, 10% YM; Group C, 20%; Group D 30%; Group E, 40%; Group F, 50%; and Group G, 100% YM. The acceptability of yeast based diets by *C. gariepinus* was studied using the time to strike index, and their growth responses studied using 'weight gain' and 'specific growth rate' while the haematological and biochemical indices were assessed using the different blood parameters. The results indicate that substituting yeast meal for soyabean meal in diets fed *C. gariepinus* juveniles slightly increased the growth response. All the catfishes fed with 100% yeast meal (diet G) died within the first week. Increasing proportion of substitutions of yeast meal in diets fed to *C. gariepinus* juveniles, led to weight increase in all dietary types with exception of diet G and higher growth induction in catfishes fed diets with exception of diet B. Diet F induced better mean growth than the control diet. Substitution of various levels of soyabean meal with yeast meal after 30 days led to better result on haemoglobin (HB), red blood cell (RBC), packed cell volume (PCV) and white blood cell (WBC) in diet C Group when compared to the control, while after 60 days, diet F Group had better HB, RBC, PCV and WBC values than the control. Substitution of varied percentages of soyabean meal with yeast meal for 60 days led to significantly higher ($P < 0.05$) serum, total protein and cholesterol in some of the groups on yeast meal inclusion when compared to the control, and significantly higher ($p < 0.05$) serum aspartate aminotransferase activity in all the groups given yeast meal based diets when compared to the control (Group A). There were however no significant differences ($p > 0.05$) between those on yeast inclusion and the control in the serum alanine aminotransferase activity. This study shows that yeast inclusion at 50% (diet F) led to significantly better growth, weight gain, haematological and biochemical profile than other diets. This study shows that yeast inclusion may have a better effect on fish diet at lower levels of inclusion. Diet F (50% yeast inclusion) was considered the best level of inclusion because of enhanced nutritional status, better blood parameters and improved health of fishes.

Key words: *Clarias gariepinus*, soyabean, yeast, growth, haematology, biochemistry, serum, total protein, cholesterol, aspartate aminotransferase, alanine aminotransferase.

INTRODUCTION

Clarias gariepinus has been widely cultured because of its high growth rate, disease resistance and amenability

to high density because of their air breathing habits (Haylor, 1993; Leung and Tisdell, 1997; Machiels and

Henken, 1985). The increase in demand for fish has led to growth in aquaculture. This increase can be attributed to factors such as increasing world population, an increase in the spending power of individuals in developing countries and the changing tastes and preferences toward sea food than meat. Fish provides the energy for life processes or provides material for restoration or replacement of worn out cell components or for growth and reproduction. As a result, most countries in the tropics have focused mainly on the development and exploitation of their fisheries resources as an avenue of providing their citizens with the needed animal protein.

The African catfish, (*C. gariepinus*) family (Clariidae) is a North African catfish that is cultured in many tropical and sub-tropical regions of the world (Anderson and Fong, 1985, 1997). The fish constitutes the largest group of cultured species after *Cyprinus carpio*, the common carp, Salmonids and Tilapia because it adapts and grows well under cultured system. The Nigeria aquaculture industry is currently faced with the problems of inadequate supply and prohibitive cost of quality fish feeds. Fagbenro and Adeparusi (2003) reported increasing attempt to develop practical diets for farmed fish in Nigeria. Omitoyin (1995) and Aderemi et al. (2004) noted that Nigeria produces large quantity of agricultural and agro-industrial by-products which serves as alternative feed sources to conventional feed ingredients. The technology of formulating, manufacturing and feeding practical diets is advanced to the stage that highly productive and cost efficient feeds are being produced.

In recent years, aquaculture researchers have focused on the development of balanced and cheap fish diets. Fish meal has been used as a source of protein. Current researchers focused on the use of alternative cheaper source of protein because of the rising cost of fish meal. In catfish diets, soybean has been used as a good replacer of fish meal with encouraging results. Soyabean meal has become the key feed ingredient among the protein supplements. In many developing countries including Nigeria, the quest for soyabean is in excess of its supply because of its use by humans and the livestock industries. The development of a sustainable and environmental friendly aquaculture demands the use of various plant proteins such as soyabeans meal and canola meal diet, which are considered promising protein sources as substitutes for fish meal in fish diet. Soyabean meal is being substituted because it contains about 45% crude protein, 56% lipid and 40% carbohydrate. It has lysine content that approaches that of fish meal for most aquaculture diets, it is however deficient in the sulphur-containing amino acids. The inclusion of soyabean meal

presents problem relating to palatability and availability of nutrients (Spinelli, 1978).

Yeast has been unexploited in the development of aqua-feed for the fisheries sector. Yeast has been considered to have some nutritional value, as it contains about 25.15% of crude protein and can be used as alternative source of protein. Yeast contains vitamins such as niacin, folic acid, riboflavin and biotin, as well as other minerals which are co-factors required for growth. Yeast also contains different quantities of essential amino acids such as thr (93), val (116), meth (63), cys (85), leu (112), phe (91), tyr (198), lys (86), arg (106) and try (141) (Tacon, 1992) (The numbers represents chemical score and limiting essential amino acids of selected protein sources). The need for these essential amino acids, vitamins and minerals is because they induce growth, elevate blood parameters and help the immune system to fight against diseases (Mustin and Lovell, 1995).

