Dietary supplementation of autolysed yeast enhances growth, liver functionality and intestinal morphology in African catfish

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Dietary supplementation of autolysed brewer's yeast enhances performance and intestinal morphology in African catfish, *Clarias gariepinus*

(**Running title**: Autolysed yeast in African catfish)

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Abstract

A feeding trial was conducted to evaluate the potential of dietary supplementation of autolysed brewer's yeast (AY) on African catfish. The catfish (22.5±1.15 g $fish^{-1}$, 20 fish 33L $tank^{-1}$) were fed with either of diets (390 g kg^{-1} crude protein, 140 g kg^{-1} lipid) supplemented with 0, 3, 6 or 10 g kg^{-1} AY (n = 3). After 49 days of feeding, the final body weight and metabolic growth rate of the catfish fed 3 g kg^{-1} AY (3-AY) diet was higher than those fed the control diet ($P < 0.05$). The lowest level ($P < 0.05$) of alanine transaminase was detected in the blood of the catfish fed 3-AY diet. The mid-intestinal histology of the catfish revealed no significant difference ($P > 0.05$) in intestinal perimeter ratio. However, an elevated ($P < 0.05$) abundance of goblet cells and intraepithelial leucocytes were found in the intestine of catfish fed 3, 6 and 10 g kg^{-1} AY diets, with the highest level of abundance recorded in the mid-intestine of the catfish fed 3-AY diet. The results suggest that dietary 3 g kg^{-1} autolysed brewer's yeast supplementation improve growth performance of African catfish without deleterious effect on liver functionality and gut morphology.

Keywords: African catfish, Brewer's yeast, functional feeds, gut morphology, hepatic function

1.0 Introduction

As the global human population continues to rise with an expected >8 billion in 2030, there will be a need to further develop sustainable food production systems to meet the increase in food demand (United Nations, 2017). In 2016, global fish production was estimated to be >171 million tonnes, with farmed fish representing ~50 % of the quantity produced (FAO, 2018). The fish aquaculture sector is expected to continue to grow in response to the global food challenge. However, the growth in the aquaculture industry is often hindered by problems associated with intensification practices and leading to suboptimal growing conditions. Some of these issues include water quality, overcrowding and nutrient imbalance. These stressful conditions can have the potential to compromise fish health and consequently lead to the fish being prone to infection and disease by opportunistic pathogens (Bondad-Reantaso *et al*., 2005).

The concept of immunonutrition is the potential of modulating the immune system through dietary means (Nakagawa *et al*., 2007; *Kiron*, 2012) and can be achieved through dietary supplementation of immunostimulants (Dawood *et al.*, 2018). Unicellular brewer's yeast (*Saccharomyces cerevisiae*) can have immunostimulatory and bioactivity effects and has been shown to enhance growth performance, health and immunity in farmed fish species (Shurson, 2018). In addition to its relatively high protein, energy and micronutrients content (e.g. vitamins and trace elements), brewer's yeast also possesses bioactive β-glucans, mannan-oligosaccharides and nucleotides (Huyben *et al*., 2017; Xue *et al*., 2017; Shurson, 2018).

The growth and health benefits of brewer's yeast and its by-products have been reported in a number of farmed fish species. These include *Labeo rohita* (Amir *et al*., 2018), rainbow trout (*Oncorhynchus mykiss*, Huyben *et al*., 2017; Jin *et al*., 2018), turbot (*Scophthalmus maximus*, Librán-Pérez *et al*., 2018), gilthead sea bream (*Sparus aurata*, Dimitroglou *et al*., 2010; Gultepe *et al*., 2011; Dawood et al., 2017), Nile tilapia (Sado *et al*., 2008; Ozório *et al*., 2012; Pilarski *et al*., 2017; Hassaan *et al*., 2018), largemouth bass (*Micropterus salmoides*, Zhou *et al*., 2018), Pacific white shrimp (*Litopanaeus vannamei*, Zhang *et al*., 2012; Qiu & Davis, 2017; Jin *et al*., 2018), Jian carp (*Cyprinus carpio* var. Jian, Yuan *et al*., 2017), gibel carp (*Carassius gibelio*, Zhang *et al*., 2018), common carp (*Cyprinus carpio*, Momeni-Moghaddam *et al*., 2015), hybrid striped bass (*Morone chrysops* x *Morone saxatilis*, Li & Gatlin, 2003), giant freshwater prawn (*Macrobrachium rosenbergii*, Prasad *et al*., 2013), Thai panga (*Pangasianodon hypophthalmus* × *Pangasius bocourti*, Pongpet *et al*., 2016), channel catfish (*Ictalurus punctatus*, Peterson *et al*., 2012), European seabass (*Dicentrarchus labrax*,

