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Effect of using low molecular weight Chitosan on water quality, stress reduction and quality indices of Nile tilapia fish flesh

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Abstract

The study investigated the efficiency of adding extracted chitosan from shrimp wastes to enhance water quality, reduce stress and enhance tilapia flesh quality. Water quality parameters showed no significant differences among all treatments in (Dissolved oxygen, Temperature, pH, NO_2 and Orthophosphate values). Treatment fish water with chitosan affected some nitrogenous parameters such as NH_3 and NO_3, where the values were (0.7, 0.6, 0.1 and 0.5 mg / l) for NH_3, and (0.62, 0.61, 0.36 and 0.59 mg / l) for NO_3, respectively. The lowest value of NH_3 and NO_3 was recorded in T_3 (15 mg / l chitosan) followed by T_4 (20 mg / l chitosan), T_2 (10 mg / l chitosan) and finally T_1 (negative control). Chemical composition of the samples revieled that the adding chitosan to the water improved moisture, protein, fat and increased ash content for fish flesh respectively, followed by control. Physiochemical quality parameters such as pH, acid value, Free amino nitrogen (FAN), Free fatty acids (FFA), total volatile bases nitrogen (TVB-N) and trimethylamine (TMA-N) showed that the addition chitosan reduce the increase in this parameters of the three treatments compared to the control. The blood chemistry indices revealed that; T_3 had the lowest AST; T_4 had the lowest ALT values compared to control and T_2, respectively. Despite the variation in Alb, Glob and ALP values among treatments they showed no significance differences. T_3 showed the lowest values among

all treatments for GLU, CHO, HDL, LDL and TG followed by T_4, T_2 and control, respectively.

Keywords: Chitosan, Tilapia, Water quality, Chemical composition, Physiochemical parameters and Stress reduction.

INTRODUCTION

Fish in production facilities are exposed to stress, disease and environmental degradation that can cause serious economic losses **(Castro *et al.*, 2008).** Currently, fish are protected from infectious diseases by vaccination or chemotherapy. However, due to the extensive use of chemical therapeutic agents, the emergence of antimicrobial resistance among pathogens and associated environmental risks has been well documented. Therefore, several alternative strategies have been proposed such as the use of various immune stimulants that, could improve fish resistance against unfavorable environmental conditions and pathogens compared to other treatments **(Sakai, 1999).**

Chitin is the second most abundant organic compound in nature after cellulose, is widely distributed in marine invertebrates, insects, fungi, and yeast. Chitosan is the structural element in the exoskeleton of crustaceans (such as crabs and shrimp) and cellular walls of the fungi.

Wibowo (**2003**) defined chitosan as a long-chained polymer of 2-deocxy-2-amino-glucose or deacetilated chitin. Chitosan has a ($C_6H_{11}NO_4$) with molecular weight of 104-106 known as β-1,4-2 amino-2-dioxy-D-glucose, a polymer with one amino (NH_2) cationic and 2 free hydroxyl (OH) in each glucose ring (positive charge), and ready to capture any negative ions.

Several methods have been reported for chitosan preparation from chitin. The major procedure for obtaining chitosan is based on the alkaline deacetylation of chitin with strong alkaline solution at high temperature (**Castelli *et al*.**, **1996**), alkali treatment through autoclaving (**Abdou *et al*.**, **2008**), and enzymatic N-deacetylation (**Martinou *et al*.**, **1995**).

The molecular weight of chitosan varies depending on the raw material sources and preparation methods **(Li *et al.*, 1992).** Most commercial chitosans have a degree of deacetylation that is greater than 70 % and a molecular weight ranging between 100,000 kDa (kilo Dalton) and 1.2 million kDa **(Li *et al.*, 1997).**

Many important variables should be taken into account when working with chitosan solutions such as the nature of the salt concentrations,

degree of acetylation, Mw, pH, ionic strength and the addition of a non-aqueous solvent **(Aranaz *et al*., 2009).**

Chitosan has been of interest in the food industry since, besides its antimicrobial effect, it possesses other functional properties including intestinal lipid binding and serum cholesterol lowering effects (**Razdan and Pettersson, 1994**), water binding (**Knorr**, **1982**), emulsifying, thickening and stabilizing agent in food industry (**Shahidi *et al*.**, **1999**), antioxidative and preservative effects in muscle foods (**Darmadji and Izumimoto, 1994**), and emulsifying capacity (**Lee *et al*.**, **1996**).