Protein is often the most expensive nutrient in manufactured diets and should be kept to a minimum, consistent with good growth and feed conversion. Dietary protein constitute therefore the principal nutritive cost associated with the formulation of fish diets, and is the primary source of nitrogen waste in culture system (Perez et al., 1997). Ayyaru and Ventakatesen (2010) observed a consistent increase in neutrophil activity and changes in the number of total white blood cell count in fish fed yeast. The increase in white blood cell count can be correlated with an antibody production which helps in survival and recovery of the fish exposed to lindane (Joshi et al., 2003). According to Wedemeyer et al. (1983), study of haematological and biochemical parameters are carried out on fish to ascertain the normal range of blood parameters, determine variation with age, sex, season, and disease conditions that affect these parameters. Decreased protein level may also be attributed to stress mediated immobilization of these compounds to fulfill an increased element for energy by fish, to cope with environmental condition exposed by the toxin (Jenkins et al., 2003). The depletion of total protein content may be due to augmented proteolysis and utilization of their product for metabolic purposes. Change in the aminotransferases, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) which promote gluconeogenesis from amino acids, as well as the effect of changes in the aminotransferase activities on liver condition, has been reported (Wieser and Hinterleitner, 1980; Ekanem and Yasuf, 2005).

The liver is the primary organ of biotransformation and serves a number of functions such as interconversion of foodstuff, synthesis of many plasma proteins and cholesterol. Haematological studies are important in clinical assessment because the blood is the major transport system of the body, and any deviations from the normal caused by invasions of the body by pathogens, other forms of injury and stress are reflected by changes in the blood picture (Schalm et al., 1975). Haematology is

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therefore indispensably important in arriving at a diagnosis, assessing the efficacy of therapy, several parasitic diseases, toxicity of drugs and chemical substances (Schalm et al., 1975; Gingerich, 1982). The assessment of the serum biochemistry profile is important because of its predictive value of pathological changes in vital internal organs of the body such as liver, kidney, pancreas, heart and muscles (Tyson and Sawhney, 1985; Coles, 1986).

The serum biochemistry is useful in evaluating the nature and extent of a disease process, response to therapeutic interventions and to forecast possible outcomes (Coles, 1986), and is of immense relevance in the clinical assessment of fishes which include: determination of serum activity of enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase; also evaluation of synthetic activity of the liver by assessment of total protein, albumin, and serum cholesterol. The study of the biochemical characteristics of cultured fish in the development of aquaculture system, particularly as regards to fish species, is important and is used in the detection of healthy from diseased or stressed animal (Rainza-Paiva et al., 2003).

The aim of this research was to evaluate the effects of varied percentages of yeast inclusion on the haematology, serum biochemistry indices and on growth of *C. gariepinus*, in order to assess the suitability of yeast inclusion in fish diet.

MATERIALS AND METHODS

Fish

105 *Clarias gariepinus* juvenile weighing 29.0 ± 0.05 g with average length of 7.5 ± 0.00 cm purchased from Unifaco Farms, Enugu, Enugu State Nigeria, were transported to the Fisheries and Hydrobiology Laboratory located within the Zoological Garden of the University of Nigeria, Nsukka. The catfish juveniles were divided into 15 plastic flow-through (60 l) plastic fish culture tanks containing 20 l of water each and acclimatized for two weeks, aerated and covered with mosquito nets to prevent the fish from jumping out. Each plastic tank had five catfishes. During the acclimatization period, the fishes were fed 5% of their body weight with 25.15% crude protein diet in (Table 1) divided rations, twice daily. Water was replaced every 48 h after feeding, in order to maintain a healthy environment for the fish during both acclimatization and experimental period. The water parameters during the experimental period were temperature: $26.0 \pm 20^\circ\text{C}$, pH: 7.2 ± 0.1 , and dissolved oxygen, 6.2 ± 0.00 mg/l.

Dietary ingredients

The dietary ingredients for the diet formulation were bought from Nsukka main market, processed and preserved.

Blood meal

Fifteen litres of fresh cow blood weighing 3 kg was purchased from the Nsukka abattoir, pickled with 10 g of NaCl and pressure cooked

to firmness for about 30 min at 100°C . The solidified blood was cut into tiny bits and sun dried. The dried blood was finely ground into powder.

Soyabean meal

3 kg of soyabean were soaked overnight and autoclaved at temperature of about 110°C for 20 min. The autoclaved soyabean was sun dried for six days and the dried soyabean was finally grounded into powder.

Yellow corn meal

Dried corn 2.6 kg was finely grounded into flour and sieved.

Fish meal

1 kg of dried fish was finely grounded into powder.

Yeast

2 kg of yeast was dried and grounded into powder.

Diets

The diets ingredients were weighed out as presented in Table 2. 1 kg of the diet ingredients was homogenously mixed with 400 ml of water to produce dough. The dough was transferred into a heat resistant polythene bag, sealed and boiled for 1 h. The heat gelatinized the starch which bound the diets. The different diets were pelletized using an improvised sieve with openings of 1 mm^2 and dried in an oven for 45 min. The resulting diets were stored separately in labeled dry air tight plastic container. Diet A, the control diet had no yeast meal (YM), diet B had 10% YM, diet C had 20% YM, diet D had 30% YM, diet E had 40% YM, diet F had 50% YM, diet G had 100% YM. In all the test diets, soyabean meal was substituted with yeast meal. Diets were proximately analyzed (AOAC, 1975).

Growth

A randomized Latin square design of seven treatments replicated thrice was employed for the growth performance. Each treatment was fed specific diet. Treatment A was fed diet A, B fed diet B etc. The record of weekly weights for all treatment was used in adjusting the feeding rate. The growth rate was calculated using the formula: specific growth rate (SGR) = $(W_1 - W_2) / t \times 100$ (Eyo, 2003); where, W_1 = final weight; W_2 = initial weight, t = time in days.

