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Influence of Hydrogen Peroxide on Performance, Body Composition and Health Status Ofmonosex Males of Nile Tilapia Fingerlings

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Abstract: This study was designed to determine the impact of hydrogen peroxide levels on water quality, growth performance and health status of monosex males Nile tilapia (*Oreochromisniloticus*) fingerlings reared in concrete ponds. Three treatments were conducted in six concrete ponds (two replicates for each treatment). Fingerlings randomly distributed into the six ponds and initial body weight and length were 2.87, 2.82 and 2.94 gm and 5.49, 5.31 and 5.37 cm for the three treatment, respectively. The first treatment (control) did not treated with hydrogen peroxide (H_2O_2) while in the second (T2) and third (T3) treatments hydrogen peroxide was added at a rate of 350 and 500 mg\m^3, respectively. The obtained results revealed that, application of hydrogen peroxide in fish ponds significantly increased dissolved oxygen in pond water and also improved the other water quality parameters. Supplementation of fish ponds with 350 and 500 mg\m^3 H_2O_2 significantly improved final body weight, length, weight gain and specific growth rate and also significantly improved survival rate of *O. niloticus*. The highest values of protein and fat contents were recorded in T3 while control group (T1) gained the highest values for dry matter and ash contents in fish bodes.Moreover, showed isolates of some external parasites; *Ichthyophthiriusmultifiliis* and *Trichodina*. So it can be concluded that the addition of hydrogen peroxide in Nile tilapia ponds by concentration 350mg/m^3 lead to an increase in growth performance of Nile tilapia, antiparasitic effect and improved survival rates in addition to more economic efficiency.

Key words: Hydrogen Peroxide · Water Quality · Nile Tilapia · Performance · Health Status · Ichthyophthirius Multifiliis and Trichodina

INTRODUCTION

Intensive fish culture in recirculating systems with intensive water reuse depends on optimal water quality. When biosecurity measures fail, water treatment strategies have to be applied to suppress pathogens. When parasites become a problem, the use of formalin is common worldwide due to its high treatment efficiency without affecting the fish at normal dosage rates. However, due to the recent focus on formalin-related worker safety issues as well as potential environmental discharge effects of formaldehyde, there are needs to apply proper management practices. As well as introducing potential alternatives such as hydrogen peroxide which has been used in aquaculture as an immersion (Bath) treatment against many different disease-causing organisms, including external parasites, bacteria and fungi, on different species and life-stages of fish. Hydrogen peroxide is produced naturally in surface water by a photochemical process involving dissolved light-absorbing organic matter and molecular oxygen [1, 2].

Hydrogen peroxide (H_2O_2) is an obvious disinfectant candidate, for it has antimicrobial effects and easily degrades to harmless by-products. At present, the use of H_2O_2 in aquaculture is relatively moderate compared to the use of other disinfectants such as formalin and salt. In order to increase the practical use of H_2O_2 fish farmers have to become familiar with its benefits and risks. When added to water, hydrogen peroxide breaks down into oxygen and water over time and the formation of these by-products is one reason that hydrogen peroxide

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Table 1: Some water quality parameters of earthen ponds.

Variable	T1	T2	T3
Temp (°C)	20.94±0.012c	21.98±0.012a	21.62±0.012b
DO oxygen	4.59±0.235c	5.15±0.235b	5.18±0.235a
pH	8.11±0.004a	7.39±0.004c	7.53±0.004b
salinity(mg/l)	1.89±0.006b	2.16±0.006a	2.13±0.006a
Nitrite	0.69±0.002a	0.33±0.004b	0.32±0.004b
Nitrate	0.72±0.002a	0.36±0.004b	0.35±0.004b
Ammonia	0.044±0.0004a	0.029±0.0004b	0.03±0.0004b
Total hardness(mg/l)	1716.0±1.91a	1680.5±2.43b	1621.7±2.67c

is considered to be relatively safe for the environment. Hydrogen peroxide's highly reactive nature, similar in some respects to the reactivity of potassium permanganate, makes it ideal for use in aquaculture against numerous external fish-disease-causing organisms but unfortunately being too expensive [3].

Some findings have documented high treatment efficiency of H_2O_2 against ectoparasites when investigated under controlled conditions. That means that H_2O_2 could be used to control important ectoparasites such as *Ichthyophthiriusmultifiliis*, which causes white spot disease in fish [4].

