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Immune and Antioxidative effects of Dietary Silver Nanoparticles on Growth of Nile Tilapia, *Oreochromis niloticus* Challenged with *Aeromonas hydrophila.*

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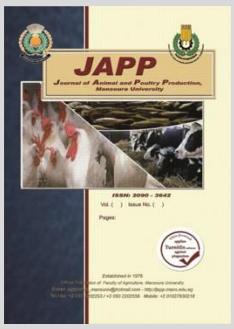

ABSTRACT

This study was conducted to study the effect of dietary supplementation of silver nanoparticles (90 nm in size) on growth performance, nutrient utilization, proximate composition, certain biochemical , antioxidant and immunity parameters and histopathological examination of sex reversed (all males) Nile tilapia (*Oreochromis niloticus*). Three treatments (Control, Sliver NPS 0.04 & 0.08 mg / kg diet). were triplicated and a total number of 288 fish Nile tilapia (56 ± 0.1 g / fish) were stocked in nine concrete ponds (1x4x1m). Fish were fed with 30% crude protein commercial diet (gross energy 4120.7 Kcal/kg). Triple times a day fish were fed for 8 weeks at 3% of their body weight. The obtained results presented an enhanced Impact of silver nanoparticles (AgNPS) on growth, response of immunity, as antioxidants, and resistance to *Aeromonas hydrophila* infection in *O. niloticus*. Thus, Ag NPs are recommended to be used for improving growth, immunity, antioxidative responses and controlling *A. hydrophila* infection in Nile tilapia.

Keywords: Silver nanoparticles, Nile tilapia, growth performance, body composition, antioxidants, innate immunity.

INTRODUCTION

Since ancient times silver has been used as an antimicrobial treatment. It has now found applications optical sensing, cosmetics, and medicine (Chen and Schluesener, 2008). Nano-silver particulate matter (AgNP) as a biocide has made it the biggest, fastest developing, class of commercially produced nanomaterials (Pinto et al. 2010). The operation of silver nanoparticles is anti-bacterial, anti-fungal and anti-virus (Panacek et al., 2009 and Galdiero et al., 2011). Silver ions (Ag+) come out to be interacted with bacteria, so that these ion can affected and kill the cells and cause microorganism death, and free radicals may also cause cell damage also (Kim et al., 2007). The colloidal use of metallic silver as silver nanoparticles (SNPs) has been widely employed. It is also clear that metallic silver has a great use, such as that of silver nanoparticles (SNPs). The (SNPs), which were recently used as an anti-microbial agent to control of many pathogens, diagnose diseases, treatments and as a material for coating (Rai et al. and Mohanty et al. 2012).

In the present study, we conducted randomized trials to investigate the effects of different levels of supplemental silver nanoparticles (NSPs) on feed utilization, growth performance, oxidative status, immunity and effects on bacterial resistance in *O. niloticus*.

MATERIALS AND METHODS

Experimental design

This experiment was conducted at the Department of Animal Production, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt. Fish were randomly allocated into nine 4 m^2 concrete ponds (1mx4mx1m, each) at a density of 8 fish /m^3. All experimental ponds were supplied with tap water (dechlorinated) and oxygen was supplied using air blower 5 HP. Fish waste and food leftover were removed everyday by siphoning and 20% of water was renewed daily. This experiment was conducted for 8 weeks after an adaptation period of two weeks. Triplicate per treatment were used in this study as following: Group 1(T1) Control: considered as control diets (basal diet without NSPs). Group 2 (T2): 0.04 mg NSPs / kg and Group 3 (T3) 0.08 NSPs mg /kg diet.

Fish

All male (sex-reversed) Nile tilapia (*O. niloticus*) fingerlings (56 ± 0.1 g / fish) were purchased from Egyptian Aquaculture Center, Kafr El-Sheikh governorate, Egypt. Fish were transported using a vehicle with aeration tools. For 14 days before the experiment, fish were acclimated to the experimental condition. At the end of the acclimatization process, a sample was collected randomly from each pond. Fish samples were weighed with a digital balance and measured with a dashboard to get initial samples for the groups. A total of 288 fish (32 fish / pond) with a mean initial body weight of (56 ± 0.1 g / fish) were randomly stocked into nine concrete ponds. After the groups have been allocated and fish adapted on the control diet (without NSPs). A completely randomized design was adopted, where three diets were fed to triplicate groups. The average initial weights were recorded. Water temperature and oxygen were recorded daily at one o'clock using thermometer and oxygen meter (HI 9146-HANNA

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interment, USA). The pH values were recorded twice a week (Orion pH meter, Abilene, Texas, USA). Ammonia, nitrite, and nitrate were measured twice weekly following APHA (1998).