Chitosan have growth and immune stimulating properties for aquatic animals and different fish species due to its solubility in water and other polar solvents more than chitin (**Romeran *et al*. 2002** and **Kim and Rajapakse, 2005**; **Dautremepuits *et al*.**, **2003**, **Dautremepuits *et al*.**, **2004**; **Wang** and **Chen 2005**, **Gopalakannan** and **Arul 2006** and **Cha *et al*.**, **2008**) and have non-toxicity, antibacterial, antioxidant, film forming ability, gel enhancer, mucus adhesiveness, encapsulating capacity, tissue engineering scaffold, wound dressing, coagulating agent, biodegradability and biocompatibility (**Alishahi**, **2012**). In addition it may have healing effects on tissue damage caused by toxicity (**Alishahi, 2012**; **Sharifinasab *et al*., 2016**).

Furthermore, chitosan and its derivatives are used to produce wide variety of functional foods and bioactive materials such, hormones, vaccines and vitamins, those compounds are susceptible to environmental factors such as oxygen, light, and temperature. Several studies showed that stress causes, blood biochemical parameters changes, osmotic regulation disorder, immunosuppression, histopathological damage in different tissues (**Mehrpak *et al*., 2015**).

Chitosan is Generally Recognized as Safe (GRAS) by the **US FDA (2001).** The largest scale of chitosan use was in the water treatment for heavy metal and radioactive pollutant. Chitosan is a coagulant that able to capture substances such as colloids and suspended solid in the water, then to sink or to float separation from the water **(Widodo, *et al*., 2005).**

This work aims to extract low molecular weight chitosan from shrimp wastes and investigate the benefit of using different concentrations to improve water quality characteristics as will as stress reduction, and quality parameters of Nile tilapia fish.

Materials and Methods

This work was conducted in Fish Production Branch, Faculty of Agriculture, Ain Shams University, Cairo, Egypt, to investigate the effect

of low molecular weight Chitosan on water quality, blood parameters and quality of Tilapia (*Oreochromis niloticus*).

The experiment in progress

An experimental design of four treatments each of two replicates; was carried out. A total of 80 *Oreochromis niloticus* (L.) of about (116 g ± 0.01) initial average weight were randomly allotted in the eight concrete ponds (2 m^3 / each), cultivation density was ten fish / pond. (T_1) was a negative control treatment, while the other three treatments exhibited different concentrations of the investigated low molecular weight chitosan as follow: 10 mg / l in T_2, 15 mg / l in T_3 and 20 mg / l in T_4 were the investigated concentrations of chitosan administered in water for 48 days. During the experimental period, all fish were fed a commercial diet (3 mm 30 % protein floating pellets; from Alleraqua Egypt) at (08:00 and 13:00 h) five days a week at a rate of 2.5 % of tilapia biomass and two days starvation till the end of the experimental period.

The experimental ponds were supplied with polluted water from intensive fishpond every 12 days beside the experimental ponds and no aeration were applied throughout the experimental period.

At the experiment wrap-up, five fish from each treatment were homogenized and frozen.

Shrimp wastes preparation method

Shrimp wastes (heads and scales) were collected from El-Obour fish market Al Qalyubia Governorate, Egypt. The wastes were packed in plastic bags and stored at -20º C until using. Shrimp wastes were washed, dried at 70°C overnight, grinded, then sieved to obtain coarse powder at the required particle size which is 60 mesh and stored in dry place until the extraction of chitosan. The chitosan was extracted according to the method mentioned by **Kurita** (**2001**).