Torrecillas *et al*., 2007, 2011, 2015; Salem *et al*., 2016) and pacu (*Piaractus mesopotamicus,* Sado *et al*., 2014). However, there is limited knowledge on the effects of brewer's yeast and its derivatives on farmed African catfish (*C. gariepinus*). This is with the exception of studies that were carried out on brewer's yeast as an alternative protein source in African catfish diets (Hoffman *et al*.,1997; Ezenwaji *et al*., 2012; Solomon *et al*., 2017). To this end, the current study evaluated the effects dietary supplementation of a commercial autolysed brewer's yeast (Leiber CeFi® Pro) has on growth performance, health and intestinal morphology in African catfish (*C. gariepinus*). The information generated would have economical importance in the sub-Saharan Africa nations (e.g. Nigeria, Ghana and Uganda), as it reperesents 91 % of the world's farmed African catfish production with a value of over USD 632 million in 2017 (FAO, 2019).

2.0 Materials and Methods

2.1 Experimental design and diet preparation

The feeding trial was performed in a freshwater flow-through aquaculture system (2.5 L min^{-1} flow rate into the fish tank) at the Department of Aquaculture and Fisheries Management, Federal University of Agriculture, Abeokuta, Nigeria. The flow-through system consists of 12 tanks (33 L) and was supplied by a freshwater spring. African catfish (*C. gariepinus*) were sourced from a local fish hatchery (Motherhood Fish Farm, Abeokuta, Nigeria) and were acclimated into the system for two weeks prior to the start of the feeding trial. For each tank, 20 fish were randomly stocked to give an average mean weight of 22.5±1.15 g $fish^{-1}$. The photoperiod (~17 h: 7 h, light: dark) and water temperature (29±0.29 ºC) were maintained at ambient condition. Water quality parameters were monitored weekly; pH, 6.85±0.34 (HI98107 pHep®, Hanna Instruments, Leighton Buzzard, UK); dissolved oxygen, >5 mg L^{-1} (HI3810, Hanna Instruments, Leighton Buzzard, UK) and total ammonia nitrogen, 0.14±0.1 mg L^{-1} (HI3824, Hanna Instruments, Leighton Buzzard, UK).

The autolysed brewer's yeast (Leiber CeFi® Pro) was supplied by Leiber GmbH, Bramsche, Germany. The nutritional value of the autolysed brewer's yeast is shown in Table 1. Four iso-nitrogenous (390 g kg^{-1} crude protein) and iso-lipidic (140 g kg^{-1} lipid) diets were formulated with the inclusion of 3 (3-AY), 6 (6-AY) and 10 (10-AY) g kg^{-1} autolysed brewer's yeast (AY) at the expense of shrimp meal (Table 2). The fourth diet was formulated without the inclusion of AY to give a basal comparison (Control). Production of the test diets involved mixing of the ingredients to give homogenous dough and subsequently cold extruded (flat die pelleting

machine-CAPSFEED, Ibadan, Nigeria) to produce 2 mm diameter sinking pellets. The diets were oven dried at 60 ^{o}C for 12 h. Dried diets were subsequently stored in airtight containers prior to use. Fish were fed with the test diets twice a day (0900 and 1600) to apparent satiation for 49 days.