Delivery of silver nanoparticles

Ag NPS solution (99.5% purity - MKN-Ag- 090) was purchased from M K Impex Corp, Canada. As per the manufacturer, AgNP particles have diameter of <100 nm. The doses of Ag NPs (0.04 and 0.08 mg /kg $^{-1}$) were selected based on previous studies (1/10 sublethal dose of 0.04 and 0.8 mg (Biplab et al., 2015). Fish were fed with 30% crude protein commercial diet (gross energy 4120.7 Kcal/kg) with varying levels of nanosilver particles. Different concentrations of silver nanoparticles was mixed with the feed particles by spraying and leaving the diets until it absorbed. Fish were randomly allocated (in triplicates) into three treatments (control without adding silver NPS, 0.04 and 0.08 Ag NP mg / kg diet). Fish were fed with a commercial diet (fishmeal 17.0%, soybean meal 30.0%, yellow corn 15.0%, wheat bran 20.0%, alfalfa hay 12.5%, oil of sunflower 3.0%, minerals mixture 0.5%, mixture of vitamin 1.0% and carboxymethyl cellulose 1.0%). The chemical composition of the feed was as follows: crude protein 30%, ether extract 4.68%, crude fiber 6.42% and gross energy 4120.7 Kcal/kg. As per the manufacturer, the following vitamin mixtures were added per Kg of feed: Vitamin A 16060 IU, B1 6 mg, B3 1500 IU, B6 9 mg, B12 6 mg, C 60 mg, E 60 mg (Skretting Egypt, Plot 170, 10th of Ramadan Rd., Belbis, Sharkia, Egypt). Fish were fed three times a day (8:00, 12:00 and 17:00 hours) at 3% of wet body weight per day. All fish were weighed every two week and food ration was adjusted after each fish weighing.

Proximate analysis of fish

Ten fish per treatment were randomly sampled at the start and at the end of the experiment for moisture, crude protein, crude fat and total ash analysis following the protocol of AOAC (2006) (Association of Official Analysis Chemists methods). Samples were stored at -4ºC till the time of analysis (Eya and Lovell, 1997).

Measurement of fish growth and feed utilization

All fishes were individually weighed to the nearest 0.1 g at the start of the experiment and subsequently fish were weighed and mortality recorded fortnightly.

Body weight (FBW), weight gain (WG), relative growth rate (RGR), specific growth rate (SGR, % day^{-1}), feed conversion ratio (FCR), and protein efficiency ratio (PER) were calculated using the following equations:

WG = Final body weight (g) - Initial body weight (g).

RGR = 100 ×(Final body weight (g) - Initial body weight (g))/ Initial body weight (g)

Specific growth rate (SGR % / day) = [Ln last body weight- Ln introductory body weight] × 100/trial period (d).

FCR = feed intake (g) /weight gain (g).

PER = weight gain (g) /protein intake (g).

Blood collection and biochemical analysis

Blood was sampled from 6 fish/treatment by caudal puncture and blood extracted into clean Eppendorf tubes at the end of the feeding experiment. Samples were centrifuged at 3000 rpm for 15 min for serum preparation without an anti-coagulant. Serum samples were collected and stored at − 20 °C for further analysis. For respiratory burst activity assay, another set of blood sample were collected using heparinized syringe. Albumin and total protein values were estimated calorimetrically according to Henry (1964) and Wotton and Freeman (1974), respectively. The globulin was computed by the subtraction of albumin from total protein. Following Reitman and Frankel (1957). Aspartate aminotransferase (AST) and alanine amino transaminase (ALT) activities were determined calorimetrically using specific commercial kits (Giza, Dokki, Egypt).

Determination of antioxidants and innate immunity assays

The antioxidant enzymes' activities such as superoxide dismutase (SOD) and catalase (CAT) activities were assessed in fish serum using the methods reported by Nishikimi et al. (1972) and Aebi (1984), respectively the using of diagnostic reagent kits following the manufacturer's instructions (My BioSource Inc., San Diego, CA, USA).