Analytical methods

Proximate analysis

Proximate analysis (Moisture, protein, lipids and ash contents) of chitosan samples and fish flesh, as well as, Acid value and Formol titration (FAN) were determined according to **AOAC** (**2000**). The molecular weight (MW) was measured and calculated according to **No *et al.*** (**2003**). The Degree of deacetylation of extracted chitosan was determined according to the method of **Qin *et al.*** (**2006**). Solubility index of chitosan was determined according to the method of **Fernandez** – **Kim** (**2004**). The pH value was estimated according to **Aitken *et al***. (**1962**).

The free fatty acids were determined according to **Egan *et al***. (**1981**). Total volatile basic nitrogen (TVBN) and Tri methyl amine nitrogen (TMAN) determination was conducted based on a semi-micro distillation procedure by **AMC** (**1979**).

Water quality

Water temperature and dissolved oxygen were measured by using oxygen meter (WPA 20 Scientific Instrument) daily at 7 am, according to **APHA (1992)**. Water samples were taken every 12 days and were analyzed in the Central Laboratory for Aquaculture Research (CLAR) to determine pH using glass electrode pH-meter (Digital Mini-pH Meter, model 55, Fisher Scientific, USA), total ammonia concentration was measured by HACH comparison apparatus using HACK kits (Hach Co., Loveland, Colorado, USA). The percentages of unionized ammonia (NH_3) calculated from multiplying the total ammonia value by the appropriate factor according to the following equation NH_3-N = A / 100 x total ammonia Where A is a coefficient related to water pH and temperature measured at the time of taking the sample. **(Boyd 1995),** Nitrate was measured by phenoldisulphonic acid method, using spectrophotometer (model Milton Roy 21D), at a wavelength of 410 nm, nitrite-nitrogen was measured by diazotization method; using spectrophotometer (model Milton Roy 21D) at wavelength of 543 nm, total phosphorus after the samples have been digested using persulfate digestion method, the concentration of total phosphorus was measured using a spectrophotometer (model, WPA Linton Cambridge UK) at a wavelength of 880 nm and orthophosphate using spectrophotometer (model Milton Roy 21D) at a wavelength of 880 nm according to **(APHA,1985).**

Blood chemistry analysis

At the end of the experiment, six *O. niloticus* were taken randomly from each experimental treatment. Clove oil was applied as anesthetic agent at a concentration of 40 mg / l of water. Blood samples were taken by puncturing each individual caudal vein using disposable-syringes, and immediately after collection, 10 µl of blood was used to measure glucose using spectrophotometric (Biomed Diagnostic, Glucose L.S, Egypt). The blood serum was collected and centrifuged at 3500 rpm for 20 min. then, aspartate transaminase (AST), alanine transaminase (ALT), total protein (TP) and albumin (Alb), alkaline phosphatase (ALP), triglyceride (TRIG) and HDL were analyzed using (Biomed Diagnostic, Egypt) spectrophotometric commercial kits, according to the manufacturer's

instructions by vitroscient semi-automated clinical chemistry analyzer model VS10. While the Low-density cholesterol LDL and globulin was calculated respectively according to the Friedewald formula (FF) [(CLDL = Cplasma - CHDL – (TG / 5)] **Friedewald *et al.*, 1972** and globulin (total protein – albumin) **Busher 1990**.

Average daily gain (ADG)

Daily gain was estimated according to the following formula:

$ADG = (Wt_2\text{-}wt_1)/ t$

Where: wt_1 = first fish weight in grams, wt_2 = following fish weight in grams, t= period in day.

Statistical analysis

The data were analyzed by using the GLM procedure with One-way analysis of variance (**SAS**, **2009**), differences among means were tested for significance according to Duncan's multiple range test (**Duncan**, **1955**). The following model was used to analyze the obtained data:

Yij=u+Ti+eij

Where:

Yij =observation, U = the overall mean, Ti= the effect of treatment, eij= random error.

Results and Discussion

Table (1). Functional properties and composition of chitosan extracted from shrimp wastes.

