EFFECTS OF SUBLETHAL NITRITE AND AMMONIA IN *OREOCHROMIS NILOTICUS* L. INFECTED WITH *EDWARDSIELLA TARDA*

Faik Sertel SECER and Hijran YAVUZCAN

[1] *Faculty of Agriculture, Ankara University, 06110 Ankara, Turkey*

Abstract

In this study, the effects of sublethal nitrite and sublethal ammonia on experimentally infected Nile Tilapia's haematological and immunological parameters have been investigated. The groups were K: control group, Group A: ammonia exposed group, AB: infected with *Edwardsiella tarda* and exposed to ammonia, Group N: nitrite exposed group, NB: infected with *E. tarda* and exposed to nitrite, B: infected with *E. tarda*. The LC_{50} value of nitrite, ammonia and *E.tarda* were determined and were 8.318, 1.304 and $1.2x10^7$ respectively. The erythrocyte sedimentation rate, haematocrit and haemoglobin, were significantly decreased in AB and NB compared with the Group K. Plasma lysozyme activity increased significantly, as did phagocytosis of the head kidney respiratory burst activities and plasma glucose concentration in Group AB and NB were higher than those in Group K. There were slightly higher mean values of plasma lactate in AB and NB than in K. In conclusion of this study suggest that sublethal concentration of nitrate and ammonia with infection have some degree of influence on the haematological and immunological characteristics of Nile Tilapia.

Keywords: *Edwardsiella tarda*, *Hematology*, *Immunology*, *Oreochromis niloticus*

Introduction

In many parts of the world aquaculture, water quality is always related to development defects. As far as commercial aspects are concerned, environmental problems are important for fish health and welfare. Environmental problems included in the parameters such as, temperature, pH and presence of undesired nitrogenous compounds which should be considered as a limiting factor in fish health. Change in the water parameters; can directly or indirectly affect fish stress, immunological response and blood parameters. Stress is an important factor affecting growth parameters negatively.

Ammonia and nitrite in aquaculture is the important water quality parameter that limit production, stocking density and water exchange (Lawson 1995). Nitrite toxicity has been reported to vary on fish species (Svobodova et al. 1994).

Changes in water quality and seasonal temperature increases have been reported to cause Edwardsiellosis (Walters and Plumb 1980; Amandi et al. 1982; Francis-Floyd et al. 1993, Baya et al. 1997). Environmental factors such as high temperatures, low water quality and high levels of organic compounds in water also have been reported to cause *Edwardsiella tarda* infection of fish (Plumb 1999; Uhland et al. 2000; Zheng et al. 2004; Pressley 2005).

Material and Methods

This trial was performed in Ankara University, Faculty of Agriculture, Department of Fisheries and Aquaculture, Laboratory of Fish Welfare. Fish were 100.64±2.80 g (89.90-110.42), 21.069±1.421 cm (18.3-23.4). Total of 280 Nile tilapia (*Oreochromis niloticus* L. 1758) were used. As an ammonia source ammonium chloride (NH_4Cl); as a nitrite source sodium nitrite ($NaNO_2$) was used. For the experimental infection *Edwardsiella tarda* (ATCC 15947) was used. Water parameters were pH 7.02±0.03, temperature 24±1°C and dissolved oxygen 4.02±0.04 (mg/L).

Semi-static procedure was used for seven days long experiments. 70 tilapia placed in 400 L filled tanks, throughout the trial period every day 10 fish was sampled. To keep the stock density constant, after each sampling proportionally reduced water from the tanks (1^{st} day 400 L, 2^{nd} day 343 L, 3^{rd} day 285 L, 4^{th} day 229 L, 5^{th} day 171 L, 6^{th} day 114 L and 7^{th} day 57 L). Fish were randomly sampled and the height and weight of the fish were measured under deep anaesthesia. Clove oil (Eugenol 25mg/L) was used as an anaesthetic (Çetinkaya and Şahin 2005). Sublethal concentration (LC_{50}) of *E. tarda* calculated with Kärber (Arda 1971; Anonymous 2000; Oliveira et

al. 2011). Ammonium chloride and sodium nitrite LC_{50X48h} was calculated with PROBIT and 30% of the value was used as an experimental dose.

