Research Article **Fouad *et al.*, 9(2): Feb., 2018:5701-5714]**
CODEN (USA): IJPLCP ISSN: 0976-7126

INTERNATIONAL JOURNAL OF PHARMACY & LIFE SCIENCES
(Int. J. of Pharm. Life Sci.)

Evaluation effects of hydrogen peroxide on health status and quality characterization of *Nile tilapia* (*Oreochromis niloticus*)

[1]A.F. Fath El-Bab, [2]Fouad I.M. [3], Shehab El-din M.T. and [4]Elgndy M.O.
[1]Animal Production, Faculty of Agriculture, Damietta University, Egypt
[2]Fish Processing and Quality Control Department, Central Laboratory for Aquaculture Research, Agriculture Research Center, Egypt.
[3]Fish Health Department; [4] Aquaculture Department; Central Laboratory for Aquaculture Research (Sakha Unit), Agriculture Research Center, Egypt.

Abstract

This study was based on investigation effects of hydrogen peroxide on health status, physicochemical, microbiological, Sensorial and nutritional quality for two stocking rates for intensive culture of tilapia mono sex males (10 and 20 fish /m^3). In which, three doses of hydrogen peroxide H_2O_2 were used; (0, 350 and 500mg/m^3) for 24 weeks to both stocks. Chemical, microbiological and sensory analyses were performed to investigate quality changes, and to determine the best sensorial and nutritional quality. The proximate, peroxide values (PV), thiobarbituric acid values (TBA), total bacterial count (TBC) and mould count were found statistically significant ($P \leq 0.05$), in the low density stocked fish treated by 350 mg H_2O_2. Finally, (after 24 weeks) 10 fish /m^3 recorded the best of nutritional quality and health to ensure safety by avoiding any threat to the health of the consumer. Protein%, fat% and ash% contents of low stocked density fish treated by 350 mg H_2O_2 samples had marked increase compared to the other treatment. The result indicated a much lower content of PV, TBA and TVB-N values in the low density stocked fish treated by 350 mg H_2O_2 in comparison with the other treated fresh fish samples other than control. These PV, TBA and TVB-N values were much lower than acceptable limit. The result also revealed that samples of low density stocked fish treated by 350mg H_2O_2 had lower TBC and mould count than the control. The organoleptic results showed that samples of low density stocked fish treated by 350mg H_2O_2 had the best acceptance, overall acceptability and were significantly different ($P \leq 0.05$), when compared to the control. Based on results obtained in this study, it could be concluded that, levels of intensive 10 fish /m^3 were better than 20 fish /m^3. In addition to that, fish treated by 350 mg H_2O_2 gave the best nutritional quality and health. While stocking 20 fish /m^3 treated with hydrogen peroxide was of best economic efficiency when compared to other treatments.

Key words:Hydrogen peroxide, Health, Fish

Introduction

Nutritional quality of a food is very important. The nutritional importance of seafood has increased substantially because of beneficial effects of eating seafood fats and oils **(Azam *et al*., 2004).** Seafood is also an important source of high quality and highly digestible protein and a respectable source of essential minerals **(Nettleton, 1992).** The nutritional quality of seafood is affected by body part of the seafood being consumed, method of handling, processing (including cooking at home), harvest, sex and species **(Krzynowek, 1988).**

Hydrogen peroxide (H_2O_2) is a perfect disinfectant item because it has antimicrobial effects. Moreover, it's easy degradable into harmless by-products. Now, the use of H_2O_2 in aquaculture is relatively moderate when compared to other disinfectants like formalin and salt **(Shehab *et al*., 2017).**

Hydrogen peroxide has also 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. The U.S. Food and Drug Administration (FDA) recently approved a hydrogen-peroxide-based aquaculture product, which has spurred greater interest in its use. In order to increase the practical use of H_2O_2, fish farmers need

*** Corresponding Author**
E.mail: Fouad_lab2020@yahoo.com

to aware about its benefits and risks. When added to water, hydrogen peroxide breaks down into oxygen and water over time, forming these by-products is one of the reasons that hydrogen peroxide is considered most safe for the environment. The highly reactive character of hydrogen peroxide is somewhat similar to potassium permanganate (an ideal therapy in aquaculture against many pathogenic organisms, but unfortunately too expensive), **(Hartmann, 2010).** In most aquaculture systems, permanent or periodic water disinfectants are used to improve fish rearing health status through preventing, controlling, and eliminating pathogenic microbes; as adding some chemicals, constant disinfectants, ultraviolet (UV) irradiation and ozone production, especially in modern recirculation systems (RAS) (**Pedersen *et al.*, 2015**).

Partial or complete recirculation aquaculture systems use a common technology, such as mechanical and microbiological filtration, without relying on UV or ozone disinfection; the latter being often neither logistical nor economically feasible. Such systems may be attacked by pathogens such as *Ichthyophthirius multifiliis* (the parasite responsible for white spot disease or Ich) and must then rely on effective water treatment strategies to avoid Severe losses (**Jørgensen *et al.*, 2014**) Formalin is an example of a commonly used therapeutic agent, however due to worker safety issues and negative environmental effects of the chemical in low retention time systems, the use of formalin may be forbidden, (**Pedersen *et al.*, 2014**).

Many researchers have examined various methods of using H_2O_2 in aquaculture (**Schmidt *et al.*, 2006**). Potency studies on H_2O_2 treatment have been recorded by (**Gaikowski *et al.*, 2004)**, analytical verification of H_2O_2 dosage by (**Saez and Bowser, 2001)**, application in biofilter aquaculture systems by **(Møller *et al.*, 2010)**. The antiparasitic effects were stated by **(Heinecke and Buchmann 2009),** especially against *Ichthyophthirius multifiliis* in a laboratory study. It should be considered that laboratory data have been obtained under conditions that are not directly compatible with practical agricultural operation. Therefore, this study aims to show the efficacy of H_2O_2 as a suitable water treatment solution in an intensive commercial freshwater Nile tilapia farm.

Material and Methods

This study was in a private fish farm (using agricultural drainage water) in Riyadh city of Kafr El-Sheikh governorate, Egypt. Fingerlings of Tilapia; *Oreochromis niloticus*, monosex males were propagated for studying two intensive culture systems of tilapia mono sex males (10 and 20 fish /m^3). In which, three doses of hydrogen peroxide H_2O_2 were used; (0, 350 and 500mg/m^3) for both stocks. Therefore, six treatments were designed in twelve concrete ponds 3x7x1 m^3, (two replicates for each treatment). At an average initial length of about 10.90, 10.80, 10.60, 11.00, 10.90 and 10.70 cm and an average initial weight of 18.80, 18.70, 18.50, 18.90, 18.80 and 18.40 gm. Fish were fed on a commercial diet containing 30% crude protein six days per week by 5% feeding rate of the average fish-weight at 9.00 am, 1.00 pm and 5.00 pm during the experimental period. Ration was broadcasted over water surface in the same place. Fish were considered satiated when it did not show any interest for feeding. Also, water quality measurements, fish sampling and data collected during harvest were recorded too. Equations and statistical methods for analyzing the growth performance parameters are illustrated.

