The growth rate and histology of Catfish (*Clarias gariepinus*) Juveniles fed Antibiotics (Oxytetracycline and Furasol) treated Feed

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Abstract: Effects of Oxytetraxycline (OTC) and Furasol (FRS) on the growth performance and stomach wall lining of *Clarias gariepinus* were investigated over a period of 10 weeks. The control group (CTR) was fed with Diet 1 which contained no treatment while antibiotics were mixed with 100 g of commercial feed at the concentrations of 0.20% OTC (Diet 2), 0.4% OTC (Diet 3), 0.15% FRS (Diet 4), and 0.3% FRS (Diet 5). Fish were fed to satiation twice daily (0830 and 1600 h) while their water was changed every other day. With the exception of fish fed Diets 3 and 4, there was no significant difference ($p > 0.05$) in the average weight gain (AWG) and specific growth rate (SGR) in treated fish compared with CTR. At 0.20 and 0.40 % OTC, SGR of fish were 2.59±0.03 and 2.1±0.21 respectively while at 0.15 and 0.30 % FRS, SGR of fish were 1.67±0.07 and 2.57±0.06 respectively. Average feed intake (AFI) and feed conversion ratio (FCR) were not significantly difference ($p > 0.05$) between the CTR group and other experimental fish with the exception of fish fed diet 4. The best (0.73±0.07) and least (1.35±0.10) performances for FCR were recorded in fish fed diets 4 and 1 respectively. Severe impairments were observed on the stomach lining at 0.40% OTC and 0.30% FRS compared with mild impairments that were recorded at 0.20% OTC and 0.15% FRS inclusion levels. Implications of these findings are discussed. **The growth rate and histology of Catfish (*Clarias gariepinus*) Juveniles fed Antibiotics (Oxytetracycline and Furasol) treated Feed.** Lawal MO, Aderolu AZ, Ezenwanne DO. *Rep Opinion* 2012;4(6):37-42]. (ISSN: 1553-9873). http://www.sciencepub.net/report. 5

Key words: Oxytetracycline, furasol, antibiotics, growth performance, *C. gariepinus*

INTRODUCTION

The World aquaculture has grown tremendously during the last years becoming an economically important industry (FAO, 2005, 2007; Subasinghe *et al*., 2009). Today, it is the fastest growing food-producing sector in the world with the *greatest* potential to meet the growing demand for aquatic food (FAO, 2006) however, a persistent goal of global aquaculture is to maximize the efficiency of production to optimize profitability.

Aquaculture animals are colonized by trillions of microorganisms that have a symbiotic relationship with their host and are distributed in gill, body surface and gastrointestinal tract (Frenkiel and Mouëza, 1995; Armstrong *et al*., 2001; Izvekova *et al*., 2007). The majority of these microbes inhabits the gastrointestinal tract and plays an important role in nutritional, physiological and pathological events (Denev *et al*., 2009). Several studies have shown that the composition of fish intestinal micro biota is highly variable and is affected by the developmental stages, diet and environmental conditions (Spanggaard *et al*., 2000; Huber *et al*., 2004).

Antibiotics are drugs of natural or synthetic origin that have the capacity to kill or inhibit the growth of micro- organisms (NOAH, 2001). Antibiotic growth promoters (AGPs) are administered at low doses in the feed and they act by specifically reducing the number of pathogenic bacteria in the gut (Dafwang *et al*., 1987). This study was conducted to evaluate the effects of inclusion of antibiotics; Furasol (Furasolidone HCl) and Oxytetracycline on the growth performance and stomach lining of *C. gariepinus* juveniles.

MATERIALS AND METHODS

Experimental site

The experiment was carried out at the Nutrition Unit of the Department of Marine Sciences Faculty of Science, University of Lagos, Akoka.

