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INFLUENCE OF ANTOX® PROBIOTIC, AS WATER ADDITIVE ON GROWTH PERFORMANCE, NUTRIENT UTILIZATIION AND BODY COMPOSITION OF THE AFRICAN CATFISH, *Clarias Gariepinus* (BURCHEL, 1822) FINGERLINGS

Umaru, J.[1] and Agbugui, M.O.[2]
1Department of Fisheries and Aquaculture, Federal University Dutsin-Ma
2Department of Biological Sciences, Edo University Iyamho, Nigeria
Correspondence Author's Email: umarujoel@gmail.com/08137121106

ABSTRACT
The study was carried out for three (3) months to determine the influence of a commercial probiotic Antox® which is a mono- strain probiotic consisting of live Saccharomyces cerevisiae at 4.125 × 10^6 cfu per 100ml on growth, nutrient utilization and body composition of Clarias gariepinus fingerlings. Fish fingerlings (7.6 – 7.9g) were randomly distributed into five triplicate treatment groups in fifteen plastic tanks (80L) at a stocking density of ten (10) fish per tank. The probiotic was administered in the culture water of the treatment groups; 0mls (control, T0), 0,5ml (T1), 1.0 (T2), 1.5 (T3), 2.0 (T4) ml probiotic/80l. Water quality parameters were regularly monitored. The growth performance, nutrients utilization and body composition of C. gariepinus fingerlings between probiotic treated groups were significantly increased (P < 0.05) with increasing dosage of the probiotic. The best Final Mean Weight (121.3g), Mean Weight Gain (113.5g), Percentage Mean Weight Gain (1456.5g), Feed Conversion Ratio (0.92g) and Protein Efficiency Ratio (2.6g), were recorded in probiotic treatment group T4 (2.0ml). Similarly, the highest increase in carcass crude protein (69.9g), moisture (10.34), ash (15.26g) and dry matter (29.46) were recorded in treatment group T4 (2.0ml). Probiotic (Antox®) is recommended for administration in C. gariepinus fingerlings culture water at 2.0mls/ 80L.
Key words: Probiotic, catfish, growth performance, carcass composition

INTRODUTION

African catfish, *Clarias gariepinus,* is of great economic importance to aquaculture in Nigeria because of their high market price, fast growth rate, its ability to withstand adverse conditions especially low dissolved oxygen, ability to practice aquatic and aerial respiration and resistance to parasites and diseases. Catfish production accounts for 80% total aquaculture production in Nigeria (Bolorunduro, 2016). One of the major challenges to increase fish production in the developing world, including Nigeria, is the improvement of production efficiency, which is hampered by high cost of imported feeds. Catfish feed constitutes over 80% of cost of production because it is mainly imported (AU-IBAR, 2013). The local feed has low digestibility, poor feed conversion efficiency with majority of them sinking to the bottom and are equally expensive. (AU-IBAR, 2013). Probiotics could be used to address the problem of low feed conversion efficiency and growth by improving food digestion and nutrient uptake.

A probiotic is "any microbial cell provided via the diet or rearing water that benefits the host fish, fish farmer or fish consumer, which is achieved, in part, by improving the microbial balance of the fish" (Llewellyn *et al*., 2014). Similarly, Verschuere *et al*. (2000) defined aquatic probiotics as "Live microorganisms that have a beneficial effect on the host by modifying the microbial community, associated with the host, by ensuring improved use of the feed or enhancing its nutritional value, by enhancing the host response towards disease, or by improving the quality of its ambient environment". The water probiotics contain multiple strains of bacteria like *Bacillus acidophilus*, *B. subtilis*, *B. licheniformis*, *Nitrobacter* sp., *Aerobacter* sp. and *Saccharomyces cerevisiae* (Ramasamy and Venkatasamy, 2015).

Even when accounting for less than 1% of the total microbial isolates in the host, yeast (*Saccharomyces cerevisiae*) can represent a major physiological contribution beyond what has been observed for probiotic bacteria. In fact, cell volume from yeast may be larger than those of bacteria by a hundredfold (Gatesoupe, 2007). Yeast is non-pathogenic, free of plasmid-encoded antibiotic resistance genes and resistant to bile and acidic pH (Abu-Elala *et al*., 2013). *Saccharomyces cerevisiae,* has been added directly to the water, administered as an additive in micro-particulated diets, or used alive to feed live food (rotifers or *Artemia*) as a possible vector to deliver it into the gut of fish larvae (Tovar-Ramirez. *et al*., 2010). There is paucity of information on the use of live yeast (*Saccharomyces cerevisiae*) as water additive in tank rearing of the African catfish fingerlings. This study was therefore designed to evaluate the efficacy of adding different concentration of Antox® probiotic consisting of live *Saccharomyces cerevisiae,* to culture tanks on growth, nutrients utilization and body composition of *Clarias gariepinus* fingerlings.

