

ASSESSMENT OF DIETARY MANNAOLIGOSACCHARIDES AND β -GLUCAN ON THE GROWTH, SOMATIC INDICES AND HAEMATOLOGICAL PARAMETERS OF AFRICAN CATFISH (*Clarias gariepinus*)

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ABSTRACT

The effects of dietary mannaoligosaccharides (MOS) and β -glucan on the production performance of African catfish, *Clarias gariepinus*, was examined in this study. Three (3) trial diets were produced viz-a-vis., Control (basal diet, BD), MOS (BD + 0.2% MOS) and β -glucan (BD + 0.02% β -glucan) and fed to catfish (11.77 ± 0.05 g fish⁻¹) for 63 days. Nine circular tanks were distinctly assigned into 3 dietary groups in triplicate, stocked with 100 fish per tank (85 L), and fed twice daily (8:00 - 9:00h and 17:00 - 18:00h). The results indicated that there was no significant difference ($p > 0.05$) in the mean final body weight, specific growth rates, feed conversion and protein efficiency ratios, and survivals among the various treatments groups. Similarly, somatic indices (condition factors, hepatosomatic indices, and viscerosomatic indices) measured were not significantly different ($p > 0.05$) across the experimental groups. However, the haematological parameters such as packed cell volume, haemoglobin as well as the erythrocyte and leucocyte counts were significantly ($p < 0.05$) lower in the catfish fed MOS diet. It could be concluded herewith that dietary supplementation of MOS and β -glucan do not have a deleterious effect on African catfish production. Further study is however warranted to establish dose-response effect of MOS and β -glucan on innate immunity of African catfish (*C. gariepinus*).

Keywords: *Clarias gariepinus*, Yeast products, Growth, Somatic indices, Haematology

INTRODUCTION

To meet global demand of seafoods, aquaculture production will need to intensify especially as over half of the global supply of finfish and shellfish is from aquaculture (FAO, 2018). As the consumption of seafoods increases and natural fish stocks decrease, there is need for the intensification of aquaculture practices to meet up with the demand for seafoods. The intensifications, however, are usually followed by sub-optimum environmental conditions such as oxygen depletion, increased nitrogen

wastes, etc., due to overfeeding of a higher number of animals in a limited and confined environment.

This condition may be stressful for fish and will likely lead to reduced growth and immune response depression thus leaving fish prone to infection and disease by opportunistic pathogens. The immunonutrition concept (i.e. development of high quality diet with growth and immune promoting effects) could enhance the performance and immunity of aquaculture species under high-density aquaculture operation (Nakagawa *et al.*, 2007; Kiron, 2012).

One of the ways of achieving the concept of immunonutrition is through dietary supplementation of immunostimulants, which are classified as functional feed additives (Dawood *et al.*, 2018). Cell wall of yeast (*Saccharomyces cerevisiae*) is rich in bioactive components and immunostimulating compounds such as nucleic acids, β -glucan and MOS, which are commonly used additives in aquaculture. The dietary impacts of MOS and beta-glucan have been reported in aquaculture species such as snapper, *Pagrus auratus* (Cook *et al.*, 2003); Rohu, *Labeo rohita* (Misra *et al.*, 2006); large yellow croaker, *Pseudosciaena crocea* (Ai *et al.*, 2007); Nile tilapia, *Oreochromis niloticus* (Sado *et al.*, 2008); gilthead seabream, *Sparus aurata* (Dimitroglou *et al.*, 2010); channel catfish, *Ictalurus punctatus* (Duncan and Klesius, 1996; Peterson *et al.*, 2012); Pacu, *Piaractus mesopotamicus* (Sado *et al.*, 2014); Rainbow trout, *Oncorhynchus mykiss* (Denji *et al.*, 2015); Sea bass, *Dicentrarchus labrax* L. (Salem *et al.*, 2016) and Asian seabass, *Lates calcarifer* (Ali *et al.*, 2017). However, there is insufficient data on the dietary effects of MOS and β -glucan on African catfish (*C. gariepinus*) except possibly for a study conducted over a decade ago in which African catfish (*C. gariepinus*) were fed commercial diets supplemented with MOS (Genç *et al.*, 2006) and β -glucan (Yoshida *et al.*, 1995). Therefore, the aim of this study is to examine the dietary effects of MOS and β -glucan supplementation on the growth performance and health of African catfish (*C. gariepinus*), an important and dominant aquaculture species in sub-Saharan Africa.

