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DIETARY OMEGA 3 NANOPARTICLES SUPPLEMENT IMPROVE GROWTH OF NILE TILAPIA, (OREOCHROMIS NILOTICUS)

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Running title: Nanoparticles improve growth and immunity of Nile Tilapia.

Key Words: Omega 3, chitosan nanoparticles, Nile Tilapia, growth performance.

ABSTRACT:

This work was planned to investigate the potential effect of omega 3 chitosan nanoparticles in Nile tilapia fingerlings. Two hundred forty Nile tilapia were used into f

our groups; control diet without omega 3- chitosan nanoparticles, omega 3- chitosan nanoparticles (0.25g/kg), omega 3- chitosan nanoparticles (0.5g/kg) and omega 3- chitosan nanoparticles (1g/kg) for 12 weeks. Our data obtained showed that, significant differences were found in growth parameters, including final body weight (g), body gain (g), body gain (%), protein efficiency ratio, specific growth rate (%) and feed conversion ratio. In addition, our data presented showed that total cholesterol, triacylglycerdes (TAG), high-density lipoprotein (HDLc), low-density lipoprotein(LDLc), total protein (TP), albumin, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were significantly affected by adding omega 3-chitosan nanoparticles in diet and showed high significant differences ($p < 0.05$). However, showed no significant differences ($p > 0.05$) on globulin (GLO) but were significant differences ($p < 0.05$) on interleukin-1 (IL1), interleukin-6 (IL6), and fish tumor necrosis factor (TNF-α). In molecular expression, omega-chitosan NPs 0.5 and 1 g/kg were the most significant treated one on interleukin-1 beta (IL1- beta) but control diet in molecular expression of toll like receptors factores 2 (TLR2) was the most significant treated one.

INTRODUCTION:

The fisheries and livestock sectors were an important agriculture subsidiary. They were recently drawing wide attention thanks to their accelerating growth and because of high market demand. In addition, fishery has becoming popular. Fish have vital food sources rich in simple, digestible animal proteins and beneficial lipids. Fish products have essential to one billion individuals for protein security and particularly vital for juvenile and pregnant women as well [1]. In addition, the development of Nano technological formulations for application in aquaculture has been a major focus of research conducted

in this industrial domain. They are suitable for multiple applications, including growth performance, administration of vaccines, antibiotics, other pharmaceuticals and nutraceuticals is a key feature of these systems [2]. Currently much of the tilapia production takes place in intensive practices. There have been problems, especially in early stages, as disease outbreaks [3]. However, the concept of functional foods has used in the food industry for production animals.in addition to meeting the nutritional requirements; they also need to improve the health of farmed fish [4, 5]**.** The functional feed additives promoted growth, immune response; induced the physiological functions and health performance of the fishes better than the normal feed additives. Moreover, functional feed additives have been included phytogenic compounds, mycotoxin binders, organic acids, immune – stimulants, yeast products, probiotics, prebiotics, enzymes [6]. Also, Chitosan has been used as natural polymers to coat different nanoparticles (NP) for their unique and outstanding bio degrable, biocompatible, and muco adhesive properties. Permeation promoted ability for the absorption of hydrophilic molecules such as insulin as well. Allowing chitosan to transiently open tight junctions among the intestinal cells to go through Para cellular pathway of absorption when administered orally. In addition, through intranasal route of administration, chitosan can improve residence time of the used nanoparticles (NP) via its bio adhesive characteristics. It also causes chitosan nanoparticles (NP) to adhere to the mucosa and increase drug bioavailability to enhance its absorption [7]. Apart from the various biological functions, has been found that chitosan is used as a food additive. Chitosan with high molecular weight is more effective as a food additive when compared to low molecular weight chitosan as well [8]. Omega-3 fatty acids represent a family of polyunsaturated fatty acids. In addition, they are called essential fatty acids. They are very important for the body [9]. It has been stated with regard to anti-inflammatory properties, omega-3 in the form of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) has been received higher prominence [10, 11]. It has been regarded that anti-inflammatory effects of omega-3 poly unsaturated fatty acids (PUFA) may decrease pro inflammatory cytokines and oxidative stress in adipocytes, leading to a lower rate of ectopic lipid accumulation in the liver [12].