Experimental Procedure

African catfish (Clarias gariepinus) juveniles were purchased from a fish farm at Cele-Egbe area of Ikotun, Lagos. The fish were put in plastic tanks (52.5 x 33.5 x 21 cm^3) under standard condition; temperature (27.5 - 29.5^oC), dissolved oxygen (4.5 - 4.8 mg/l), and pH (7.3 - 8.0) as described by Aderolu and Akpabio (2009). The fish were allowed to acclimatize for two weeks in plastic tanks, and fed with 2 mm Coppens feed before the commencement of the experimental work. The fish were sorted by size and reweighed using a sensitive scale (Camry EK 5055). A total of 150 catfish juveniles were randomly stocked into the tanks at the rate of ten (10) fish per tank on the bases of their

body weight into fifteen tanks, with an average weight of 19.00 g per fish.

Feed formulation and Feeding Trials

A total of fifteen tanks with five experimental diets were used. Diet 1, the control diet (CTR), is an untreated 3 mm imported floating Coppens feed. Diet 2, (3 mm imported floating feed treated with OTC antibiotic, at an inclusion rate of 0.2 g OTC/100 g feed). Diet 3 was treated at inclusion rate of 0.4 g OTC/100 g feed. Diets 4 and 5 were treated with FRS antibiotic, at inclusion rate of 0.15 and 0.3 FRS/100 g feed respectively. Feed were treated with antibiotics by dissolving known concentration of antibiotic in 3 ml of water, missing the solution with 100 g of feed and air dried. Each of the treatment was set up in triplicates.

Fish were fed with one of the five experimental diets twice daily (0830 and 1600 h) to apparent satiation for 10 weeks. The amount of feed consumed was recorded weekly. The water of the tanks was changed regularly at every 2 days to maintain good water quality while fish mortality was monitored daily. The fish were bulk weighed on weekly basis after which the mean body weight and mean feed intake were determined accordingly.

Histological analysis

Histological study was carried out at the Morbid Anatomy Department of the College of Medicine, University of Lagos, Idiaraba, Lagos according to the process described by Belelander and Ramaley (1979). Two fish from each of the tank were randomly selected the end of the tenth week. Fishes were dissected and pieces of tissue samples from the stomach lining were excised, rinsed in physiological saline and immediately fixed in Bouin's fluid before embedding in paraffin wax of melting point 56^{o}C. Section were cut using microtome and sizes of sections averaged 10 µm. Sections were left stained in haemoxylin for 10minutes and later transferred to acid alcohol for 20 seconds.

Sections were left in tap water for 10 minutes to remove the acid alcohol and transferred into 10 % aqueous eosin for 2 minutes, while excess eosin was washed in running tap water. Dehydration of sections with 70 %, 90 % and absolute alcohol was carried out. Finally sections were mounted in DPX chemical and dried at room temperature. Sections were viewed under binocular microscope after injecting 95 % ethanol between the glass slides for better light refraction (Ezenwa and Kusemiju, 1985). An ocular micrometer was mounted with the right eye piece, with a conversion factor of 0.006mm for each unit. This conversion factor was calculated from the graduated and absolute units of stage micrometer. The photomicrographs of the final stage of the sections were taken.

Growth and Nutrient Utilization Parameters

Growth and nutrient utilization indices were calculated as stated below:

1) Average Initial Weight (AIW) (g)

$$= \frac{\text{Total weight of fish at the start of experiment}}{\text{Total Number of fish}}$$

2) Average Final Weight (AFW) (g)

$$= \frac{\text{Total weight of fish at the end of experiment}}{\text{Total number of fish}}$$

3) Average Weight Gain (AWG)

AWG =Average final body weight (AFW) (g) - Average initial body weight (AIW) (g)

4) Specific Growth Rate (SGR)

$$SGR = \frac{(\text{Log}_e W_1\ (g) - \text{Log}_e W_2\ (g) \times 100}{T_2 - T_1\ (\text{day})}$$

Where, e = natural logarithm,

$T_2 - T_1$ = experimental period

W_1 = initial weight,

W_2 = final weight

5) Average Feed Intake (AFI)

$$AFI = \frac{\text{Feed intake for the Experimental Period}}{\text{No of days in the Period}}$$

6) Feed Conversion Ratio (FCR)

$$FCR = \frac{\text{Feed eaten in dry mass (g)}}{\text{Weight gain (g)}}$$

Statistical analysis

The data collected were statistically analyzed with one-way ANOVA using SPSS version 10.0 for windows and means were statistically compared for the significant difference ($p<0.05$) following Ogbeibu (2005).