MATERIALS AND METHODS

Experimental Design and Diets

All experimental procedure involving fish complies with the ethical provision of the Federal University of Agriculture, Abeokuta, Nigeria.

The experimental trial was run in a flow-through aquaculture system of the Aquaculture & Fisheries Department, Federal University of Agriculture, Abeokuta, Nigeria. The system contains nine circular tanks (875 L capacity each) supplied with freshwater from a deep well. Nine hundred African catfish (*C. gariepinus*) of average weight 11.77 ± 0.05 g were obtained from a reputable hatchery (Motherhood Fish Farm, Abeokuta) and were randomly distributed (100 fish per tank) into the nine tanks

after two weeks of acclimatisation. The photoperiod (~17 h: 7h, light: dark) and water temperature (29±0.29 °C) were maintained at ambient condition.

Three iso-proteic and iso-lipidic diets were formulated (Table 1) as Control (containing neither MOS nor β -glucan), MOS (containing 0.2% MOS) and β -glucan (containing 0.02% β -glucan) diets. The feed ingredients were thoroughly mixed, moistened, and cold press extruded to produce a 2mm pellets. The diets were air-dried and stored in airtight containers until use. The fish were hand fed twice a day with their respective diets to apparent satiation for 63 days (0900h and 1600h). The proximate composition of the diets were determined (Table 1) following AOAC protocols (AOAC, 1996). Briefly, samples were dried in a convection oven at 103°C for 12 h to determine moisture level according to AOAC (AOAC 930.15). Dried samples were finely ground with mortar and pestle and analyzed for crude protein (total nitrogen x 6.25) using a Kjeldahl method (AOAC 2001.11). Ether extract was analyzed following AOAC 920.39 method. Ash was analyzed by incineration at 550°C in a muffle furnace for 3 h (AOAC 942.05). The fibre content was analysed by filtration method (AACC 32-10). All samples were analysed in triplicate.

Table 1. Formulation and Composition of the Experimental Diets

Ingredients (%)	Control diet	MOS diet	β -glucan diet
Fishmeal	22.00	22.00	22.00
Shrimp meal	1.00	1.00	1.00
Soybean meal	40.00	40.00	40.00
Groundnut cake	14.00	13.80	13.98
Maize	9.49	9.49	9.49
Whole wheat meal	7.50	7.50	7.50
Vegetable oil	4.00	4.00	4.00
Fish oil	1.00	1.00	1.00
^a Vitamin mineral premix	1.00	1.00	1.00
^b Mannaoligosaccharides	0.00	0.20	0.00
^c β -glucan	0.00	0.00	0.02
Antioxidant (Sodium metabisulfite)	0.01	0.01	0.001
Total	100.00	100.00	100.00
Composition (% dry weight)			
Dry matter	88.30	88.10	88.13
Crude protein	36.10	38.5	38.50
Lipid	12.3	12.0	11.95
Ash	8.11	8.11	8.11
Crude fibre	3.48	2.72	2.72
^d Nitrogen-free extracts (NFE)	28.3	26.9	26.86
^e Gross energy (MJ/kg)	18.19	18.40	18.37

^aVitamin mineral premix contains (per 2.5kg) 20,000,000IU vitamin A, 4,000,000IU vitamin D3, 200,000 vitamin E, 8,000mg vitamin K3, 20,500mg vitamin B1, 15,000 mg vitamin B2, 19,500 mg vitamin B6, 15mcg vitamin B12, 90,000 mg Nicotinic Acid, 40,000 mg Pantothenic Acid, 500 mg Folic Acid, 600,000 mcg Biotin, 40,000 mg Choline Chloride, 4,000 mg Iron, 500 mg Copper, 30,000 mg Manganese, 40,000 mg Zinc, 2,000 mg Iodine, 200 mcg Selenium, 300,000 mg coated Vitamin C, 50,000 mg Inositol, 750 mg Cobalt, 50,000 mg Lysine, 50,000 mg Methionine and 125,000 mg Antioxidant; ^bBiolex[®] MB40 from Leiber GmbH, Bramsche, Germany; ^cLeiber[®] Beta-S from Leiber GmbH, Bramsche, Germany; ^dNitrogen-free extract (NFE) = 100 - (Crude protein + Lipid + ash + Moisture); ^eGross energy (MJ/ kg) = (Crude protein, % X 23.6) + (Lipid, % X 39.5) + (NFE, % X 17).