Technical or food grade (35% active ingredient) H_2O_2 is presently considered a therapeutant of "Low regulatory priority" by the U.S. Food and Drug Administration (FDA) to control mortalities associated with external fungal infections on all species and life-stages of fish when administered at concentrations ranging from 250 to 500 mg/L. The treatment concentrations on the proposed label range from as low as 50 mg for fish to a maximum of 1,000 mg/L for fish eggs [5-15]. The disease claims presently included on the proposed H_2O_2 label include the control of mortality associated with saprolegniasis on freshwater-reared finfish eggs and the control of mortality associated with certain external bacterial infections on freshwater-reared finfish (Table 1). Preliminary studies and hatchery field trials with H_2O_2 suggested that H_2O_2 was also efficacious for the control of external parasitic infestations [8] and fungal infections [11] in a variety of cultured fish.

Moreover, the most economic use of water is in this study as we use agriculture drainage water which is considered waste water; meeting the countries policy for careful use of our share of water. Therefore, the study is a demonstration of an applied field trial for increasing both quality and quantity of fish production [16].

MATERIALS AND METHODS

Experimental Design: The present experiment had been conducted in a private farm at Tollumbat No. 7 (using agriculture drainage water) in Riyad area, Kafr El-Sheikh governorate, Egypt. Fish were randomly distributed in 6 concrete ponds (2.7, 7.0, 1.25m length, width and depth for each pond) to represent three treatments (Two ponds each). The first treatment (Control) was under normal fish culture conditions without hydrogen peroxide addition and the second treatment (T1) in which hydrogen peroxide was added at level 350mg/m^3/once a week). The third treatment (T2) hydrogen peroxide was added at level 500mg/m^3/once a week).

The ponds were stocked with all male monosex Nile tilapia, *Oreochromisniloticus* fingerlings at an average initial length of about 5.49, 5.31 and 5.37 cm and an average initial weight of 2.78, 2.82 and 2.94 g for control, T1 and T2, respectively. Each pond was stocked with 100 fingerlings/m^3. Nile tilapia fingerlings were fed on a commercial diet containing 35% crude protein six days/week at a daily feeding rate of 3% of an average fish-body weight twice at 9.0 am and 3.0 pm during the experimental period in each pond. At the end of the experiment (After 16 weeks) ponds were drained and fish were harvested, counted and the individual weight and length were measured.

Laboratory Analysis: Regarding water quality criteria; Hydrogen ion concentration (pH) was measured with pH meter (Model 25, Fisher Scientific). Total dissolved solids (TDS as g/l) were determined using a salinity-conductivity meter (Model, YSI EC 300).Temperature and dissolved oxygen were measured by using a digital oxygen meter (Model YSI 55). The concentration of total hardness (mg/l as Ca CO_3), total ammonia (NH_4-N+NH_3-N), unionized ammonia (NH_3-N), nitrite (NO_2-N) and nitrate (NO_3-N) were measured by methods described inDacie and Lewis [17].

Growth Parameters: Random samples of 60 fish from each treatment were taken biweekly till theend of the experimental period. Body measurements; body weight (g) and body length (cm). Growth performance parameters were calculated by the following equations:

Condition factor (K) = (Wt/L^3) × 100; where Wt is the total gutted weight of the fish (g) and L is the total length (cm) according to Schreck and Moyle [18].

Specific growth rate (SGR) was calculated according to Jauncey and Rose [19] as SGR= (Ln W2 – Ln W1 × 100) / t,

Daily weight gain (DWG) = [Average W2 (g) – Average W1 (g)] / t, where W1 = first fish weight in grams, W2 = final fish weight in grams, t= period in day.

Clinical Signs and Post-mortum Examination: Freshly collected fishes were grossly examined by naked eye and with the aid of hand lens to detect abnormal changes on external body surface, skin discoloration on the fins and gills, swellings, hemorrhages, ulcerations, parasitic cataract or exophthalmia. The examinations were carried out immediately according to the methods described by Woo [20].

Parasitological Examinations

Examination of Skin, Gills and Fins: Direct smears were obtained from the two lateral sides of the body, as well as the fins. The skin and fin scrapings were obtained; mixed with few drops of water and examined microscopically, using both low and high magnification powers. Each gill arch was dissected separately and placed in a Petri dish and examined by naked eye and by using magnification lens to detect the presence of spots, cysts of ciliated protozoa. The gill arch was placed on a slide and proceeded to cut away the cartilaginous arch using needles to separate gill filaments. Few drops of saline were added to obtain a uniform distribution under the entire cover slip; and to facilitate the observation of parasites under the microscope according to Lucky [21].

