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Research Article

Aeromonas Septicemia Infection in Cultured Nile tilapia, Oreochromis niloticus L.

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ABSTRACT

The study was conducted at Gudar Experimental Aquaculture farm, from November 2011 to March 2012. The main objectives of the present study were to investigate host-parasite relationship of Aeromonas bacteria in Nile tilapia (Oreochromis. niloticus) in aquaculture production system and evaluate pathogenicity and hematological change through experimental infection. The bacterial isolates were identified according to the biochemical reactions scheme. The pathogenicity test was performed for Aeromonas bacteria through intra-peritonium injection (IP) using 21/gauge sterile needle at ventral parts of the body of experimental fish. After 24 hours post injection of bacteria (1.4 x 106 CFU ml-1), all fish fingerlings became less active, showed decreased feeding rate and erratic swimming behavior, and remained at the bottom of the aquarium. External clinical signs observed were darkened dorsal part of body and mild hyperemia of the pectoral and ventral fin bases. Significant hyperemia on the base of the fins, severe fin rot and fin erosion were observed but there was no loss of scales, no sore development on the body part, and no excess mucus secretion around the gills and fin parts that are some of the clinical symptoms of Aeromonas infection. The results indicated that fish fingerlings developed apparent symptoms of Aeromonas bacterial infection and the intensity increased due to stress factors like local irritation, disturbances in the aquaria, handling and crowding and there by developed the clinical symptoms of the disease. In this study, all clinical findings except free mucus defecation, scale protruding, edema within scale pockets, skin ulceration and abdominal distension were observed. Macroscopic findings revealed the presence of pale gills, serious fluid accumulation in intestine, pale gonads with matured eggs, pale liver and enlarged gall bladder filled with emerald-black secretion. Fish injected with Aeromonas bacteria (1.4x106 CFU ml-1) showed increased WBC which was believed to be caused by migration of white blood cells from the spleen to the blood circulation and cause leukocytosis. This fact shows more production of leucocytes in the bacterial injected fish that enhances the defense mechanism of the fish.

Keywords: Aeromonas bacteria; Aquaculture farm; Nile tilapia

INTRODUCTION

Aquaculture is growing rapidly worldwide, with fish being the primary source of animal protein in many countries. In Ethiopia, aquaculture remains more potential than actual practice, despite the fact that the country's physical and socio-economic conditions support its development. Considering its significance in enhancing the livelihood of rural farmers' aquaculture gained importance in recent years in Ethiopia. It is in this context factors that limit aquaculture production has to be identified and measures taken to solve such issues. Among other factors the factor that assumes exceptional significance is bacterial diseases in cultivable fishes. Ventura and Grizzle (1987) reported that bacterial gill disease, hemorrhagic septicemia, and furunculosis are infectious diseases known to be precipitated by adverse environmental changes. The incidence of microbial pathogens, especially those of bacterial origin is one of the most significant factors affecting fish culture [1]. Bacterial diseases were considered as major cause of mortality among cultivable

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stocks causing great economic loss [2]. Although extensive work has been carried out on bacterial diseases elsewhere [3]. Not much work has been carried out on fish diseases especially microbial diseases.

Aeromonas bacteria are opportunistic gram-negative pathogen, rod shaped bacterium, naturally occurring in aquatic environment [4]. Aeromonas spp have been isolated from fishes infected with hemorrahagic septicemia, epizootic ulcerative syndrome, and tail and fin rot [5]. However, they frequently cause problems to both natural and cultured fish [6] leading to heavy economic loss [7] and deterioration of product quality [8].There is very little available literature on Aeromonas infection in fish of Ethiopia, and the present paper describes the Aeromonas infection in O. niloticus reared in Gudar farm in Ambo, Ethiopia. Excepting a very few studies on parasites and bacterial diseases of food fishes [9], there seems to be no detailed study on bacterial infection in fishes of Ethiopia.

MATERIALS AND METHODS

Collection of fish samples: Fifty five health O. niloticus fingerlings with a mean weight of 44.9g and mean length of 15.4cm, were collected from 300m2 Experimental Aquaculture Farm of Ambo University. Collected fish were brought to laboratory in clean and aseptic plastic bags with ice to biology laboratory for clinical and bacteriological examinations. Lesions on body surface were observed and data regarding the same were maintained.