The reason for conducting this study was the massive availability, use of nanoparticles element such as omega 3 and chitosan as growth promotors in Nile Tilapia. This experiment was designed to determine the possible application of omega 3- chitosan nanoparticles as a potential dietary supplement for Nile Tilapia through determination of: growth performance parameters , total protein (TP), plasma albumin, plasma globulin , alanine transaminase (ALT) , aspartate transaminase (AST)

,total cholesterol , triacylglycerdes (TAG) , high-density lipoprotein (HDLc) , low-density lipoprotein (LDLc), fish interleukin-1 (IL1), fish interleukin-6 (IL6) , fish tumor necrosis factor α (TNF-α) and molecular studying of toll like receptors 2 (TLR2) and interleukin 1 beta (IL1-beta) by using real time polymerase chain reaction .

MATERIAL AND METHODS*:*

Preparation of Omega Chitosan Nanoparticles

The samples were irradiation with Co^{60} (Ge4000A Cell) Indian irradiation facility gamma rays at a dose rate ranged (1.144 KGy/h). The irradiation facility was constructed by the National Center for Radiation Research and Technology, Atomic Energy Authority of Egypt. The infrared spectra were performed by using FTIR spectrophotometer, Mattson 100 UniCam, England, over the range 400–4000 cm^{-1}. A dry constant weight from each composite was ground with 20µg of KBr and then pressed to form transparent disks. The samples for IR analysis were first dried in a vacuum oven at 80^{o}C for 2 hr.

Fish and experimental design:

They were carried out at fish research unit (F.R.U), and Biochemistry Department, Faculty of Veterinary Medicine, Zagazig University. Nile tilapia with average initial weight of (18.42 g) were purchased from a local fish hatchery (Central laboratory for aquaculture research, Abbassa, Abu-Hammad, Sharkia, Egypt). The fish were dived into equal 4 triplicate of the fish groups (each replicate group contained 20 fish) and each replicate of the fish groups was stocked in its corresponding glass aquarium for two weeks to be acclimatized before the start of the experiment. The fish were fed is nitrogenous (37 %CP), isochoric (DE, 2900Kcal) diet contained (omega 3- chitosan nanoparticles) by three levels (0.25g, 0. 5g, and 1g/kg), the diet of Nile tilapia [13]. The fish in the experiment were located in 12 rectangular glass aquaria (150x 40x 30 cm) filled with dechlorinated tap water which continuously aerated by a small air compressor which were used to represent 4 experimental treatments (3 replicates per treatment) and each aquarium was stocked with 20 fish .The institutional fish care and use committee of the Faculty of Veterinary Medicine; Zagazig University approved our study **(ZU-IACUC/2/F/53/2020).** All fish were adapted for a small period of two week prior to start the beginning of the study. Nile tilapia were used into four triplicate groups; control diet, omega 3-chitosan nanoparticles 0.25(g/kg), omega 3- chitosan nanoparticles (0.5g/kg) and omega 3- chitosan nanoparticles (1g/kg).All groups were given respective orally treatment via diet which was performed in glass aquaria for duration of 12 weeks. Fishmeal, soybeans meal, poultry-by-

products meal, yellow corn, corn gluten, wheat bran and vegetable oils were used as the main feeds ingredients in diets formulation besides lysine, methionine, calcium dibasic phosphate and vitamins and minerals mixture were added. Isonitrogenous (37 %CP), isochoric (DE, 2900Kcal) diets were formulated according to [14] as with addition of omega 3 loaded chitosan nanoparticle as mash diet and mixed with ingredient .

Water characteristics

Dissolved oxygen (D.O):- It was 5.4 ± 0.1 mg / L. PH: - It was 7.5 ± 0.07. Water temperature: - The average water temperature during experiment was 24°c ± 2 °c (adapted by using water heater). Nitrite (NO2): - The nitrite level in fishponds was 0.024± 0.02 mg / L. 5- Nitrate (NO3):- It was 7± 0.2 mg /L. Ammonium (NH4): The ammonium level in fishponds was 0.4± 0.2 mg / L. Total hardness: - It was 160.25 ± 0.07mg / L. Phosphate (PO4):- It was 0.25± 0.01 mg / L.