Haematological profile

Blood sample were collected by cardiac puncture using a separate heparinized plastic syringe, fitted with 21 gauge hypodermic needle and preserved in disodium salt of ethylene diamine tetra-acetic acid (EDTA) bottles for analysis. Approximately 2 ml of blood from each fish was put separately into a bottle and were used in the assessment of the various blood parameters. The packed cell volume (PCV) of the fish was obtained by filling the heparinized capillary tubes (Marienfeld, Germany) with whole blood, sealed at one end with plasticine and centrifuge for 5 min in a Hawksley microhaematocrit centrifuge (Hawksley, England) at 10000

Table 1. Data on the proximate analysis of the feed ingredients.

Feed stuff	Protein	Fiber	Ash	Moisture	Fat	Carbohydrate
Fish meal	65	0.48	10.35	14.95	2.13	7.30
Soyabean meal	50.35	2.05	2.95	7.35	28.61	8.69
Yellow corn	9.76	4.15	4.40	10.05	5.36	66.28
Yeast	64.45	Nil	4.95	9.65	3.00	17.95

Table 2. Compositions of the dietary ingredients per kg.

Diet	Diet A	Diet B	Diet C	Diet D	Diet E	Diet F	Diet G
Substitution of soyabean with yeast	0%	10%	20%	30%	40%	50%	100%
Fish meal	154.5	154.5	154.5	154.5	154.5	154.5	154.5
Blood meal	154.5	154.5	154.5	154.5	154.5	154.5	154.5
Soyabean meal	154.5	139.1	123.6	108.2	92.7	77.3	-
Yellow corn	506.5	506.5	506.5	506.5	506.5	506.5	506.5
Vitamin premix	13	13	13	13	13	13	13
Mineral premix	17	17	17	17	17	17	17
Yeast	-	15.3	30.9	46.3	61.8	77.2	154.5
Percentage nutrient composition per kilogram diet							
Crude protein	25.15	35.53	34.75	33.97	33.19	27.97	28.52
Carbohydrates	37.03	36.90	36.77	36.63	36.50	36.36	35.69
Fat	5.09	7.10	6.65	6.21	5.77	5.33	3.11
Ash	2.22	4.87	4.82	4.78	4.73	4.68	4.46
Fiber	2.56	2.52	2.49	2.46	2.43	2.40	2.24
Moisture	8.66	8.55	8.43	8.45	8.21	8.09	7.53

revolution per minute (rpm), and the PCV read off the haemocrit reader (Hawksley, England). Haemoglobin concentration was estimated using the cyano-methaemoglobin method (Brown, 1980; Dacie and Lewis, 1979). The white blood cell and red blood counts were done as described by Schalm (1975). The leucocytes differential cell counts were done using leishman counter stain (Bemac Science and Chemical Company, Nigeria) techniques (Vallada, 1988). The cells were counted using a light microscope (Leica Inc. USA) at $\times 10$ objective.

Biochemical analysis

Blood for biochemical analysis were collected by cardiac puncture using separate disposable syringes. Approximately 5 ml of blood was collected from each fish. The blood was allowed to clot and serum was separated from clot by centrifugation.

Serum total protein

The serum total protein was analyzed by Biuret method. Commercially available test kits products of Randox U.K (Randox, England) were used and manufacturers' instructions strictly adhered to.

Cholesterol determination

The determination of cholesterol was carried out using enzymatic colorimetric test (Chop-pap method) for the *in vitro* determination of

cholesterol in serum using Quimica Clinica Aplicada (QCA) test kit (QCA, S. A Spain) as described by Allain et al. (1974).

Aspartate aminotransferase (AST)

AST determination was done by the Reitman-Frankel colorimetric method for *in vitro* determination of AST in serum using a Quimica Clinica applicada (QCA) test kit (QCA, Spain) (Reitman and Frankel, 1975).

Alanine aminotransferase (ALT)

ALT was carried out using Reitman and Frankels method. The assay kit was Quimica Clinica Aplicada (QCA) test kit (QCA, Spain) (Reitman and Frankel, 1975).

Statistical analysis

Data obtained was analyzed for statistical difference using Anova at 95% confidence level, and mean values were separated using Duncan new multiple range test (Duncan, 1955; Ritcher, 1975).

RESULTS

Growth studies

The effect of increasing levels of yeast meal in diets on

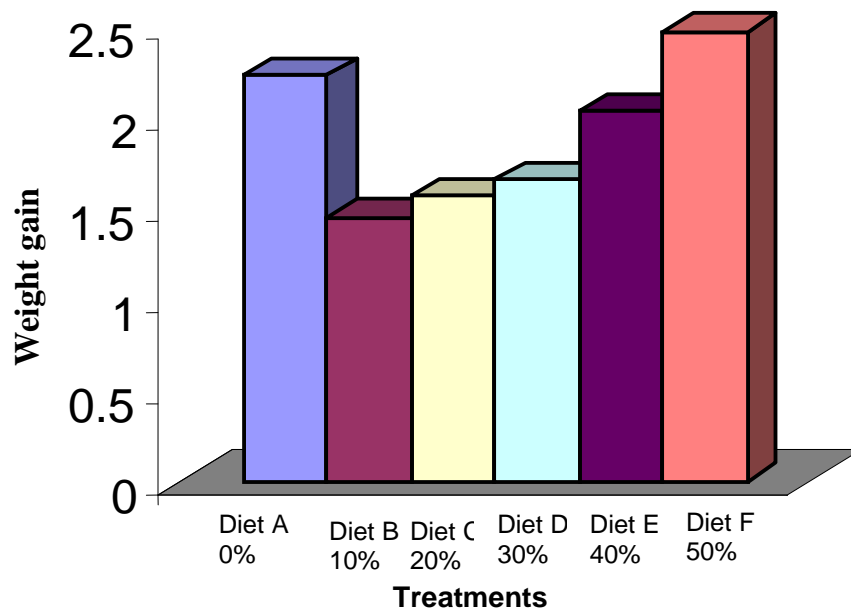


Figure 1. Weight gain (g) of *Clarias gariepinus* juveniles fed yeast meal base diet.