Parameters	**Chitosan**
Molecular weight (M.W)	$1.62*10^4$# 16200
Degree of Deacetylation (DDA)	94%
Solubility	63%
Total nitrogen (T. N) percentage	2.76%
Ash content percentage	3.06%

Data presented in Table (1) showed that, total nitrogen and ash contents of the extracted chitosan were 2.76 and 3.06 %, receptively as determined on dry weight bases. Also, the deacetylation degree of chitosan was 94 %, while solubility was 63 % and molecular weight measure was 1.62kDa. From the previous results, it could be noticed that the chitosan extracted contained low total nitrogen and ash, While, the highest values were found for degree of deacetylation and solubility. The chitosan extracted from shrimp wastes could be classified as a low molecular weight and more effective than chitosan extracted from the

other resources according to **Jing *et al.*** (**2007**). The obtained results are in accordance with that mentioned by **Li *et al.*** (**1992**).

Table (2). Means of the investigated water quality parameters.

	DO (mg/l)	**Temp. °C**	**pH**	**NH_3 (mg/l)**	**NO_2 mg/l**	**NO_3 mg/l**	**(OP) (mg/l)**
T1 (Control)	$1.6^{NS} \pm 0.02$	$23.24^{NS} \pm 0.1$	$7.53^{NS} \pm 0.01$	$0.7^{a} \pm 0.05$	$0.2^{NS} \pm 0.03$	$0.62^{a} \pm 0.1$	$0.47^{NS} \pm 0.2$
T2	1.6 ± 0.1	23.61 ± 0.05	7.56 ± 0.11	$0.6^{a} \pm 0.11$	0.2 ± 0.12	$0.61^{a} \pm 0.5$	0.45 ± 0.01
T3	1.7 ± 0.2	23.65 ± 0.2	7.55 ± 0.01	$0.1^{b} \pm 0.01$	0.1 ± 0.11	$0.36^{b} \pm 0.2$	0.43 ± 0.3
T4	1.5 ± 0.11	23.69 ± 0.3	7.53 ± 0.07	$0.5^{a} \pm 0.2$	0.2 ± 0.02	$0.59^{a} \pm 0.3$	0.47 ± 0.02

Means in the same column having the same superscript letters are not significantly different ($P < 0.05$).

Water quality parameters shown in Table (2) revealed that; there were no statistical significance differences among the different treatments concerning Dissolved oxygen, Temperature, pH, NO_2 and Orthophosphate values alongside the experimental period while there were significant differences among all treatments in concern to NH_3 and NO_3**,** the lowest value was recorded in T_3 (0.1 and 0.36) followed by T_4 (0.5 and 0.59), T_2 (0.6 and 0.61) and control (0.7 and 0.62) mg / l, respectively. As NH_3 concentration of less than 2 mg / l is recommended during the cultivation process (**Zweig *et al.*, 1999)**, the removal of NH_3 may be due to the formation of hydrogen bonds between chitosan and NH_3 or the mechanism of partial charge neutralization (**Chung *et al.*, 2007)**. They revealed that, If chitosan of molecular weight (3.62×10^5) was added at 12 mg / l to simultaneously remove NH_3, results exceeding the discharge standard were reached. (**Patil *et al.*, 2013)** concluded that, when adding chitosan in powder form with concentrations of 0.5, 1 and 1.5 g respectively, to a 250 ml sample of groundwater the feasibility of removing nitrate was noticed. **The same author** revealed that, in concentration of 0.5 g chitosan the concentration of NO_3 decreased from almost 24 mg / l to 8 mg / l. From the previous results it can be concluded that chitosan has an effect on Non-ionized ammonia and Nitrate.

Table (3). Growth parameters of the experimental treatments.