Bacteriological examination: The collected fish were killed, and to prevent secondary infection by microorganisms, 70% ethanol was applied on the body surface of the fish before opening the body to remove the organs of the fish. Specimens for isolation taken from the gills, intestine, kidney and skin of fish were cultured on nutrient agar which was incubated at 250C for 48 hours. The concentration of heterotrophic bacterial load in different organs was counted under dark field colony counter. A random selection of colonies from various samples were picked up and re-streaked into agar media. The bacterial isolates were identified according to the biochemical reactions scheme provided by Bergey's Manual of Systemic Bacteriology [10].

Experimental infection: A total of thirty healthy O. niloticus fingerlings with a mean weight of 15.03g and mean length of 9.6 cm were equally divided into three groups: non-treated, treated with saline solution (0.85%) and treated with bacterial isolate. They were reared in rectangular glass aquaria (0.91 x 0.30 x 0.38) with 50-60L dechlorinated water. The fish were acclimatized for a week in the laboratory at 25 + 10C and during this period they were fed with pelleted feed at a rate of 3% of body weight per day. Water change was done once in two days and aerated.

The pathogenicity test was performed following the method of for Aeromonas bacteria. Each fish in the treatment group, 0.1ml of 1.4x104 CFUml-1 of the isolated Aeromonas bacteria was injected through intra-peritoneum (IP) using 21/gauge sterile needle at ventral part of the body according to Matushima and Mariano (1996) and Martins et al. [12]. A total of 10 fish were injected with 1ml sterile saline solution 0.85% using the same procedure and another 10 fish were held as control without injection. Twelve hours after injection, the fish were observed based on clinical manifestation and post mortem examination of internal organs.

Hematological analysis: Blood samples were drawn by severance of dorsal aorta of fish in the way of physical stunning to evaluate blood parameters. Hematocrit (Hct) values were obtained by centrifuging samples obtained ¾ directly via 75µl micro hematology tubes or blood capillary tube (Hematology centrifuge, SR10000, Thailand); blood glucose (touch screen glucose reader, Accu-Chek Advantage 2 Roche) according to [13]; total count of red blood cells (RBC) and white blood cell with haemocytometer [14]. For differential counting of leucocytes like neutrophiles, lymphocyte and monocytes, the smears were stained by Giemsa stain in which a hundred cells were counted for the establishment of each cell contents. While the values of hemoglobin and mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated .

Drug Sensitivity Test: The drug sensitivity testing of the isolated bacteria was performed by the disk diffusion techniques as modified by the Clinical and Laboratory Standard Institution (CLSI formerly NCCLS, 2004). The recommended antibacterial drugs like Amoxicillin (25µg), Ampicillin (10µg), Gentamycin (10µg), or Tetracycline (30µg) were applied on the agar surface using disk dispenser. The resistant and sensitive patterns of the isolates were determined by measuring the zone of inhibition diameter for each antibiotic disc and the interpretation of zone of inhibition was estimated and recorded.

Data Analysis: Bacterial density and hematological data were transformed into Microsoft Excel spread sheet before statistical analysis. The means of bacterial load were compared by using ANOVA followed by Tukey's post hoc for multiple comparisons. Statistical Package for Social Sciences (SPSS) software version 16.0 windows were used to analyze the data with the level of significance at $P<0.05$.

RESULTS AND DISCUSSION

The distribution of heterotrophic bacteria in different organs of O. niloticus is presented in Table 1. The results show that, bacterial population in gill filaments ranged from 1.06 ± 1.10x104 to 1.74 ± 10.8x104 CFU g-1; in intestine it ranged from 1.62 ± 11.2x104 to 2.82 ± 13.0x104 CFU g-1; in kidney it ranged from 0.82 ± 5.9x104 to 1.27 ± 22.6x104 CFU g-1; and in skin it ranged between 0.48 ± 5.0 and 0.77 ± 4.1x104 CFU g-1. The distribution showed no significant difference ($P>0.05$) during November and December but significantly increased in February ($P<0.05$). This may be related to ambient water temperature. Similar observation was reported by Ferguson et al. [16] who reported that changes in water parameters have a positive correlation to total heterotrophic bacterial population. Pal and Das Gupta [17] established that environment could influence the micro flora of the fish and pond system.