Experiment was designed as control group (Group K), nitrite exposed group (Group N). nitrite exposed infected group (Group NB) and bacterial infected group (Group B). The experiment was 7 days long. The experiment has 10 iterations, each fish in each group was a recurrence. The test area was illuminated 12 hours a light, 12 hours a dark (Philips. 900 lumens, 2700K). The oxygen requirement of the fish was provided with a dry air motor (SONIC P-65. USA). Fish were infected by i.p injection of 0.2ml of LC_{50}.

Blood was taken under deep anaesthesia from caudal vein with 21G needle which heparinized (Nevparin, Injectable 25000 IU / 5 mL) before to use. Erythrosine sedimentation rate was performed Micro-Wintrobe method (Blaxhall and Daisly 1973; Akinrotimi 2012). Haematocrit (Ht) was determined as Siwicki and Anderson (1993) and Yildiz (2009). Haemoglobin (Hb) was colorimetrical determined. Blood plasma was collected after centrifuged of whole blood on 10000 rpm at 5 minute and preserve at -20°C until used (Siwicki and Anderson 1993; Yildiz 2009). Plasma biochemical parameters as plasma glycose (Medispec Diagnostics), plasma lactate (TECO Diagnostics) was determined with the kits. Plasma Lysosome Activity was spectrophotometricly determined (Siwicki and Anderson 1993). Head kidney macrophage respiratory burst activity was determined as Secombes (1990).

Molecular procedures and protocols were optimized according to Sambrook et al. (1989). Specific primers for PCR were used from the divergent region of the partial gyrB gene of *E. tarda*. The forward primer, gyrBF1, was 5'-GCATGGAGACCTTCAGCAAT-3'; the reverse primer, gyrBR1, was 5'-GCGGAGATTTTGCTCTTCTT-3' Lan et al. (2008). The expected PCR is 415 bp in length.

Statistics test calculated with DUNCAN Test in SPSS. The required application has been submitted to ethics committee of T.C. Ankara University Animal Experiments Local Ethics Committee was granted with the dated 10.08.2011 and numbered 20011-117/454.

Results

In our study LC_{50x48h} of ammonia, nitrite and LC_{50} of pathogenic *E. tarda* bacteria were firstly determined for *O. niloticus*. The LC_{50x48h} of ammonia and nitrite was 1.304 and 8.318 mg/L whereas *E. tarta*'s LC_{50} was $1.2x10^7$ CFU/mL.

Erythrocyte sedimentation rate was decreased ($P < 0.05$) (Table 1).

Table 1. Erythrosine sedimentation rate (mm/Hour)

	1st Day	2nd Day	3rd Day	4th Day	5th Day	6th Day	7th Day
Group K	3.412 ±0.029^{Aa*}	3.412 ±0.022^{Aa}	3.406 ±0.018^{Aa}	3.410 ±0.016^{Aa}	3.408 ±0.036^{Aa}	3.406 ±0.018^{Aa}	3.406 ±0.019^{Aa}
Group A	3.234 ±0.038^{Db}	3.244 ±0.042^{CDb}	3.248 ±0.024^{BCDb}	3.262 ±0.023^{ABCDb}	3.278 ±0.016^{ABCb}	3.282 ±0.008^{ABb}	3.298 ±0.013^{Ab}
Group AB	2.608 ±0.035^{De}	2.634 ±0.029^{CDd}	2.630 ±0.023^{CDe}	2.646 ±0.030^{Ce}	2.658 ±0.016^{BCe}	2.682 ±0.016^{ABe}	2.698 ±0.015^{Ae}
Group N	3.180 ±0.031^{Bc}	3.212 ±0.040^{ABb}	3.206 ±0.022^{ABc}	3.222 ±0.019^{Ac}	3.220 ±0.023^{ABc}	3.230 ±0.024^{Ac}	3.232 ±0.036^{Ac}
Group NB	2.598 ±0.031^{Ce}	2.604 ±0.019^{Cd}	2.622 ±0.016^{BCe}	2.626 ±0.021^{BCe}	2.650 ±0.019^{ABe}	2.650 ±0.016^{ABf}	2.664 ±0.024^{Af}
Group B	2.776 ±0.021^{Bd}	2.776 ±0.036^{Bc}	2.802 ±0.040^{ABd}	2.808 ±0.013^{ABd}	2.826 ±0.021^{Ad}	2.832 ±0.015^{Ad}	2.838 ±0.019^{Ad}