The method of Rook et al. (1985) was used to evaluate the superoxide ion production by leukocytes through the decreasing of Nitro blue tetrazolium (NBT, Sigma-Aldrich Chemical, St. Louis, MO, USA). Lysozyme activity was estimated using turbidity measurements, ten µl of serum was added in cuvettes to 200 µl of *Micrococcus lysodeikticus* suspension prepared by adding of 35 mg of dry powder Micrococcus in 95 mL phosphate buffer 0.15 M phosphate + 5.0 mL NaCl solution. At the beginning of the reaction and after 20-minute incubation at 40 um the shift in extinguishment was measured spectrophotometrically at 546 nm. Based on the calibration curve, the extinction was measured and the content of lysozymes was determined according to Schäperclaus *et al.* (1992).

Challenge test

A. hydrophila was isolated from moribund fish. The isolate was identified and was confirmed by the API20E system (Biomerieux, France) . After experimental period of 60 days, 10 fish/treatment were randomly allocated into three subgroups and stocked into 100-L aquaria. The first subgroup was challenged with 0.1 ml dose of virulent *Aeromonas hydrophila* (5×10^5 CFU/mL) using intraperitoneal route (Schäperclaus, 1992). The control subgroup was injected with 0.1 ml of saline solution. Challenged fish were kept for 14 days under observation. Infected fish were observed for morbidity, postmortem lesions, clinical symptoms, and mortality for 14 days. Cumulative mortality rate was estimated following Anderson *et al*. (1980).

Histopathological examination

Fish liver and intestine specimens were taken and immediately fixed in 10% buffered neutral formalin solution for 48 hours, dehydrated in gradual ascending ethanol (70, 80, 95, 95 and 100%), cleared in xylene, and embedded in paraffin. Paraffin blocks were sectioned into five-micron thickness using a microtome (Leica RM 2155, England). The sections from the specimens were prepared and then routinely stained with Hämatoxylin and Eosin stains and examined microscopically (Suvarna *et al*., 2013).

Statistical analyses

Statistical analysis system (SAS, 2002) was used to perform one-way analysis of variance (ANOVA) at a 95% confidence limit. Differences among means were tested using Duncan's Multiple Range test (Duncan, 1955).

RESULTS AND DISCUSSION

Results

Growth performance and feed utilization

The Effect of different levels of dietary Nanosilver particles on growth parameters are shown in Table 1. Comparison of means of body weight at the start of the experimental period indicated that the distribution of individual fish between treatments was random. There was significant difference in final body weight, body weight gain and daily weight among the three treatment groups with the 0.04 mg NSPs / kg diet treatment being highest, followed by 0.08 mg NSPs / kg diet treatment and with the control group being the lowest.

Table 1.The Effect of different levels of dietary Nanosilver particles on growth performance of Nile tilapia reared in concrete ponds.

Items	Control (T1)	0.04 mg NSPs /kg diet (T2)	0.08 mg NSPs /kg diet (T3)
IW	56.34 ±0.29	56.35 ±0.23	56.37 ±0.82
FBW	141.01 ±3.79^{c}	191.83 ±2.1^{a}	173.81 ±0.94^{b}
BWG	84.67 ±3.78^{c}	135.47 ±2.17^{a}	117.44 ±0.95^{b}
DWG	1.51 ±0.67^{c}	2.42 ±0.40^{a}	2.10 ±0.15^{b}

Values in the same row with different superscripts are significantly different at p<0.05. IW= initial weight, FBW=final body weight, BWG= body weight gain and DWG= daily weight gain.

The effect of treatment with different levels of AgNP on feed utilization in *O. niloticus* is shown in Table 2. There were significant differences (P<0.05) among different treatments in feed and protein intake. The highest FI and PI were observed in 0.08 mg NSPs / kg treatment (204.39 and 61.32g), respectively while the lowest FI and PI values were recorded in control treatment (187.64 and 56.29 g), respectively. The 0.08 mg NSPs / kg diet treatment has the highest Feed conversion ratio of 1.51 followed by 1.64 in 0.04 mg NSPs / kg diet treatment and 2.22 in control treatment. The 0.08 mg NSPs / kg diet treatment has significantly highest values of FER and PER (0.66 and 2.21), followed by (0.61 and 2.03), in the 0.04 mg NSPs / kg diet treatment and significantly lowest values were recorded in the control group (0.45 and 1.50).

Table 2.The Effect of different levels of Nanosilver particles on feed utilization of Nile tilapia reared in concrete ponds.