	Initial Weight (g)	**Final Weight** (g)	**ADG** (g)	**% Survival**
T1 (Control)	$116.5^{NS} \pm 0.02$	$97.5^{c} \pm 0.04$	$-0.4^{c} \pm 0.01$	85^{c}
T2	116.6 ± 0.01	$121.2^{b} \pm 0.02$	$0.1^{b} \pm 0.07$	100^{a}
T3	116.2 ± 0.03	$129.5^{a} \pm 0.01$	$0.28^{a} \pm 0.06$	100^{a}
T4	117 ± 0.02	$123.4^{b} \pm 0.03$	$0.13^{b} \pm 0.05$	95^{b}

Means in the same column having the same superscript letters are not significantly different ($P < 0.05$).

The effects due to chitin and chitosan on growth performance of any aquatic animals are somewhat controversial (**Niu *et al.*, 2013**). Several studies have proven some benefited effects of the dietary chitosan on growth performance and immunity of the aquatic (**Niu *et al.*, 2011**; **Niu *et al.*, 2013**; **Wang and Chen, 2005**).

In the present study, the treatments with chitosan had significantly higher Final Weight (FW) and Average Daily Gain (ADG) than the control. There were significant differences found among chitosan treatments. The highest value of the FW and ADG within chitosan treatments was in T_3 followed by T_4 and T_2, respectively. **Niu *et al.*** (**2011**) reported that medium chitosan level gave benefited effects on the growth and the survival of the *Litopenaeus vannamei* and the optimum supplement dietary of the chitosan level should be in a range between 2.13 and 2.67 g kg^{-1} diet. The use of chitosan in the diet at a medium concentration could have taken place as a part in the biosynthesis of the organism at a rapid speed (**Niu *et al.*, 2011**). Chitosan supplemented diet was also reported to enhance the growth of fish such as olive flounder, *Paralichthys olivaceus* (**Cha *et al.*, 2008**) and common carp, *Cyprinus carpio* (**Gopalakannan** and **Arul, 2006**). From the previous data, it was clear that chitosan treatments recoded high survival rate compared to control, which the highest survival rate was in T_2 and T_3 while the lowest one was recorded T_1 (control). These results were in accordance with those by (**Maqsood *et al*. 2010**) who found that, the inclusion of chitosan at a high level of 20 g kg^{-1} in the diet of European carp (*Cyprinus carpio*) decreased the fish mortality and enhanced the growth under stress conditions.

Table (4). Approximate chemical composition of Tilapia fish flesh on dry weight bases at the end of the study.

	Moisture	Protein	Fat	Ash
T1(Control)	$78.2^{NS} \pm 0.44$	$75.62^{NS} \pm 1.93$	$14.01^{NS} \pm 0.13$	$9.92^{NS} \pm 0.13$
T2	78.57 ± 0.4	75.75 ± 1.51	13.32 ± 0.53	9.72 ± 0.28
T3	79.04 ± 0.33	76.97 ± 1.23	12.69 ± 0.91	9.82 ± 0.3
T4	78.41 ± 0.17	76.64 ± 0.38	12.83 ± 0.46	9.93 ± 0.54

Means in the same column having the same superscript letters are not significantly different ($P < 0.05$).

The % moisture tended to have a downward pattern of change since it started by 78.2, 78.41 and 78.57 % in control, T_4 and T_2, while, moisture content for T3 was 79.04 % as shown in Table (4). The % of total protein and fat of fish samples were (75.62, 75.75, 76.97 and 76.64) and (14.01, 13.32, 12.69 and 12.83) at the end of the experiment period for control, T_2, T_3 and T_4, respectively. It was evident from the protein and fat results that there were no observed significant differences among treatments. Also, the ash content of the Tilapia samples recorded 9.92, 9.72, 9.82 and 9.93 % for control, T_2, T_3 and T_4, respectively. It was clear from the data that there were no significant differences among treatments concerning the ash content. Whole-body moisture, crude protein and crude fat were significantly influenced by chitosan supplementation. There was a significant ($P \leq 0.05$) change in ash content associated with diets supplemented with different levels of chitosan. A general tendency of increased crude protein was observed in all treatments evident by increasing growth rate and age. Fat decrease with increasing chitosan level (**Zaki *et al*., 2015**).