Hydrogen peroxide is the chemical compound H2O2. Hydrogen peroxide is a highly reactive, strong oxidizing and bleaching (whitening) agent that is classified as corrosive at concentrations higher than 20%. Hydrogen peroxide has numerous non-medical and medical uses because of these properties. 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 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 with similar concerns regarding toxicity. The FDA-approved product, 35% PEROX-AID® (Eka Chemicals, Marietta, Georgia), is available at a strength of 35% weight/weight (e.g., 35% active ingredient). Over-the-counter products used for human health are typically sold at 3% active ingredient, (**Russo and Yanong, 2007**).

Analytical methods:

Moisture content, total protein, lipids and ash were determined according to methods described in **(AOAC, 1990)**. Growth parameters were recorded according to **(Schreck and Moyle, 1990)**. Specific growth rate (SGR) was recorded according to the method used by **(Jauncey and Rose, 1982).** Microbial Count was detected according to the methods described by **(Harrigan and MacCance, 1976) and (APHA, 1992).** Total volatile base-nitrogen (TVB-N) values were measured according to **(Goudlas and Kantians, 2005).** The results are expressed as mg

TVB/N 100 g-1 muscle. Peroxide value (PV) was determined in the lipid extract according to the method described by **(AOAC, 2005).** Results are expressed as milliequivalents oxygen per kg lipid (meq O2/kg lipid). Thio-barbituric acid (TBA) was determined calorimetrically by the Porkony and Dieffenbacher method as described by **(Kirk and Sawyer, 1991).** Results are expressed as mg malonaldehyde/kg (mg MAL/kg fish muscle). Clinical pathological examinations were determined according to methods described by (**Dumas and Biggs, 1972).** Clinical signs and post-mortem examination were described by **(Woo, 2002).** Parasitological examinations were recorded according to **(Lucky, 1977).** Mounting and fixation of external protozoa were recorded according to **(Kabata, 1985).** Identification of the parasites was described by **(Paperna, 1996).**

Samples were organoleptically evaluated for appearance, color, odour and overall acceptability every month during storage as described by **(Teeny and Miyauchi, 1972)** according to the following scheme:

Description	Score	Description	Score
Fair	4	Ideal	10
Poorly fair	3	Excellent	9
Poor	2	Very good	8
Very poor	1	Good	7
Repulsive	0	Fairly good	6
		Acceptable	5

Statistical analysis:

Statistical analysis of the collected data was carried out by a computer program (**SAS, 1996**) using the following fixed model: $xij = \mu.. + \alpha i + \beta j + \alpha\beta ij$.

Where: Xij = observation of the ijkl-th fish μ = Overall mean. αi = the effect of I-th level of intensive culture. βj = the effect of J-th levels of hydrogen peroxide.

$\alpha\beta ij$ = the effect of interaction between I-th level of intensive culture and levels of hydrogen peroxide J-th. Significance was determined according to Duncan's multiple range tests (**Duncan, 1955**).

Results and Discussion

Body composition:

Body composition is an important aspect of nutritional quality and affects the nutritional value and consumption quality of fish. Despite the usefulness of the length-weight relationship and body composition, as well as the great economic importance of Nile tilapia (Oreochromis niloticus) to global aquaculture **(Kamal *et al*,. 2007) and (Azam *et al*., 2004).**

Table 1: Effect of stocking density and hydrogen peroxide concentrations on body composition % DM basis of Nile tilapia.

Variable	Moisture%	Protein%	Fat%	Ash%	carbohydrate %	Energy (calories)
(T1)Control m3/10	73.63±0.21^{a}	63.83±0.25^{c}	18.03±0.13^{bc}	15.12±0.08^{a}	3.02±0.04^{a}	429.67± 1.21^{b}
(T2)350mg/m3/10	72.56±0.22^{b}	65.95±0.22^{a}	19.96±0.11^{a}	13.30±0.06^{b}	0.79±0.02^{d}	446.6±1.7^{a}
(T3)500mg/m3/10	71.45±0.20^{c}	65.31±0.23^{a}	19.44±0.10^{a}	13.45±0.07^{b}	1.8±0.03^{c}	443.4±1.4^{a}
(T1*)Control m3/20	73.97±0.23^{a}	63.51±0.25^{c}	18.15±0.11^{b}	15.22±0.08^{a}	3.12±0.04^{a}	429.87± 1.3^{b}
(T2*)350mg/m3/20	72.66±0.20^{b}	64.54±0.20^{b}	19.38±0.12^{a}	13.42±0.06^{b}	2.66±0.04^{b}	443.22±1.8^{a}
(T3*)350mg/m3/20	71.51±0.22^{c}	64.44±0.24^{b}	19.31±0.10^{a}	13.76±0.07^{b}	2.49±0.03^{b}	441.51±1.5^{ab}

$^{a-d}$ Means with the same letter in each column are not significantly different (P≥ 0.05).

The body composition of fish has recently received attention in studies on nutrition, genetics, and health because of the increasing interest in the quality and safety of fish products (**Dumas *et al*., 2010) and (Tobin *et al*., 2006).**

Finally, the chemical analysis is usually used to study the effect of food on the body composition of cultured fish. According to **(Hanley, 1991),** intrinsic characters (size, sex and steps of life cycle) and exogenous characters (ration formulation, feeding regime, climate, etc.) could alter the fish body composition. Sometimes, with endogenous factors, like food composition; could influence the chemical composition of fish.

Mean values for the proximate chemical composition % DM basis of Nile tilapia treated through stocking density and different concentrations of hydrogen peroxide are described in **Table (1).** The moisture content was significantly reduced, while protein and fat were significantly increased. These results are nearly accordance with obtained by **(Seok-Ryel *et al.*, 2008).**

Fish when stocked at low density (10 /m3 released the highest values of protein and fat which significantly different from the density (20 /m3) for protein content only, addition of hydrogen peroxide concentrations in experimental ponds also significantly affected protein and fat content of fish when treated by 350mg / m3 hydrogen peroxide concentration released the highest protein and fat percentages, also gave the best nutritional quality. The interaction between the two factors studied stocking density and hydrogen peroxide concentration show that released the highest protein and fat content.

Body weight:

Table 2: Least-square means and tested standard error of the factors affecting on total body weight (gm) and body length (cm).

\	$10/m^3$			$20/m^3$		
	Control (T1)	**350mg/m^3 (T2)**	**500mg/m^3 (T3)**	**Control (T1*)**	**350mg/m^3 (T2*)**	**500mg/m^3 (T3*)**
Initial weight	**18.80±0.27^{a}**	**18.70±0.27^{a}**	**18.50±0.27^{a}**	**18.90±0.27^{a}**	**18.80±0.27^{a}**	**18.40±0.27^{a}**
Final weight	**287.80±2.78^{c}**	**321.20±2.78^{a}**	**310.40±2.78^{ab}**	**271.50±2.78^{d}**	**318.40±2.78^{a}**	**302.50±2.78^{b}**
Initial length	**10.90±0.23^{a}**	**10.80±0.23^{a}**	**10.60±0.23 ^{c}**	**11.00±0.23^{a}**	**10.90±0.23^{a}**	**10.70±0.23^{b}**
Final length	**29.50±0.97^{ab}**	**30.10±0.97^{a}**	**29.80±0.97^{ab}**	**30.69±0.97^{a}**	**30.80±0.97^{a}**	**29.70±0.97^{ab}**

$^{a-d}$ Means with the same letter in each raw are not significantly different (P≥0.05).