Items	Control (T1)	0.04 mg NSPs /kg diet (T2)	0.08 mg NSPs /kg diet (T3)
FI	187.64 ±3.47^{c}	192.99 ±1.52^{b}	204.39 ±2.15^{a}
PI	56.29 ±1.04^{bc}	57.90 ±0.46^{b}	61.32 ±0.65^{a}
FCR	2.22 ±0.63^{a}	1.64 ±0.003^{b}	1.51 ±0.34^{c}
FER	0.45 ±0.12^{c}	0.61 ±0.00^{b}	0.66 ±0.14^{a}
PER	1.50 ±0.40^{c}	2.03 ±0.003^{b}	2.21 ±0.05^{a}

Values in the same row with different superscripts are significantly different at p<0.05. FI= feed intake PI protein intake FCR= feed conversion ratio FER= feed efficiency ratio PER= protein efficiency ratio.

The effect of supplementation of different levels of AgNP on body chemical composition of *O. niloticus* is shown in Table 3. The DM was similar in all treatment groups with no significant differences (P>0.05). CP was significantly different in all treatment groups, with the 0.04 mg/kg diet being the highest and the control treatment the lowest.

Ether extract (EE) was not significantly different (P>0.05) among the treatments. There was a significant difference in ash content among the treatment groups with the control group having the highest ash content and the 0.04 mg/kg having the lowest ash content.

Table 3. The Effect of different additives of Nanosilver particles on chemical composition of Nile tilapia reared in concrete ponds.

Items	Control (T1)	0.04 mg NSPs /kg diet (T2)	0.08 mg NSPs /kg diet (T3)
DM	23.4 ±0.2	24.2 ±0.8	23.5 ±0.5
CP	70.17 ±0.48^{c}	72.37 ±0.18^{a}	71.43 ±0.19^{b}
EE	13.4 ±0.25	14.03 ±0.27	14.03 ±0.23
Ash	16.43 ±0.23^{a}	12.6 ±0.58^{c}	13.6 ±0.58^{b}

Values in the same row with different superscripts are significantly different at p<0.05. DM=dry mater, CP =crude protein and EE= ether extract.

Biochemical analysis

It was found that serum protein (total protein, albumin, and globulin) values increased significantly in 0.04 mg Ag NP group and decreased in 0.08 mg group (Table 4). By the end of the experiment, there was a remarkable elevation (P < 0.05) in serum total protein (5.13 ±0.03 g/dL) in the group fed on 0.04 mg AgNP when compared to control one (4.23 ±0.01 g/dL). The total protein values in fish fed on 0.0 (control), 0.04 and 0.08 mg Ag NP groups were 4.23 ±0.01, 5.13 ±0.03 and 4.01 ±0.04 g/dL, respectively. The albumin values were 2.21±0.04, 2.31±0.11and 2.11±0.21 g/dL, respectively. Meanwhile, the globulin values (g/dL) were 2.02±0.05, 2.82±0.03 and 1.90±0.06, respectively. The values of liver enzymes (AST and ALT) are represented in Table 4 and showed significant difference (P<0.05) between the fish groups that fed with Ag NP supplemented diets and those fed with the control diet. The values of AST and ALT were significantly higher in fish sera in all treatment groups compared to control group.

Table 4. Biochemical analysis of Nile tilapia, *O. niloticus*, fed different levels of silver Nano particles (Ag NPS) for 60 days.

Items	Control (T1)	0.04 mg AgNP/kg diet (T2)	0.08 mg AgNP/kg diet (T3)
Total protein, g/dL	4.23 ±0.01^{b}	5.13 ±0.03^{a}	4.01 ±0.04^{c}
Albumin, g/dL	2.21±0.04^{b}	2.31±0.11^{a}	2.11±0.21^{c}
Globulin, g/dL	2.02±0.05^{b}	2.82±0.03^{a}	1.90±0.06^{c}
AST, U/L	20.15 ±0.05^{c}	26.29 ±0.03^{b}	28.18 ±0.06^{a}
ALT, U/L	18.23 ±0.02^{c}	25.89 ±0.21^{b}	27.27.05^{a}

Values in the same row with different superscripts are significantly different at p<0.05.

Antioxidants and innate immunity assays

Significantly elevated activities of SOD and CAT was recorded on fish fed on 0.04 mg AgNP (19.3±0.05 U L^{-1} and 18.3 ±0.01 U L^{-1}) compared to fish fed on control diet (15.6.01 and 14.1 ±0.02) respectively (Table 5). SOD and CAT levels decreased in the group fed on (0.08 mg Ag NP). There was a tendency of increase in serum nonspecific immune parameters such as serum lysozyme activity and respiratory burst activities in fish group fed on 0.04 mg AgNP. On the other hand fish groups fed on 0.08 mg and control groups have showed decreased values (Table 5).