Month	Gill (x104 CFU g-1)	Intestine(x104 CFU g-1)	Kidney (x104 CFU g-1)	Skin (x104 CFU g-1)

November	1.06 ± 1.10	1.62 ± 11.2	1.06 ± 1.5	0.76 ± 2.6
December	1.13 ± 10.6	2.03 ± 3.40	1.07 ± 1.3	0.68 ± 4.9
January	1.14 ± 2.10	1.67 ± 9.80	0.82 ± 5.9	0.48 ± 5.0
February	1.49 ± 12.8	2.82 ± 13.0	1.60 ± 12.1	0.77 ± 4.1
March	1.74 ± 10.8	2.36 ± 14.5	1.27 ± 22.6	0.75 ± 4.0

Note: mean ± SE

Table 1: Total heterotrophic bacterial population in different organs of fish.

When bacterial count of different organs were compared, the count in the month of February was significantly increased ($p<0.05$) in intestine and gills. The presence of high bacterial population in the gills and intestine of fish might be due to the high metabolic activity of fish associated with increased feeding rates at higher water temperatures. The bacterial population observed in fish samples was highest in intestine. This may be due to the voracious feeding behavior of Tilapia which feeds on detritus, organic matter as reported by Beveridge et al. [18]. It is generally presumed that those bacteria which were consumed by fishes like tilapia are particle-bound [19]. Next to intestine, higher bacterial load was found in the gills. This is mainly because of the role played by gills in filtering microscopic organisms [20].

Evidences from recent studies of feeding in tilapia suggest that small particles are entrapped among the gill apparatus in a mucous film [21]. Histological studies of the buccopharyngeal cavity showed that the mucous cells of the gill rackers produce highly negatively charged mucus [22] which may facilitate flocculation and retention of very small particles. Next to intestine and gills, the bacterial count was more in kidney. Kidney being an excretory organ, the bacterial population might have trapped inside the kidney in the process of excretion. Skin showed the minimum bacterial load. The reason for minimum load of bacteria in the skin may be due to its frequent contact with the contaminated water and sediment in the aquatic media.

Experimental infection of Aeromonas bacteria

Clinical findings: After post injection either the inoculum or saline solution (0.85%), some of the fingerlings showed non clinical symptoms, inactive and surface breathing in the aquaria. After 12-16 hours following the injections of bacteria (1.4x106 CFU ml-1) all fish fingerlings become less active and mortality of a single fish was recorded. The rate of feeding decreased. After 24 hours, the dorsal part of the body became darkened and mild hyperemia of the pectoral and ventral fin bases were observed. Later, hyperemia extended to the pectoral and ventral fin bases as well as at the margins of the vent. Fin rot developed especially on the tips of the caudal fin which was more obvious.

The injected fish developed extensive hemorrhages at the ventral body part and the fish stopped eating and showed erratic swimming behavior, and remained at the bottom of the aquarium. Significant hyperemia on the base of the fins, severe fin rot and fin erosion were observed but there was no loss of scales, no sore development on the body part, and no excess mucus secretion around the gills and fin parts that are some of the clinical symptoms of Aeromonas infection (Figure 1a-e).

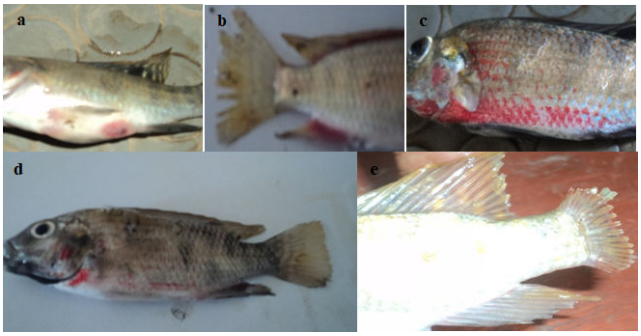

Figure 1: Photograph showing clinical symptoms on the external part fish injected bacteria: a) Hyperemia of ventral and inflammation of the vent; b) Hyperemia at the bases of the pectoral fin and fin rot in caudal part; c) Extensive hemorrhage and exophthalmia (protruding eyes); d) Darkness of skin and extensive hemorrhage on ventral part, scale detachment with excess mucus; d) Erosion of caudal fin.