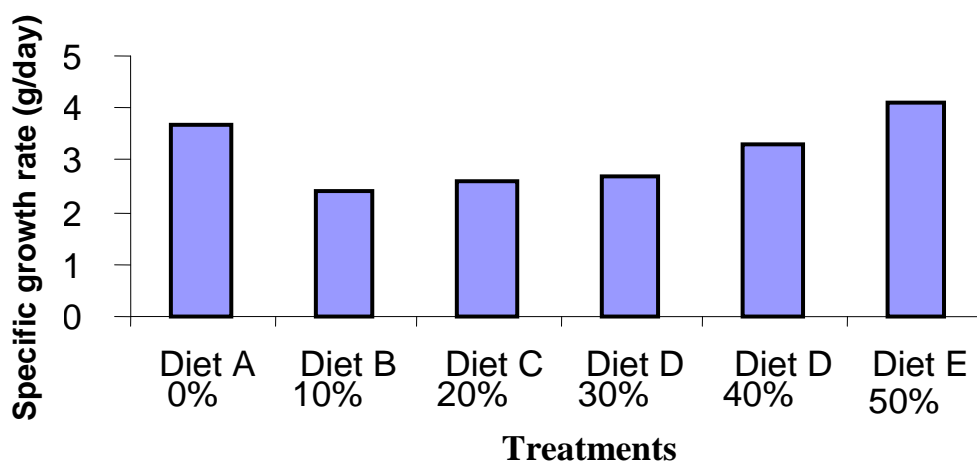


Figure 2. Specific growth rate in (g/day) of *C. gariepinus* juveniles fed yeast meal base diet.

the weight gain of fish species are shown in Figure 1. It was observed that fish species in treatment F fed diet containing 50% yeast meal gained more weight (2.46 ± 0.05 g) when compared with the mean weights of fishes fed diets A to E. The results of other treatments were diet B (1.45 ± 0.03 g), diet C (1.56 ± 0.07 g), diet D (1.65 ± 0.03 g), diet E (2.03 ± 0.03 g), and diet A (2.22 ± 0.03 g) (Figures 1 and 2). The effect of increasing substitution of soyabean meal for yeast meal on mean weight gain of *C. gariepinus* juveniles fed for 60 days showed growth difference with different dietary types. The catfish fed 100% yeast meal inclusion all died within the first week of the experiment (Figure 1).

Specific growth rate (SGR)

The effect of yeast meal based diets on specific growth rate of fish species are shown in Figure 2. It was observed that the catfish juveniles fed diet F (4.11 ± 0.40 g/day) grew better than those fed diet A (3.70 ± 0.91 g/day), followed by diet E (3.38 ± 0.60 g/day), diet D (2.75 ± 0.10 g/day), diet C (2.61 ± 0.03 g/day), and diet B (2.43 ± 0.18 g/day).

Haematological profile

The haematological profile of substituting yeast meal for

soyabean meal in different fish groups after 30 days of feeding the experimental diets is presented in Table 3 while the profile after 60 days is presented in Table 4. The mean haemoglobin (HB) concentrations of Groups B, C and E (10, 20 and 40% substitution) were significantly higher ($p < 0.05$) than that of control (Group A) but the mean haemoglobin of Group F (50%) was significantly lower ($p < 0.05$) than that of the control after 30 days. However, the mean HB of the Groups B, C, D and E were significantly higher ($p < 0.05$) than that of the control (Table 4) after 60 days. The effect of the treatment of yeast meal on the mean red blood cell count of Groups B, C, D, E and F (10, 20, 30, 40 and 50% substitution), respectively were significantly higher ($p < 0.05$) than that of the control after 30 days (Table 3). The mean red blood cell count of the Groups B, C and F (10, 20 and 50% substitution) were significantly higher ($p < 0.05$) than that of the control (Group A) but Group E (40%) was significantly lower ($p < 0.05$) than that of the control after 60 days (Table 4).

The mean PCV level of Groups B, C, D, and E (10, 20, 30 and 40% substitution) were significantly higher ($p < 0.05$) than that of the control (Group A), after 30 days (Table 3) while the mean PCV level of Groups B, C and F were significantly higher ($p < 0.05$) than that of the control after 60 days (Table 4). The effect of dietary substitution on the experimental fish groups of *C. gariepinus* after 30 days showed that the mean white blood cell count of Groups B, D, E and F (10, 30, 40 and 50% substitution) were significantly lower ($p < 0.05$) than the control (Group A and C) (Table 3). After 60 days, however, the mean white blood cell count of the Groups B, C, D, E and F (10, 20, 30, 40 and 50% substitution) were significantly lower ($p < 0.05$) than that of the control (Table 3).

Microscopic examination of blood smears revealed the presence of neutrophils, monocytes and lymphocytes. Under the condition employed here, no basophils or eosinophils were found in blood smears of *C. gariepinus*. However, the mean neutrophil concentrations of Groups D and F were significantly higher ($p < 0.05$) than that of the control while Groups B, C and E were significantly lower ($p < 0.05$) than the control after 30 days (Table 3), but after 60 days of yeast inclusion, neutrophil concentration in Groups D, E and F were significantly higher ($p < 0.05$) than the control. The result also showed that the mean monocyte concentrations of Groups B, C, E and F (10, 20, 40 and 50% substitution) were significantly higher ($p < 0.05$) than that of the control after 30 days (Table 3), while the mean monocyte concentrations of the Groups B, C, E and F were significantly higher ($p < 0.05$) than the control (Group A) after 60 days (Table 4).