Mounting and Fixation of External Protozoa: Gills wereexamined immediately to avoid the disintegration or escape of the external protozoa. Smears for protozoal examination were taken very thin and allowed to dry for 2-3 minutes and fixed with absolute methyl alcohol for 5 minutes. The fixed slides were stained with freshly diluted Giemsa stain for 30-45 minutes and impregnated in dense canda balsam then left to dry in cubator at 37± 1C for 24 hours for driving any bubbles. Examinations of both fresh and stained smears were carried out under low, high objectives and oil immersion lens**es** accordingto the methods adopted byKabata [22].

Identification of Collected Parasites: The collected parasites were identified according to the identification keys of Paperna [23].

Histopathological Examination: Tissue specimens were taken from the infected areas (Gills and skin) fixed in 10% buffered formaline saline and dehydrated through different concentrations of ethyl alcohol, treated with xylol then blocked in paraffin boxes. Sections of 4-5 microns thickness were mounted on cleaned slides, stained by Haematoxyline and Eosin technique according to Schaperclaus*et al.*[24].

Chemical Composition: At experiment termination, three fish were chosen at random from each treatment and exposed to the proximate analysis of whole fish body according to the methods of AOAC [25]. Fish samples were oven-dried 105°C for 24 h, ground and stored at -20°C for subsequent analysis.Dry matter was determined after drying fish samples in an oven (105°C) for 24 h. Ash by incineration at 550°C for 12 hour. Crude protein was determined by micro-Kjeldhal method, N×6.25 (Using Kjeltech auto analyzer, Model 1030, Tecator, Höganäs, Sweden) and crude fat by Soxhlet extraction with diethyl ether (40 - 60°C).

Statistical Analysis: One-way ANOVA and Duncan's multiple range tests were used to evaluate the significant difference of the data which were computed by applying the computer program SAS [26]. Significant differences are stated at $P<0.05$. Data were statistically analyzed according to Duncan [27].

RESULTS AND DISCUSSION

Water Quality Parameters: Water quality parameters are presented in Table 1. Water temperatures were significantly varied among treatments throughout the experimental period and ranged from 20.94°C in control to 21.98°C in T1. Application of hydrogen peroxide caused a significant increase ($P<0.05$) in dissolved oxygen (DO) from 4.59 mg/l for control (T1) to 5.15 and 5.18 mg/l for T2 and T3, respectively. DO attained lower value in control, the highest values of DO in T2 may be attributed to good water quality conditions caused by hydrogen peroxide. Water pH was significantly ($P<0.05$) affected by treatments and it was higher (8.11 mg/l) in control followed by T2 and T3 (7.39 and 7.53 mg/l).

Oxygen is one of the critical factors for the aquatic life. Though some amount of Dissolved Oxygen (DO) is always there in the ponds, under semi-intensive fish farming the rate of depletion of DO is quite high. But such drops make the culture weak, susceptible to diseases and reduce their growth rate [28]. On the other hand, such conditions favor the growth of harmful bacteria and promote the anaerobic decomposition of feed and other organic matter including culture excreta and wastes leading to production of toxic materials [29].

As described by Larry *et al.*[30] H_2O_2 has been used to reduce the biological oxygen demand (BOD) and chemical oxygen demand (COD) of industrial wastewaters in fish farms for many years. While the cost of removing BOD/COD through chemical oxidation is typically greater

Table 2: Growth parameters of Nile Tilapia under both culture systems.

Variable	No.	T0	T1	T2
Initial body weight (g)	60	2.87±0.003b	2.82±0.012c	2.94±0.0032a
Final body weight (g)	60	22.549±0.079	28.37±0.151a	24.50±0.079b
Initial body length (cm)	60	5.49±0.057a	5.31±0.057a	5.37±0.057a
Final body length (cm)	60	10.85±0.062c	11.74±0.062a	11.17±0.062b
Daily weight gain (g)	60	0.18±0.0007c	0.23±0.001a	0.19±0.0007b
Specific growth rate	60	1.84±0.003c	2.06±0.005a	1.89±0.003b
Initial condition factor (K)	60	1.76±0.054a	1.91±0.062a	1.92±0.061a
Final condition factor (K)	60	1.77±0.026a	1.76±0.027a	1.76±0.025a
Survival rate (%)	76.13%	92.26%	93.05%	

than that through physical or biological means, there are nonetheless situations which justify its use. H_2O_2 can be used as a stand alone treatment or as an enhancement to existing physical or biological treatment processes.H_2O_2 can be used alone or with catalysts such as iron, UV light, ozone (O3) and alkali to oxidize BOD/COD contributing compounds in wastewaters**.**

$$2\ H_2O_2 \rightarrow 2H_2O + O_2$$

Boyd [31] reported that waters with a pH range of 6.5 – 9.0 are the most suitable for fish production.