Fish injected with normal saline solution (0.85%) did not develop visible clinical signs but a single fish died after 12 hours without showing any apparent clinical symptoms. The control group was also normal without any visible clinical symptoms. The results indicated that fish fingerlings developed apparent symptoms of Aeromonas bacterial infection and the intensity increased due to stress factors like local irritation, disturbances in the aquaria, handling and crowding and there by developed the clinical symptoms of the disease. It is reported that, fish disease related to Aeromonas infections can cause losses seldom exceed 50%, however, mortality is strongly influenced by the health status of fish, stress conditions, and virulence of infecting bacterial strain [23].

Similar results have also been reported by Leung et al.[24] and Roberts who reported that abrupt temperature change, handling, hauling, crowding, inadequate feed and oxygen are known to be the predisposing factors which contribute to the infection of Aeromonas spp. Such stressors are most commonly associated with environmental and physiological parameters that adversely affect fish under intensive condition. In conformity with the findings of earlier reports [25], motile Aeromonads appropriately receive much attention as pathogens of fish, and also as part of the normal intestinal micro flora of healthy fish. Therefore, the presence of these bacteria, by itself, is not indicative of disease and, consequently, stress is often considered to be a contributing factor in outbreaks of disease caused by these bacteria. In this study all clinical findings except free mucus defecation, scale protruding, edema within scale pockets, skin ulceration and abdominal distension was observed. Macroscopic findings revealed the presence of pale gills, serious fluid accumulation in intestine, pale gonads with matured eggs, pale liver and enlarged gall bladder filled with emerald-black secretion (Figure 2a-d).

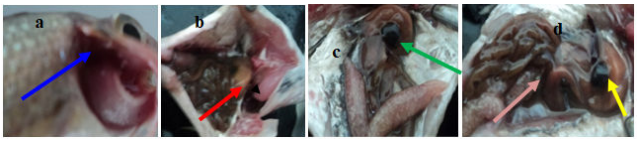

Figure 2: Photograph showing clinical symptoms of internal organs while post mortem examination: a) Pale colors of gills; b) Intense fluid in intestine; c) Pale colors of gonads containing matured eggs; d) Pale liver and enlarged gall bladder filled with emerald-black secretion.`

Haematological changes: Glucose concentration was not significantly different among the treatments (Table 2). White blood cells were significantly increased in fish injected with 1.4x106 CFU ml-1 of Aeromonas bacteria when compared to non-injected control. The hematocrit percentage, RBC and Hgb decreased in fish injected with 1.4x106 ml-1 of Aeromonas bacteria and injected with saline solution.

Treatment	PCV(%)	RBC(x10 6.µl-1)	WBC (x103.µl-1)	Hgb (mg.dl-1)	Glucose (mg.dl-1)
NI	26.4± 0.65	4.40 ± 0.1	1.36 ± 0.03	8.80 ± 0.21	49.8 ± 0.69
Saline	23.8 ± 0.62	3.96 ± 0.1	1.38 ± 0.07	7.94 ± 0.21	47.1 ± 0.56
1.4x106	14.0 ± 0.70	2.32 ± 0.1	2.46 ± 0.05	4.68 ± 0.22	45.9 ± 0.45

Table 2: Haematological parameters in O. niloticus non injected (NI), injected with saline solution, and injected with 1.4x106 CFU ml-1 of Aeromonas bacteria. Packed cell volume (PCV), Red blood cell (RBC), White blood cell (WBC), Hemoglobin (Hgb) and Glucose.

Differential leukocyte counts were characterized by predominance of neutrophils followed by lymphocytes (Table 3). Three types of leucocytes, namely lymphocytes, neutrophils and monocytes were identified in the circulating blood of O. niloticus. Mean corpuscular value also calculated from circulating blood. The number of lymphocytes in fish injected with 1x106 CFU ml-1 of Aeromonas bacteria was significantly ($P<0.05$) higher than that of the other treatments. There was a significant increase in the number of neutrophils in saline injected fish when compared with bacterial injection. On the other hand, a significantly reduced number ($P<0.05$) of monocytes was found after injection with 1.4x106 CFU ml-1 of Aeromonas bacteria. MCV, MCH and MCHC counts were not significantly different among the treatments.