Table (2) shows the averages of initial weights of *O. niloticus* were 18.70, 18.50, 18.80 and 18.40 g for T2, T3, T2*and T3*, respectively; while at the end of the experiment, the averages of body weight for *O. niloticus* were 321.20, 310.40, 318.40 and 302.50g for T2, T3, T2*and T3*, respectively. The body weight for stocking rate 10 fish /m^3 was higher than 20 fish /m^3. The differences between both stocks were significant (P<0.05). **(Ntanzi *et al.*, 2014)** found that higher stocking rates affect fish growth. In this study, the first sample showed no significant effect for stocking densities on growth rate. While the second and third (further sampling) showed a great significant effect of stocking densities on growth. This can be attributed to that appropriate stocking density before attainment of carrying capacity; the fish grow properly during early culture. More interpretation could be 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 **(Cooper *et al.*, 1988) and (Szymczak and Waite, 1988**). Increasing the phytoplankton in fish ponds may be the cause of improving growth performance of aquatic animals (**Cooper *et al.*, 1994)**. Also variations were significant (P<0.05) due to the interaction between the stocking density and hydrogen peroxide concentrations. Indicating that these two factors act dependently on each other and also each of them had its own significant effect.

In addition to that the best final weight was obtained from the second treatment (T2, being 321.20g) at 350mg /m3 hydrogen peroxide concentration, (**Masser *et al.*, 1999**). A key to successful RAS is using of cheap water treatment system components for eliminating the adverse effects of waste products (**Losordo *et al.*, 1998**). In recirculating tank systems, proper water quality is maintained by pumping tank water through special filtration and aeration and/or oxygenation equipment. Each component should work in conjunction with others in the system; to maintain uniform flow rates (water and air/oxygen), fixed water levels and uninterrupted operation. (**Summerfelt *et al.*, 2004)** found that, recent freshwater recirculating systems are used to rear high value species of fish such as: Salmon smolt and ornamental fishes, as well as fingerlings and food-sized tilapia, hybrid-striped bass, yellow perch, eels,

rainbow trout, African catfish, Channel catfish, and Arctic charr; to name just a few.

Changes in pH, TVB-N, PV and TBA of Nile tilapia treated after stocking density and different concentrations of hydrogen peroxide are described in Table (3).

Changes in pH:

The pH of freshwater fish flesh at fresh condition is almost neutral (**Seher *et al.*, 1985**). The Intracellular PH of treated Nile tilapia fishes by different concentrations of hydrogen peroxide were significantly reduced when compared with control. These results are accordance with obtained by **(Doris Abele-Oeschger *et at.* 1997)**, who reported that hydrogen peroxide causes a decrease in intracellular pH in the musculature of an animal that may very well be exposed to high ambient peroxide levels in its natural environment. A reduction in pH is discussed to be a main factor in causing a decrease in metabolic rate **(Hand, S.C. and Gnaiger, 1988).** The reason may be that the decrease in metabolic rate in vivo is largely caused by metabolic depression in the musculature with possibly less effect of H_2O_2 on the other tissues.

Table 3. Changes in pH, TVB-N, PV and TBA values of Nile tilapia samples after treated by stocking density and hydrogen peroxide concentrations.

\	10/m³			20/m³		
	Control (T1)	350mg/m³ (T2)	500mg/m³ (T3)	Control (T1*)	350mg/m³ (T2*)	500mg/m³ (T3*)
pH	7.33±0.15[d]	7.26±0.11[a]	7. 16±0.10[a]	7.33±0.12[a]	7.20±0.14[a]	7.10±0.11[a]
PV (meq/kg)	3.4±0.07[b]	3.7±0.06[b]	3.9±0.07[b]	3.4±0.08[b]	4.1±0.08[a]	4.3±0.08[a]
TBA (meq/kg fish muscle)	0.2±0.02[d]	0.5±0.03[c]	0.9±0.04[ab]	0.2±0.02[d]	0.7±0.03[b]	1.1±0.04[a]
TVB-N(mg/100fish muscle)	6.1±0.10[c]	6.7±0.12[c]	7.8±0.11[b]	6.9±0.11b[c]	7.1±0.11[b]	8.4±0.11[a]

[a-d] Means with the same letter in each raw are not significantly different (P≥0.05).

Changes in peroxide values (meq O_2/kg lipid) and thiobarbituric acid (meq/kg fish muscle).

Peroxide values (PV) and Thiobarbituric acid values (TBA) is widely used as an indicator of degree of lipid oxidation, and the presence of TBA reactive substances is due to the second stage auto-oxidation **(Rezaei and Hosseini, 2008)** during which peroxides are oxidized to aldehydes and ketones **(Lindsay, 1991).**

PV and TBA values of the fresh samples of Nile tilapia varied between 3.4 to 4.3 meq O_2/kg lipid and 0.2 to 1.1 meq/kg fish muscle for (T1) and (T3*), respectively. This result is in agreement with reported by **(Wayne *et al.*, 1988).** Which showed that as hydrogen peroxide is an uncharged molecule, it easily passes through cell membranes by diffusion, inside the cells highly reactive hydroxyl radicals are liberated ($2\ H_2O_2 + 2e \rightarrow OH^{\cdot} + OH^{-}$). In a reaction catalyzed by transition metals or other cellular reductions at high concentrations, these radicals induce peroxidation of membrane lipids and proteins.

Changes in TVB-N, values (mg/100 g fish muscle):

Measurement of TVB-N indicates the freshness of fish. TVB-N content of the fresh samples of Nile tilapia varied between (T1) 6.1±1.2 to (T3) 7.8±2.1 mg/100 fish muscle , respectively. These results indicated a much lower content of (TVB-N) in comparison with the other fresh samples. These TVB-N values were much lower than acceptable limit which ranged between 30 – 40mg /100 g; this result is in agreement with reported by **(Connell, 1976).**

Water activity:

Changes in water activity (aW) are shown in **Table 4.** Initially, the Aw for all samples was in the range of 0.85 to 0.93, which increased significantly ($P<0.05$). The control samples showed higher water activity (aw) value than the counter-part samples. The difference in moisture content is a confirmation of the fact that fishes has high water content which predisposes it to high microbial spoilage if not well preserved after harvest. Also, water activity (aW) has its most useful application in predicting the growth of bacteria, yeast and mold. The knowledge of water activity is very important factor to guarantee the required stability towards microbial spoilage of the product and to ensure safety by avoiding any threat to the health of the consumer, this is because micro-organisms generally grow best between aW values of 0.995-0.980, while most microbes cease growth at Aw <0.900. It is also necessary for the transport of nutrients and the removal of waste materials, to carry

out enzymatic reactions, to synthesize cellular materials, and to take part in other biochemical reactions **(Fijelu *et al.*, 2014).**

Table 4. The Water activity and Microbiological Characteristic Log $_{10}$ CFU/g of Nile tilapia samples after treated by stocking density and hydrogen peroxide concentrations.