Table 5. Antioxidants and immunity parameters of *O. niloticus*, fed different levels of (Ag NPS)) for 60 days.

Parameter	Control (T1)	0.04 NSPs /kg diet (T2)	0.08 NSPs /kg diet (T3)
SOD (U/L)	15.6 ±0.01^{b}	19.3±0.05^{a}	13.2 ±0.04^{c}
CAT (U/L)	14.1 ±0.02^{b}	18.3 ±0.01^{a}	13.7 ±0.13^{c}
Lyz activity (µg/ mL)	0.978 ±0.04^{b}	1.215 ±0.05^{a}	0.892 ±0.06^{c}
RB (mg/mL)	0.465 ±0.02^{b}	0.743±0.07^{a}	0.322±0.12^{c}

Values in the same row with different superscripts are significantly different at p<0.05.

Challenge test

Fish challenged with *A. hydrophila* showed ascites, detached scales and hemorrhagic patches all over the body. Congestion was noticed in all internal organs, particularly in the control group. The 0.04 Ag NPs group has the highest survival rate of 60% followed by 60% survival rate in the AgNPs (0.08) group and the control group had a survival rate of only 20% (Figure 1).

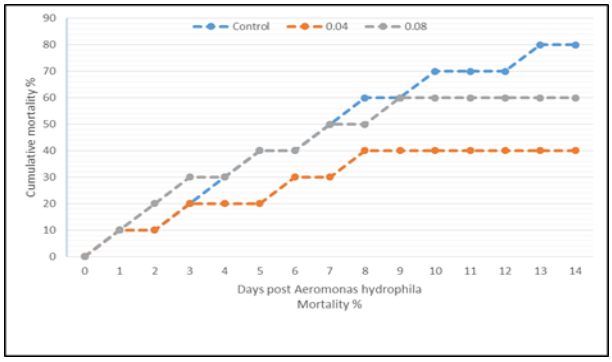

Figure 1. Cumulative mortality rate of *O. niloticus* receiving various levels of Ag NPS incorporated diets for 14 days.

Histological findings

Figure 2A. shows a normal histological structures in liver. The liver of the control group showed normal histological structure conformed as evenly distributed polygonal hepatocytes with central spherical nucleus, as long and intact blood vessels and sinusoids of different size (Figure 2 B). Fish intestine samples showed normal intestinal structure.