MATERIALS AND METHODS

Formulation of experimental diets

The ingredient/proximate composition of the experimental diet is presented in Table 1. The basal feed comprising standard amounts of fish meal, yellow maize, soybean meal, vegetable oil, salt, vitamin premix and starch, was formulated according to Pearson square method with pre-determined values of 42% protein content. All the feed ingredients were milled and integrated into computing the required quantities to make up 100 units of the feed. The ingredients were thoroughly mixed, then hot water was added until stiff dough was formed. The dough was placed into a grinder for thorough mixing and extruded in a pelletizing machine through 2.0 mm diameter strand in a commercial feed mill. The pellets were dried at ambient temperature (27 -30°C).and stored in airtight jars at room temperature. Furthermore, proximate composition of experimental diets were determined according to Association of Official Analytical Chemist (A.O.A.C. 1990).

One hundred and fifty (150) *Clarias gariepinus* fingerlings were obtained from the hatchery unit, Department of Biology, Ahmadu Bello University Zaria, Kaduna State Nigeria. The fish were acclimated for two weeks in a concrete tank during which they were fed 3% of their body weight with coppens pelleted fish feed (42% crude protein) twice daily, morning (8:00am) and evening (5:00pm). The physico-chemical parameters (water pH, temperature, electrical conductivity and dissolved oxygen) were determined using LaMotte fresh water aquaculture test kit (Model: AQ-2, Code 363303).

Determination of Growth performance and nutrient utilization

Growth parameters were calculated according to Panasea *et al*., (2018): as shown below;

Mean body weight Gain: Weight gain was determined between the final weight and initial weight of experimental fish.

Weight gain = Final weight -Initial weight

Specific Growth Rate (SGR);

It is the percentage rate of change in the logarithmic body weight and was computed as;

$$SGR = \frac{Log_{c\ final\ weight} - Log_{c\ initial\ weight}}{\text{Initial weight}} \times 100$$

Mean Percentage Weight Gain (MPWG):

The percentage (%) weight gain was determined as follows;

$$MPWG = \frac{\text{Mean weight gain}}{\text{Mean initial weight}} \times 100$$

Feed Conversion Ratio (FCR):

This was calculated using the formula

$$FCR = \frac{\text{Feed fed}}{\text{Fish weight gain}}$$

Protein Efficiency Ratio (PER):

It is calculated from the relationship between the increments in the weight of fish and protein consumed.

$$\textbf{PER} = \frac{\text{Mean weight gain (g)}}{\text{Protein intake}}$$

Where protein intake = $\frac{\text{Total feed consumed}}{\text{Crude protein in feed}} \times 100$

Mean Protein Intake (MPI);

$$MPI\ (g) = \frac{\text{Protein (\%) in feed} \times \text{total weight (g) of diet consumed}}{100}$$

Motality

$$\text{Motality} = \frac{N_t \times 100}{N_0}$$

Where N_t and N_0 are the initial and final numbers of fish respectively

Carcass analysis of experimental fish

Before the experiment, five fingerlings were randomly chosen for proximate analysis. After the 92 days experiment, 1 fishes per tank (in triplicate) were sacrificed for proximate analysis. The proximate composition of the fish carcass was carried out according to AOAC (1990).

Determination of Physico-Chemical Parameters

The water pH, temperature, electrical conductivity and dissolved oxygen were determined using LaMotte fresh water aquaculture test kit (Model: AQ-2, Code 363303).

Data Analysis

All data collected were subjected to One way analysis of variance (ANOVA) to test for significant differences among treatments using IBM SPSS version 23, followed by Turkey's' Post-Hoc test which was used to separate significantly different means. The level of significance set for treatments was $P \leq 0.05$.

Table 1. Ingredients composition of formulated diets (% Dry weight)

Ingredients (%)	Control	T1(0.5ml)	T2(1.0ml)	T3(1.5ml)	T4(2.0ml)
Fish meal	35	35	35	35	35
Soybean meal	17	17	17	17	17
Yellow Maize	43	43	43	43	43
Cassava	2	2	2	2	2
Vitamin premix (a)	1.5	1.5	1.5	1.5	1.5
Mineral premix (b)	1.5	1.5	1.5	1.5	1.5
Total	100	100	100	100	100