\	10/m^3			20/m^3		
	Control (T1)	350mg/m^3 (T2)	500mg/m^3 (T3)	Control (T1*)	350mg/m^3 (T2*)	500mg/m^3 (T3*)
aW	0. 93±0.013^a	0. 87±0.01^b	0. 84±0.012^b	0.93±0.011^a	0. 89±0.012^b	0. 85±0.010^b
Total viable count bacteria (cfu/g)	3.40 ±0.025^a	2.73±0.023^{ab}	2. 09 ±0.021^b	3.40±0.024^a	2.97±0.012^{ab}	2. 35 ±0.023^b
E .coli	0	0	0	0	0	0
Proteolytic bacteria	1.73±0.014^a	1.36±0.015^{ab}	1.04±0.013^b	1.73±0.012^a	1.48±0.013^{ab}	1.17±0.011^b
Lipolytic bacteria	0.84±0.013^a	0.68±0.012^{ab}	0.52 ± 0.010^b	0.84±0.013^a	0.74 ± 0.011^{ab}	0.58 ± 0.010^b
Yeasts & moulds (cfu/g)	2.9±0.022^a	1.7 ±0.016^b	1.4 ±0.011^b	2.9±0.023^a	1.9±0.015^b	1.5±0.011^b

$^{a-b}$Means with the same letter in each raw are not significantly different (P≥0.05).

The Microbiological Characteristic:

Hydrogen peroxide (H_2O_2) is highly effective against pathogenic bacteria, fungi, algae, viruses and amoebae. Hydrogen peroxide (H_2O_2) acts as the oxidizing agent which is an excellent enhancement of the disinfecting effect on pathogens **(Fallik *et al.*, 1994).**

The results of the microbiological analyses included the load of proteolytic bacteria, yeasts, moulds, Lipolytic bacteria and pathogenic microorganisms (*E .coli*) in various samples. Highest total viable count of bacteria (3.40 Log $_{10}$ CFU/g) was observed in control and the lowest (2. 09 Log $_{10}$ CFU/g) was observed in samples of fish stocked at low density treated by 500mg of hydrogen peroxide. Highest proteolytic bacteria (1.73 Log $_{10}$ CFU/g) was observed in control and lowest (1.04 CFU /g) was observed in samples of fish stocked at low density treated by 500mg of hydrogen peroxide. Highest lipolytic of bacteria (0.84 Log $_{10}$ CFU/g) was observed in control and lowest (0.52 Log $_{10}$ CFU/g) was observed in samples of fish stocked at low density treated by 500mg of hydrogen peroxide. Highest yeasts and moulds of bacteria (2.9 CFU /g) were observed in control and the lowest (1.4 CFU/g) was observed in samples of fish stocked at low density treated by 500mg of hydrogen peroxide. Pathogenic microorganisms (*E .coli*) were not detected in any samples of Nile tilapia. This result is in agreement with report by **(Mohammad saeed and Mohammad reza, 2017)** which showed that hydrogen peroxide is an active antimicrobial agent and can eradicate the bacteria from the water, therefore may be a useful chemical and for hygienic procedures of water against the bacterium. Also, this result is in agreement with report by **(Alexandra *et al.*, 2015)** which showed that hydrogen peroxide used against fish pathogens to assist fish farmers in the effective and safe disinfection of eyed eggs.

Table 5: Sensory evaluation of Nile tilapia samples after treated by stocking density and hydrogen peroxide concentrations.

\	10/m^3			20/m^3		
	Control (T1)	350mg/m^3 (T2)	500mg/m^3 (T3)	Control (T1*)	350mg/m^3 (T2*)	500mg/m^3 (T3*)
Appearance	8.7±0.15^b	8.7±0.16^b	9.2±0.17^a	8.7±0.16^b	8.6±0.15^b	9.0±0.17^a
Texture	8.8 ±0.13^a	8.8±0.14^a	8.9 ±0.15^a	8.8 ±0.14^a	8.7 ±0.11^a	8.9±0.13^a
Colour	8.1 ±0.14^a	8.4 ±0.15^a	8.6±0.14^a	8.1 ±0.13^a	8.2 ±0.12^a	8.5±0.14^a
Overall acceptability	8.6 ±0.13^b	8.9 ±0.11^{ab}	9.3±0.12^a	8.6 ±0.12^b	8.8±0.14^b	9.0 ±0.15^{ab}

[a- b]Means with the same letter in each raw are not significantly different (P≥0.05).

The sensory evaluation is presented in **Table 5**. The result obtained showed that, there were little changes in all the sensory parameters after treated the fish with different concentration of hydrogen peroxide. There were significant changes in appearance, colour, texture and overall acceptability in all the fish. The best appearance and overall acceptability were recorded for all fish samples that were fish stocked at low density treated by 500mg of hydrogen peroxide. The most attractive appearance was recorded for fish stocked at low density treated by 500mg of hydrogen peroxide samples while the least attractive was recorded for fish stocked at high density treated by 350mg of hydrogen peroxide samples, this result is in agreement with reported by **(Priya *et al.*, 2011).**

Biochemical Parameters:

Table (6). Effect of stocking density and hydrogen peroxide concentrations on biochemical and hematological parameters of *O. niloticus*.

\	10/m^3			20/m^3		
	Control (T1)	350mg/m^3 (T2)	500mg/m^3 (T3)	Control (T1*)	350mg/m^3 (T2*)	500mg/m^3 (T3*)
Total Lipids (g/L)	14.70±0.32^{d}	14.90±0.32^{d}	15.70±0.32^{bc}	16.60±0.32^{b}	18.90±0.32^{ab}	19.80±0.32^{a}
Total Protein (g/L)	2.07±0.01^{b}	2.64±0.01^{b}	3.45±0.01^{a}	2.33±0.01^{b}	2.71±0.01^{b}	3.58±0.01^{a}
Glucose (mg/L)	64.9±1.72^{d}	89.5±1.72^{c}	124.2±1.72^{a}	108.2±1.72^{a}	119.4±1.72^{ab}	128.4±1.72^{a}
Cholesterol (mg/L)	31.80±0.59^{b}	30.30 0.59^{b}	28.90±0.59^{c}	34.80±0.59^{a}	32.40±0.59^{b}	31.20±0.59^{b}
AST (IU/L	17.40±0.13^{d}	22.70±0.13^{c}	26.50±0.13^{a}	21.60±0.13^{c}	24.80±0.13^{b}	26.90±0.13^{a}
ALT (IU/L	6.10±0.07^{c}	6.30±0.07^{c}	7.80±0.07^{b}	6.10±0.07^{c}	7.40±0.07^{b}	8.80±0.07^{a}
RBCs (×10^6/µL)	1.26±0.09^{b}	1.30±0.09^{ab}	1.53±0.09^{a}	1.31±0.09^{b}	1.52±0.09^{a}	1.61±0.09^{a}
Hb (g/L)	6.06±0.47^{cd}	6.57±0.47^{c}	7.95±0.47^{b}	6.69±0.47^{c}	7.64±0.47^{b}	8.40±0.47^{a}
Ht (%)	13.80±0.178^{d}	15.00±0.178^{bc}	15.80±0.178^{b}	14.30±0.178^{c}	15.60±0.178^{ab}	16.50±0.178^{a}

[a - d] Means with the same letter in each raw are not significantly different (P≥ 0.05).

