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Growth Response of Juvenile Catfish (*Clarias gariepinus*) Fed Diets Supplemented with *Lactobacillus sp.* Inclusion into Feeds and Cultured Water.

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SUMMARY

Growth and nutrient utilization by catfish (*Clarias gariepinus*) through the inclusion of *Lactobaccillus* in either the feed or culture water were studied. A total of sixty three juvenile *C. gariepinus* average weight 17±1.2 g in a flow through system over a period of 42 days were fed with a conventional feed containing either *Lactobacillus sp.* added to feed (TF) or culture water (CW) and compared to control diet (without *Lactobacillus*) in a randomised complete design. The result revealed the values of feed conversion ratio (FCR) in TF, CW and control to be 0.82, 0.96 and 1.17 while specific growth rate (SGR%/day) values were 1.09, 1.12 and 0.93 respectively. No significant difference ($p>0.05$) was observed in feed intake, mean weight gain and relative growth rate between TF and CW. The inclusion of *Lactobacillus* in the diet of *C.gariepinus* juvenile gave better FCR an indication that profitable and sustainable aquaculture can be successfully achieved by the inclusion of *Lactobacillus* in feeds.

KEY WORDS: Bacteria, growth, haematology, probiotics, feed, *clarias gariepinus*. Fish, Feed, Probiotics, growth, Haematology

INTRODUCTION

Many feed ingredients are not fully digested by fish and livestock. Addition of enzymes or probiotics to feed can enhance digestibility of feed components (Doyle 2001). This allows feed producers to extend the range of raw materials used in feed, and also improves the efficiency of existing formulations. Probiotics are described as beneficial microorganisms employed in aquaculture in order to control disease, as well as supplements for improving growth and in some cases as a mean of replacing antibiotics in feeds. Some probiotics produces vitamins and detoxify the compounds in the diets or break down the indigestible compounds, which may lead into the nutritional improvement and stimulate appetite (Irianto and Austin 2002).

Cereals such as wheat, barley and rye are source of energy in animal feeds. Much of the energy remains unavailable to monogastric animals due to the presence of non-starch polysaccharides (NSP) which interfere with digestion. This also prevents animals own digestive enzymes to the nutritional components in cereals. Although, NSP can become solubilised in the gut and cause problems of high gut viscosity, which further interfere with digestion. The addition of selected probiotics or enzymes, can NSP, releasing nutrients (carbohydrate and protein) as well as reducing the viscosity of the gut

contents, overall effect is improved feed utilisation and healthy digestive system for monogastric animals.

Lactobacilli are non-pathogen to aquatic organisms and widely accepted as probiotics in aquaculture (Moriarty1998, Gullian *et al.* 2004). They produce antimicrobial substances (acids, hydrogen peroxide, bacteriocins) and enzymes (Ogunbanwo *et al.* 2003). Consequently, their application as probiotics may have positive nutritional effects on fish diet and reproduction. This study therefore investigated the effect of *Lactobacillus* as probiotic through administration via diets or culture water by comparative monitoring of *C. gariepinus* for nutrient utilization, growth and yield for economic viability.

MATERIALS AND METHODS

Bacterial culture propagation

Lactobacillus UF10 was obtained from the Department of Microbiology, University of Lagos, Nigeria culture collection. This bacterium was previously isolated earlier from the gastrointestinal tract of matured catfish cultured in the Department of Marine Science, University of Lagos, Nigeria. The 10^9 CFU/ml cells of *Lactobacillus* UF10 was inoculated into 10ml MRS broth medium (Oxoid, Hampshire, UK), incubated at 37 °C for 24 hours. The 10ml cultured broth was added into 90ml of sterile MRS broth (Oxoid, Hampshire, UK) and incubated at 37°C for 24hours and subsequently scaled up to obtain 250ml broth culture. Cells were harvested by centrifugation at 5000x g for 10 min, supernatant discarded and rinsed twice with sterile distilled water before suspension in 250ml to obtain 10^9CFU/ml culture meant for inoculation of each of the fish tank. The cell suspensions were inoculated into the fish feed at 2 % inclusion rate in feed. Coppens 3mm fish feed obtained from Coppens International Feed Manufacturing Company Holand was used for the experiment.