Quality validation of the finished diets was performed through proximate analysis according to AOAC (2012) protocols and values are presented in Table 2. Moisture was determined by drying samples in oven set to 105°C until constant weight was achieved. Samples were transferred to desiccator to cool, re-weighed and moisture content determined. For ash analysis, samples were weighed and placed in muffle furnace at 550°C for 8 h until a light grey ash resulted. After cooling in desiccator, samples were re-weighed, and ash content was determined. The Soxhlet ether method was used for lipid analysis. The Kjeldahl method was used to determine the nitrogen content of the samples. The crude protein content was determined by multiplying the nitrogen by a factor of 6.25 for animal proteins and 5.95 for proteins of plant origin. All samples were analysed in triplicate.

2.2 Growth, feed efficiency and somatic indices

To assess the effects of the test diets on the fish, the following morphological parameters were measured: body weight (BW), full length (FL), liver weight (LW) and visceral weight (VW). In addition, growth performance, feed efficiency and somatic indices were calculated (Adeoye *et al*., 2016; Fawole *et al*., 2018).

Feed Intake, **FI** = Total feed consumed (g)/ Number of fish harvested

Specific Growth Rate, **SGR** = $((lnFBW - lnIBW)/T)X\ 100$

where FBW = final body weight (g) and IBW = initial body weight (g)

Metabolic Growth Rate, **MGR**

= (Net weight gain in g) / [{(IBW/1000)$^{0.8}$ + (FBW/1000)$^{0.8}$}/2]/ feeding duration in days

where FBW = final body weight (g) and IBW = initial body weight (g)

Feed Conversion Ratio, **FCR** = FI/WG

where FI = feed intake (g) and WG = wet weight gain (g)

Protein Efficiency Ratio, **PER** = WG/PI

where WG = wet weight gain (g) and PI = protein ingested (g)

Hepatosomatic Index, **HSI** = $(LW/BW)X\ 100$

where LW = liver weight (g) and BW = body weight (g)

Viscerosomatic Index, VSI = $(VW/BW)X\ 100$

where VW = visceral weight (g)

Condition Factor, **K** = $(100\ X\ BW)/\ 〚TL〛$ ^3

where BW = body weight (g) and TL = total length (cm)

Survival = (Total number of fish harvested/ total number of fish stocked) X 100

2.3 Haematological-biochemical parameters

At the end of the feeding trial, two fish per tank (n = 6 per treatment) were anaesthetised with clove oil at a concentration of 100 mg L^{-1} followed by cerebral percussion and disruption of the brain prior to sampling. Blood collection was carried out through the caudal arch using 25-gauge needle and 1 mL syringe. Blood smears were prepared for determination of differential leucocytes count and additional blood was left to stand in a slanted position at room temperature to isolate serum. Packed cell volume, haemoglobin, erythrocyte blood cell count, leucocyte count, and differential leucocyte proportions were determined according to standard methods. Packed cell volume of the whole blood was assessed in triplicate using microhaematocrit method (Brown, 1980). Haemoglobin was determined using Drabkin's cyanide-ferricyanide solution (1/250 dilution factor) measured after 5 min of incubation using a spectrophotometer set to 540 nm wavelength and the haemoglobin levels (g dL^{-1}) calculated using the following formula below.

Haemoglobin concentration (g dL^{-1}) =

$$\frac{Absorbance\ of\ sample}{Absorbance\ of\ standard}\ X\ Dilution\ factor$$

Enumeration of erythrocytes and leucocytes was conducted as described by Dacie and Lewis (1975). Twenty microliters of whole blood was mixed with 980 µL of Dacies solution (1/50 dilution factor), mixed for 60 seconds to ensure a homogenous solution. A 5 µL of the homogenous solution was aliquoted to haemacytometer and minimum of 500 cells counted for a ststistically valid data. Blood smears for differential leucocytes count were air-dried, fixed

in methanol for 15 min and stained using May Grünwald stain (diluted 1:1 with Sorensen's buffer, pH 6.8). The smears were then rinsed in Sorensen's buffer and counter stained with Giemsa stain (diluted 1:9 with Sorensen's buffer, pH 6.8). After a final rinse in buffer, slides were left to dry. Once dried, the slides were mounted in DPX. Neutrophil, lymphocytes, basophil, eosinophil and monocytes were identified as described by Rowley (1990). A minimum of 200 cells per sample were counted and the values expressed as percentage of the total leucocytes. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated as previously described by Adeoye *et al*. (2016). The sera were centrifuged (3,000 g, 10 min at 4 ^{o}C) and transferred into another tube and kept at -20 ^{o}C for immediate use. Serum aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) were estimated as described by Fawole *et al*. (2018) using a commercial kit (Randox Laboratories Limited, Crumlin, United Kingdom).