The mean lymphocytes concentrations of Groups B, E and F (10, 40, 50% inclusion), respectively were significantly lower ($p < 0.05$) than the control (Group A) but that of Group D (30%) was significantly higher ($p < 0.05$) than that of the control after 30 days (Table 3). After

60 days however, the mean lymphocytes concentrations of the Groups B, E and F were significantly higher ($p < 0.05$) than the control (Table 4).

Biochemical profile

The biochemical profile of fish groups after 30 days of feeding the experimental diets is presented in Table 5, while the profile after 60 days is presented on Table 6. After 30 days of feeding the experimental diets, the serum total protein of Groups C, D and F (20, 30, and 50% inclusion), respectively was significantly higher ($p < 0.05$) than that of Groups A (0%) and E (40%), but after 60 days the serum total protein of Groups A, C and F (0, 20, and 50%) were significantly higher ($p < 0.05$) than that of Group B, D and E (10, 30, and 40% inclusion) (Tables 5 and 6). The mean serum cholesterol of Group D fishes was significantly higher ($p < 0.05$) than those of all other fish groups after 30 days of feeding while that of Group B fishes was significantly lower ($p < 0.05$) than those of all other groups (Table 5). After 60 days of feeding however, the mean serum cholesterol of the Group F (50%) fishes was significantly higher ($p < 0.05$) than that of other fish groups and that of Group E (40%) was significantly lower ($p < 0.05$) than those of all other groups (Table 6). The serum AST activity of the fish groups fed diets (Groups B to F) were significantly higher ($p < 0.05$) than that of the control (Group A) after both 30 and 60 days of feeding on the experimental diets (Tables 5 and 6).

In contrasts, there were no significant variations ($p > 0.05$) in the serum ALT activity of all the fish groups (Tables 5 and 6).

DISCUSSION

Based on reported proximate analysis of yeast meal based diets in Table 2, it was noted that yeast's crude protein (25.15%) and corn meal's carbohydrate (66.28%) could be utilized for catfish diet, considering their levels of protein and energy. The quantity of protein: carbohydrate ratio in each diet was found to be proportional and correspond to the nutritional need of the catfish fed. However, the result obtained for the control diet A (0%) 2.22 ± 0.03 g weight gain was statistically not significant ($p > 0.05$) when compared with the 50% result obtained for yeast meal based diet (Diet F) 2.46 ± 0.05 g weight gain. The increase meal weight gain and specific growth rate observed in catfish fed 50% yeast meal based diet may be due to effect of protein.

Eyo (2005) demonstrated in the hybrid catfish *Heterobranchus bidorsalis* (♂) × *C. gariepinus* (♀) that during energy starvation, protein molecules are mobilized and as such growth is affected. Increasing substitution of soyabean meal for yeast meal lead to increase in protein contents of diets. The diet with 50% (Diet F) yeast

Table 3. Effect of dietary substitution of various levels of soyabean meal with yeast on *Clarias gariepinus* after feeding for 30 days (Results are presented as Mean \pm SEM).

Parameter	Diet A	Diet B	Diet C	Diet D	Diet E	Diet F
Hb (g/dl)	9.52 \pm 0.03 ^a	10.72 \pm 0.03 ^b	12.47 \pm 0.03 ^c	9.80 \pm 0.06 ^a	10.37 \pm 0.03 ^b	8.53 \pm 0.03 ^d
RBC ($\times 10^6$)	4.00 \pm 0.03 ^a	5.00 \pm 0.00 ^b	6.12 \pm 0.00 ^c	4.97 \pm 0.00 ^b	5.15 \pm 0.00 ^b	4.29 \pm 0.00 ^c
PCV (%)	26.00 \pm 0.01 ^a	29.00 \pm 0.00 ^b	36.00 \pm 0.00 ^c	29.67 \pm 0.35 ^b	31.00 \pm 0.00 ^d	26.33 \pm 0.33 ^a
WBC ($\times 10^3$)	28.73 \pm 0.31 ^a	19.80 \pm 0.33 ^b	28.73 \pm 0.33 ^a	20.90 \pm 0.33 ^b	22.07 \pm 0.33 ^c	22067.20 \pm 0.33 ^a
Neutrophil (%)	8.31 \pm 0.33 ^a	2.33 \pm 0.33 ^b	3.33 \pm 0.33 ^b	5.33 \pm 0.33 ^c	4.33 \pm 0.33 ^{bc}	6.33 \pm 0.33 ^c
Monocytes (%)	5.00 \pm 0.03 ^a	15.67 \pm 0.33 ^b	9.67 \pm 0.33 ^c	5.00 \pm 0.00 ^a	13.67 \pm 0.33 ^d	10.00 \pm 0.00 ^e
Lymphocytes (%)	87.00 \pm 0.03 ^a	82.00 \pm 0.33 ^b	87.00 \pm 0.00 ^a	90.00 \pm 0.00 ^c	82.00 \pm 0.00 ^b	84.33 \pm 0.33 ^{ab}

^{abcd}, Different superscripts in a row indicate significant difference between the means ($P < 0.05$). The composition of the different diets is given in Table 2.

Table 4. Effect of dietary substitution of various levels of soyabean meal with yeast on *C. gariepinus* after feeding for 60 days.