* Different superscript capital latters in the same line and small case latters in the same column indicate the significance of the difference ($p < 0.05$).

Haematocrit ratio decreased in all groups except the control group (P<0.05) (Table 2).

Table 2. Haematocrit (Ht) rate (%)

	1st Day	2nd Day	3rd Day	4th Day	5th Day	6th Day	7th Day
Group K	35,836 ±1,875^{Aa*}	35,94 ±2,622^{Aa}	35,905 ±1,059^{Aa}	35,771 ±2,482^{Aa}	35,877 ±3,300^{Aa}	35,899 ±2,073^{Aa}	35,827 ±2,568^{Aa}
Group A	35,45 ±2,574^{Aa}	34,794 ±1,989^{ABab}	33,934 ±3,826^{ABCab}	33,312 ±2,758^{ABCab}	33,343 ±3,093^{ABCab}	32,329 ±2,504^{BCb}	31,346 ±2,231^{Cb}
Group AB	33,436 ±3,040^{Aa}	32,704 ±3,518^{Ab}	31,996 ±2,606^{Ab}	32,399 ±4,278^{Ab}	31,333 ±3,860^{Ab}	28,104 ±1,973^{Bc}	27,905 ±2,854^{Bc}
Group N	33,954 ±3,146^{Aa}	33,852 ±3,130^{Aab}	33,774 ±1,815^{Aab}	33,971 ±2,782^{Aab}	33,703 ±3,084^{Aab}	33,402 ±3,263^{Aab}	33,256 ±3,139^{Aab}
Group NB	34,379 ±2,864^{Aa}	33,914 ±1,497^{Aab}	33,518 ±2,425^{Aab}	33,169 ±2,677^{ABab}	32,894 ±2,823^{ABab}	31,825 ±3,428^{ABb}	30,613 ±3,616^{Bbc}
Group B	29,758 ±3,256^{Ab}	28,723 ±4,241^{Ac}	27,906 ±3,501^{Abc}	27,009 ±3,605^{ABCc}	26,166 ±3,149^{ABCa}	24,867 ±3,660^{BCd}	23,994 ±4,182^{Cd}

* Different superscript capital latters in the same line and small case latters in the same column indicate the significance of the difference (p <0.05).

There was a decrease in the haemoglobin in the experiment groups compared with control groups (P<0.05) (Table 3).

Table 3 Haemoglobin (Hb) (g/100ml)

	1st Day	2nd Day	3rd Day	4th Day	5th Day	6th Day	7th Day
Group K	10,51 ±0,325^{Aa*}	10,5 ±0,403^{Aa}	10,52 ±0,286^{Aa}	10,52 ±0,355^{Aa}	10,5 ±0,462^{Aa}	10,51 ±0,401^{Aa}	10,5 ±0,455^{Aa}
Group A	9,75 ±0,481^{Ab}	9,67 ±0,340^{Ab}	9,64 ±0,350^{Ab}	9,41 ±0,495^{ABa}	9,38 ±0,394^{ABb}	9,05 ±0,506^{Bb}	8,55 ±0,506^{Cb}
Group AB	9,52 ±0,661^{Abc}	9,48 ±0,461^{Ab}	9,39 ±0,657^{ABb}	9,31 ±0,348^{ABa}	9,23 ±0,492^{ABb}	9,19 ±0,351^{ABb}	8,86 ±0,752^{Bb}
Group N	9,39 ±0,448^{Abc}	8,93 ±0,380^{Bc}	8,87 ±0,455^{Bc}	8,77 ±0,531^{BCc}	8,69 ±0,647^{BCc}	8,59 ±0,387^{BCc}	8,36 ±0,433^{Cb}
Group NB	9,15 ±0,726^{Ac}	8,96 ±0,878^{ABc}	8,45 ±0,570^{BCc}	8,33 ±0,419^{Cd}	8,26 ±0,453^{Cc}	8,13 ±0,558^{Cd}	7,56 ±0,580^{Dc}
Group B	8,38 ±0,454^{Ad}	7,81 ±0,662^{Bd}	7,36 ±0,822^{BCd}	7,26 ±0,613^{BCe}	6,98 ±0,527^{CDd}	6,59 ±0,644^{De}	6,51 ±0,446^{Dd}