Vitamin Premix:

a. Provided the following vitamins: Vitamin A 8,500,000.00IU; Vitamin D3 1,500,000.00 IU; Vitamin E 10,000.00IU; Vitamin K3 1500.00mg, Vitamin B1 1600.00mg; Vitamin B2 4000.00mg; Niacin 20,000.00mg; Pantothenic acid 5,000.00mg, Vitamin B6 1,500.00mg; Vitamin B12 10.00mg; Folic acid 500.00mg; BiotinH2 750.00mg; Choline Chloride 175,000.00mg; Cobalt 200.00mg; Copper 3,000.00mg; Iodine 1000.00mg; Iron 20,000.00mg; Manganese 40,000.00mg; Selenium 200.00mg; Zinc 30,000.00mg; Antioxidant 1,250.00mg
b. Provided the following minerals (mg kg-1 diet): zinc (as ZnSO4·7H2O), 150; iron (as FeSO4·7H2O), 40; manganese (as MnSO4·H2O), 25; copper (as CuCl2), 3; iodine (as KI), 5; cobalt (as CoCl2·6H2O), 0.05; selenium (as Na2SeO3), 0.0

Table 2. Proximate composition of experimental diet

Parameters					
Crude protein	40.53	40.53	40.53	40.53	40.53
Crude fiber	1.19	1.19	1.19	1.19	1.19
Oil	4.69	4.69	4.69	4.69	4.69
Ash	8.04	8.04	8.04	8.04	8.04
Nitrogen free extract	45.10	45.10	45.10	45.10	45.10

RESULTS

Growth performance and nutrients utilization increased with increase concentration of the probiotic administration within the probiotic treatment groups as presented in table 1. The best FMW (121.3g), MWG (113.5g) and PMWG (1456.5%) was recorded in T4 (2.0ml/80L). Similarly, the better MPI (640.9g), FCR (0.92) and PER (2.6g), were recorded in T4.

Table 3 Influence of Antox® probiotic administration in culture water, on growth performance and nutrients utilization

Parameters	(T0) Control	T1(0.5ml)	T2(1.0ml)	T3(1.5ml)	T4(2.0ml)
IMW(g)	7.6±0.09^{a}	7.9±0.26 ^{a}	7.6±0.07 ^{a}	7.5±0.20 ^{a}	7.8±0.17 ^{a}
FMW(g)	90.5±2.62^{c}	100.0±1.42^{b}	103.8±0.47^{b}	115.5±2.54^{a}	121.3±1.62^{a}
MWG(g)	83.3±2.45^{c}	92.1±1.38^{b}	96.2±0.53^{b}	107.9±2.39^{a}	113.5±1.6 ^{a}
PMWG (%)	1102±45.5^{d}	1172±42.4^{cd}	1272.1±18.0^{bc}	1433.5±26.0^{ab}	1456.5±37.0^{a}
SGR	1.51±0.01^{c}	1.50±0.01d^{bc}	1.53±0.01^{b}	1.57±0.01^{a}	1.57±0.01b^{a}
MPI(g)	507.5±2.89^{d}	527.6±11.50^{cd}	585.4±5.78^{bc}	608.3±4.62^{bc}	640.9±11.55
FCR	1.63±0.02^{a}	1.52±0.04a^{bc}	1.57±0.01^{ab}	1.46±0.00^{c}	1.34±0.01^{c}
PER	2.2±0.0^{c}	2.4±0.09^{bc}	2.3±0.02d^{bc}	2.5±0.01^{ab}	2.6±0.04^{a}
NPU	17.6±6.42^{c}	20.3±0.63ed^{bc}	21.4±0.17b^{ab}	22.8±0.45^{ab}	24.6±0.75^{a}
SURV (%).	88.9±2.20^{c}	91.1±2.20^{c}	93.3±0.00^{ab}	95.5±2.23^{ab}	100±0.00^{a}

Values with different superscripts across the rows are significantly different at P ≤. 0.05. Data are means ± Standard error of mean (SEM).

Key;

IMW: Initial Mean Weight **SGR:** Specific Growth Rate

FMW: Final Mean Weight **MWG**: Mean Weight Gain

PMWG: Percentage Mean Weight Gain **MPI:** Mean Protein Intake

PER: Protein Efficiency Ratio **FCR:** Feed Conversion Ratio

SUR (%).; Percentage Survival

There was no significant difference (P < 0.05) in carcass composition (Table 3), within the probiotic treated groups and also, between probiotic treated groups and the control. However, the highest crude protein (69.08 ± 5.06g) was recorded in T4.