2.4 Intestinal histology

At the end of the trial, two fish per tank (n = 6 per treatment) were sampled for mid-intestine histological examination. The samples were fixed in 10 % neutral buffered formalin and embedded in paraffin wax for sectioning. Sample sections were subsequently stained with haematoxylin and eosin and Periodic acid–Schiff stains. The mid-intestines were imaged using a light microscope (BX53, Olympus Life Science, Tokyo, Japan) and morphological measurements were carried out through ImageJ (version 1.51, National Institute of Health, Bethesda, Maryland, USA). The intestinal perimeter ratio (PR) was assessed as described in Adeoye et al. (2016). PR was calculated as the ratio between the internal perimeter (IP) of the intestinal lumen (villi and mucosal folding length) and the external perimeter (EP) of the intestine (PR = IP/ EP, arbitrary units, AU). The number of intraepithelial leucocytes (IELs) and goblet cells in the epithelium, across a standardized distance of 100 μm (10 folds per specimen) was calculated by averaging the numbers from all specimens.

2.5 Statistical analysis

All data are presented as mean values and with its corresponding standard deviation. Data were analysed using one-way analysis of variance (ANOVA). *Post-hoc* multiple comparisons test was performed using Duncan's new multiple range test. Differences were considered significant for each parameter when $P < 0.05$.

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3.0 Results

3.1 Growth, feed efficiency and somatic indices

From the 49 days feeding trial, the African catfish (*C. gariepinus*) growth performance, feed efficiency and somatic indices were assessed through FBW, SGR, MGR, FCR, PER, K-factor, HSI, VSI and survival (Table 3). At the end of the trial, the catfish fed with the test diets had increase in weight by up to 4.8-fold. It was observed that catfish FBW (15 %) and MGR (10 %) had significantly increased in 3 g kg^{-1} AY dietary inclusion level, when compared to the dietary control group ($P < 0.05$). The VSI was also significantly higher (24 %) among the catfish that were fed with 10 g kg^{-1} AY supplementation than among those fed the control diet ($P < 0.05$). The dietary treatment did not have a significant effect on other parameters (FI, SGR, FCR, PER, K, HSI and survival) in the African catfish.

3.2 Haemato-biochemical parameters

The results of the haemato-biochemical parameters from the African catfish fed with the experimental diets are displayed in Table 4. No differences were observed between dietary treatments in any measured haematological parameters. However, the level of blood alanine transaminase (ALT) activity was found to be significantly lower in catfish fed either 3 g kg^{-1} or 6 g kg^{-1} AY dietary supplementation, when compared with the control group ($P < 0.05$). The largest decrease in ALT activity was by 45 % in 3 g kg^{-1} AY dietary treatment, while 6 g kg^{-1} AY inclusion gave only 39 % reduction.

3.3 Intestinal histology

The mid-intestine of the African catfish fed each of the experimental diets was examined by light microscopy (Figure 1). The African catfish from all treatments showed intact epithelial barriers with extensive mucosal folds extending into the lumen. Each fold consisted of simple lamina propria with abundant intraepithelial leucocytes (IELs) and mucous-secreting goblet cells. There was no significant difference in the intestinal perimeter ratios of African catfish fed with AY supplemented diets ($P > 0.05$, Table 5). However, there was a significant increase in the abundance of goblet cells and IELs in the catfish intestine when fed with AY supplemented diets (i.e. 3 g kg^{-1}, 6 g kg^{-1} and 10 g kg^{-1} AY diets). The highest increase was found in 6 g kg^{-1} AY dietary group, with goblet cell and IELs levels elevated by 28 and 24 % respectively.