RESULTS

The results of growth performance and nutrient utilization are presented in Table 1. There was no significant difference ($P > 0.05$) in the initial weight of the experimental fish. Also, no significant difference ($P > 0.05$) was recorded in the average final weight gain (AFWG) between diet 1(CTR) and other diets except diets 3(0.4% OTC) and 4 (0.15 % FRS). The fish fed CTR recorded the highest value (117.62 ± 2.29 g) for AFWG while the least value (61.66 ± 3.39 g) was recorded for fish fed diet 4(0.15 % FRS). Similarly, average weight gain (AWG) and specific growth rate (SGR) followed the same trend. At lower concentration of OTC (0.20 %, diet 2)

inclusion in the fish diet, AFWG of fish was 116.89 ± 3.23 g while at higher concentration (0.40 %, diet 3) it was (89.59 ± 13.86 g). However, the converse was observed with Furasol inclusion in the fish diet; at lesser concentration (0.15 % FRS, diet 4), fish AFWG was 61.66 ± 3.39 g while at higher concentration (0.30 % FRS, diet 5), AFWG of fish was 115.33 ± 5.56 g. Similar pattern was recorded for AWG and SGR of fish. There was no significant difference (P > 0.05) in the average feed intake (AFI) and Food conversion ratio (FCR) of the test fish with the exception of fish fed Diet 4. The best performance in food conversion ratio (FCR) was recorded in fish fed diet 4(0.15 % FRS, 0.73 ± 0.07) while the least performance was recorded in fish fed diet 1 (CTR, 1.35± 0.10).

The results of histological analysis of stomach wall are presented in Plates 1 – 8. The Photomicrograph of a normal gastric tissue or stomach lining consisting of an inner mucosa (blue arrow), middle smooth muscle cell layer (black arrow) and outer serosa are shown in Plate 1(Fish on CTR). At minimum concentration of OTC (0.2 %/100 g of feed), mild changes were observed in the stomach lining of the *C. gariepinus* juveniles. The mucosa wall of these fish were intact and well distinct, but focal degenerative epithelium changes and glandular necrosis were present (Plate 2). However, at a higher concentration of OTC (0.4 %/100 g of feed), severe focal degenerative changes and mucosal necrosis were observed (Plate 3), with diffuse area of necrosis of the muscular walls and mucosa lining of the tissue (Plate 4). At minimum concentration of FRS (0.15 %/100 g of feed), there were moderate changes on the stomach wall. The glands of the stomach were pulled away from the muscle walls and disconnected from the underline muscularis propria while glands also appeared necrotic (Plate 5). Fragmented walls and glands with necrosis were also observed in Plate 6, while glands were also separated from the muscle (Plate 6). At maximum concentration of FRS (0.30 %/100 g of feed), severe changes were observed in the stomach lining. Gland cells were vacuolated; the glands were partly damaged and necrotic while vacuoles occurred everywhere (Plate 7). Mucosa walls and muscles were necrotic while erosion of the mucosa (in viable area) was also noticed (Plate 8).