Treatment	Lymphocyte (%)	Neutrophils (%)	Monocytes (%)	MCV (cµ)	MCH (µg)	MCHC (%)
NI	10.0 ± 0.25	86.7 ± 0.33	2.3 ± 0.21	60.05 ± 0.08	20.06 ± 0.03	33.3 ± 0.0
Saline	10.4 ± 0.54	87.5 ± 0.59	1.7 ± 0.30	60.05 ± 0.05	20.00 ± 0.00	33.3 ± 0.0
1.4x106	13.1 ± 0.48	85.7 ± 0.42	1.2 ± 0.32	60.36 ± 0.22	20.17 ± 0.11	33.3 ± 0.0

Table 3: Differential counts of leukocytes in the blood of O. niloticus non injected (NI), injected with saline solution and injected with 1.4x106 CFU ml-1 of Aeromonas bacteria. Lymphocytes, Neutrophils, Monocytes, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC).

The variation in the degree of haematological response is an important tool to fish health diagnosis and may vary according to stress stimulus, treatment, parasitic or infectious diseases [26]. Several factors like sex, age, and size, environmental and physiological conditions also have been reported to affect haematological responses in fish [27]. The assay is aimed to verify the effects of experimentally injected bacteria on blood parameters. In this study, the values of hematocrit percentage, RBC and Hgb of fish injected with Aeromonas bacteria were lower than the control. The results indicated that the total numbers of RBC, Hgb and hematocrit percentages were affected by the bacterial injection. The reduction in these parameters is an indication of severe anaemia caused by bacterial infection in the exposed fish. The anaemic response could be as a result of disruption in erythrocyte production [28], haemodilution, and destruction of intestinal cells involved in the production of vitamin B-12 used in the production of the hemoglobinportion of the red cells. Similar studies have also confirmed that decreased RBC, hemoglobin and hematocrit in chum salmon infected with V. anguillarum, in rainbow trout infected with Aeromonas bacteria and Streptococcus and in cichlid fish with epizootic ulcerative syndrome were previously reported. Contrarily to that observed in this study, glucose did not alter with the bacterial injections. It can be suggested that the inoculum neither was sufficient to alter this parameters nor affected the haemopoiesis.

On the other hand, fish injected with Aeromonas bacteria (1.4x106 CFU ml-1) showed increased WBC which was believed to be caused by migration of white blood cells from the spleen to the blood circulation and cause leucocytosis. This fact shows more production of leucocytes in the bacterial injected fish that enhances the defense mechanism of the fish. Changes in white blood cells and the differential counts, neutrophils, lymphocytes and monocytes indicated a stress condition in O. niloticus. The distribution of differential leucocytes was also affected. Fish injected with Aeromonas bacteria (1.4x106 CFU ml-1) were found to have the highest values of lymphocytes when compared with other treatment. Leucocytes belong to an important cell involved in the immune response. In fact, under severe infection, the organism produces more white blood cells. It can be added that lymphocytes have been reported as immune-competent cells. Fish injected with saline solution was responsible for increased number of neutrophils, possibly might be due to the effect of stress as also commented. Another result observed was decreased number of monocytes in fish injected with bacteria 1.4x106 CFU ml-1. This is because the cells are being recruited to the lesion site as commented.