Hematology is an important factor that could be considered for the fish diet quality assessment, consequently nutritional quality. **Hari *et al.* (2006)** reported that the hematology would be useful for the assessment of suitability of diets and nutritional quality, feed mixtures, evaluation of fish conditions, determination of toxic effect of substances, as well as the diagnosis of disease **(Avnimelech *et al.*, 1994)** reported that one of the most common blood variables consistently influenced by diet is the Hct and Hb levels.

As shown in Table (6) the stocking density (20 / m^3) significantly increased blood glucose, cholesterol, total protein (g/L) and total lipids compared with the stocking density (10 / m^3). Also the complete hydrogen peroxide concentrations significantly (P<0.05) increased these parameters. The interaction between stocking density and hydrogen peroxide concentrations in the present study showed that, fish when stocked (20 / m^3) with treated by hydrogen peroxide showed the highest blood glucose, cholesterol, total Protein and total lipids compared with the low stocking density (10 / m^3).

Chen *et al.*, (2004) studied the comparative blood chemistry and histopathology of tilapia infected with *Vibrio vulnificus or Streptococcus* exposed to carbon tetrachloride, gentamicin, or copper sulphate. He found that, the hematological parameters are an important tool of diagnosis that reveals the state of health of fish. For example, decreased red blood cells and hematocrit were found in Nile tilapia experimentally infected with Streptococcus.

As shown in Table (6) the high stocking density (20 / m3) significantly increased hemoglobin while liver enzymes values (AST and ALT) significantly affected compared to the low stocking density (10 / m3). Also the addition of hydrogen peroxide the experimental ponds significantly (P<0.05) increased

hemoglobin, AST and ALT enzymes values significantly affected. The interaction between stocking density and hydrogen peroxide in the present study showed that, fish when stocked the high density (20 / m^3) with highly hydrogen peroxide concentration (500mg/m^3) showed the highest hemoglobin, compared with 350mg/m3 and control. Shows decreasing in the erythrocyte count especially for the control group indicating an anaemic case. On the other the second treatment (350 mg/m3 H_2O_2) gained the best health condition (**Lahnsteiner and Weismann, 2007**).

Table 7 : Survival rate of *O. niloticus* and least-square means and standard error of the tested factors affecting on condition factor (K), DWG , SGR and Total mass.

\	10/m^3			20/m^3		
	Control (T1)	350mg/m^3 (T2)	500mg/m^3 (T3)	Control (T1*)	350mg/m^3 (T2*)	500mg/m^3 (T3*)
Total mass (Kg/m3)	2.88±0.021^{d}	3.21±0.022^{c}	3.10±0.024^{c}	5.43±0.023$_{b}$	6.37±0.022^{a}	6.05±0.025^{a}
Survival rate%	90.20±0.25^{ab}	93.40±0.24^{a}	93.20±0.25^{ab}	88.30±0.26$_{b}$	90.90±0.25^{ab}	91.30±0.23^{ab}
Initial K	1.73±0.013^{ab}	1.78±0.014^{a}	1.87±0.013^{a}	1.50±0.012$_{b}$	1.43±0.013^{c}	1.54±0.013^{b}
Final K	0.72±0.011^{c}	0.74±0.010^{c}	0.88±0.011^{b}	1.03±0.012$_{a}$	0.95±0.011^{ab}	0.80±0.010^{b}
Daily weight gain(DWG), G/fish	1.60±0.012^{b}	1.80±0.012^{a}	1.74±0.013^{ab}	1.50±0.014$_{c}$	1.78±0.014^{a}	1.69±0.015^{b}
Specific growth rate (SGR), /day%	1.62±0.011^{ab}	1.69±0.012^{a}	1.68±0.011^{a}	1.59±0.012$_{b}$	1.69±0.010^{a}	1.67±0.013^{a}

$^{a-d}$ Means with the same letter in each raw are not significantly different (P≥0.05).

Total mass and Survival rate:

Table (7) illustrates that the highest total mass (Kg/m^3) and Survival rate (3.21kg/m^3 – 93.40%) were in ponds stocked by 10 fish / m^3 compared with (6.37kg/m^3 – 90.90%) stocking 20 fish / m^3, this results seems close to the recorded by **(Ntanzi *et al.*, 2014)** who found that, the high survival rates of tilapia fry at high stocking density (82.9% at 5330 fry/m^3) indicate amenability of tilapia to intensive culture. Worthy to mention, this could also be due to favorable environmental conditions during the study.

The averages of total mass of *O. niloticus* during the whole period were 2.88 kg/m^3 – 90.2%, 3.12 kg/m^3 – 93.40%, 3.10 kg/m^3 – 93.20%, 5.43 kg/m^3 – 88.30%, 6.37 kg/m^3 – 90.90% and 6.05 kg/m^3 – 91.30% for all treatments T1, T2, T3, T1*, T2* and T3*, respectively. While, the best total mass was obtained from the second treatment (6.37 kg/m^3 – 90.90%) at stocking rate 20 fish /m^3.

Shehab El-din *et al.*, (2017) found during studying a similar approach to improve tilapia health; adding Hydrogen peroxide in fish ponds showed the highest survival rate. In additional tests with fathead minnows (*Pintphalespromales*), bluegill sunfish (*Lepomis macrochirns*), and channel catfish (*Ictalurns punctatus*) fingerlings, no mortality was observed for exposures up to 350, 500 mg, respectively, for 45-min exposures. Walleye (*Sander vitreum*) fish were the most sensitive species tested; some mortality was observed even at the lowest exposure concentration (1.13 mg) as described by **(Gaikowski *et al.*, 2004).**

Rach *et al.*, (2003) 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 (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*), and lake trout (*Salvelinus namaycush*) fingerlings showed no mortality during growth, when exposured to concentrations reaching 283, 283 and 1.132 mg/L, respectively.

Condition factor (K):

As recorded in Table (7), the averages of initial (K) were 1.73, 1.78, 1.87, 1.50, 1.43 and 1.54. While, the averages of final (K) were 0.72, 0.74, 0.88, 1.03, 0.95 and 0.80, respectively for all treatments. So, the best (K) value was obtained from the second group (T2, being 0.95) by the higher stock.

Shehab El-din *et al.*, (2017) when studied influence of hydrogen peroxide on performance, body composition and health status of monosex males of nile tilapia fingerlings found that, the condition factor was positively better by additing it on water for fish.

Moreover, health status was improved. Significant (P<0.05) changes may be due to the interaction between stocking density (10 and 20 fish/m^3) and hydrogen peroxide treatments (control, 350 and 500mg/m3, respectively). Condition factor of fish is an important measure of relative musculature to bony growth. Various responses of these tissues to feeding regime may be reflected by changes in condition factor **(Soltan *et al*., 1999 and Ibrahim *et al*., 2000)**. It is mostly assumed to reflect not only characteristics of fish such as health, reproductive state and growth but also the environment condition such as habitat, water quality and prey availability.