The addition of hydrogen peroxide in fish ponds significantly decreased all the inorganic dissolved nitrogen, namely total ammonia (NH_3-N + NH_4+-N), nitrite (NO_2-N) and nitrate (NO_3-N) content in the water. The levels of these nutrients in the three treatments were significantly (P<0.05) different with highest values in control ponds.

The values of the total hardness found to be 1716.0, 1680.5 and 1621.7 mg/l, for T1, T2, T3, respectively. The obtained results showed that all parameters of water quality were in the suitable range required for Nile tilapia [32].

Anderson [33] reported that, H_2O_2 is a safe, convenient alternative for many sanitary processing operations and brings the assurance of a new life for farms and ponds. Also it generates and releases the lifesaving oxygen and helps maintain the Dissolved Oxygen levels of the pond. Hydrogen Peroxide will be very useful in increasing the Dissolved Oxygen levels, especially during like rainy days, cloudy weather, high bacterial count in water and summer when oxygen levels decrease in the ponds.

Growth Parameters: Growth and survivability which together determine the ultimate yield are influenced by a number of biological parameters such as genetic materials and managerial practices, including water and food quality, energy content of the food and stocking density [34].

As described in Table (2), the initial body weight found to be 2.87, 2.82 and 2.94g and body length 5.49, 5.31 and 5.37 cm for monosex Nile tilapia received T1, T2and T3, respectively and the differences in initial BW and BL among the different treatments were insignificant indicating the random distribution of fish around the different experimental treatments. At the end of the experimental period the averages of BW were 22.54, 28.37 and 24.50g and BL found to be 10.85, 11.74 and 11.17 cm for Nile tilapia for the three treatments T1, T2 and T3, respectively and the differences among treatments were significant (P<0.05). This result may be attributed to the positive effect of hydrogen peroxide in reducing stress and microbial load in fish ponds.

Hydrogen peroxide is naturally produced in the surface water through the photovoltaic process, which involves the solution of organic materials that absorb light and molecular oxygen, which leads to the production of phytoplankton [35,36]**.** Increasing the phytoplankton in fish ponds may be the cause of improving growth performance of aquatic animals [37].

Condition Factor: Condition factor of fish is essentially a measure of relative muscle to bone growth and the differing growth responses of these tissues to diet treatment may be reflected by changes in condition factor [38,39]. It is frequently assumed to reflect not only characteristics of fish such as health, reproductive state and growth but also characteristics of the environment such as habitat, water quality and prey availability. As described in Table (2), the averages of initial (K) for Nile tilapia were 1.76, 1.91 and 1.92. While at the end of this experiment became 1.77, 1.76 and 1.76 for control, T1 and T2, respectively and the differences between treatments were significant (P<0.05). K values in the present study relatively similar to those obtained by Abdel-Hakim *et al.*[40], Abdel-Hakim *et al.* [41] and Abdel-Hakim *et al.* [42].

Daily Weight Gain (DWG): The averages DWG of *O. niloticus* fingerlings were 0.18, 0.23 and 0.19 g/fish for the

three treatments; T1, T2 and T3, respectively (Table 2). Analysis of variance for the obtained data indicates that, the differences among treatments were significant ($P<0.05$). More phytoplankton in fish ponds may be the cause of improving DWG of reared fingerlings [37].

Specific Growth Rate (SGR): The averages of (SGR) of *O. niloticus* fingerlings were 1.84, 1.91 and 1.92 %/day for the three treatments, respectively (Table 2). These results indicate that, SGR for T3 recorded the highest values compared to control (T1) and T2 and the differences among treatments were significant ($P<0.05$). SGR values obtained in the present study in the normal range for the same specie obtained by many authors[43,44]. Rach*et al.*[10] also conducted tests on rainbow trout, channel catfish and bluegill sunfish using 15-min exposures, for which the NOEC values for mortality were approximately 2 to 3 times as great (1,132 to 3,396 mg/l). All of the above treatments were "dip" treatments, where fish were immersed in treatment water for the desired exposure period, then removed and placed into well water for recovery immediately after the exposure period. Specific growth rate was increased in the same study, the 24-h LC, values for rainbow trout, channel catfish and bluegill sunfish were 48,63 and 81 mg/L, respectively.