REFERENCES

1. Austin B, Austin DA. Bacterial Fish Pathogens. Environ Sci.2007.
2. Azevedo TMP, Martins ML, Bozzo FR, Moraes FR. Haematological and gill responses in parasitized tilapia from Valley of Tijucas river, SC, Brazil. Sci agric. 2006; 63(2):115-120.
3. Beveridge MCM, Briggs MRP, Northcott ME, Ross LG. The occurrence, structure and development of microbranchiospines among the tilapias (Cichlidae: Tilapiini). Canadian J Zool. 1988; 66:2564-2572.
4. Camus AC, Durborow RM, Hemstreet WG, Thune RL, Hawke JP. Aeromonas Bacterial Infections, Motile Aeromonad Septicemia. South Reg Aqua Center. 1998.
5. Chen CY, Wooster GA, Bowser PR. Comparative blood chemistry and histopathology of tilapia infected with Vibrio vulnificus or Streptococcus iniae or exposed to carbon tetrachloride, gentamicin, or copper sulphate. Aquaculture. 2004; 239(4):421-443.
6. Cipriano CR. Aeromonas hydrophila and Motile Aeromonas Septicemias of fish. 2001.
7. Ellis AE. Immunity to bacteria in fish. Fish Shellfish Immunol. 1999; 9(4): 291-308.
8. Eshetu Yimer. Preliminarily survey of parasites and bacterial pathogen of fish. J Ethopia Sci. 2000; 23(1):25-33.
9. Ferguson HW. Systemic Pathology of Fish, Iowa State Press, Ames, Iowa. CAB Direct. 1989.
10. Ferguson CM, Coote BG, Ashbolt NJ, Stevenson IM. Relation between indicators, pathogen and water quality in an estuarine system. Water Res. 1996; 30:2045-2050.
11. Gardner GR, Yevich PP. Histological and haematological response of an estuarine teleost to cadmium. J Fish Res. 1970; 27:2185-2196.
12. Groff JM. Lapatra SR. Infectious diseases impacting the commercial salmonids. J Applied Aqua. 2000; 10(4): 17-90.
13. Hampl A, Jirasek J, Sirotek D. Growth morphology of the filtering apparatus of silver carp (Hypophthalmichthys molitrix) Microscopic anatomy. Aquaculture. 1983; 31(2):153-158.
14. Harbell SC, Hodgins HO, Schiewe MH. Studies on the pathogenesis of vibriosis in Coho salmon Oncorhynchus kisutch (Walbaum). J Fish Dis. 1979; 2(5): 391-404.
15. Karunasagar I, Otta SK. Disease problems affecting fish in tropical environments. J Applied Aquaculture. 2003; 13(4):231-249.
16. Khalil AH, Mansour EH. Toxicity of crude extracellular products of Aeromonas hydrophila in tilapia, Tilapia nilotica. Lett Appl Microbiol. 1997; 25:269-273.
17. Kompanets EV, Isaeva NM, Balakhnin IA. Bacteria of genus Aeromonas and their role in Aquaculture. Microbiol Zh. 1992; 54(4): 89-99.
18. Lafrentz BR, Lapatra Jones SR, Congleton GRJL, Sun B, Cain KD. Characterization of serum and mucosal antibody responses and relative percent survival in rain bow trout, Oncorhynchus mykiss (Walbaum), following immunization and challenge with flavobacterium psychrophilum. J Fish Dis. 2002; 25:703-713.
19. Len PP. Mesophilic spoilage of marine fish: bay trout (Arripis trutta), bream (Acanthopagrus butcheri) and mullet (Aldrichetta forsteri). Food Technol Aust. 1987; 39:277-282.
20. Leung KY, Yeab IV, Lam TJ, Sin YM. Serum resistance as a good indicator for virulence in Aeromonas hydrophila strains isolated from diseased fish in South East Asia. J Fish Dis. 1994; 18:511-518.
21. Martins ML, Tavares-dias M, Fujimoto RY, Onaka EM, Nomura DT. Haematological alterations of Leporinus macrocephalus (Osteichthyes: Anostomidae) naturally infected by Goezia leporine (Nematoda: Anisakidae) in fish pond. Arq Bras Med Vet Zoot. 2004a; 56(5): 640-646.
22. Martins ML, Pilarsky F, Onaka EM, Nomura DT, Fenerick J, Ribeiro K, et al. Hematologia e resposta inflamatória em Oreochromis niloticus submetida aos estímulos único econsecutivode estresse de captura. Bol. Inst. Pesca. 2004b; 30(1):71-80.
23. Martins ML, Moraes FR, Fujimoto RY, Onaka EM, Bozzo FR, Moraes JRE. Carrageenin induced inflammation in cultured Piaractus mesopotamicus (Osteichthyes: Characidae) in Brazil. Bol Inst Pesca. 2006; 32(1):31-39.
24. Matushima ER, Mariano M. Kinetics of the inflammatory reaction induced by carragin in the swim bladder of Oreochromis niloticus (Nile tilapia). Braz J Vet Res Anim Sci. 1996; (33):5-10.
25. Noga EJ. Fish disease diagnosis and treatment. Iowa. 2000.
26. Northcott ME, Beveridge MCM. The development and structure of pharyngeal apparatus associated with filter feeding in tilapias (Oreochromis niloticus). J Zool. 1988; 215:133-49.
27. Omoregie E. Changes in the haematology of the Nile Tilapia (Orochronis niloticus) under the effect of crude oil. Hydrobiol. 1995; 40(4):287-292.
28. Opuzynski K. The influence of the silver carp (Hypophthalmichthys molitrix Val.) on eutrophication of the environment of carp ponds. Part VII. Recapitulation. Rocz Nauk Roln. Ser. 1981; 94:127-151.