Sampling

Under hygienic condition blood sample were collected once without using anesthesia from a random sample of fish/group at the end the experiment. Blood samples were collected from caudal vein were collected from a random sample of fish /group and pooling to the blood sample from different fish in the same sample [15]. Immediately after scarifying, take liver, intestine and muscle. Every sample was divided to 2 parts; one was wrapped in aluminum foil and put immediately in liquid nitrogen container to make snap freezing for molecular investigation. Second part kept at -20 ^{o}C to be homogenized for antioxidants measurements.

Growth performance determination:

According to Merrifield *et al* [16] were calculated weight gain (g /fish), weight gain (WG, %) , specific growth rate % , protein efficiency ratio and feed conversion ratio (FCR).

Biochemical determinations:

Determination of plasma total protein (TP) according to Yatzidis *et al* [17]. Plasma albumin, plasma globulin outlined by Doumas *et al* [18]. Plasma alanine aminotransferase (ALT) reported by Breuer [19]. Plasma aspartate aminotransferase (AST) reported by Sherwin *et al* [20]. Determination of total cholesterol described by Naito and Kaplan [21]. Triacylglycerdes (TAG) was assayed according to Burtis *et al* [22]. high-density lipoprotein (HDLc) and low-density lipoprotein (LDLc) according to Naito and Kaplan [21]. Determination of Interleukin-1 (IL-1) was assayed using Kit (Cat.No: MBS042749). This Quantitative Sandwich ELISA kit was for lab reagent/research use only, not for drug, household, therapeutic or diagnostic applications! This kit is intended to

be used to determine the level of Interleukin-1 (IL-1) (hereafter termed "analyte") in undiluted original fish serum, plasma or tissue homogenates samples. Determination of fish Interleukin-6 (IL-6) was assayed using Elisa Kit (Catalog Number. MBS702353). This assay employs the competitive inhibition enzyme immunoassay technique. The microtiter plate provided in this kit has been pre-coated with an antibody specific to IL-6. Determination of fish tumor necrosis factor α (TNF-α) was assayed using Kit (Cat.No: MBS281612). This assay employs a two-site sandwich ELISA Kit to quantitate fish tumor necrosis factor α (TNF-α) in Fish serum, plasma. An antibody specific for fish tumor necrosis factor α (TNF-α) were pre-coated onto a microplate.

Molecular determinations:

Determination of the levels of expression of interleukin-1 beta (IL1- beta) and toll like receptors 2 (TLR2)، using Real Time-PCR**.** Using Pure Link® RNA Mini Kit obtained from Ambion by life technologies by Thermo Scientific, Catalog numbers: 12183018A and using the manufacture instructions. The formation of cDNA was done by using High Capacity cDNA Reverse Transcription Kit obtained from Thermo Scientific, code 4374966.Amplification was done using SYBR Green qPCR Master Mix (2X) kit obtained from Thermo scientific, catalog #K0251. The amount of target gene expression levels was estimated using the formula of $2^{-\Delta\Delta ct}$ Livak and Schmittgen, [23] and using internal control GAPDH. The primer sequences were designed as fellow: Toll-Like Receptors 2 (TLR2) [24] primer sequence (5′-3′), F-CAGCCATTGACTCTCTGCCT` \ R-CACCAGTGGCATGACCTTCA and interleukin-1 beta (IL-1β) [25] primer sequence (5′-3′), F-TGCTGAGCACAGAATTCCAG \ R-GCTGTGGAGAAGAACCAAGC.

Statistical analysis:

The results were done using mean and standard error (Mean ± SEM).ANOVA test has been done to test the significant changes among different groups. Duncan multiple range test was considered as a post hoc test. The statistical analysis was done using IBM SPSS version 25.