Parameter	Diet A	Diet B	Diet C	Diet D	Diet E	Diet F
Hb (g/dl)	9.30 \pm 0.05 ^a	11.47 \pm 0.03 ^b	11.33 \pm 0.33 ^b	9.93 \pm 0.08 ^c	9.30 \pm 0.06 ^a	12.17 \pm 0.03 ^d
RBC ($\times 10^6$)	5.01 \pm 0.03 ^a	5.60 \pm 0.01 ^b	5.41 \pm 0.00 ^b	5.00 \pm 0.00 ^a	4.71 \pm 0.00 ^c	6.17 \pm 0.00 ^d
PCV (%)	30.65 \pm 0.33 ^a	33.00 \pm 0.00 ^b	32.00 \pm 0.00 ^c	30.67 \pm 0.33 ^a	29.33 \pm 0.33 ^a	35.00 \pm 0.33 ^d
WBC ($\times 10^3$)	21.50 \pm 0.33 ^a	24.10 \pm 0.67 ^b	21.50 \pm 0.58 ^a	20.80 \pm 1.86 ^a	23.40 \pm 0.33 ^b	25.20 \pm 0.33 ^b
Neutrophil (%)	3.2 \pm 0.03 ^a	1.33 \pm 0.33 ^b	1.67 \pm 0.33 ^b	5.67 \pm 0.88 ^c	3.33 \pm 0.33 ^a	7.00 \pm 0.00 ^d
Monocytes (%)	5.33 \pm 0.03 ^a	12.33 \pm 0.33 ^b	9.33 \pm 0.33 ^c	4.33 \pm 0.33 ^a	10.33 \pm 0.33 ^d	10.67 \pm 0.33 ^d
Lymphocytes (%)	92.03 \pm 0.01 ^a	87.33 \pm 0.33 ^b	90.33 \pm 0.33 ^a	89.33 \pm 0.33 ^a	85.67 \pm 0.33 ^b	83.90 \pm 0.00 ^c

^{abcd}, Different superscripts in a row indicate significant difference between the means ($P < 0.05$). The composition of the different diets is given in Table 2.

inclusion substituted for soya bean meal has the best performance. The substitutions of the soyabean meal with yeast meal offer much prospects of conversion of yeast meal into fish protein, and boost the blood parameters and health of fish. Statistical analysis showed that the mean weight increase and specific growth rates for the different incorporation levels were significantly different, except for 10% yeast inclusion. Jarmolowicz et al. (2012) reported the effect of brewer's yeast extract on growth while Yuan et al. (2012) reported a significant negative

relationship between growth response and the level of fish meal protein replacement with fermented soyabean meal.

Ayyaru and Venkatesan (2010) reported the effects of dietary (beta) 1,3 glucan and whole cell yeast (*Sachacromyces uvarum*) on immune response and disease resistance in *C. carpio* with encouraging results; stated that dietary supplementation of glucan significantly increase the white blood cell count in fish on the 60th day (2.91 \pm 0.04 \times 10⁴). The utilization of yeast inclusion in *C. gariepinus* was in line with this

works of Ayyaru and Ventakatesan (2010). The effect of the substitution of soyabean meal with yeast meal on mean haemoglobin value after 30 days showed similar range values reported in Nile Tilapia (10.40 g/dl) (Nilza de Lucas et al., 2003). The outstanding increase in red blood cell may be attributed to the high protein content of soyabean and yeast based diet. Kenari et al. (2012) in their study on how low dietary nucleotide (NT) supplements influence the growth, haemato-immunological parameters and stress responses in endangered Caspian brown trout (*Salmon trutta*

Table 5. Effect of dietary substitution of various levels of soyabean with yeast meal on *C. gariepinus* after feeding for 30 days.

Parameter	Diet A	Diet B	Diet C	Diet D	Diet E	Diet F
Serum total protein (g/dl)	5.33±0.50 ^a	6.03±0.03 ^b	6.33±0.03 ^b	6.90±0.06 ^c	4.40±0.06 ^d	6.93±0.03 ^c
Cholesterol mg/dl	150.33±0.33 ^a	154.33±3.33 ^a	139.67±0.33 ^b	161.00±0.58 ^c	145.67±0.03 ^d	155.67±0.33 ^a
Aspartate aminotransferase (AST IU)	71.50±0.03 ^a	132.00±0.68 ^b	134.33±0.33 ^b	135.33±0.33 ^b	135.33±0.33 ^b	136.67±0.33 ^b
Alanine aminotransferase (IU)	34.66±0.33 ^a	34.67±0.33 ^a	35.07±0.33 ^a	35.09±0.67 ^a	35.67±0.35 ^a	36.33±0.67 ^a

^{abcd}Different superscripts in a row indicate significant different between the means ($p < 0.05$). The composition of the different diets is given in Table 2.

Table 6. Effect of dietary substitution of various levels of soyabean with yeast meal on *C. gariepinus* after feeding for 60 days.