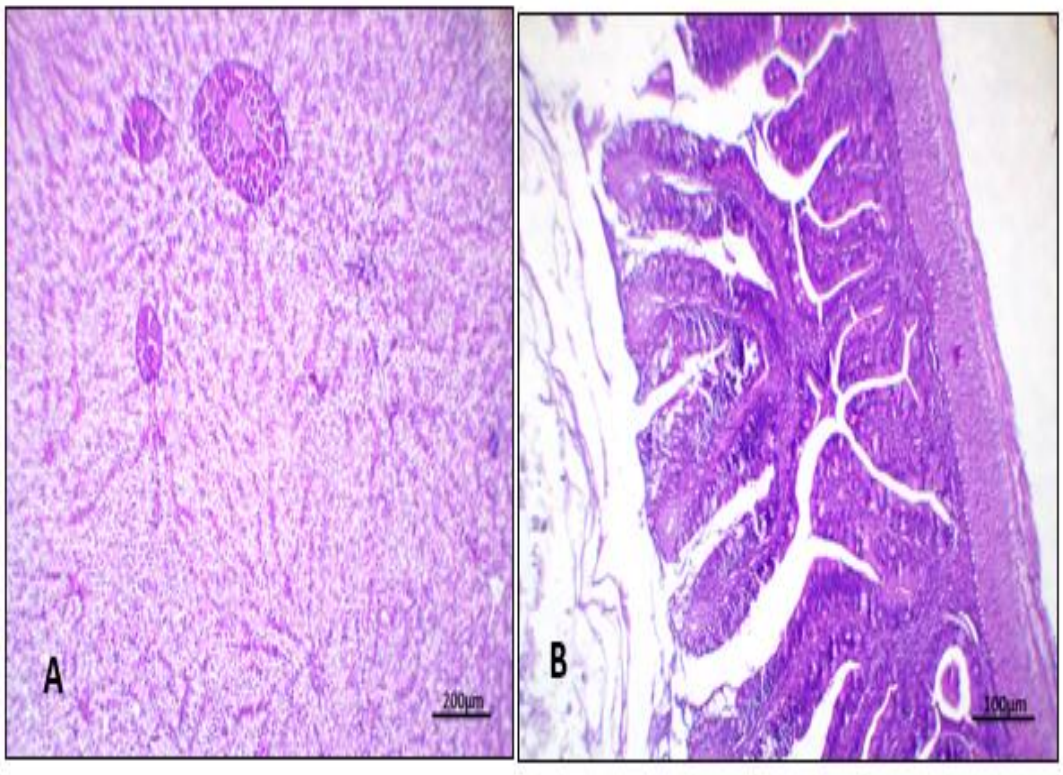

Figure 2. Normal histological findings were seen in liver (Fig. 2. A). Representative photomicrograph of Fish liver (control group) showing normal hepatic sinusoids. Fish (Fig 2. B). Intestine (control group) showing normal intestinal structures. Hämatoxylin and eosin stain.

The histopathological results

The liver samples of control group fish challenged with *A. hydrophila* showed marked congested portal blood vessels (arrows) surrounded inflammatory cells infiltrations (star) besides fatty hepatocytes (Figure 3 A1). Normal hepatic cells (star) hepatopancreas structures and hepatic sinusoids (arrow) in 0.04 Ag NPs group (Figure 3 A2). Nearly normal fatty hepatocytes (star) and sinusoids with mild congested central vein (arrow) were seen in fish liver in 0.08 Ag NPS group (Figure 3 A3). Fish intestine of control group showed tall villi and severe widening of lacteal (arrow) (Figure 3 B1). Tall and broad villus tips represented cup shaped (arrow) were observed in fish intestine in 0.04 Ag NPS group (Figure 3 B2). Fish intestine in 0.08 Ag NPS group showed apparently normal intestinal villi with marked increase goblet cells (metaplasia). (Figure 3. B3). Hämatoxylin and Eosin x200.