RESULTS

Impact of omega 3- chitosan nanoparticles on Growth performance:

Our results showed that in table (1) fish groups fed on diet contained omega 3 -chitosan nanoparticles 1 g/kg revealed significant increase ($P < 0.05$) in final body weight (g), body gain (g), body gain (%) , specific growth rate (%) , feed conversion ratio and protein efficiency ratio when compared with omega 3- chitosan nanoparticles 0.5 g/kg but there were no

significance difference (P >0.05) between fish groups feed on diet contained control diet and omega 3- chitosan nanoparticles 0.25 g/kg.

Table (1): Impact of Omega – Chitosan Nanoparticles on Growth performance:

Treatment	Control	omega 3 – Chitosan Nanoparticles 0.25 g/kg	omega 3 – Chitosan Nanoparticles 0.5 g/kg	omega 3 – Chitosan Nanoparticles 1 g/kg
Initial body weight (g)	24.65 ±0.35	24.53 ±0.20	25.00 ±0.33	24.15±0.17
Final body weight (g)	64.54 ±1.00^{c}	64.00±0.40 ^{c}	68.81 ±0.95 ^{b}	74.29 ±0.95 ^{a}
Body gain (g)	39.89 ±0.73 ^{c}	39.47 ±0.60 ^{c}	43.80 ±0.63 ^{b}	50.14 ±0.99 ^{a}
Body gain (%)	161.83 ±2.00 ^{c}	160.95 ±3.80 ^{c}	175.22 ±0.60 ^{b}	207.70±4.80 ^{a}
Specific growth rate (%)	1.15 ±0.01 ^{c}	1.14 ±0.01 ^{c}	1.21 ±0.01 ^{b}	1.34 ±0.02^{a}
Feed conversion ratio	2.00±0.03^{c}	1.98±0.01^{c}	1.91±0.04^{b}	1.74±0.02^{a}
Protein efficiency ratio	1.53±0.01^{c}	1.55±0.01^{c}	1.64±0.04^{b}	1.79±0.01^{a}
Means of variables having different superscripts in the same raw are significantly different.				

Impact of omega 3- chitosan nanoparticles on total cholesterol, triacylglycerdes (TAG), high-density lipoprotein (HDLc) and low-density lipoprotein LDLc:

Data presented in table (2) showed that fish groups fed on diet-contained omega 3- chitosan nanoparticles (1 g /kg) in total cholesterol revealed significant increase (P <0.05) when compared with control diet while fish groups fed on diet-contained omega 3- chitosan nanoparticles (0.5, 1 g /kg) in triacylglycerdes (TAG) revealed significant increase (P <0.05) when compared with control diet but fish groups fed on diet-contained omega 3- chitosan nanoparticles (0.5, 1 g /kg) in high-density lipoprotein (HDLc) and low-density lipoprotein LDLc revealed significant decrease (P <0.05) when compared with control diet.

Table (2): Impact of omega – Chitosan Nanoparticles on total cholesterol, triacylglycerdes (TAG), high-density lipoprotein (HDLc) and low-density lipoprotein LDLc:

Treatment	Control	Omega- Chitosan Nanoparticles 0.25 g /kg	Omega - Chitosan Nanoparticles 0.5 g /kg	Omega - Chitosan Nanoparticles 1 g /kg
Total Cholesterol	150.33^{a}	146.83^{a}	140.90^{ab}	138.20^{b}
Triacylglycerdes (TAG)	209.66^{a}	184.96^{b}	89.00^{c}	86.06^{c}
High-density lipoprotein(HDLc)	33.60^{c}	37.66^{bc}	43.63^{a}	43.60^{a}
low-density lipoprotein(LDLc)	74.80^{abc}	72.17^{c}	79.46^{a}	77.38^{ab}
Means of variables having different superscripts in the same raw are significantly different.				