Parameter	Diet A	Diet B	Diet C	Diet D	Diet E	Diet F
Serum total protein (g/dl)	5.65±0.03 ^a	4.67±0.03 ^b	5.67±0.03 ^a	4.80±0.03 ^b	5.13±0.03 ^b	6.00±0.03 ^a
Cholesterol (mg/dl)	150.03±0.03 ^a	156.33±3.67 ^b	160.67±0.33 ^b	159.67±0.33 ^b	136.33±0.33 ^c	169.67±0.33 ^d
Aspartate aminotransferase (AST /IU)	72.05±0.33 ^a	133.00±0.58 ^b	134.06±0.33 ^b	135.33±0.33 ^b	135.67±0.88 ^b	136.67±0.33 ^b
Alanine aminotransferase (AIT/ IU)	34.00±0.33 ^a	35.33±0.33 ^a	35.67±0.33 ^a	36.03±0.33 ^a	36.33±0.33 ^a	37.73±0.33 ^a

^{abcd}, Different superscripts in a row indicate significant difference between the means ($P < 0.05$). The composition of the different diets is given in Table 2.

caspius) reported that fish fed diet with 2.5 g dietary (NT) kg⁻¹ had higher blood protein, albumin, red blood cells, lymphocyte content and lower alkaline phosphatase similar to this study. The incorporation of yeast into the diet boosted the PCV level (Kenari et al., 2012). This was similar to the values described for Nile Tilapia (Nilza et al., 2003) but lower than the value reported by Gabriel et al. (2001, 2004) on *C. gariepinus*. Neutrophils increased with increase in yeast inclusion observed in this study, similar to Ayyaru and Ventakatesen (2010).

This study showed that yeast inclusion may have a better effect on fish diet at lower levels of inclusion. Ramesh and Savananan (2000) reported that serum total protein value for fresh water fish *C. carpio* was 6.705 ± 0.653 g/dl. This is similar to the values obtained in the present study at 20% yeast inclusion 6.33 ± 0.03 g/dl after 60 days. This is however lower than the value

reported by Nilza de Lucas (2003) on *Oreochromis niloticus*. Yeast inclusion boosted the cholesterol level. This was in line with the values described for millet (Fatma et al., 2008). The cholesterol level observed in this study would be as a result of increased utilization for corticosteroid synthesis. It may also indicate impairment of the liver synthesis, but there is no direct evidence for this in the literature. Marked increases in cholesterol observed at 50% yeast inclusion may be as a result of enhanced production by the liver and other organs. Enzymes such as aspartate aminotransferase and alanine aminotransferase are well known transaminases that play important role in metabolism. In the present study, exposure of *C. gariepinus* to yeast after 30 and 60 days, respectively gave a similar reference range to that of Kristofferson et al. (2009) and Osman et al. (2009) on pollution with benzo-a-pyrene in catfish (*C. gariepinus*). The study on alanine

aminotransferase is different from the work of Myburgh et al. (2008). Elevation gives an indication of cytotoxicity while decreases observed could suggest leakage of enzyme into the serum from liver.

Conclusion

Yeast could be substituted for soyabean as protein in fish diet at low level of inclusion. Diet F (50% yeast inclusion) was considered the best level of inclusion because of enhanced nutritional status, better blood parameters and improved health of fish.