* Different superscript capital latters in the same line and small case latters in the same column indicate the significance of the difference (p <0.05).

As an immunological parameter; head kidney macrophage respiratory burst activity counts and plasma lysosome activity were given in table 4 and table 5.

Table 4 Head kidney macrophage respiratory burst activity

	1st Day	2nd Day	3rd Day	4th Day	5th Day	6th Day	7th Day
Group K	0,643 ±0,025^{Ed*}	0,669 ±0,015^{De}	0,713 ±0,017^{Cc}	0,734 ±0,022^{BCe}	0,755 ±0,02^{Bd}	0,802 ±0,014^{Ad}	0,815 ±0,015^{Ac}
Group A	1,306 ±0,051^{Db}	1,328 ±0,027^{Dc}	1,345 ±0,045^{Db}	1,394 ±0,02^{Cc}	1,45 ±0,011^{Bb}	1,463 ±0,016^{ABb}	1,499 ±0,021^{Aa}
Group AB	1,362 ±0,014^{Ea}	1,385 ±0,024^{DEab}	1,391 ±0,013^{Da}	1,427 ±0,015^{Cb}	1,457 ±0,019^{Bb}	1,493 ±0,021^{Aa}	1,514 ±0,02^{Aa}
Group N	1,266 ±0,037^{Fc}	1,298 ±0,02^{Ed}	1,338 ±0,024^{Db}	1,348 ±0,015^{CDd}	1,374 ±0,019^{BCc}	1,406 ±0,018^{Ac}	1,403 ±0,016^{ABb}
Group NB	1,36 ±0,019^{Da}	1,373 ±0,019^{Db}	1,408 ±0,019^{Ca}	1,419 ±0,016^{Cb}	1,465 ±0,025^{Bab}	1,5 ±0,023^{Aa}	1,516 ±0,033^{Aa}
Group B	1,358 ±0,014^{Da}	1,407 ±0,014^{Ca}	1,427 ±0,039^{Ca}	1,472 ±0,024^{Ba}	1,489 ±0,03^{ABa}	1,493 ±0,02^{ABa}	1,51 ±0,025^{Aa}

* Different superscript capital latters in the same line and small case latters in the same column indicate the significance of the difference (p <0.05).

Table 5 Plasma Lysosome Activity

	1st Day	2nd Day	3rd Day	4th Day	5th Day	6th Day	7th Day
Group K	$119\pm4^{Bc*}$	138 ± 6^{Ac}	140 ± 9^{Ac}	134 ± 9^{Ac}	135 ± 5^{Ad}	134 ± 8^{Ad}	133 ± 9^{Ad}
Group A	187 ± 8^{Bb}	197 ± 3^{ABbc}	197 ± 10^{ABb}	193 ± 10^{ABc}	200 ± 4^{Ac}	199 ± 4^{Ac}	203 ± 10^{Abc}
Group AB	202 ± 4^{Aa}	205 ± 7^{Aab}	203 ± 14^{Ab}	205 ± 4^{Abc}	210 ± 5^{Ab}	211 ± 8^{Ab}	213 ± 14^{Ab}
Group N	189 ± 10^{Bb}	195 ± 8^{ABb}	195 ± 8^{ABb}	194 ± 4^{ABb}	197 ± 6^{ABc}	202 ± 6^{Abc}	198 ± 6^{ABc}
Group NB	203 ± 10^{Aa}	203 ± 8^{Aabc}	206 ± 7^{Aab}	208 ± 12^{Aab}	212 ± 8^{Ab}	209 ± 8^{Ab}	213 ± 6^{Ab}
Group B	212 ± 10^{Ba}	210 ± 7^{Ba}	218 ± 10^{Ba}	218 ± 11^{Ba}	231 ± 4^{Aa}	234 ± 7^{Aa}	238 ± 7^{Aa}

* Different superscript capital latters in the same line and small case latters in the same column indicate the significance of the difference ($p < 0.05$).