Impact of omega 3- chitosan nanoparticles on total protein (TP), albumin, globulin (GLO), alanine aminotransferase (ALT) and aspartate aminotransferase (AST):

Our obtained data in table (3) showed that fish groups fed on diet-contained omega 3- chitosan nanoparticles (0.5, 1 g /kg) in total protein (TP), albumin revealed significant increase ($P < 0.05$) when compared with control diet while no significance difference ($P > 0.05$) among differ experimental groups fed on diets contained different levels of omega 3-chitosan nanoparticles in globulin but fish groups fed on diet-contained omega 3- chitosan nanoparticles (0.5, 1 g /kg) in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) revealed significant decrease ($P < 0.05$) when compared with control diet.

Table (3): Impact of Omega – Chitosan Nanoparticles on total protein (TP), albumin, globulin (GLO), alanine aminotransferase (ALT) and aspartate aminotransferase (AST):

Treatment	Control	Omega- Chitosan Nanoparticles 0.25 g /kg	Omega - Chitosan Nanoparticles 0.5 g /kg	Omega - Chitosan Nanoparticles 1 g /kg
Total protein (TP)	5.23^{d}	5.75^{b}	5.80^{b}	6.15^{a}
albumin	2.14^{c}	2.83^{b}	3.45^{a}	3.40^{a}
Globulin (GLO)	3.19	2.83	2.39	2.70
Alanine aminotransferase (ALT)	22.10^{ab}	21.97^{ab}	20.50^{bc}	19.63^{c}
Aspartate aminotransferase (AST)	43.33^{ab}	43.66^{ab}	41.33^{abc}	38.43^{c}

Means of variables having different superscripts in the same raw are significantly different.

Impact of omega 3- chitosan nanoparticles on interleukin-1 (IL1), interleukin-6 (IL6) and fish tumor necrosis factor (TNF-α):

Our obtained data in table (4) showed that fish groups fed on diet contained omega 3- chitosan nanoparticles (0.5, 1 g /kg) in interleukin-1(IL1), interleukin-6 (IL6) and fish tumor necrosis factor (TNF-α) revealed significant increase ($P < 0.05$) when compared with control diet.

Table (4): Impact of omega – Chitosan Nanoparticles on interleukin-1 (IL1), interleukin-6 (IL6) and fish tumor necrosis Factor (TNF-α):

Treatment	Control	Omega- Chitosan Nanoparticles 0.25 g /kg	Omega - Chitosan Nanoparticles 0.5 g /kg	Omega - Chitosan Nanoparticles 1 g /kg
interleukin-1 (IL1)	3.72^{c}	3.82^{c}	4.20^{b}	4.21^{b}
interleukin-6 (IL6)	23.40^{b}	24.56^{a}	24.86^{a}	24.90^{a}
fish tumor necrosis Factor (TNF-α)	16.13^{c}	19.58^{b}	21.35^{a}	21.27^{a}
Means of variables having different superscripts in the same raw are significantly different.				

Impact of omega 3- chitosan nanoparticles on Interleukin-1 beta (IL1-beta) and Toll-Like Receptors 2 (TLR2) in Nile tilapia by using real time PCR:

Data presented showed that in table (5) fish groups fed on diets contained omega 3- chitosan nanoparticles 0.5 and 1 g/kg revealed up regulated expression of interleukin-1beta (IL-1β) ($P < 0.05$) followed by group fed on diet contained omega 3- chitosan nanoparticles 0.25 g/kg when compared with group fed on control diet. while groups fed on diets contained omega 3- chitosan nanoparticles 0.25 g/kg revealed down regulated expression level of toll-like receptors 2 (TLR2) ($P < 0.05$) followed by group fed on diet contained omega 3- chitosan nanoparticles 0.5 g/kg then group fed on omega 3- chitosan nanoparticles 1 g/kg when compared with group fed on control diet.