4.0 Discussion and Conclusion

Unlike conventional brewer's yeast, the proteins, amino acids, energy and other nutrients (e.g. vitamins and trace metals) can be found bounded to the cell wall. Consequently, this would result in in a lower nutrient digestibility for fish (Ferreira *et al.*, 2010; Shurson, 2018). In contrast, autolysed brewer's yeast would have the cell wall degraded, thereby increasing nutrient bioavailability and potentially having higher bioactivity. The potential of autolysed brewer's yeast to enhance growth performance was confirmed in this study, with improved final body weight and metabolic growth rate. This was particularly evident in African catfish fed with a diet that has 3 g kg^{-1} autolysed brewer's yeast inclusion. The findings in this study concur with the results in the feeding trial study of Yuan *et al*., (2017) on Jian carp (*Cyprinus carpio* var. Jian) using hydrolysed yeast. The authors reported that 30 g kg^{-1} inclusion of yeast hydrolysate resulted in significantly improved fish final weight and weight gain by up to 21 and 24 %, respectively. This ten-fold difference in brewer's yeast inclusion level between the feeding trials, could be the result of varying manufacturing processes being used to produce the degraded brewer's yeast. In contrast, largemouth bass (*M. salmoides*) fed with diets supplemented with lower levels up to 2 g kg^{-1} hydrolysed yeast showed no enhancements in growth performance, feed efficiency or morphometric parameters (Zhou *et al*., 2018).

Haematological parameters of fish species are useful tools for assessing the health status and function of internal organs. In this present study, the measured haematological parameters showed that there were no discernible changes in the fish health or welfare (e.g. white blood cells count and white blood cells differentiation), regardless of whether the fish received dietary autolysed brewer's yeast. The health of the liver can be assessed by several key enzyme activities both in the organ and in the blood (e.g. aspartate transaminase, AST; alanine transaminase, ALT; alkaline phosphatase, ALP). Basically, ALT and AST function in transferring amine groups in trans-amination reactions in liver for non-essential amino acid synthesis and de-amination pathways. Aspartate aminotransferase (AST) catalyses a key metabolic step of the molecular rearrangement involving amino acids associated with the citric acid cycle (ketogenic) whereas alanine aminotransferase (ALT) predominates in tissues and organs with intensive gluconeogenesis, such as in the liver (Urich, 1994; Torre *et al*., 2000). The determination of plasma or serum enzyme activity levels of alanine-amino transferase (ALT) and aspartate-amino transferase (AST) and alkaline phosphatase (ALP) may be indicative of hepatic function and status in animals including fish. Hence, ALT and AST are standard activity measurements for 'liver function' tests in clinical diagnosis of hepatic health

in humans and animals. Elevations in the serum AST and ALT enzyme activity can indicate liver damage or inflammation to environmental contaminants, disease, stress and nutrients (Wan *et al.*, 2016). For the current study, catfish fed diets supplemented with 3 g kg^{-1} or 6 g kg^{-1} yeast autolysate showed significantly lower serum ALT activity but not at the highest level of 10g Kg^{-1} inclusion rate. Since ALT was lowered in plasma of catfish fed dietary autolysed yeast, it may be inferred that this natural and bioactive supplement could help protect the membrane integrity of the liver cells and optimize hepatic function within a specific range. Dimitroglou *et al*. (2010) reported enhancement the intestinal system integrity and immune function by yeast fraction components (i.e. β-glucans and MOS) in other species like sea bream. Future work will test this hypothesis in more detail to examine hepatic function of catfish fed AY in terms of both histomorphology and histocytochemistry for selected enzyme activities.