Plasma lysozyme activity values were found increased between days according to groups and in the head kidney macrophage respiratory burst activity groups compared to the days ($P<0.05$). The increase in plasma lactate (table 6) among the groups according to the days was parallel to plasma glucose (table 7).

Table 6 Plasma lactate

	1st Day	2nd Day	3rd Day	4th Day	5th Day	6th Day	7th Day
Group K	6,693 $\pm0,494^{Bab*}$	6,848 $\pm0,52^{ABa}$	6,925 $\pm0,582^{ABa}$	7,029 $\pm0,719^{ABa}$	7,054 $\pm0,69^{ABa}$	7,158 $\pm0,572^{ABa}$	7,39 $\pm0,669^{Aa}$
Group A	6,331 $\pm0,534^{Cb}$	6,564 $\pm0,669^{BCa}$	6,693 $\pm0,672^{BCa}$	6,822 $\pm0,561^{ABCa}$	7,158 $\pm0,751^{ABa}$	7,313 $\pm0,572^{Aa}$	7,39 $\pm0,599^{Aa}$
Group AB	6,693 $\pm0,447^{Bab}$	6,693 $\pm0,59^{Ba}$	6,925 $\pm0,631^{ABa}$	6,951 $\pm0,745^{ABa}$	7,08 $\pm0,475^{ABa}$	7,158 $\pm0,572^{ABa}$	7,39 $\pm0,863^{Aa}$
Group N	6,719 $\pm0,531^{Cab}$	6,925 $\pm0,499^{BCa}$	7,184 $\pm0,619^{ABCa}$	7,339 $\pm0,68^{ABa}$	7,416 $\pm0,634^{ABa}$	7,675 $\pm0,771^{Aa}$	7,726 $\pm0,694^{Aa}$
Group NB	6,848 $\pm0,52^{Ba}$	6,951 $\pm0,755^{Ba}$	7,054 $\pm0,751^{Ba}$	7,054 $\pm0,545^{Ba}$	7,158 $\pm0,559^{ABa}$	7,287 $\pm0,594^{ABa}$	7,701 $\pm0,687^{Aa}$
Group B	6,796 $\pm0,457^{Cab}$	6,977 $\pm0,558^{BCa}$	7,184 $\pm0,665^{ABCa}$	7,339 $\pm0,443^{ABa}$	7,494 $\pm0,472^{ABa}$	7,494 $\pm0,502^{ABa}$	7,545 $\pm0,729^{Aa}$

* Different superscript capital latters in the same line and small case latters in the same column indicate the significance of the difference ($p < 0.05$).