Table (5): Impact of omega – Chitosan Nanoparticles on interleukin-1 beta (IL1- beta) and toll like receptors factores 2 (TLR2) in Nile tilapia by using real time PCR:

Treatment	interleukin-1 beta (IL1- beta)	toll like receptors factores 2 (TLR2)
Control	$1.000^{c} \pm 0.07$	$1.000^{a} \pm 0.08$
Omega- Chitosan Nanoparticles 0.25 g/kg	$1.13^{b} \pm 0.03$	$0.547^{b} \pm 0.16$
Omega- Chitosan Nanoparticles 0.5 g/kg	$1.38^{a} \pm 0.07$	$0.500^{bc} \pm 0.25$
Omega- Chitosan Nanoparticles 1 g/kg	$1.363^{a} \pm 0.15$	$0.427^{c} \pm 0.07$
Means of variables having different superscripts in the same raw are significantly different.		

DISCUSSION:

This study was designed to investigate the effects of adding omega 3 loaded chitosan nanoparticles in the diet of Nile tilapia and its effect on

growth performance, body composition, some immunological and biochemical parameters and their economic efficiency.

The use of functional feed additives in aquaculture industry have dual benefit of improved fish growth performance also it have posted immune response of cultured fish .Moreover , the development of Nano technological formulations for application in aquaculture has a major focus of research conducted in this industrial domain. They were suitable for multiple applications; including growth performance, administration of vaccines, antibiotics, other pharmaceuticals in addition, nutraceuticals is a key feature of these systems Rather *et al* [26]. Among this respective feed additive were omega 3 and chitosan. It has been found application of this Nano formulation will enhance fish growth also promote immune statue. Dawood and Koshio , Ringø *et al* [27,28] said that these feed additives has help increase immune status, feed efficiency, and growth performance of fish and shellfish.

I) Using of Omega 3 Chitosan Nano particle as feed additive:

In this study, we have been investigated the effect of a nanoparticle on growth performance mainly, which was produced, from two known immune stimulants, namely Omega 3 and Chitosan. Through our experiment, we observed that Omega 3-loaded chitosan nanoparticles have stimulatory effect on growth performance, body composition, and some immunological parameters, molecular studies, antioxidant status, lipid profile and the anti-inflammatory effect in Nile tilapia.

It has been showed that Microencapsulation could be applied to encapsulate natural compounds such as essential oils as omega 3 fatty acid and plant extracts [29]. In aquaculture, found that many of oral delivery systems of bioactive materials meet three main barriers while passing through the gastrointestinal tract involving the enzymes, immunological cells and the physical barrier of the epithelial cells [30]. Therefore, the encapsulation of bioactive compounds and functional foods has a promising way to overcome these problems. Moreover, chitosan can be acted as an encapsulating agent because of its characteristics, such as non-toxicity, biocompatibility, mucus adhesiveness also biodegradability characteristics [31].

II) Growth performance finding:

In our study, our presented data showed that fish groups fed on diet contained omega 3 -chitosan NPs 1 g/kg showed significant increase in final body weight (g), body gain (g), body gain (%), specific growth rate (%), feed conversion ratio and protein efficiency ratio. There was an

increase in growth performance when there is an increase omega 3- chitosan nanoparticles in diet. The higher condition factor showed that the fish were gaining more mass relative to their length, which was positive for the aquaculture industry as the fillets will be more robust per fish in agreement with following findings.

Tilapia growth rate increased during the 90-day period and omega 3- chitosan nanoparticles 0.5 and 1 mg /kg revealed the most significant in specific growth rate (%) and feed intake (g).

In agreement of our result, used omega 3- fatty acid (n-3 FA) sources were either fishmeal or fish oil (FO), linseed or linseed oil (LO). In addition, the use of fishmeal included at greater than 5% has been reported to affect the performance parameters of broilers e.g. body weight and feed conversion ratio (FCR) that resulted in reduced growth rate causing subsequent economic losses for the producer Scaife *et al* [32]. Previous studies have also indicated that feeding juvenile Nile tilapia fish oil FO rich on omega 3- chitosan nanoparticles for 90 days Justi *et al* [33] and at different levels (from 0.25g\kg to1g\kg) Visentainer *et al* [34] stated satisfactory incorporation results for n- 3 fatty acids into muscle tissue. However, those shorter studies were been conducted with juvenile Nile tilapias. Omega-3 UN saturated fatty acids (UFAs) has important nutritional factors. They modulate immune functions. Moreover, has a great importance for nervous system development and for lowering blood platelet aggregation and the incidence of thrombosis, hypertension, and atherosclerosis, and have anti-tumor, anti-inflammatory, growth performance and cardio protective effects Ahmad *et al* [35]. It has been proved that chitosan has more additional biological properties. It works as an immune adjuvant and a bacteriostatic agent Abu-Elala *et al*; Sun *et al* and Wang *et al* [36, 37, and 38].