Daily weight gain (DWG):

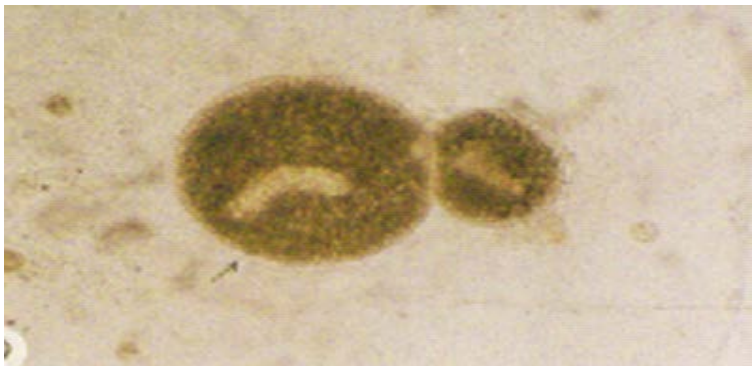
As recorded in **Table (7),** the averages of (DWG) for *O. niloticus* were 1.60, 1.80, 1.74, 1.50, 1.78 and 1.69 g/fish for both stocking densities respectively, for all treatments. The differences between the two stocking densities were significant (P<0.05). Obviously the dose 350 mg/m^3 H_2O^2 was higher than the lower ones. The obtained results agreement with **(Szymczak and Waite, 1988 and Cooper *et al*., 1994)** who reported that Hydrogen peroxide aids in the solution of organic materials that absorb light and molecular oxygen, thus producing more nutritive planktons.

Specific growth rate (SGR):

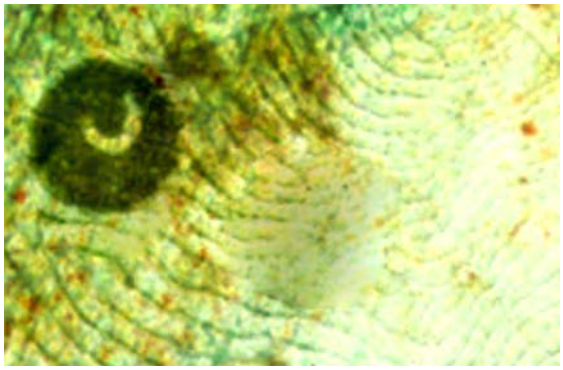
As recorded in Table (7), the averages of (SGR) for *O. niloticus* were 1.62, 1.69, 1.68, 1.59, 1.69 and 1.67 g/fish for both stocking densities respectively, for all treatments. The differences between the two stocking densities were significant (P<0.05). Obviously the dose 350 mg/m^3 H_2O^2 was higher than the lower ones.In which giving 350 mg/m^3 hydrogen peroxide was found to be higher than the other treatments. Analysis of variance of these results indicates that, the differences among treatments were significant (P<0.05). So the best (SGR) was obtained for second treatment (350mg/m^3, being 1.69) for stocking rates 10 and 20 fish /m^3. **Shehab El-din *et al*., (2017)** found that, the averages of (SGR) of O. niloticus fingerlings were 1.84, 1.91 and 1.92 %/day for the three treatments, respectively. 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 (**Abou Zead *et al*., 2008) and (Hassaan and Soltan, 2016**).

Clinical signs, post-mortem examination and Parasitological examinations of fish:

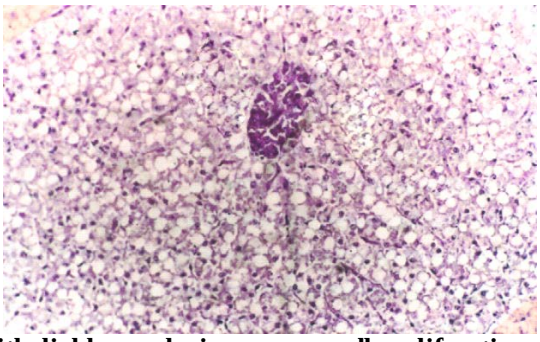
Few fish from the control group were dull in appearance with slimness due to the excessive amounts of mucous over the whole body surface. Scale detachment (loose scales), hemorrhages, wounds (faded whitish spots on the external body) and ulcers, were also noticed. Which might be caused by an irritation (itching); due to ciliated protozoal act on the fish, while the mucous was released to relief is ithch, inflammatory reaction), (**Woo, 2002**) Microscopical smears were taken from gills, skin and fins of examined fish 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. Such ciliated protozoans were related to family Icthyophthiridae, genus Icthyophthirius multifilliis (commonly known as ich), pictures (1 and 2) **(Paperna, 1996).** Ich is a widespread ectoparasitic ciliate that occurs in temperate, subtropical, and tropical zones, and may cause considerable loss of fish, particularly under farm or hatchery conditions (1,2). According to **(Hines and Spira, 1974)**, *ichthyophthiriasis* is probably the most devastating parasitic disease of cultured fish. The parasitic ciliate *Ichthyophthirius multifiliis* (Ich) is one of the most important protozoan pathogens of freshwater fish worldwide. The *ectoparasites* have an economic impact on fish production through fish mortalities, low body weight gain, reduced quality fish meats plus the marketing value. Furthermore, the adverse environmental conditions in the water of the ponds may weaken the immunity status of the fish and favor the growth and spread of the ectoparasites. Indeed, the *ectoparasites* may cause skin injuries which facilitate the invasion of other fish pathogens, (**Eissa, 2002**).



Picture, (1): Ichthyophthirius multifiliis wet mount high power



Picture (2): Ichthyophthirius multifiliis stained Giemsa, high power



Picture, (3): showing epithelial hyperplasia, mucous cell proliferations and necrosis in the skin

Histopathological results:

Epithelial hyperplasia, mucous cell proliferations and necrosis, **(Jørgensen *et al.*, 2014)** in the skin could limit the osmoregulatory gas and ion exchanges in the fish leading to metabolic disturbances being lethal for fish in the control untreated group, picture (3).

References

1. Abou Zead, M. Y., Soltan, M. A. and Ibrahim, M. S. (2008): Effect of replacing Soybean meal by sunflower meal in the diets of Nile tilapia, Oreochromis niloticus. (L.). Proceeding of the 8th International Symposium on Tilapia in Aquaculture, 12-14/10/2008, Cairo, Egypt., 787-799.
2. Alexandra Grasteau , Thomas Guiraud, Patrick Daniel, Ségolène Calvez, Valérie

Chesneau and Michel Le Hénaff (2015). Evaluation of Glutaraldehyde, Chloramine-T, Bronopol, Incimaxx Aquatic® and Hydrogen Peroxide as Biocides against Flavobacterium psychrophilum for Sanitization of Rainbow Trout Eyed Eggs .J Aquac Res Development, Vol. 6(12), pp. 1-8.