Table 4 Influence of probiotic administration in culture water, on Carcass composition of *Clarias gariepinus* fingerlings

Parameters	Control	T1(0.5ml)	T2(1.0ml)	T3(1.5ml)	T4(2.0ml)
Moisture	9.53±0.71 ^{a}	10.37±0.65 ^{a}	9.99±1.16 ^{a}	10.06±0.98 ^{a}	10.34±0.50 ^{a}
Dry matter	31.54±1.27^{a}	26.98±0.49^{b}	25.32±1.91^{bc}	27.89±3.11 ^{ab}	29.46±0.66 ^{ab}
Crude protein	62.48±1.53 ^{a}	62.18±4.12 ^{a}	62.61±1.56 ^{a}	64.90±2.10 ^{a}	69.08±5.06^{a}
OIL	10.15±0.71 ^{a}	9.41±0.55 ^{a}	10.02±0.35 ^{a}	9.51±0.52 ^{a}	8.89±0.39 ^{a}
ASH	14.84±0.64 ^{a}	15.84±0.63^{a}	14.89±0.20 ^{a}	15.82±0.51^{a}	15.26±1.07 ^{a}
Nitrogen Free extract	9.80±0.38 ^{b}	9.10±0.52^{b}	9.20±1.06^{b}	8.87±0.87^{b}	8.57±0.49^{b}

Values within each row not sharing a common superscript letter are significantly different. Data are means ± SEM of triplicate tanks.

Physico-chemical parameters

The mean values of water quality parameters recorded during the research period were temperature 26.4 ± 3.96^{0}C, dissolved oxygen 6.68 ± 0.76 mg/L and pH 7.21 ± 7.07.

Table. 5; Mean values of monthly in some physico-chemical parameters during the research period

Parameter	**Mean value**
Temperature (^{0}C)	26.4 ± 3.96^{0}C
Dissolved oxygen	6.68 ± 0.76 mg/L
pH	7.21 ± 7.07

DISCUSSION

Growth performance and nutrients utilization

Administration of single strain *Saccharomyces cerevisiae* (Antox® probiotic) in the culture water enhanced growth and nutrient utilization of *Clarias gariepinus* fingerlings with increase concentration in the present study. This agrees with EL-Dahhar *et al.* (2014), who reported a better growth, feed utilization, and survival rate of sea bream larvae administered liquid probiotics in the culture water, in comparison to the control.

A probiotic acts by reducing the feed conversion ratio, resulting in an increase in daily live weight gain, which is achieved through a natural physiological way and improvement of digestion by balancing the resident gut microflora as reported by Fuller, (1989); Enyidi and Onuoha (2016). This may explain relatively lower feed conversion ratio recorded in the probiotic treated groups. Yeast probiotic has beneficial effects of promoting a healthy gastrointestinal tract environment by nourishing the enterocytes, improving ideal mucosal development and reinforcing mucosal barrier function through maintaining epithelial integrity which improve growth and nutrient utilization (Aluwong *et al*., 2013). Yeast acts as a source of enzymes, i.e. amylase, protease and lipase that improve food digestion and consequently food utilization, resulting in growth increased. Yeast is also a very good source of vitamin B6 as reported by Mc Dowell (1989), which act as a stimulator of growth hormone (Hassan, 2007).

Carcass Composition

Concerning crude protein content, the result revealed that, all the treatments exhibited higher values compared to the control group. Similar result was reported in *Clarias garipinus* (El-feky *et al*., 2017) and *Mystus cavasius* (Banu, *et al*., 2020). The high carcass protein observed could be due to good protein retention for growth and also because the energy available in the diets was adequate to spare the protein (Banu, *et al*., 2020). Furthermore, the difference in values of carcass protein and lipid in the present study shows that, there were different levels of utilization which could be linked to the changes in their synthesis and deposition rate in the fish muscles (Aluwong *et al*. (2013). It is also, very likely that *Saccharomyces cerevisiae* administration assisted in improving protein syntheses which also increased growth of fish in all the probiotic treatment groups in comparison to the control.

Physico-chemical parameters

The water quality parameters in which the fish were reared were ideal for their survival and growth, especially for *Clarias gariepinus*, Sainai *et al*. (2015). Probiotics have been shown to be useful in improving water quality in a variety of ways. They increased the amount of dissolved oxygen in the culture environment by enhancing organic matter decomposition, lowering nitrogen and phosphorus concentrations, and controlling ammonia, nitrite, and hydrogen sulphide (Cha *et al*., 2013). In addition, warm water fish as shown in this work, grow best at temperature between 25- 32^0C (Boyd and Lichtkoppler, 1979).

CONCLUSION/ RECOMMENDATIONS

Administration of single strain *Saccharomyces cerevisiae* (Antox® probiotic) in the culture water improved growth and nutrient utilization of *Clarias gariepinus* fingerlings than the control group without probiotic administration. Similarly, there was increased in carcass crude protein in all probiotic treated groups in comparison to the control, although the increase was not significant ($P < 0.05$). The highest increase in carcass crude protein (69.08 ± 5.06) was recorded in T4

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