Fish Culture

A total of 63 juvenile fish with average weight of 17g per fish was purchased from a local fish farm in Alimosho, Local Government, Lagos State, Nigeria. Each fish was considered healthy based on examination of their physical appearance. The fish were acclimatised for 2 weeks at the nutritional laboratory of the Department of Marine Sciences, University of Lagos, Nigeria. The juvenile fish were randomly allocated on the basis of their body weight to 9 culture plastics measuring 40 x 56 x 31cm, filled with 25 l of dechlorinated water. Each treatment contain 7 fish per tank and 3 triplicate per treatment, they were then divided into 3 groups: (A-C) Fish to be fed control feed; (A) Fish to be fed with microbial laden feed; (B) Fish that had LAB added to their culture water (C). All fish culture treatments were kept under a natural photoperiod of an approximately 12/12 hour's light/ dark cycle. Each tank was continuously supplied with de-chlorinated tap water from an overhead reservoir tank fitted with a flow-through device system supplying water at 1.5 l min^{-1} with continuous aeration. On weekly basis the fish in each tank were bulk weighed using a digital scale(Camry EH5055) and the average from the 3 tanks on the same treatment were used to compute growth and other performance parameters. Water quality parameters (pH 7.3-8.0,Temperature 27.5-29 °C and DO 4.5-4.8 mg/l) were also monitored and ensured to be within tolerance level as previously stated by Aderolu and Akpabio (2009).

Feeding method and regime

The most popular and acceptable commercial fish feed Coppens™ (3mm) was used during the acclimatization and the feeding trials. The composition of the feed (in % dry matter) includes: crude protein 42%; crude fat 13%; crude fibre 1.9%; crude ash 9.5%. The juvenile fish in all the experimental tanks were fed *ad-libitum* three times daily (0800, 1200 and 1600hr) for 6 weeks. The average weight of the experimental fish was taken at the end of every

week and the weight of feed consumed per week was recorded per treatment group. Treatment one is the control without addition of *Lactobacilus*, treatment two had the probiotics *lactobacillus* added to feed (TF), while the third treatment had the probiotics added to the culture water (CW)

Carcass analysis

Duplicate samples were taken from the control and test samples for carcass proximate analysis using the methods of A.O.A.C(1990)

Haematological Analysis

Blood samples were taken at the end of the feeding trial (42days) using 2ml syringes from the caudal vein of a set of *Clarias gariepinus* juveniles from each treatment, taken to the laboratory for determination of haemoglobin (Hb), Packed Cell Volume (PCV), Total Plasma Protein and Blood Cholesterol level according to the methods of Joshi *et al.* (2002).

Growth and Nutrient Utilization Parameter

All fish per tank were weighed using digital electronic scale (Camry EK5055 Max. 5kg/11lb d=1g/0.05oz Guangdong, China) on a weekly basis after the commencement of the feeding trials. The effect of *Lactobacillus* doses supplementation on parameters of growth performance in relation to feeds were statistically analysed.

Statistical analysis

Analysis of variance test (ANOVA) and Duncan's multiple range test was used to evaluate the mean differences, which were deemed significant at $p < 0.05$.

Growth and Nutrient Utilization Parameters

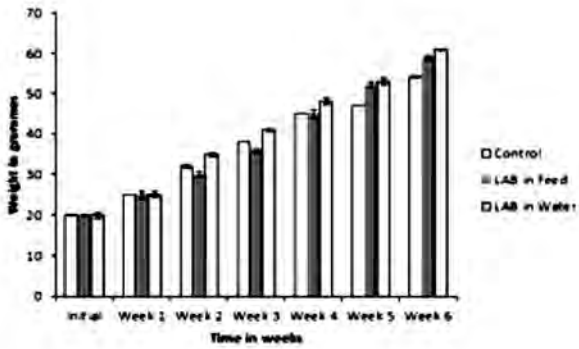
Mean Weight Gain (g) = Mean Final weight – Mean Initial weight. Specific Growth Rate (%day) = $(\text{Ln}W_2 - \text{Ln}W_1)/T \times 100$. Where W2 and W1 represent the mean final and mean initial weight (g) per fish in each tank, T= duration of the trial (days) Feed conversion Ratio (FCR) Dry Feed fed (g) / Fish live body mass gain (g) Protein Efficiency Ratio (PER) = mean weight gain (g)/ protein intake (g) The cost was based on the current prices of feed ingredients in Nigeria at the time of purchase. The economic evaluations of the diets were calculated according to the methods of New (1989)