Survival Rate: Table (2) showed that, addition of Hydrogen peroxide in fish ponds showed the highest survival rate. In additional tests with fathead minnows (*Pintphalespromales*), bluegill sunfish (*Lepomismacrochirns*) and channel catfish (*Ictalurnspunctatus*) fingerlings, no mortality was observed for exposures of 566, 1,132and 1,132 mg, respectively, after 45-min exposures. Walleye (*Sander vitreum*) were the most sensitive species tested, with two fish mortalities being observed even at the lowest exposure concentration (1.13 mg) as described by Gaikowski*et al*. [13].

Rach*et al*. [9] investigated the toxicity of H_2O_2 to various species of freshwater fish and observed that, most species are quite tolerant to exposure. Rainbow trout (*Oncorhynchusmykiss*), brown trout (*Salmotrutta*) and lake trout (*Salvelinusnamaycush*) fingerlings showed no mortality and the increasing of weight gain at exposure concentrations of 283, 283and 1,132 mg/L, respectively.

Clinical and Post Mortem Examinations: Some protozoa are worldwide ectoparasites and have agreat role in causing fish parasitic diseases. The ectoparasites have an economic impact on fish production especially when it predisposes mortalities, poor body weight gain, spoiled fish meats plus reducing marketing value. Furthermore, the adverse environmental conditions in ponds may weaken the immune status of the fish and favor both growth and spread of ectoparasites. Thus, ectoparasites may cause skin injuries which facilitate secondary fish pathogens. Massive colonization of sessilian protozoan ectoparasites on the gills may disturb the gaseous exchange through the gills leading to asphyxia leading to mortalities.

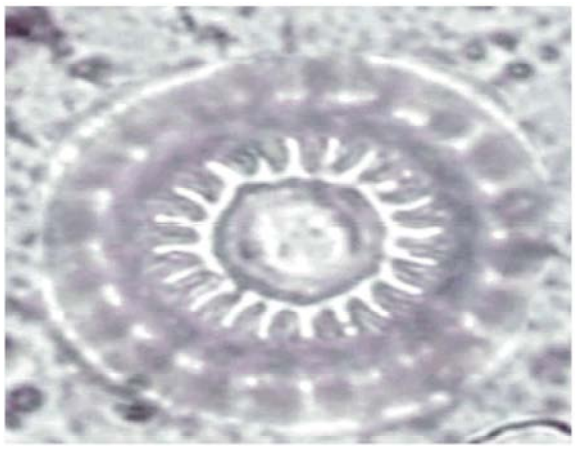

Fig. 1: Trichodinageimsa stain high power

Untreated fish group (T1) showed obvious restlessness, rubbing against hard objects in fish ponds, reduced appetite and emaciation. As well as, respiratory difficulties manifested by surface swimming and gasping of air. On advanced stages, the fish became dull with loss of escape reflex and sunk-deep in ponds and these results agreed to that obtained by Kenawy [45].

Parasitological Examination: Microscopic smears were taken from gills, skin and fins of examined fishes, showed some ciliated protozoan. The first ones with complex structures. On their adhesive discs with flat lateral projections were related to family *Trichodina*, Figure 1.

Microscopical smears from skin and fins of examined fishes showed large round to oval shape ciliated parasites from 0.5 up to 1 mm in diameter. They have macronucleus embedded in the protoplasm and characterized by a horseshoe, cresented or C-shape. The micronucleus is spherical, very small (Fig 2. Such ciliated protozoans were related to family *Icthyophthiridae,* genus *Icthyophthiriusmultifilliis*[47].In Egypt increasing the parasitic infestation attributed to using agriculture drainage water mixed with domestic sewage water. Both types are considered as an important stress ecological factors affecting the prevalence of either external or internal parasitic diseases in cultured fishes [48].