Also, the current study showed a trend for elevated serum AST and ALP. However, although not deemed to be statistically significant due to high variation in the data, these enzyme activities were higher in the control diet without hydrolysed yeast. We know that yeast contains quite high levels of nucleotides that may affect metabolism in animals and fish. It might be interesting in a future study to test if yeast nucleotides can be assimilated with liver hepatocytes and raise protein synthesis and metabolism and thus leading to enzyme activation of ALT, AST, and ALP. These may show some leakage into the systemic circulation of the catfish but may not be due to liver impairment *per se*. Exogenous dietary nucleotides as found in yeast play an important role in the repair and regeneration of damage in liver; since deprivation of nucleotides significantly reduces the hepatic protein synthesis rate as shown in the cirrhotic rat model by Perez *et al*. (2004).

Factors such as stress, contaminants, and diets can all play a role in disrupting the normal morphology and function of the gut. A deterioration in alimentary canal exposes the fish to opportunistic pathogens as an entry site to gain access to the rest of the body (Segner *et al.*, 2012). The current study observed that the perimeter ratio of catfish mid-intestine (indicative of surface for nutrient absorption) remained unchanged when fed with brewer's yeast autolysate. However, the abundance of mucous-secreting goblet cells in the mid-intestine were significantly elevated compared to those catfish that were not fed with yeast supplementation. This could suggest that autolysed yeast supplemented diets could enhance the intestinal barrier interface secretory dynamics of the catfish as in other fish species (Sweetman *et al.* 2010). The observed increase in the number of goblet cells found in the current study was comparable to the results reported by Zhu *et al.* (2012). The authors found that 40 g kg^{-1} dietary

supplementation of yeast polysaccharides in channel catfish (*I. punctatus*) increased goblet cells count by up to 40 %. It was also reported that the channel catfish had higher intestinal folds in yeast polysaccharide supplementation treatment groups, which was not observed in the present study by the perimeter ratio of mid-intestine measurements.

The morphological examination of the catfish gut revealed there were enhancements in the abundance of intraepithelial leucocytes (a component of gut-associated lymphoid tissue) and goblet cells in autolysed yeast supplemented dietary group. This could be attributed to the higher exposure of nutraceutical compounds (e.g. β-glucans, mannan-oligosaccharides and nucleotides) present in the degraded cell wall of autolysed brewer's yeast. Furthermore, the trends in the proliferation of goblet cells and IELs in AY supplemented groups might be associated with increased immune response, however, further study is required to validate this assertion. While the present study has shown brewer's yeast hydrolysate can affect the physiological function of the fish intestinal tract, Zhou *et al*. (2018) found that degraded yeast can also decrease several potential pathogen species (*Plesiomonas, Mycoplasmas, Synechococcus* and *Peptostreptococcus*) in the gut of largemouth bass. Further assessment on effects of autolysed yeast on African catfish gut microbiome would be warranted, in order to fully appraise this functional feed ingredient as an enhancer of gut robustness.

It could be concluded from this feeding study that dietary supplementation of autolysed brewer's yeast can improve growth performance and enhance intestinal morphology in African catfish, *C. gariepinus*. This can have important consequences in the health management of the species in intensive production systems. The use of prophylactic farming strategies associated with enriched diet formulations using autolysed brewer's yeast would not impair the liver function and may mitigate husbandry-related and environmental stresses. This will help to minimise the use of therapeutic agents with obvious economic and environmental benefits for this important farmed fish species in Africa and other parts of the African catfish farming regions of the world as in Asia for related species.

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Data Availability Statement

559 The data published in this research study is available upon reasonable request from the
560 corresponding author, adeoyeaa@funaab.edu.ng.
561

562 # Tables

563 **Table 1**. Nutritional composition of autolysed brewer's yeast (g kg^{-1}, dry weight)

Variables (g kg^{-1})	**Autolysed brewer's yeast**
Crude protein	500.00
Crude oils and fats	30.00
Crude fibre	10.00
Crude ash	66.00
Lysine	36.00
Methionine	8.00
Glutathione	6.00
Choline	3.20
Nucleic acid protein (in CP)	120.00

564 The autolysed brewer's yeast (Leiber CeFi® Pro) was supplied by Leiber GmbH, Bramsche,
565 Germany
566

567 **Table 2**. Formulation and composition of the experimental diets (g kg^{-1}, dry weight)