Table 1: Growth Performance and Nutrient Utilisation of *C. gariepinus* fed diets incorporated with Antibiotics (Oxytetracycline and Furasol)

Parameters	Control (Diet 1)	0.20% Oxy. (Diet 2)	0.4% Oxy. (Diet 3)	0.15% Furasol (Diet 4)	0.30% Furasol (Diet 5)
Average Initial Weight (g/fish)	19.00±0.00	19.00±0.00	19.00±0.00	19.00±0.00	19.00±0.00
Average Final Weight (g/fish)	117.62±2.29^{a}	116.89±3.23^{a}	89.59± 13.86^{b}	61.66±3.39^{c}	115.33±5.56^{a}
Average Weight Gain (g/fish)	98.62 ± 2.29^{a}	97.89 ± 3.23^{a}	70.59± 13.86^{b}	42.66± 3.39^{c}	96.33±5.56^{a}
Specific Growth Rate (%/day)	2.60 ± 0.27^{a}	2.59 ± 0.03^{a}	2.18± 0.21^{b}	1.67 ± 0.07^{c}	2.57 ± 0.06^{a}
Average Feed Intake (g)	74.01± 7.76^{ab}	72.64± 3.60^{ab}	62.13± 7.74^{ab}	58.56± 2.14^{b}	77.74 ± 3.88^{ab}
Feed Conversion Ratio	1.35 ± 0.10^{a}	1.35 ± 0.07^{a}	1.11± 0.08^{a}	0.73 ± 0.07^{b}	1.23 ± 0.01^{a}

Figures in each row with different superscript are significantly different (P < 0.05) from each other

Plate 1 (H&E x400): Photomicrograph of control (without antibiotics), showing a normal gastric tissue of *C. gariepinus* consisting of an inner mucosa (blue arrow), middle smooth muscle cell layer (black line arrow) and outer serosa.

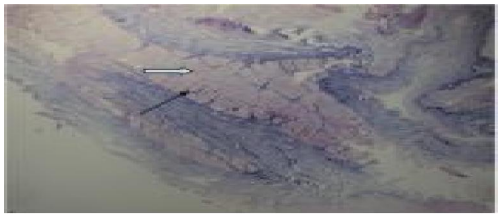

Plate 2 (H&E x400): Photomicrograph of *C. gariepinus* gastric tissue fed oxytetracycline (0.2%/100g of feed). Mucosa wall was intact and well distinct (black arrow), but focal degenerative epithelium changes of glandular necrosis (blue arrow) were present.

Plate 3 (H&E x100): Photomicrograph of *C. gariepinus* gastric tissue fed oxytetracycline (0.4%/100g of feed). Focal degenerative epithelium changes and necrotic mucosa areas (arrow) were observed with epithelial wall sloughing off (blue arrow).

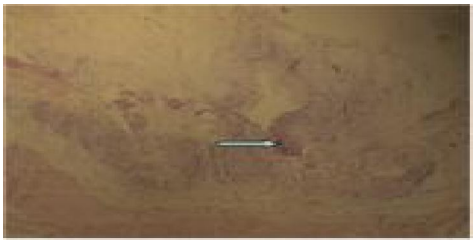

Plate 4 (H&E x100): Photomicrograph of *C. gariepinus* gastric tissue fed oxytetracycline (0.4%/100g of feed). Area of focal degenerative changes, diffuse area of necrosis of the muscular walls and mucosa lining (blue arrow) of tissue were observed.

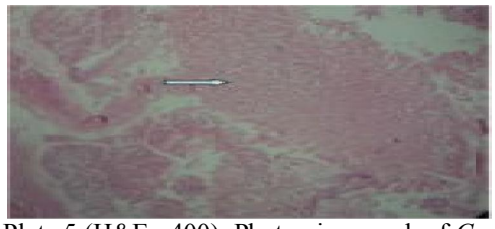

Plate 5 (H&E x400): Photomicrograph of *C. gariepinus* gastric tissue fed furasol (0.15%/100g of feed). Glands were preserved, but pulled away from the muscle walls and generally disconnected from the underline muscularis propria. Glands appeared necrotic (blue arrow).

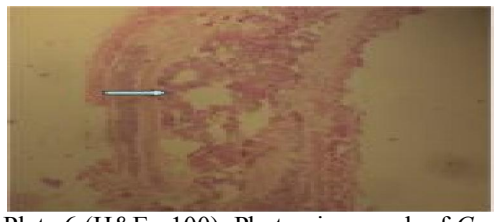

Plate 6 (H&E x100): Photomicrograph of *C. gariepinus* gastric tissue fed furasol (0.15%/100g of feed). Fragmented walls and glands with necrosis (blue arrow) were observed. Glands were also separated from the muscle.