Table (5). physical and chemical quality parameters of Tilapia fish flesh.

	pH	Acid value mg / g	Free amino nitrogen mg / 100g	Free fatty acids %	TVB-N mg / 100 g	TMA-N mg / 100 g
T1 (Control)	6.24^{NS}±0.11	0.31^{ab}±0.01	1.47^{c}±0.17	0.11^{b}±0.01	1.52^{c}±0.07	0.27^{NS}±0.02
T2	6.21±0.08	0.27^{b}±0.06	1.64^{b}±0.35	0.13^{ab}±0.04	1.70^{b}±0.08	0.30±0.02
T3	6.38±0.12	0.31^{ab}±0.01	1.92^{a}±0.06	0.16^{a}±0.01	1.91^{ab}±0.01	0.33±0.00
T4	6.32±0.08	0.35^{a}±0.01	1.90^{a}±0.30	0.08^{c}±0.04	1.96^{a}±0.03	0.35±0.01

Means in the same column having the same superscript letters are not significantly different ($P < 0.05$).

Results in Table (5) showed that, pH, acid value, free amino nitrogen and free fatty acid values for all treatments were (6.24, 6.21, 6.38 and 6.32); (0.31, 0.27, 0.31 and 0.35); (1.47, 1.64, 1.92 and 1.9) and (0.11, 0.13, 0.16 and 0.08) for control, T_2, T_3 and T_4, respectively. This is indicative of the high degree of quality and safety in all treatments, and the most value of quality was in T_3, although the best coefficients in terms of free fatty acids content were in (T_4). The TVB-N contents of different Nile tilapia fish samples ranged from 1.7 to 1.96 mg / 100 g while values of TMA-N ranged from 0.27 to 0.35 mg / 100 g for control, T_2, T_3 and T_4, respectively. In spite of this, the values of the TVB-N and TMA-N for all coefficients are below the permissible limits and according to the results they considered excellent, accordingly the best treatment found to be (T_4). These results are consistent with that approved by **Bennour *et al.*, (1991)** who stated that, the standard of fish freshness is very good, and intermediate when trimethylamine (TMA-N) = < 1 - 10 mg / 100 g, 10 - 30 mg / 100 g, and 30 - 50 mg / 100 g, respectively, Abd-EL-Aziz (1996) suggested that the critical level of free amino nitrogen (FAN) was 53.2 mg / 100 g in imported fish and Shen (1996) for fresh fish, sub-fresh fish, and deteriorated fish when total volatile bases nitrogen (TVB-N) = <15 mg / 100 g, 15 - 25 mg / 100 g, and >25 mg / 100 g, respectively. The average TVB-N values for fresh crustaceans are often higher than that of fish.

Table (6). Experimental treatments blood chemistry indices ±SE

	T1 (Control)	T2	T3	T4
AST (U/l)	$138^{a} \pm 23.05$	$125.71^{a} \pm 13.78$	$52.25^{b} \pm 7.94$	$97.83^{a} \pm 18.41$
ALT (U/l)	$38.5^{a} \pm 4.05$	$21^{bc} \pm 3.03$	$28^{b} \pm 0.87$	$18.83^{c} \pm 2.24$
TP (g/dl)	$3.05^{b} \pm 0.39$	$3.69^{ab} \pm 0.45$	$5.08^{a} \pm 0.79$	$4.21^{ab} \pm 0.7$
Alb (g/dl)	$1.6^{NS} \pm 0.06$	1.82 ± 0.1	1.86 ± 0.11	1.67 ± 0.04
Glob (g/dl)	$1.46^{NS} \pm 0.39$	1.87 ± 0.46	3.22 ± 0.68	2.54 ± 0.71
ALP(U/l)	$53.5^{NS} \pm 5.97$	62.75 ± 5.39	66.86 ± 3.36	56 ± 5.7
GLU (mg/dl)	$80^{a} \pm 4.14$	$71.87^{ab} \pm 3.3$	$67.29^{b} \pm 1.91L$	$67.75^{b} \pm 4.71$
CHO (mg/dl)	$163.66^{a} \pm 6.36$	$131.5^{b} \pm 4.2$	$98.14^{c} \pm 9.53$	$114^{bc} \pm 5.93$
TG (mg/dl)	$138.83^{a} \pm 29.19$	$109.62^{b} \pm 10.05$	$88.71^{d} \pm 4.73$	$100.25^{c} \pm 20.51$
HDL (mg/dl)	$51.17^{a} \pm 1.99$	$43.5^{b} \pm 1.04$	$35.85^{c} \pm 1.92$	$43.25^{b} \pm 2.53$
LDL (mg/dl)	$84.73^{a} \pm 7$	$66.32^{ab} \pm 3.43$	$44.54^{c} \pm 7.76$	$50.45^{bc} \pm 4.37$