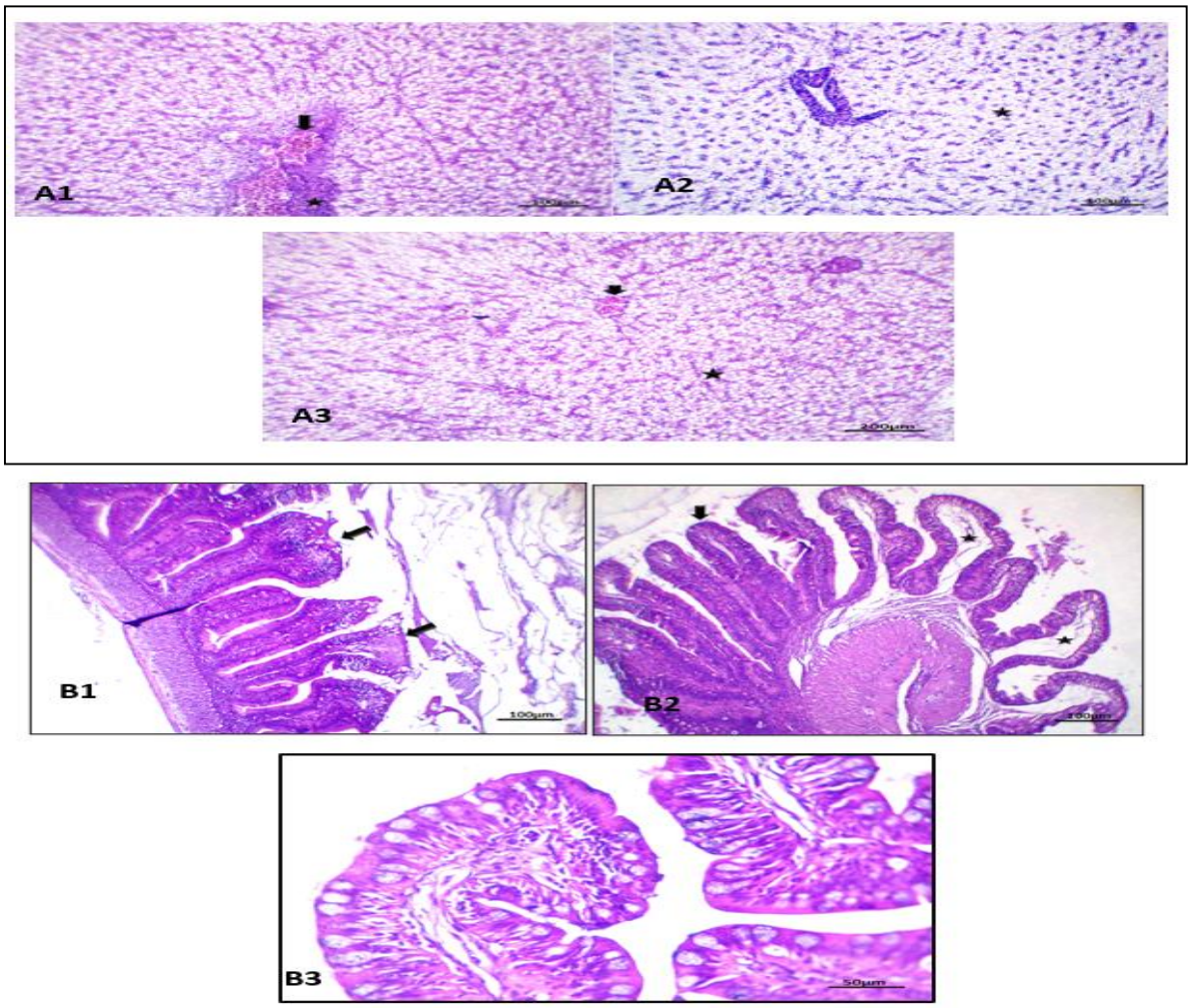

Figure 3. A Photomicrograph of liver of different experimental groups after bacterial challenge (Figure A1) fish liver of control group showing marked congested portal blood vessels (arrows) surrounded inflammatory cells infiltrations (star) besides fatty hepatocytes (H&E X200). Figure A2: fish liver of 0.04 AgNP group showing apparently normal hepatic cells (star) hepatopancreas structures and hepatic sinusoids (arrow). Figure A3: liver fish of 0.08 Ag NP group showing nearly normal fatty hepatocytes (star) and sinusoids with mild congested central vein (arrow). Figure 3 B1: intestine fish (control) showing marked tall villi and severe widening of lacteal (arrow). Figure 3 B2: in 0.04 Ag NPS group showing marked tall and broad villus tips represented cup shaped (arrow). Figure 3 B3: in 0.08 Ag NPS group showing apparently normal intestinal villi with marked increase goblet cells metaplasia. Hämatoxylin and eosin stain.

Discussion

Silver nanoparticles which are mainly used as anti-microbial agents have had very great importance in the applications of medical devices, building materials, the clothing industry etc. Immunostimulants not only enhances the immune system but also help in preventing infectious diseases (Watanuki et al., 2006). Serum proteins (albumin, and globulin) have a substantial part in fish immunity (Kumar et al., 2007). and the elevation of their values are an important indicator that fish have excellent immunity (Wiegertjes et al., 1996). In the present study, fish fed a diet containing 0.04 mg AgNPS / kg^{-1} diet exhibited significantly ($P<0.05$) higher total protein, albumin, and globulin as compared with that fed the control diet. These results might be attributed to the improvement of fish health status when fed AgNPS enriched diets, contrary with Abdel-Khalek et al. (2015). However, the 0.08 Ag NPS supplementation group resulted in significant decrease in serum total protein. These results are in accordance with Alkaladi et al. (2015) who reported a significant decrease in total proteins, albumin, and globulin contents of *O. niloticus* after exposure to copper and zinc oxide nanoparticles, respectively. AST and ALT activities ($P<0.05$) increased at the end of the feeding experiment compared with the control. These findings are in agreement with Monfared and Soltani (2013). The elevation of antioxidant enzymes including SOD and CAT might be needed not only for detoxification of hydrogen peroxide (H2o2) but also as inhibition the action of reactive oxygen species (ROS). These results are corroborated by Kim and Ryu (2013) that silver NPS induce enhance the activity of catalase enzyme in cell lines. Engstad et al. (1992) who reported that the high values of lysozyme in fish sera are correlated to the increasing the phagocytes production or lysozymes and hence lysosome activity is a crucial enzymatic parameter in nonspecific immunity in fish. Ji et. al. (2017) further elucidated that phagocytic cells can be activated to produce antimicrobial substances such as lysosomal enzymes, the complement system and production of reactive oxygen metabolites (ROS). Our results indicated that Ag NPS level