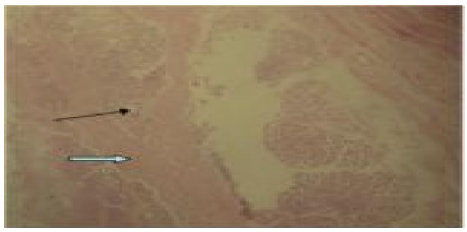

Plate 7 (H&E x400): Photomicrograph of *C. gariepinus* gastric tissue fed furasol (0.30%/100g of feed). Vacuolation of gland cells (black arrow), muscles were preserved or intact (blue arrow) but gland were partly damaged and necrotic.

Plate 8 (H&E x400): Photomicrograph of *C. gariepinus* gastric tissue fed furasol (0.3%/100g of feed). Mucosa generally necrotic (blue arrow) and eroded (in viable area). Muscles were also necrotic (tissue cannot be made out) but not detached.

DISCUSSION

The weight gain recorded amongst the fish fed the experimental diets treated with antibiotics (OTC and FRS) showed a dose dependent fashion. The non significant difference ($p < 0.05$) in weight gain recorded between the CTR and diets 2 and 5 may be due to better utilization of nutrients uptake in the diets. This result is in agreement with FAO (2005), who reported that antibiotics may prevent irritation of intestinal lining and may enhance the uptake of nutrients from the intestine by thinning of the mucosal layer. This result was also corroborated by Sarmah *et al.* (2006), that antibiotics have been used as growth promoters, and to improve feeds nutritional efficiency. Also, there was no significant difference ($p < 0.05$) in average feed intake of the three diets mentioned above, which could be the reason why fish fed those diets recorded similar specific growth rates. It was also observed that fish fed diets 2 (0.2 g OTC/100 g feed) and 5 (0.3 g FRS/100 g feed) recorded better feed conversion ratio compared to fish in the control group. This result is in agreement with Anderson *et al.* (1999) who observed that the increase in nutrient uptake in broiler chickens consequently increased the growth of the animal. The stimulated growth of the fish which fed diets 2 and 5 may be explained to some extent by a higher feed intake, but the enhanced digestibility of dietary components by the antibiotics might also contribute to the increased

growth.

At minimal concentration of OTC inclusion in the fish diet 2 (0.2%/100g feed), the average final weight gain of fish was greater than the weight at higher concentration. This could be due to the fact that at higher concentration, antibiotics that eliminate disease-causing bacteria could also eliminate the beneficial ones, thereby leading to reduction in nutrient utilization in such animal and concomitantly reduced growth. This is supported by WHO (2006), that antibiotics inhibit or kill beneficial microbiota in the gastrointestinal (GI) ecosystem. This could be suggested that for effective growth rate to be achieved with the use of OTC, lower inclusion of it should be administered in the feed. However, the converse was observed with Furasol inclusion in the fish diet; the highest AWG was recorded at higher inclusion level (Diet 5). Indicating that Furasol is directly proportional to its concentration though, this could be as a result of deteriorative changes over long usage of the antibiotics.

The histological changes and impairments observed in the stomach lining of the experimental fish due to prolong dosage of the antibiotics result in mild to severe necrosis of the stomach lining. This impairment was the response of the fish to the direct effect of the high concentration of the antibiotics as reported by Pachcco and Stantos (2002). The result is further corroborated by Burka *et al.* (1997) who observed that antibiotic such as polymixins at topical use damage cell membrane.

CONCLUSION

Dose-response effects of antibiotics showed enhanced growth with mild effect of antibiotics on the stomach lining of the fish at minimal concentrations. Hence the inclusion levels of oxytetracycline and furasol at 0.2 and 0.15%/100g of feed respectively may be well tolerated in the diet of *C. gariepinus*.

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