3. American Public Health Association (APHA) (1992). Compendium methods for microbiological examination of foods, 2nd Ed.,Washington D.C.
4. Anderson, I. G. (2012). The use of chemotherapeutic agents in finfish and shellfish culture in Australia. pp. 493-504. In: Sheriff, M., Subasinghe, R. P., and Arthur, J.R. (eds.). Diseases in Asian Aquaculture I. Fish Health Section, Asian Fisheries Society, Manila, Philippines. 587 pp.
5. AOAC, (1990). Official Methods of Analysis, Association of Official Analytical Chemists. Washington, D. C.
6. AOAC. (2005). Official methods of analysis (18th ed.), Association of Official Analytical Chemists International, Maryland, USA.
7. Avnimelech, Y., Kochva, M. and Diab, S. (1994). Development of controlled intensive aquaculture systems with a limited water exchange and adjusted carbon to nitrogen ratio. Bamidgeh 46:119-131.
8. Azam, K.; Ali, M. Y.; Asaduzzaman, M.; Basher, M. Z. and Hossain, M. M. (2004). Biochemical Assessment of Selected Fresh Fish. Journal of Biological Sciences 4:9-10.
9. Boyed, C. E. (1998). Water quality for pond aquaculture. Research and development series No. 43. pp. 37. International Centre for aquaculture and aquatic Environments. Alabama Agricultural Experiment Station. Auburn University.
10. Chen, C., Wooster, G. and Bowser, P., (2004). Comparative blood chemistry and histopathology of tilapia infected with Vibrio vulnificus or Streptococcus iniae or exposed to carbon tetrachloride, gentamicin, or copper sulphate. Aquaculture, vol. 239, no. 1-4, p. 421-443.
11. Cooper W. J., C. Shao, D. R. S. Lean, A. S. Gordon, and F. E. Scully. (1994). Factors affecting the distribution of H_2O_2 in surface waters. Pages 391 -422 in L.A. Baker, editor. Environmental Chemistry of Lakes and Reservoirs. American Chemical Symposium Series 237, Washington, D.C.
12. Cooper, W. J., R. G. Zika, R. G. Petasne, and A. M. Fischer. (1988). Sunlight induced photochemistry of humic substances in natural waters: Major reactive species. Pages 333-362 in P. McCarthy and I.H. Suffet, editors. Influence of aquatic humic substances on fate and treatment of pollutants. American Chemical Society Symposium Series 219. Washington, D.C.
13. Diana, J. S., and Lin, C. K. (1998). The effects of fertilization and water management on growth and production of Nile tilapia in deep ponds during the dry season. J. of the World Aquaculture Society, 29 (4): 405 – 413.
14. Dumas, A.; France, J. and Bureau, D. (2010). Modeling of growth and body composition in fishnutrition:wherehavewebeenandwhere are we going? Aquaculture Research 41:161-181.
15. Dumas, B.T. and Biggs, H.G. (1972). Standard Methods of Clinical Chemistry. Ed., Academic Press, New York.
16. Duncan, D.B., (1955). Multiple ranges and multiple F. test. Biometrics, 11: 1-42.
17. Eissa I. A. M. (2002). Parasitic fish diseases in Egypt". Dar El-Nahdda El-Arabia publishing, Cairo, Egypt.
18. Fallik, E., Y. Aharoni, S. Grinberg, A. Copel and JD Kelin (1994). Postharvest hydrogen peroxide treatment inhibits decay in eggplant and sweet red pepper. Crop Protection 13(6): 451-454.
19. Fijelu Frank, Yanshun Xu, Qixing jiang and Wenshui Xia (2014). Protective effects of garlic (*Allium sativum*) and ginger (*Zingiber officinale*) on physicochemical and microbial attributes of liquid smoked silver carp (*Hypophthalmichthys molitrix*) wrapped in aluminium foil during chilled storage African Journal of Food Science.Vol. 8(1), pp. 1-8.
20. Gaikowski, M.P., J.J.Rach and R.T.Ramsay (2004). Acute toxicity of hydrogen peroxide treatments to selected life stages of cold-,cool-,and warm water fish. Aquaculture 178:191–207.
21. Hanley, F. (1991). Effects of feeding supplementary diets containing varying levels of lipid on growth, food conversion,

and body-composition of Nile tilapia, Oreochromis niloticus (L). Aquaculture, 93(4):323-334.
22. Hari, B., Kurup, B. M., Varghese, J. T., Schrama, J. W. and Verdegem, M. C. J. (2006). The effect of carbohydrate addition on water quality and the nitrogen budget in extensive shrimp culture systems. Aquaculture, 252(2-4):248-263.
23. Harrigan, W.F. and M.E. MacCance (1976).Laboratory Methods in Food and Dairy Microbiology. Academic press, London.
24. Hartmann, A.C. (2010). Hydrogen peroxide keeps salmon healthy. On Site the Eka Chemicals staff magazine No4 December, pp: 14.
25. Hassaan, M. S. and M. A. Sultan (2016). Evaluation of Essential Oil of Fennel and Garlic Separately or Combined with Bacillus licheniformis on the Growth, Feeding Behaviour, Hemato-biochemical Indices of Oreochromis niloticus (L.) Fry. Journal of Aquaculture, Research & Development. 7:3. http://dx.doi.org/10.4172/2155-9546.1000422.
26. Heinecke, R. D., and K. Buchmann. (2009). Control of Ichthyophthirius multifiliis using a combination of water filtration and sodium percarbonate: dose–response studies. Aquaculture 288:32–35.
27. Hines, R.S. and D.T. Spira (1974). Ichthyophthiriasis in the mirror carp *Cyprinus carpio* (L.). III. Pathology. J. Fish Biol., 6: 189-196.
28. Ibrahim, M. K., Fatma, A. Hafez and M. A. Soltan (2000). Effect of organic fertilization, supplementary feeding and stocking rate on growth performance of Nile tilapia and silver carp. Egypt. J. Agric., Res., 78 (4):1775-1799.
29. Jauncey, K. and B. Rose (1982). A guide to tilapia feeds and feeding". Institute of Aquaculture, University of Sterling, Scotland. GB: Institute of Aquaculture.
30. Jørgensen, T. R., T. B. Larsen and K. Buchmann (2014). Parasite infections in recirculated rainbow trout (*Oncorhynchus mykiss*) farms. Aquaculture 306:91–94.
31. Kabata, Z., (1985). Parasites and diseases of fish culture in the tropics. Printed in Great Britain by Taylor and Franks (Ltd Basingstoke Hants), pp: 127-161.
32. Kamal, R., A. N. Khan, M. A. Rahmanand F. Ahamed (2007). Biochemical composition of some small indigenous fresh water fishes from the river Mouri, Klulna, Bangladesh. Pakistan Journal of Biological Sciences 10:1559-1561.
33. Krzynowek, J. (1988). Effects of handling, processing and storage of fish and shellfish. In Karmas E, Harris R.S. Eds. Nutritional Evaluation of Food Processing. 3rd Ed. New York: Van Nostrand Reinhold: 245- 265.
34. Kuzirian, A.M., E.C.S. Terry and D.L. Rechtel (2001). Hydrogen peroxide: An effective treatment for ballast water. Biological Bulletin, 201: 297-299.
35. Lahnsteiner, F. and T. Weismann (2007). Treatment of ichthyophthiriasis in rainbow trout and common carp with common and alternative therapeutics. J. Aquat. Anim. Health, 19: 186-194.
36. Larisch, B.C. and S.J.B. Duff (2007). Effect of H202 on characteristics and biological treatment of TCF bleached pulp mill effluent. Water Research, 31: 1694-1700.
37. Larry J. S., Mark P. G. and H. G. William (2006). Environmental Assessment for the Use of Hydrogen Peroxide in Aquaculture for Treating External Fungal and Bacterial Diseases of Cultured Fish and Fish Eggs. U.S. Geological Survey, Biological Resources Division Upper Midwest Environmental Sciences Center 2630 Fanta Reed Road La Crosse, Wisconsin 54603 (608) 783-645:1.
38. Lindsay, R.C. (1991). Flavour of fish. Paper presented at 8th World Congress of Food Science & Technology, 29th September–4th October, Toronto, Canada.
39. Losordo, T.M., Masser, M.P. and Rakocy, J. (1998). Recirculating Aquaculture Tank Production Systems: An Overview of Critical Considerations. SRAC Publication No. 451, 6 p.
40. Lucky, Z., (1977). Methods for the diagnosis of fish diseases. Amerind Publishing Co., Pvt. Ltd. New Delhi, Bombay Calcutta and New York.
41. Masser, M.P., J. Rakocy and T.M. Losordo (1999). Recirculating Aquaculture Tank Production Systems: Management of