Growth and Somatic Indices

Growth performance, feed utilisation and somatic indices parameters were assessed using the formulae stated below. All the fish were measured for final body weight, total length and number of surviving fish were recorded.

- **Specific Growth Rate (SGR, % day⁻¹)** = $100 \times (\text{Ln final body weight (g)} - \text{Ln initial body weight (g)}) / \text{trial duration}$
- **Feed Conversion Ratio (FCR)** = $\text{feed given (g)} / \text{wet weight gain (g)}$
- **Protein Efficiency Ratio (PER)** = $\text{wet weight gain (g)} / \text{protein intake (g)}$
- **Hepatosomatic Index (HSI)** = $100 \times (\text{liver weight (g)} / \text{whole body weight (g)})$
- **Viscerosomatic Index (VSI)** = $100 \times (\text{visceral weight (g)} / \text{whole body weight (g)})$
- **Condition Factor (k)** = $\text{whole body weight (g)} / (\text{total length})^3$

Three fish per tank were sampled and used to record viscera weight and whole-body weight to calculate the somatic indices (condition factor, HSI and VSI).

Haematological Parameters

At the end of the feeding trial, 3 fish per tank (n = 9 per treatment) were randomly sampled and anaesthetized with clove oil at 100 mg L⁻¹. The blood was collected via the caudal vein using a 1 mL syringe, placed in an EDTA-coated tube, and analysed to determine packed cell volume, haemoglobin, white blood cells (leucocyte) count, red blood cells (erythrocyte) count, mean corpuscular volume, mean corpuscular haemoglobin as well as mean corpuscular haemoglobin concentration were calculated as described by Adeoye *et al.* (2020). Enumeration of erythrocytes and leucocytes was conducted as described by Dacie and Lewis (1975). Neutrophil, lymphocytes, basophil, eosinophil and monocytes were identified as described by Rowley (1990).

Statistical Analysis

Data were analysed using one-way analysis of variance (ANOVA) to test for differences in mean. Duncan Multiple Range Test (DMRT) was used to separate mean when significant differences were found. Differences were considered significant at a value of $P < 0.05$. The statistical analysis was carried out using SPSS

for Windows (SPSS Inc., 23.0, Chicago, IL, USA). Data are presented as mean \pm standard deviation.

RESULTS

Table 2. Growth, feed utilisation and somatic indices of African catfish (*C. gariepinus*) fed the experimental diets

	Control diet	MOS diet	β -glucan diet
IBW (g fish ⁻¹)	11.87 \pm 0.06	11.77 \pm 0.06	11.77 \pm 0.06
FBW (g fish ⁻¹)	65.42 \pm 2.96	61.38 \pm 6.09	64.83 \pm 3.26
SGR (% day ⁻¹)	3.18 \pm 0.09	3.05 \pm 0.19	3.16 \pm 0.09
FCR	1.31 \pm 0.05	1.32 \pm 0.09	1.27 \pm 0.03
PER	1.66 \pm 0.08	1.64 \pm 0.14	1.72 \pm 0.06
K-factor	0.80 \pm 0.04	0.83 \pm 0.09	0.75 \pm 0.07
HSI	0.87 \pm 0.17	0.94 \pm 0.14	1.01 \pm 0.16
VSI	10.75 \pm 0.83	12.02 \pm 1.62	10.41 \pm 0.55
Survival (%)	88.33 \pm 1.15	92.33 \pm 2.31	87.33 \pm 8.02

Means in the same row with no superscripts are not significantly different ($P > 0.05$). IBW, initial mean body weight; FBW, final mean body weight; SGR, specific growth rate; FCR, feed conversion ratio; PER, protein efficient ratio; K-factor, condition factor; HSI, hepatosomatic index and VSI, viscera-somatic index.