Net Profit=Sales-Expenditure

Benefit Cost Ratio (BCR)= Total Sales

RESULT

At the end of the experimental period, relative growth rate of 197.36% and 188.36% were recorded for the test groups against 156.04% for the fish on control diet (Table 1). Although mean weight gain was not significantly different ($p>0.05$) between test groups but this compared to the control was significant ($p \leq 0.1$). Although the fish on control diet ate more feed (38.99g) compared to the test group (31.68 and



Data represent mean value of triplicate determination with error bars indicating standard deviation.

FIGURE 1. Comparative weekly weight gain of fish fed diets supplemented with LAB. with control diet; LAB in feed and LAB in water

34.23g), both the feed conversion ratio and protein efficiency ratio were significantly ($p<0.05$) poorer for the control diet (1.17 and 37.14) and best in the diet that had the *Lactobacillus* added to feed (0.828 and 52.65 respectively). On weekly basis, weight gain of the fish were on the increase

TABLE 1: Growth performance and nutrient utilization of *C. gariepinus* fed diets supplemented with LAB in in feed, culture water and control diet

Parameters	LAB in feed	Control	LAB in water
Mean Initial weight (g/fish)	20.63±0.25	20.66±0.31	20.63±0.11
Mean Final weight (g/fish)	59.47±1.76^{a}	52.16±1.45^{b}	61.53±2.62^{a}
Mean weight gain (g/fish)	38.83±2.01^{a}	31.50±1.81^{b}	40.77±2.32^{a}
Specific growth rate (%/day)	1.09±0.05^{a}	0.93±0.03^{b}	1.12±0.11^{a}
Average feed intake(g)	31.68±1.01^{b}	38.99±1.68^{a}	34.28±0.91^{b}
Voluntary feed intake (g)	8.45±0.61^{b}	10.27±0.56^{a}	10.00±0.51^{a}
Feed conversion ratio	0.828±0.05^{c}	1.17±0.11^{a}	0.96±0.08^{b}
Protein intake (g)	0.754±0.04^{b}	0.816±0.05^{b}	0.928±0.04^{a}
Protein efficiency ratio	52.650.68^{a}	37.140.50^{c}	44.29±0.72^{b}
Net profit value (N)	11425.2±294^{b}	12021.0±608^{a}	11351.7±556^{b}
Investment cost analysis (N)	289.5±0.78^{b}	290.28±0.76^{b}	291.70±1.52^{a}
Incidence of cost	0.249±0.13^{c}	0.346±0.18^{a}	0.288±0.06^{b}
Profit Index	1424.4±61^{b}	1374.0±134^{b}	1883.9±60^{a}

throughout the experimental period with the fish that had *Lactobacillus* added to their culture water having the highest weekly weight gain (Figure 1).

Both the gross profit and the net profit value were not significantly different among the *Lactobacillus* treated group, but were all significantly different ($p \leq 0.1$) from the control diet. No mortality was recorded throughout the experimental period. The specific growth rate (%/day) values of 1.09 and 1.12 obtained for the test diets were significantly different from 0.93 recorded for the control group (Table 1).

The addition of *Lactobacillus* to either the feed or culture water caused an increase in PCV(%), HB(g/dl) and blood protein (g/dl) when compared to the control diet, but the values obtained for these parameters were not significantly different ($p>0.05$) between the control and the *Lactobacillus* laden feed diet.

While the lipid and ash percentage (14.80 and 0.20%) were significantly lower ($p<0.05$) for the *Lactobacillus* laden feed, the dry matter (DM) and crude protein percentage values of 75.22 and 30.81% were significantly higher ($p<0.05$) than both the control and the group that had the *Lactobacillus* added to the culture water (Table II).