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP), Serum Albumen (Alb), Serum Globulin (Glob), Alkaline phosphatase (ALP), Glucose (GLU), Cholesterol (CHO), Triglycerides (TG), High density lipid (HDL), Low density (LDL). Means in raw with different superscript are significantly different ($P< 0.05$).

Blood chemistry indices shown in Table (6) revealed that; T_3 had the lowest AST; T_4 had the lowest ALT values compared to control and T_2 respectively. The lowest TP value was acquired by control and the highest was recorded by the T_3 followed by T_4 and T_2, respectively. Despite the variation in Alb, Glob and ALP values among treatments they showed no statistical significance differences. T_3 showed the lowest values among all treatments compared to control for the following blood indices GLU, CHO, HDL, LDL and TG, followed by T_4, T_2 and control respectively.

From the collected data there was a significant decrease in T_2 liver enzymes activities compared to control and other treatments. These findings are supported by **Shin *et al*., (2016)** and **Kim *et al*., (2017)** who revealed that ALT and AST values were substantially increased by the ammonia exposure, whereas total protein was significantly decreased.

Moreover, **Sinha *et al.*, (2013)**; stated that, AST and ALT were notably elevated in carp and goldfish while only ALT was upregulated in trout exposed to high ammonia levels as adaptive defense mechanism. While, **Abbas, (2006)**; found that, there was a significant increase on stress enzymes activities "ALT & AST of common carp is indicative of some degree of liver and kidney tissue necrosis or dysfunction. **Labarrère *et al.* (2013**) stated that, hyperglycemia related to stress is reported in several teleosts through mobilization of energy sources such as glucose and free fatty acids to cope with unfavorable conditions. **The author also stated that**, the blood glucose level is used in fish farming research as an efficient indicator of stress. The obtained data showed that there were significant differences between control which had the highest values for CHO 163.66 ± 6.36 mg / dl, HDL 51.17 ± 1.99 mg / dl and LDL 84.73 ± 7 mg / dl and TG 138.83 ± 29.19 mg / dl compared to the T_3 which had the lowest obtained values for CHO 98.14 ± 9.53 mg / dl, HDL 35.85 ± 1.92 mg / dl and LDL 44.54 ± 7.76 mg / dl and TG 88.71 ± 4.73 mg / dl. Our assumption that due to the detoxifying effect of chitosan, the liver functions were enhanced the lipidogram (CHO, HDL, LDL and TG) in the experimental treatments, where they were reduced significantly due to the reduction of liver stress. The same trend supported by the findings of (**Xu *et al.*, 2018**) who stated that, although our comprehension to the physiological roles that cholesterol can play in bony fish health under stress conditions is insufficient. However, the cholesterol is closely linked to fish stress through the hypothalamic-pituitaryinterrenal axis (HPI axis). In fish, cortisol is the main hormone in the HPI axis that maintains the balance of physiological and biochemical processes when fish dealing with stress; and promotes the secretion of adrenocorticotropic hormone derived from (ACTH) in the anterior pituitary gland. Moreover, the finding of (**Tao *et al.*, 2018**) stated that, the increase in cholesterol in blood serum mainly produced either by high fat diet content or increase in oxidative stress, AST and ALT in liver during inflammation or damage that, may lead to lipid metabolism disorder and induce hyperlipidemia. This also supported by the findings of (**Hernández-Pérez *et al.*, 2019**) who showed that, when *O. mykiss* and Atlantic salmon and other teleost species is subjected to stress, the liver displays changes in activity such, decreasing lipogenic activity, and increase fatty acid release from the liver into the blood. These changes occur in order to provide energy resources that allow the organism to handle the adverse situation.