Table 2 shows the growth performance, feed utilisation and somatic indices of African catfish (*C. gariepinus*) fed the experimental diets. There was no significant difference ($p > 0.05$) observed in the measured growth parameters (final body weight and specific growth rate) of the catfish fed the control, MOS and β -glucan diets. However, by the end of the 63 days of feeding the African catfish (*C. gariepinus*) with the respective experimental diets, the catfish increase in weight by more than five folds. Similarly, there was no significant difference ($p > 0.05$) observed in the feed efficiency (feed conversion ratio) and nutrient utilisation (protein efficiency ratio) parameters measured as well as the somatic indices (K-factor, hepatosomatic indices, and viscerosomatic indices) and survivals among the groups fed the control, MOS and β -glucan diets.

Table 3. Haematological parameters of African catfish (*C. gariepinus*) fed the experimental diets

	Control diet	MOS diet	β -glucan diet
Haematocrit (%PCV)	28.22±0.69 ^a	25.89±1.07 ^b	28.55±0.69 ^a
Haemoglobin (g dL ⁻¹)	9.47±0.36 ^a	8.75±0.31 ^b	9.58±0.25 ^a
WBC (10 ⁹ L ⁻¹)	110.09±15.84 ^a	83.09±5.94 ^b	114.18±16.16 ^a
RBC (10 ¹² L ⁻¹)	1.63±0.01 ^a	1.47±0.09 ^b	1.68±0.07 ^a
Neutrophil (%)	20.22±6.74	21.55±7.90	24.45±5.87
Lymphocytes (%)	76.11±5.93	75.55±7.25	70.44±7.96
Basophil (%)	0.56±0.20	0.78±0.51	0.67±0.34
Eosinophil (%)	1.67±1.20	1.22±0.70	2.55±0.69
Monocytes (%)	1.45±0.39	0.89±0.38	1.89±1.34
MCV (fL)	173.32±3.44	174.86±2.56	170.23±4.74
MCH (pg)	58.09±1.87	59.79±1.66	57.03±2.00
MCHC g dL ⁻¹)	33.50±0.41	33.90±0.34	33.56±0.19

Means in the same row with different superscripts are significantly different ($P < 0.05$). WBC, leucocytes; RBC, red blood cells; %, mean percentage of total leucocytes; MCV, mean corpuscular volume (haematocrit (%PCV) \times 10) / RBC (10⁹ L⁻¹); MCH, mean corpuscular haemoglobin (haemoglobin (g dL⁻¹) \times 10) / RBC (10¹² L⁻¹); MCHC, mean corpuscular haemoglobin concentration (haemoglobin (g dL⁻¹) \times 100) / haematocrit (%PCV).

The haematological indices of African catfish fed the experimental diets are shown in Table 3. Haematological parameters such as packed cell volume, haemoglobin as well as the erythrocyte and leucocyte counts were significantly ($p < 0.05$) lower in fish fed MOS-based diet compared to those fed the control and β -glucan diets. No significant difference was observed in other haematological parameters measured ($p > 0.05$).

DISCUSSION

Cell wall of yeast (*Saccharomyces cerevisiae*) is rich in bioactive components and immunostimulating compounds like nucleic acids, β -glucan and mannanoligosaccharides (MOS). MOS has been reported to have prebiotic effect with potential benefit on host by selectively stimulating the growth of and/ or activating the metabolism of health-promoting bacteria in the intestinal tract (Gibson and Roberfroid,

1995; Akrami *et al.*, 2008) and subsequently leading to increased growth rate and better health of the host fish (Ahmadifar *et al.*, 2009). On the other hand, the intestinal uptake and distribution of β -glucan may be transferred through epithelial cells in the intestine and cleared from the blood in the lymphoid tissues. As the intestine contains lymphoid tissue, oral administration of β -glucan is possibly processed by the mucosal immune system (MIS) of the gastrointestinal tract, which is made up of connective tissue, the mononuclear phagocyte system and gut-associated lymphoid tissue (Castro, 1993). Hence, both the undigested and digested β -glucan is possibly processed by the mononuclear phagocyte and thus stimulate an immune response, thereby reducing the host metabolic energy and subsequently improve the growth performance and health condition of the fish.