III) Effect of omega 3- chitosan nanoparticles on Immunity and Biochemical parameters:

Our results documented that total cholesterol, triacylglycerdes (TAG), high-density lipoprotein (HDLc), low-density lipoprotein (LDLc), total protein (TP), albumin, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were significantly affected and showed high significant differences ($p < 0.05$). However, showed no significant differences on globulin (GLO), interleukin-1 (IL1), interleukin-6 (IL6), and fish tumor necrosis factor (TNF-α). In molecular expression, omega 3- chitosan nanoparticles 0.5 and 1 g/kg were the most

significant treated one on interleukin-1 beta (IL1- beta) but control diet in molecular expression of toll like receptors factores 2 (TLR2)was the most significant treated one.

Additionally, oxidative stress, immunological and hematological variables were essential blood parameters. It has been found they are essential for monitoring the conditions of fish breeding Cecchini *et al*, Fazio *et al* [39 , 40]. Also these blood parameters were been used to estimate the effects of feed additives on fish as mentioned by Acar *et al* , Yilmaz [41 , 42]. Omega-3 fatty acids were been regarded to be cardio protective Lemaitre *et al*; Hansen and Harris [43 , 44] and Anil [45] showed a positive effect on both the blood lipid profile promote immune statue in Nile Tilapia . Thus, Burk *et al*; Kumar *et al* [46 , 47] , have been reported that an increased amount of these proteins will present a more protective role and in turn promotes the immunity status of fish .Therefore, several studies have shown the synergism between the use of essential oils encapsulated in biodegradable nanoparticles according to Chifiriuc *et al*; Pavela *et al* [48 , 49]. Moreover , several studies have been shown the synergism between the use of essential oils encapsulated in biodegradable nanoparticles according to Chifiriuc *et al* ; Pavela *et al* [48 , 49]. In contrast with our results, has been found that using poly un saturated fatty acids (PUFAs) in poultry diets significantly have reduced the cholesterol and total lipid content in the blood. Therefore, several studies have been conducted to minimize the harmful effects of triglycerides and total cholesterol in poultry products Konieczka *et al* [50]. In our study, high significant differences in interleukin-1 beta (IL1-beta) in molecular expression level of omega 3 chitosan nanoparticles but significantly lower in molecular expression level of toll like receptors factores 2 (TLR2)with increase concentration of omega 3 chitosan nanoparticles. Nanoparticles are able to exhibit a high rate of uptake in the gastrointestinal tract even though the extent of absorption depends on the nature of the particles employed, their surface charge, their colloidal stability, the dose given and the species of animal [51]. Moreover, changing chitosan to nanoparticles could be result in positive effects on absorption surface area. In addition, increased absorption surface can be enhance intestinal efficiency in digestion, absorption and consequently feed conversion ratio and growth performance [16]. [52] has been observed more intraepithelial lymphocytes in tilapia intestine after feeding Lactobacillus rhamnosus GC-supplemented diet for 30 days. In fact, teleost fish gut associated lymphoid tissue (GALT) lack the

organized Peyer Patches and mesenteric lymph nodes present in the mammals GALT [53]. Intraepithelial mononuclear leukocytes, a component of the gut associated lymphoid tissue, plays a major role in mucosal defense mechanisms against intraluminal foreign antigens [54]. In previous study [55] due to the special characters of chitosan nanoparticles, more efficient have been uptake by phagocytic cells induced stronger systemic and mucosal immune responses in comparison with chitosan. More studies are warranted to evaluate the effects of Nano chitosan/Omega 3 fatty acid composites on immune system but this preliminary study can show the potential of these additives on mucosal immunity of fish intestine.