Recirculating Systems. SRAC Publication No. 452, 12 p.
42. Mohammad saeed and Mohammad reza (2017). Inhibitory Effect of Hydrogen Peroxide (H2O2) and Ionic Silver (Sanosil-25®) on Growth of a Pathogenic Bacterium (*Vibrio harveyi*) Isolated From Shrimp (*Litopenaeus vannamei*).Oceanography & Fisheries open access Jornal. Vol. 2(3): 1-4.
43. Møller, M., E. Arvin, and L. F. Pedersen. (2010). Degradation and effect of hydrogen peroxide in small-scale recirculation aquaculture system biofilters. Aquaculture Research 41:1113–1122.
44. Nettleton JA., (1992). Seafood Nutrition in the 1990s, Issues for the Consumer In: Bligh EG. Ed. Seafood Science and Technology London: Fishing New Books. pp. 32–39.
45. Ntanzi, R., G. Bwanika and G. Eriku (2014). The Effects of Stocking Density on the Growth and Survival of Nile Tilapia (*Oreochromis niloticus*) Fry at Son Fish Farm, Uganda. J Aquac Res Development, 5:2 :1-7.
46. Paperna, I. (1996). Parasites infection & diseases of fish. Book 6.in.S.F.Sneiszko and H. R. Axelord eds. Diseases of fishes.T.F.A. publications.Inc. Ltd.
47. Pedersen, L. F., P. B. Pedersen, J. L. Nielsen, and P. H. Nielsen (2014). Peracetic acid degradation and effects on nitrification in recirculating aquaculture systems. Aquaculture 308:246–254.
48. Pedersen, L. F., P. B. Pedersen, J. L. Nielsen, and P. H. Nielsen (2015). Long-term/low-dose formalin exposure to small-scale recirculation aquaculture systems. Aquacultural Engineering 42:1–7.
49. Priya, P.K., S. Niraimathi, S. Megala and M. Govindarajan (2011). An analysis of bio chemical with minerals composition of three different species fish by drying methods (bonga spp., sardinella spp. and oreochromis nilotieus).international journal of recent scientific research vol. 2, issue, 11:279 -282.
50. Rach, J.J., S.M. Schleis, M.P. Gaikowski, and A. Johnson. (2003). Efficacy of hydrogen peroxide in controlling mortality associated with external columnaris on walleye and channel catfish fingerlings. North American Journal of Aquaculture 65:300-305.
51. Rezaei, M. and F.S. Hosseini (2008). Quality Assessment of Farmed Rainbow Trout (Oncorhynchus mykiss) during Chilled Storage. J. Food Sci. 73(6): H93-H96.
52. Russo, R., E. W. Curtis and R. P. E. Yanong (2007). Preliminary investigations of hydrogen peroxide treatment of selected ornamental fishes and efficacy against external bacteria and parasites in green swordtails. Journal of Aquatic Animal Health 19:121–127.
53. Saez, J. A., and P. R. Bowser (2001). Hydrogen peroxide concentrations in hatchery culture units and effluent during and aftert reatment.North American Journal of Aquaculture 63:74–78.
54. SAS, (1996). SAS procedure guide version 6.12 Ed. SAS Institute Inc., Gary, NC, USA.
55. Schaperclaus, W., H. Kulow and K. Schreckenbach (1992). FishDiseases"Vol. 1. A. A. Balkema Rotterdam.
56. Schmidt, L. J., M. P. Gaikowski, and W. H. Gingerich (2006). Environmental assessment for the use of hydrogen peroxide in aquaculture for treating external fungal and bacterial diseases of cultured fish and fish eggs. U.S. Geological Survey, Washington, D.C.
57. Schreck, C.B. and P.B. Moyle (1990). Methods of Fish Biology". American Fisheries Society; Bethesda, Maryland, USA.
58. Seok-Ryel Kim, Kyung-Hee Park, Duwoon Kim, Sung-Ju Jung, So-Yong Kang, and Myung-Joo Oh (2008).Antimicrobial Effects of Chemical Disinfectants on Fish Pathogenic .Food Sci. Biotechnol. Vol. 17, No. 5: 971 - 975
59. Shehab El-Din, M.T., Fouad, I.M. and Fath El-Bab, A.F. (2017). Influence of hydrogen peroxide on performance, body composition and health status of monosex males of Nile tilapia fingerlings. Global Veterinaria 18 (1): 05-13, 2017.
60. Soltan, M. A., N. F. Abdel-Hakim and M. N. Bakeer (1999). Effect of stocking rate, organic fertilization and supplementary feed on growth performance, carcass and chemical analysis of Nile tilapia, Oreochromis niloticus. Egyptian J. Nutrition and Feeds 2:765-778.

61. Summerfelt, S.T. and M.J. Sharrer (2004). Design implication of carbon dioxide production within biofilters contained in recirculating salmonid culture systems. Aquacultural Engineering 32: 171 – 182.
62. Szymczak, R., and T. D. Waite (1988). Generation and decay of hydrogen peroxide in estuarine waters. Marine and Freshwater Research, 39(3), 289-299.
63. Tobin, D., Kause, A. Mntysaari, E. A. Martin, S. A. M. Houlihan, D. F. Dobly, A. Kiessling, A. Rungruangsak-Torrissen, K. O. Ritola and K Ruohonen (2006). The quantitative genetic basis for selection strategies of muscle and body composition traits in breeding schemes of rainbow trout (Oncorhynchus mykiss). Aquaculture 261:510-521
64. Wayne, F., J. Beyer and I. Fridovich (1988).Catalases with and without heme. Basic Life Sci. 49:651–661;
65. Woo, P.T.K. (2002). Fish diseases and disorders. World Journal CAB, Int. Wallingford, Oxon, UK.

How to cite this article
El-Bab A.F. F., Fouad I.M., Shehab El-din M.T. and Elgndy M.O. (2018). Evaluation effects of hydrogen peroxide on health status and quality characterization of *Nile tilapia* (*Oreochromis niloticus*). *Int. J. Pharm. Life Sci.*, 9(2):5701-5714.
Source of Support: Nil; Conflict of Interest: None declared

Received: 31.12.17; Revised: 20.01.18; Accepted: 10.02.18