CONCLUSION

The addition of chitosan at different concentrations led to improve water quality, reducing stress through the detoxification effect that could indicate better metabolic activity which was reflected in the high quality properties of tilapia fish and their suitability for human consumption.

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تأثير استخدام الشيتوزان ذو الوزن الجزيئي المنخفض على جودة المياه وتقليل الإجهاد ومؤشرات جودة لحم أسماك البلطي النيلي

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الملخص العربي

بحثت الدراسة في كفاءة إضافة الشيتوزان المستخرج من مخلفات الجمبري لتحسين جودة المياه وتقليل الإجهاد وزيادة جودة لحم أسماك البلطي. أظهرت قياسات جودة المياه عدم وجود فروق ذات دلالة إحصائية بين المعاملات المختلفة في قيم الأوكسجين المذاب، درجة الحرارة، درجة الحموضة، النتيريت و الأورثوفوسفات. أثرت معاملة مياه الأسماك بالشيتوزان على بعض القياسات النيتروجينية مثل الأمونيا والنترات، حيث كانت القيم على النحو التالي (٠,٧ و ٠,٦ و ٠,١ و ٠,٥ ملجم / لتر) للأمونيا و (٠,٦٢ و ٠,٦١ و ٠,٣٦ و ٠,٥٩ ملجم / لتر) للنترات، على التوالي. تم تسجيل أقل قيمة للأمونيا والنترات في المعاملة الثالثة (١٥ ملجم / لتر من الشيتوزان) متبوعه بالمعاملة الرابعة (20 ملجم / لتر من الشيتوزان) و المعاملة الثانية (١٠ ملجم / لتر من الشيتوزان) وأخيرًا المعاملة التجريبية. أظهر التركيب الكيميائي للعينات أن إضافة الشيتوزان إلى الماء يحسن من محتوى لحم الأسمك من الرطوبة والبروتين والدهون وزيادة محتوى الرماد على التوالي، يليه المعاملة التجريبية. أظهرت معايير الجودة الفيزيوكيميائية مثل الأس الهيدروجيني، قيمة الحموضة، النيتروجين الأميني الحر، الأحماض الدهنيه الحره، القواعد النيتروجينيه الطياره وثلاثي ميثيل الأمين في لحم أسماك البلطي أن إضافة الشيتوزان أدت إلى الحفاظ على جودة اللحم وتقليل معدل الزيادة في هذه القياسات في المعاملات الثلاثة مقارنة مع المعاملة التجريبية. كشفت مؤشرات كيمياء الدم أن أقل قيمة ل AST كان في المعاملة الثالثة، وكانت أدنى قيمه ALT في المعاملة الرابعة مقارنة بالمعاملة التجريبية و المعاملة الثانيه على التوالي. على الرغم من التباين في قيم Alb و Glob و ALP بين المعاملات إلا أنهم لم يظهروا أي اختلافات ذات دلالة إحصائية. أظهرت المعاملة الثالثة أدنى قيم بين جميع المعاملات في قياسات الدم التالية GLU ، CHO ، HDL ، LDL ، TG متبوعه بالمعاملة الرابعة، المعاملة الثانية ثم المعاملة التجريبية على التوالي.

الكلمات المفتاحية: الشيتوزان، البلطي النيلي، جودة المياه، التركيب الكيميائي، القياسات الفيزيائية الكيميائية وتقليل الأجهاد.