Ingredients (g kg^{-1}**)**	**Control**	**3-AY**	**6-AY**	**10-AY**
Fish meal (72% CP)[a]	100.00	100.00	100.00	100.00
Poultry meal (66% CP)[a]	200.00	200.00	200.00	200.00
Shrimp meal (56% CP)[a]	50.00	47.00	44.00	40.00
Soybean meal (45% CP)[a]	350.00	350.00	350.00	350.00
Maize flour[a]	200.00	200.00	200.00	200.00
Vegetable oil[a]	79.90	79.90	79.90	79.90
Vitamin mineral premix[b]	10.00	10.00	10.00	10.00
Autolysed brewer's yeast	0.00	3.00	6.00	10.00
Anti-oxidant	0.10	0.10	0.10	0.10
Binder (Cassava starch)	10.00	10.00	10.00	10.00
Total	1000.00	1000.00	1000.00	1000.00
Composition (g kg^{-1}***, dry weight)***				
Dry matter	906.70	908.70	904.70	907.30
Crude protein	389.00	389.00	386.00	391.80
Lipid	136.00	137.00	133.00	139.00
Ash	65.50	68.40	69.60	67.90
NFE	361.00	356.00	356.00	357.00
Crude fibre	43.90	43.50	49.40	40.20

568 [a]Ingredients were sourced from local feed ingredients' market (ABMN LTD, Ibadan, Nigeria). [b]Vitamin mineral
569 premix contains (per 2.5kg) 20,000,000IU vitamin A, 4,000,000IU vitamin D3, 200,000 vitamin E, 8,000mg
570 vitamin K3, 20,500mg vitamin B1, 15,000 mg vitamin B2, 19,500 mg vitamin B6, 15mcg vitamin B12, 90,000
571 mg Nicotinic Acid, 40,000 mg Pantothenic Acid, 500 mg Folic Acid, 600,000 mcg Biotin, 40,000 mg Choline
572 Chloride, 4,000 mg Iron, 500 mg Copper, 30,000 mg Manganese, 40,000 mg Zinc, 2,000 mg Iodine, 200 mcg
573 Selenium, 300,000 mg coated Vitamin C, 50,000 mg Inositol, 750 mg Cobalt, 50,000 mg Lysine, 50,000 mg
574 Methionine and 125,000 mg Antioxidant. CP, crude protein.
575

576 **Table 3**. Growth, feed efficiency and somatic indices of African catfish fed diets containing
577 different levels of autolysed brewer's yeast (AY) for 49 days (*n*=3, ±SD)

	Control	**3-AY**	**6-AY**	**10-AY**
IBW (g $fish^{-1}$)	22.00±0.71	22.20±0.85	23.50±1.41	22.30±0.47
FBW (g $fish^{-1}$)	91.00±1.90^{a}	106.05±6.46^{b}	98.12±8.21^{ab}	98.01±8.21^{ab}
Feed intake (g $fish^{-1}$)	90.69±10.11	91.16±4.68	87.48±6.10	88.73±2.41
MWG (g $fish^{-1}$)	72.37±3.65	84.18±5.94	76.34±4.80	78.40±6.10
PWG (%)	301.74±22.52	374.03±31.20	309.98±29.21	306.30±52.39
SGR (% day^{-1})	2.90±0.03	3.19±0.11	2.92±0.12	3.01±0.19
MGR (g $kg^{-0.8}$ day^{-1})	14.51±0.08^{a}	16.01±0.54^{b}	14.80±0.47^{ab}	15.12±0.91^{ab}
FCR	1.25±0.12	1.08±0.02	1.15±0.10	1.14±0.06
PER	1.53±0.15	1.82±0.06	1.67±0.18	1.70±0.13
Condition factor	0.72±0.07	0.84±0.10	0.82±0.09	0.81±0.10
HSI	1.06±0.11	1.12±0.16	1.30±0.12	1.18±0.22
VSI	10.19±1.15^{a}	11.55±1.44^{ab}	10.87±1.67^{ab}	12.95±0.54^{b}
Survival (%)	91.67±4.71	98.33±2.36	95.00±0.00	86.67±8.50