TABLE II: Haematological parameters and carcass analysis of *C. gariepinus* fed diets supplemented with LAB either in feed or culture water

Parameters	Control	LAB in feed	LAB in water
Haematology			
PCV (%)	28.50±1.53 [b]	30.00±1.00 [b]	40±1.53 [a]
Hb (g/dl)	9.30±1.00 [c]	9.70±0.49 [b]	13±1.22 [a]
Serum Protein (g/l)	3.800.58 [b]	3.90±0.37 [b]	4.9±0.47 [a]
Blood Cholesterol Level (mM/l)	0.57±0.05 [b]	0.49±0.02 [c]	0.69±0.02 [a]
Carcass			
DM (gKg-1)	720±6.58 b	752.2 ±3.95a	655.5±2.27c
Crude Protein (gKg-1)	289.4±3.58 b	308.1 ±2.00a	294.4±5.45ab
Lipids (gKg - 1)	207±5.68' b	148.0 ±1.53c	262.6 ±1.91a
Ash (gKg-1)	2.4±0.66 b	2.0 ±0.15c	2.8±0.21 a

DISCUSSION

The results of this study showed the probiotic effect of *Lactobacillus* strain on the growth performance, carcass analysis and heamatological parameters of catfish fed with *Lactobacillus* either in culture water or feed compared to a control diet without *Lactobacillus*. Since the first use of probiotics in aquaculture, a growing number of studies have demonstrated their ability to increase the growth rate and welfare of farmed aquatic animals(Wang and Xu 2006).

Higher growth rate in terms of mean weight gain, relative weight gain and weekly weight gain obtained for the test diets could be attributed to the fact that probiotics are sometimes expected to have a direct growth promoting effect on fish either by involvement in nutrient uptake or by providing nutrients to the fish (Ringo and Gatesoupe 1998). All experimental fish had an increase in weight gain confirming the nutrient adequacy of all diets tested. The results from this study showed microbial supplemented diets had better growth rate than the control, this is in agreement with Douillet & Langdon(1994) that used microbial probiotic supplementation to increase the yield of Pacific Oyster.

Probiotics are suspected to stimulate appetite and improve nutrition by production of vitamins, detoxificaion of compounds in the diet, and by breakdown of indigestible component(Williams 1991, Roth 2000).

Although, the fish on control diet ate more than the treated group, but this did not translate into better FCR. Similar results were reported by Prabhu *et al.* (1999), lower FCR (1.58) was obsterved in the test diets of *Oreochromis niloticus* supplemented with *Micrococcus*

luteus compared to FCR value of 1.69 obtained for the control group. On the overall, the specific growth rate, protein efficiency ratio and feed conversion ratio of the treated groups were better than the control, this attested to the fact that *Lactobacillus* spp has a growth promoting effect on fish, this result is also in agreement with the findings of Wang & Zirong (2006). No mortality was recorded in the control and the *Lactobacillus* supplemented diet groups. Tovav-Romirez *et al.* (2004) had earlier noticed that larvae of sea bass fed with probiotics feed had better survival rate than the control group without probiotics and claimed that the *Lactobacillus* fed larvae had improved immunity to pathogenic microorganisms,this could be responsible for the for the zero mortality rate in probiotic *Lactobacillus* fed group in our study.

Results of this study showed that *Lactobacillus* in the diet of *Clarias gariepinus* caused an increase in most haematological parameters. This result was in agreement with Irianto & Austin (2002) who reported increase in erythrocytic count of fish fed on probiotics bacteria than control group. The result of the biochemical index indicated an elevated level PCV and Hb ($p<0.05$) in the treated groups compared to the control, similar findings was also reported by Choudhury *et al.* (2005).
The proximate composition of fish carcass on both diets agreed with the work of Rosvitz *et al.*(1998) Both the dry matter and lipid content decreased, while the crude protein content of the *Lactobacillus* fed fish was on the increase. Decrease in carcass lipids and increase in carcass protein might also explain why the blood protein increased but the blood cholesterol level decreases, similar pattern of result was obtained by Moharrerey (2005) when he fed Malic acid to both male and female broiler chicks.

In conclusion, *Lactobacillus* strain of bacteria could be seen as having growth promoting effect on *C. gariepinus* when added directly to feed or culture water with a tendency of improving nutrient utilization, carcass proximate composition and improved haematological parameters in juvenille catfish.

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