In the present study, improvement in growth performance was not observed when African catfish (*C. gariepinus*) were fed diet supplemented with MOS (at 0.2% level of inclusion) and β -glucan (0.02% level of inclusion). Similar observation was made in African catfish, *C. gariepinus* fed a commercial trout diet supplemented with MOS (Genç *et al.*, 2006); channel catfish, *Ictalurus punctatus* (Peterson *et al.*, 2010; Peterson *et al.*, 2012); common carp, *Cyprinus carpio* (Momeni-Moghaddam *et al.*, 2015); sea bream, *Sparus aurata* (Dimitroglou *et al.*, 2010); Nile tilapia, *Oreochromis niloticus* (Sado *et al.*, 2008); and pacu, *Piaractus mesopotamicus* (Sado *et al.*, 2014) when fed diets supplemented with MOS at different levels of inclusion over a period of time. On the other hand, studies on Rainbow trout, *Oncorhynchus mykiss* (Denji *et al.*, 2015); seabass, *Dicentrarchus labrax* L. (Salem *et al.*, 2016); and Asian seabass, *Lates calcarifer* (Ali *et al.*, 2017) reported significant improvement in growth performance when the fish species were fed diets supplemented with MOS at varied inclusion levels. The observed increase in growth was partly attributed to the ability of MOS to promote the gut microflora (mainly the beneficial lactic acid bacteria) and subsequent production of extracellular enzymes by the gut microflora (Andrews *et al.*, 2009) which in turn enhanced nutrient digestion. Similarly, Cook *et al.* (2003), Misra *et al.* (2006) and Ai *et al.* (2007) reported improvement in growth performance of snapper (*Pagrus auratus*), *Labeo rohita*, and large yellow croaker (*Pseudosciaena crocea*) respectively when fed diets supplemented with β -glucan. Since the growth capacity of fish species is conditioned by several factors, the variations and contradictory effects of dietary MOS and β -glucan on the growth of fish species could be due to the type of yeast-derived mannans, dosage, feeding duration, fish rearing conditions (such as stocking densities, water quality or environmental variables), different life stage and/ or fish species (Akrami *et al.*, 2009; Torrecillas *et al.*, 2014). Cook *et al.* (2003) were of the opinion that when fish are held at conditions and temperature lower than optimum. The application of immunostimulants may result in improved growth performance. Hence, dietary supplementation of MOS and β -glucan may be more beneficial when the aquaculture species are more susceptible to infection.

Haematological parameters such as packed cell volume, haemoglobin, leucocyte counts, erythrocyte counts, neutrophil, lymphocytes, basophil, eosinophil and monocytes are efficient indicators of physiological condition and welfare in cultured fish (Ayoola *et al.*, 2013; Fawole *et al.*, 2017; Lawal *et al.*, 2019). Increased production of oxygen radicals by monocytes and neutrophils is also a good indicator of activation of non-specific defence mechanisms in fish (Jeney and Anderson, 1993; Siwicki *et al.*, 1994; Jørgensen and Robertsen, 1995) especially as the phagocytic function of monocytes and neutrophils is important in preventing the establishment of bacteria infection in host tissue. In the present study, packed cell volume, haemoglobin, leucocyte counts and erythrocyte counts were significantly lower in fish fed MOS-based diet compared to higher value recorded for other dietary groups. However, other haematological parameters measured were not significantly different among the dietary groups ($p > 0.05$). Denji *et al.* (2015) and Ali *et al.* (2017) reported similar effect in the haematology of Rainbow trout (*O. mykiss*) and Asian seabass (*L. calcarifer*) respectively when fed diet supplemented with MOS. On the contrary, Yoshida *et al.* (1995) reported enhanced phagocytic functions of neutrophils and monocytes as well as the elimination of bacteria in *Clarias gariepinus* when fed a commercial diet supplemented with β -glucan (at 1 g kg^{-1} inclusion level). Duncan and Klesius (1996) also reported lower percentage of erythrocytes, higher percentage of lymphocytes, enhanced non-specific immune functions of macrophages and neutrophils in channel catfish when fed diet supplemented with 0.2% β -glucan. The inconsistency reported in the effects of MOS and β -glucan could be attributed to a range of factors which include species, size, age, physiological status, environmental conditions and dietary regime (Osuigwe *et al.*, 2005).

CONCLUSION AND RECOMMENDATIONS

In conclusion, it could be inferred from the present study that MOS (0.2%) and β -glucan (0.02%) at the current level of inclusion do not improve the growth performance nor haematological parameters of African catfish (*C. gariepinus*). Hence, further studies could consider graded levels of inclusion of MOS and β -glucan above the level tested in this study in the diet of African catfish (*C. gariepinus*) for possible effects on growth, haematology and intestinal health.

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