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REFERENCES

- Aderemi FA, Ladokun OA, Tewe OO (2004). Study on haematological and layers fed biodegraded cassava root sieviate. *Bowen J. Agric.* 1(1):79-83
- Allain CC, Poon LS, Chan CS, Richmond W, Fu PC (1974). Enzymatic determination of total cholesterol. *Clin. Chem.* 20(4):470-475.
- Anderson JL, Fong QSW (1985) Private aquaculture and commercial fisheries, bioeconomics of salmon ranching. *Int. J. Econ. Manag.* 112:353-370.
- Anderson JL, Fong QSW (1997). Aquaculture, Economics and Management J. Int. Assoc. Aquacult. Econ. Manag. 1(2):112.
- AOAC (1975). Association of Official Analytical chemists) Official Method of analysis. AOAC, Arlington, Virginia.
- Ayyaru G, Ventakatesan A (2010). Enhancement of the Innate immune system and disease-resistance activity in *Cyprinus carpio* by oral administration of *B. glucan* and whole cell yeast. *J. Tocs* 3(4):1-4.
- Brown BA (1980). Haematology. Principles and Procedures (3rd ed), Lea and Fabiger, Philadelphia. p. 356.
- Coles EH (1986). Determination of Packed cell volume in Coles E.H Ed. *Veterinary Clinical Pathology*, W.B. Saunders Co; Philadelphia. pp. 17-19.
- Dacie JV, Lewis SM (1979). *Practical haematology*. Longman Group, Ltd.
- Duncan RM (1955). Multiple range and multiple F-tests. *Biometrics* 11:1-42.
- Ekanem JI, Yusuf OK (2005). Activities of alkaline phosphates, glutamate oxaloacetic and glutamate pyruvate transaminases in liver and serum of rats treated with honey. *Biokem Niger Soc. Exp. Bio.* 17:2.
- Eyo JE (2003). Acceptability, growth performance and cost analysis of diets enriched with lipids from varied plants and animal sources fed to finger lings of *Clarias gariepinus* (Teleostei, clariidae) Burchell, No. 22. *Bio. Res.* 1(2):87-99.
- Eyo JE (2005). Effects of substituting soyabean meal for maggot meal in acceptability of diets, growth performance and cost benefit of diets fed to hybrid catfish-*Heterobranchus Bidorsalis* (♂)X *Clarias gariepinus* (♀). *J. Sci. Technol. Res.* 4:38-43.
- Fagbenro AO, Adeparusi E (2003). Feedstuff and dietary substitution for armed fish in Nigeria. Paper presented at Pan African Fish and Fisheries Conference Contonou Benin Republic. pp. 276-278.
- Fatma AS, Mohamed N, Nahed SG (2008). Environmental Pollution – Induced Biochemical Changes in Tissues of *Tilapia zilli*, *Solea vulgaris* and *Mugil capito* from lake Qarum, Egypt. *Glob. Vet.* 2(6):327-336.
- Gabriel UU, Alagoa JK, Allison ME (2001). Effects of dispersed crude oil water dispersion on the haemoglobin and haematocrit of *Clarias gariepinus* J. *Aqua. Sci. Environ. Manag.* 5(2):9-11.
- Gabriel UU, Ezeri GNO, Opabunmi OO (2004). Influence of sex, source, health status and acclimation on the haematology of *Clarias gariepinus* (Burch, 1822) *Afr. J. Biotechnol.* 3(9):463-487.
- Gingerich WH (1982). Hepatic toxicology of fishes, in *Aquatic Toxicology*. Weber, L., Ed., Karen Press, New York. p. 55.
- Haylor GS (1993). Aspects of the biology and culture of the African catfish *Clarias gariepinus* with particular reference to developing African countries. *Aquacult. Res.* 4:235-294.
- Jarmolowicz S, Zakes SI, Wicki A, Kowalska A, Hopko M, Glabski E, Demska-Zakes K, Partyka K (2012). Effects of brewer's yeast extract on growth performance and health of juvenile pikeperch *Sander lucioperca* (L). *Aquacult. Nutr.* 18:457-464.
- Kenari, AA, Mahmoudi N, Soltani M, Abediankenari S (2012). Dietary nucleotide supplement influence the growth, haemato-immunological parameters and stress responses in endangered Caspian brown trout (*Salmo trutta caspius* Kessler, 1877). *Aquacult. Nutr.* 10:1365-2095.
- Kristoffersson R, Broberg S, Oikuri A, Peickarinen M (2009). Effect of a sublethal concentration of phenol on some blood enzyme activities in the pike (*Esox lusius*) in brackish water. *Ann. Zoo. Fea.* 11:220.
- Leung PS, Tisdell C (1997). Aquaculture, Economics and Management. *J. Int. Assoc. Aqua. Econ. Manag.* 1:1-3.
- Machiels MAM, Henken AM (1985). Growth rate, feed utilization and energy metabolism of the African catfish, *Clarias gariepinus* as affected by dietary protein and energy content. *J. aquacult.* 44:271-284
- Mustin WG, Lovell RI (1995). Dietary Protein Concentration and daily allowance influence response of channel Catfish *Ictalurus punctatus* (Rafinesque), to ractopanone. *Aqua. Nutr.* 1:21-26.
- Myburgh JG, Botha CJ, Booysse DG, Reyers F (2008). Provisional clinical chemistry parameters in the African Sharptooth catfish (*Clarias gariepinus*) *Tyd. S. Afr. Vet.* 79(4):156-160.
- Nilza de lucas RB, Ligia MM, Denise de Oliveiras, Rassa BP, Celso VN, Benicio AF, Benedito POF (2003). Haematological and Biochemical values of Nile *Tilapia*, *Oreochromis niloticus* culture in semi-intensive system. *Acta Sci.* 25(2):385-389.
- Omitoyin BO (1995). Utilization of poultry by products (feathers and offals) in the diet of african catfish *Clarias gariepinus* (BURCHELL) P.hD dissertation, University Ibadan, Ibadan, Nigeria.
- Osman HAM, Mona M, Ismaiel, Wafaa TA, Taghreed BI (2009). An approach to the Interaction Between Trichodiniasis and Pollution with Benzo-a-Pyrene in catfish (*Clarias gariepinus*) *J. Fish Mar. Sci.* 1(4):283-289.
- Perez L, Gonzalez H, Jover M (1997). Growth of European Sea bass fingerlings (*Dicentrarchus labox*). Fed extruded diets containing varying levels of protein, lipid and carbohydrate. *Aquaculture* 136:183-193.
- Rainza-Paiva MJ (2003). Haematological characteristics and relative conditions factor (kn) associated with parasitism in *Schizodon borelli* (Osteichthyes) Anostomidae abd *Prochilodus lineatus* (Osteichthyes Prochilodontidae) from Parana River, Parto, Rico Region, Parana, Brasil *Act. Sci.* 2(22):515-521.
- Ramesh M, Saravanan M (2000). Haematological and biochemical responses in fresh water fish *Cyprinus carpio* exposed to chlorpyrifos. *Int. J. Int.* 3:1-6.
- Reitman S, Frankel S (1975). A colorimetric method for determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *Am. Clin. Pathol.* 28:56-62.
- Ritcher D (1975). Multiple ranges and multiple F–test. *Biometrics* 11:1-42.
- Schalma OW, Jain NC, Carroll EJ (1975). *Veterinary haematology* 3rd ed. Lea & Febiger, Philadelphia. pp. 19-25.
- Spinelli J (1978). *Fish Faced Technology*. Aqua. Dev. Co. Pro. FAO/UNDP 11:191-200.
- Tacon AGJ (1992). Nutritional fish pathology, morphological signs of nutrient deficiency and toxicity in farmed fish. *FAO Fish Tech. Pap.* 75:1-4.
- Tyson CA, Sawhney DS (1985). Organ function tests in toxicology evaluation. Noyes publication, New Jersey USA.
- Wedemeyer GA, Gould RW, Yasutake WT (1983). Some potentials and limits of the leucocrit test as a fish health assessment method. *J. Fish Biol.* 23:711-716.
- Wieser W, Hinterleitner S (1980). Serum enzymes in rainbow trout as tools in the diagnostic of water quality. *Bull. Environ. Con. Toxicol.* 25:88.
- Yuan YC, Lin YC, Yang HS, Gong Y, Gong SY, Yu DH (2012). Evaluation of fermented soyabean meal in the practical diets for juvenile Chinese sucker, *Myoyprinus asiaticus*. *Aquacult. Nutr.* 10:1365-2095.