(0.04 mg/kg) enhanced the immunity in Nile tilapia and we believe that level might be optimal dose although the mechanism of action of silver NPS on immunity is still unknown. The results of Ag NPS (0.08 mg) group showed decrease in lysozyme values which is in agreement with Li et al. (2006) who observed the role of lymphocytes and the formation of phagolysosomes in trout B cells, possibly indicating their role in bacterial killing. In the current study, significant increase in respiratory burst activity was observed treatment group 0.04 mg AgNP group. And the activity of phagocytosis was significantly increased at concentration 0.04 Ag NPS. Contrary to our finding, Aboud (2010) found that phagocytic activity in Nile tilapia was significantly decreased in metal exposed fish. Similarly, monitoring effects of different heavy metals (cadmium, lead, mercury, silver) on the activity of phagocytic cells in the bivalve *Mytilus edulis* found that silver ions induced decreased phagocytosis (Rault et al. 2013) . Our findings indicate that Phagocytosis decreased in group fed 0.08 concentration of Ag NPS. We suggest that concentration of 0.08 mg might have induced a negative feedback while 0.04 might have a positive effect possibly indicating that it is optimal level for *O. niloticus*.

The histopathology of hepatic lesions is often evaluated in toxicological studies and used as markers of the pollution of the environment (Altinok and Capkin 2007; Dabrowska et al. 2012). The histological analysis of the current study suggests that no liver damage was attributed because of the supplementation of 0.04 Ag NPS. This finding is suggestive that 0.04 treatment was optimal while the 0.08 had adverse effects because of the high concentration. Assessment of the histology of the intestine of the experimental groups showed significant increases in the length of intestinal villi which leads to an increase in the absorptive surface area of the intestine which in turn increases the body weight gain and decreases the FCR in 0.04 AgNPs group. Apparently normal intestinal villi with marked increase goblet cells metaplasia were seen in 0.08 mg Ag NPS. But very few reports are available on histology of organs changes because of silver nanoparticles administration on commercial fish varieties. Accumulation of Ag NPS in the intestine is possibly an important factor for alteration of histological makeup. Prior studied have reported penetration of Ag NPS into chorion of Japanese meddaka (*Oryzias latipes*) (Ghosh and Adhikari, 2006). Our results indicate that fish groups fed on silver nanoparticles exhibited the lower mortality (40% in 0.04 group and 60% in 0.08 group) compared to control group (80%) after challenge with *A. hydrophila*. These results are in agreement with Prabhu and Poulose (2012) who demonstrated that physical and chemical properties that AgNPs have helped to increase the efficiency of silver, principally in control disease area.

CONCLUSION

The results show an improved effect of Silver nanoparticles (AgNPS) on growth, immune response, antioxidants, and resistance to *Aeromonas hydrophila* infection in *O. niloticus*. Better results of different parameters were obtained in the 0.04 treatment compared to 0.08. We suggest further research using different concentrations but our study can be used as a baseline and we suggest that concentration of 0.04 of Ag NPs is an optimal level and can be applied to improve growth, immunity, antioxidative responses and controlling *A. hydrophila* infection in Nile tilapia.

Conflicts of interest: All authors declare that they do not have any conflict of interest.

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التأثيرات المناعية والمضادة للاكسده لجسيمات نانو الفضة فى عليقة البلطي النيلي على النمو ومقاومه العدوى لبكتريا الايرموناس هيدروفلا

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صممت هذه الدراسة لمعرفة تأثير استخدام جسيمات الفضة النانوية (بحجم 90 نانومتر) على أداء النمو ، والاستفاده من الغذاء وتكوين الجسم والمناعة والاستجابات المضادة للأكسدة والسيطرة على العدوى ببكتريا الايرموناس في البلطي النيلى. تم تغذية البلطي النيلي بوزن ابتدائى (56 ± 0.1 جم / سمكة) على عليقة تجارية (30٪ بروتين خام وطاقة إجمالية 4120.7 كيلو كالوري / كجم) مدعمة بمستويات مختلفة من جزيئات النانوفضه. تم توزيع الأسماك عشوائياً (بثلاث مكررات) على 3 معاملات (كنترول، 0.04 ، 0.08 نانوفضه مجم / كجم علف). تم إجراء المعاملات في 9 أحواض خرسانية (1 م x 4م x 1م). تم تغذية الأسماك ثلاث مرات في اليوم لمدة 8 أسابيع بمعدل3 ٪ من وزن الجسم. أظهرت النتائج التي تم الحصول عليها تأثيرًا محسّنًا لجسيمات النانو فضة على النمو والاستجابة المناعية كمضادات للأكسدة ومقاومة العدوى بالأيروموناس. ولذلك يوصى باستخدام جزيئات النانوفضه لتحسين النمو والمناعة والاستجابات المضادة للأكسدة والسيطرة على العدوى ببكتريا الايرموناس هيدروفيلا في البلطي النيلي.