578 Values with different superscripts on the same row indicates there is a significant difference ($P < 0.05$). IBW,
579 initial mean body weight; FBW, final mean body weight; MWG, mean weight gain; PWG, percentage weight
580 gain; SGR, specific growth rate; MGR, metabolic growth rate; FCR, feed conversion ratio; PER, protein efficient
581 ratio; HSI, hepatosomatic index; VSI, viscerosomatic index.
582

583 **Table 4**. Haematological-biochemical parameters of African catfish fed diets containing
584 different levels of autolysed brewer's yeast (AY) for 49 days (*n*=3, ±SD)

	Control	**3-AY**	**6-AY**	**10-AY**
PCV (%)	35.00±2.78	36.30±1.76	35.50±1.00	37.30±1.04
Haemoglobin (g dL^{-1})	11.70±0.98	12.30±0.65	11.90±0.36	12.50±0.31
RBC (10^{12} L^{-1})	2.50±0.45	2.55±0.13	2.40±0.18	2.86±0.43
WBC (10^{9} L^{-1})	143.00±24.40	101.00±64.80	208.00±82.20	113.00±66.90
Neutrophil (%)	24.60±6.45	23.20±7.77	24.40±3.61	27.00±11.70
Lymphocytes (%)	72.20±6.43	68.30±8.13	71.50±3.12	70.50±11.40
Basophil (%)	0.67±0.76	0.33±0.58	0.67±0.76	0.33±0.58
Eosinophil (%)	1.50±1.32	2.00±1.50	2.00±1.73	1.00±0.76
Monocytes (%)	3.50±1.80	2.17±1.76	3.83±0.76	2.83±0.29
MCV (fL)	144.00±23.1	142.00±0.40	149.00±9.77	133.00±16.3
MCH (pg)	48.40±7.38	48.20±0.26	50.00±3.58	44.80±5.51
MCHC (g dL^{-1})	33.60±0.54	33.80±0.26	33.50±0.24	33.60±0.15
AST (IU L^{-1})	160.00±19.70	174.00±14.60	146.00±19.60	185.00±32.00
ALT (IU L^{-1})	$24.90±2.17^{a}$	$15.70±3.9^{c}$	$16.80±1.53^{bc}$	$22.40±2.67^{ab}$
ALP (IU L^{-1})	60.00±8.40	62.70±12.50	75.70±15.20	69.80±11.60

585 Values with different superscripts on the same row indicates there is a significant difference (*P < 0.05*). PCV,
586 packed cells volume; RBC, red blood cells; WBC, leucocytes; %, mean percentage of total leucocytes; MCV,
587 mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin
588 concentration; AST, aspartate transaminase (IU L^{-1}); ALT, alanine transaminase (IU L^{-1}); ALP, alkaline
589 phosphatase (IU L^{-1})

Table 5. Intestinal histology of African catfish fed diets containing different levels of autolysed brewer's yeast (AY) for 49 days (*n*=3, ±SD)

	Control	**3-AY**	**6-AY**	**10-AY**
Perimeter ratio (AU)	2.93±0.63	3.12±0.87	2.30±0.60	3.24±1.51
Goblet cells (per 100 μm)	4.78±0.87[a]	5.75±1.49[b]	6.32±1.28[c]	6.62±1.28[c]
IELs (per 100 μm)	42.00±7.33[a]	47.94±7.87[b]	53.71±8.23[c]	53.36±9.56[c]

Values with different superscripts on the same row indicates there is a significant difference ($P < 0.05$). AU, arbitrary units and IELs, Intraepithelial leucocytes

Figure Legend

Figure 1. Light micrograph of the mid-intestine of African catfish fed the Control (a), 3 g kg^{-1} AY (b), 6 g kg^{-1} AY (c) and 10 g kg^{-1} AY (d) diets; Goblet cells (arrows) and abundant IELs (arrowheads) are present in the epithelia. Abbreviations are E enterocytes, LP lamina propria and L lumen. Light microscopic staining: Periodic Acid Schiff, scale bar = 100 μm

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