CONCLUSIONS:

In conclusion, these findings of the present study indicate that feeding Nile tilapia with a diet containing omega 3- chitosan nanoparticles (0.25g, 0. 5g, and 1g/kg), on a period of 90 days might be adequate to improve fish growth, immune parameters, antioxidant status . In addition, Nano diet improved growth performance and faster growth rate through nutritional manipulation in Nile tilapia fish via improving oxidative stress and improves immune function. Hence, the responses of fish are likely to change depending on different doses. Therefore, further investigations are encouraged in order to evaluate and understand the effects of different levels of omega 3- chitosan nanoparticles on various Nile tilapia fingerlings, as well as their reactions to different fish pathogens. Omega 3 is a very important element for counteracting stress responses in fish and livestock and showed significant efficiency at nanoscale level. Animal and fishery researchers can initiate Nano-chitosan work because of its more bioavailability, bio efficacy, and low toxicity. Industry can select this multipurpose feed additive against diseases like cancer, gastro enteritis, etc. or as a common antidote and immunomodulatory molecule against any single or composite stressors. These findings suggest that omega 3- chitosan nanoparticles possess a potential effect in Promoting growth in Nile tilapia fish via improving oxidative stress and improves immune function. Future investigations regarding omega 3- chitosan nanoparticles acts as an immunomodulatory when used as a dietary supplement. omega 3- chitosan nanoparticles may influence fish intestinal health by stimulating immunological parameters and affecting the diversity of the microflora in the intestine.

Disclosure statement:

The authors declare no conflicts of interest, financial or otherwise.

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تأثير استخدام جزيئات الشيتوزان النانو المحملة بالاوميجا علي النمو في السمك البلطي النيلي

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تم التخطيط لهذا العمل لدراسة التأثير المحتمل لجسيمات أوميجا شيتوزان النانوية في إصبعيات البلطي النيلي. تم استخدام مائتين وأربعين من إصبعيات البلطي النيلي في أربع مجموعات. نظام غذائي خالي من جزيئات أوميجا 3 – الشيتوزان النانوية ، أوميجا 3 – جزيئات الشيتوزان النانوية (0.25 جم / كجم) ، جزيئات أوميجا 3 – الشيتوزان النانوية (0.5 جم / كجم) جزيئات أوميجا 3 – الشيتوزان النانوية (1 جم / كجم) لمدة 12 أسبوعًا. أظهرت بياناتنا أنه تم العثور على فروق ذات دلالة إحصائية في متغيرات النمو ، بما في ذلك وزن الجسم النهائي (جم) ، وكسب الجسم (جم) ، وكسب الجسم (٪) ، ونسبة كفاءة البروتين ، ومعدل النمو النوعي (٪) ونسبة التحويل الغذائي. بالإضافة إلى ذلك، أظهرت بياناتنا المقدمة

أن الكوليسترول الكلي، وثلاثي الجلسريد (TAG) ، والبروتين الدهني عالي الكثافة (HDLc) ، تأثر البروتين الدهني منخفض الكثافة (LDLc) والبروتين الكلي (TP) والألبومين و alanine aminotransferase (ALT) و aspartate aminotransferase (AST) معنويا بإضافة جزيئات أوميجا 3 – الشيتوزان النانوية في النظام الغذائي وأظهرت فروقا معنوية عالية (p <0.05). ومع ذلك، لم تظهر فروق ذات دلالة إحصائية (p> 0.05) على الجلوبيولين (GLO) ولكن كانت هناك فروق ذات دلالة إحصائية (P <0.05) في إنترلوكين 1 (IL1)، وإنترلوكين 6 (IL6)، وعامل نخر ورم الأسماك (TNF-α). في التعبير الجزيئي، كانت omega-chitosan NPs 0.5 و g / kg 1 هي أهم معاملة تمت معالجتها على interleukin-1 beta (IL1- beta) ولكن النظام الغذائي الضابط في التعبير الجزيئي عن مستقبلات شبيهة بالحصيلة 2 (TLR2) كان الأكثر علاجًا.