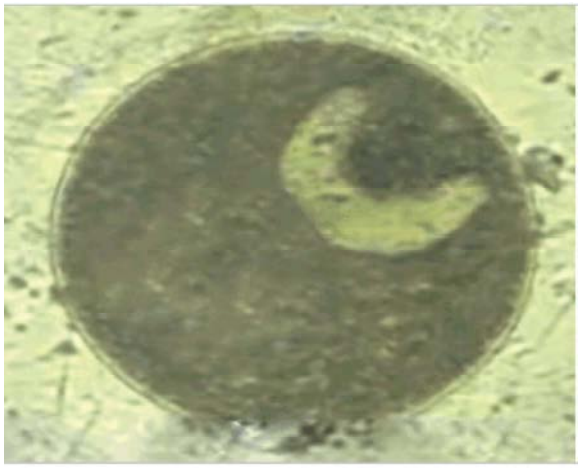

Fig. 2: Ich wet mount high power

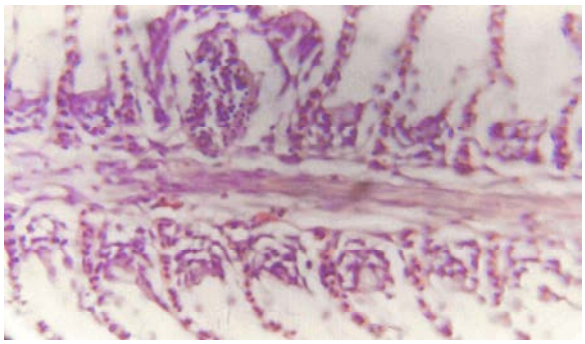

Fig. 3: Gill suffering Trichodinosis

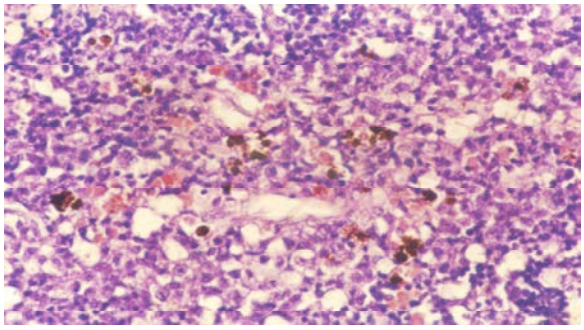

Fig. 4: Skin suffering Ichthyophthiriosis

Table 4: Least-square means and tested standard error of the factors affecting on chemical composition % DM basis of Nile tilapia

Variable	No.	T0	T1	T2
Moisture	6	70.02±1.22a	69.66±0.91a	69.67±1.09a
Protein	6	57.75±0.66b	59.56±1.28a	57.23±1.67b
E. ext.	6	28.23±1.05a	26.51±0.32c	27.66±0.13b
Ash		13.87±0.60a	13.39±0.73a	14.31±0.27a

Histopathological Findings: Histopathological alterations caused by ciliates on gills of examined fishes showed many degenerative changes in both primary and secondary gill lamellae in the form of hyperplasia of epithelial cell proliferations especially in the secondary gill lamellae. In addition, to leucocytic cell infiltrations. In addition to congestion and granulosis in the gill arch (Fig 3). This picture was also reported by Bullock [49], C rosbie and Munday [50] and Del Carmen *et al.* [51].

Moreover, *Ichthyophthiriusmultifiliis* infection caused multifocal coalescing areas of degeneration, epithelial necrosis and ulceration of the skin. Indeed, progressive cellular destruction and hyperplasia of epithelial cells especially the mucous cells, (Fig 4). The skin ulcers and gill damage might cause a portal of entry to secondary infections, indeed, the epithelial hyperplasia, mucous cell proliferations and necrosis in the gill tissues might limit the osmoregulatory gas and ion exchanges in the fish leading to metabolic disturbances being lethal to the host [52]**.**

Chemical Composition of Fish: . The main tissues involved in the whole-body growth are bones, muscles and adipose tissues. The relative development of these tissues is very important for the conformation of fish and thus its yield in processing [39,53]. Proximate analysis shows significant ($P<0.001$) effects in the three treatments. Fish in T2 released the highest values of protein while the control group released the highest values of fat (Table 4). Chemical analysis at the end of a feeding trial is frequently used to determine the influence of feed on fish body composition. According to Hanley [54] endogenous factors (Fish size, sex and stage of life cycle) and exogenous factors (Diet composition, feeding frequency, temperature etc.) affect the body composition of fish. It should be noted that within endogenous factors, the composition of the feed is only the factor, which could have influenced the chemical composition of fish body.

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