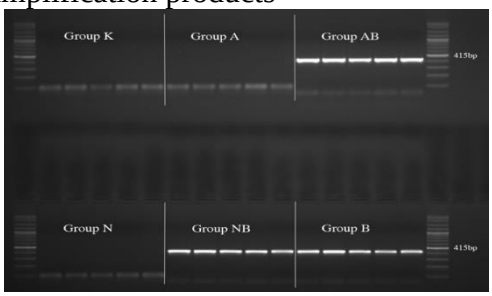
Table 7 Plasma glycose

	1st Day	2nd Day	3rd Day	4th Day	5th Day	6th Day	7th Day
Group K	82,314 $\pm6,499^{Ac*}$	77,289 $\pm6,678^{ABc}$	75,614 $\pm9,451^{Bc}$	75,375 $\pm7,245^{Bc}$	72,025 $\pm7,262^{Bb}$	64,461 $\pm3,171^{Cc}$	62,743 $\pm4,367^{Cd}$
Group A	97,15 $\pm5,774^{Bab}$	97,868 $\pm5,345^{Bab}$	99,064 $\pm5,883^{Bb}$	100,979 $\pm9,69^{Bb}$	104,568 $\pm9,028^{ABa}$	104,568 $\pm8,138^{ABb}$	108,157 $\pm6,26^{Ac}$
Group AB	91,168 $\pm9,991^{Db}$	100,739 $\pm8,77^{Cab}$	100,979 $\pm8,04^{Cab}$	103,371 $\pm8,65^{Cab}$	103,611 $\pm7,487^{Ca}$	116,532 $\pm7,819^{Ba}$	127,061 $\pm7,688^{Aa}$
Group N	90,929 $\pm6,577^{Db}$	95,954 $\pm6,995^{CDb}$	99,064 $\pm7,152^{BCb}$	101,457 $\pm5,194^{ABCb}$	103,371 $\pm8,65^{ABa}$	104,089 $\pm8,535^{ABb}$	107,918 $\pm7,174^{Ac}$
Group NB	100,739 $\pm7,349^{Ba}$	101,457 $\pm9,451^{Bab}$	102,654 $\pm6,121^{ABab}$	105,046 $\pm7,77^{ABab}$	106,961 $\pm7,487^{ABa}$	108,636 $\pm10,412^{ABb}$	110,071 $\pm4,786^{Abc}$
Group B	103,132 $\pm8,697^{Ba}$	104,329 $\pm7,414^{Ba}$	107,439 $\pm9,332^{ABa}$	109,593 $\pm6,558^{ABa}$	109,832 $\pm8,324^{ABa}$	110,789 $\pm7,401^{ABab}$	114,139 $\pm6,582^{Aa}$

* Different superscript capital latters in the same line and small case latters in the same column indicate the significance of the difference ($p < 0.05$).

Changes in the plasma lactate between days were not statistically significant whereas changes in plasma glucose levels between days were found to be significant ($P<0.05$). Illumination of PCR amplification products was shown Figure 1.

Figure 1. Illumination of PCR amplification products



Discussion

In this study, environmental factors such as low water quality like nitrite toxicity was observed in fish infected with *E. tarda* pathogens, had a significant change in the blood parameters of the stress.

Between experimental groups at the erythrocyte sedimentation decreased ($P<0.05$). Nitrite and pathogenic bacteria were firstly determined by our current study and value measured for *O. niloticus*. The decrease of parameter compared to the days between groups, is statistically significant. The values in the control group remained stable during the trial period but in the treatment groups values were generally lower. The reduction in values were compatible with the previous studies (Öztürk and Egemen 2003).

Yu et al. (2010) were found in *Silurus asotus*. which was experimentally infected with *E. tarda*. Sebastião et al. (2011) in *O. niloticus* infected with *Flavobacterium columnare*. Welker et al. (2012) and Akinrotimi et al. (2013) *Tilapia guineensis* in toxic substance concentration and Kpundeh et al. (2013) reported for *O. niloticus* that increased stocking density in unchanged waters was caused a decrease in the rate of haematocrit. Findings of Azevedo et al. (2004), Yildiz et al. (2006), El-Sherif & El-Feky (2008), Benli and Gulen (2009), Yu et al. (2010), Sebastião et al. (2011), Welker et al. (2012) and Akinrotimi et al. (2013) were consistent with our results of haematocrit rates.

Kpundeh et al. (2013) reported that increased in stocking density in waterless water in *O. niloticus* caused a decrease in the haemoglobin. Haemoglobin calculations of infected group were significantly different from the control. The values we have obtained are in accordance with results in the literature.

Balfry et al. (1997) reported an increase in serum lysozyme activity and head kidney macrophage respiratory burst activity in intraperitoneal infected *O. niloticus*. Gi-Hong et al. (2006) found an increase of head kidney macrophage respiratory burst activity in *E. tarda* infected *O. mosambicus*. Yu et al. (2010) reported an increase in plasma lysozyme activity in the *Silurus asotus*. experimentally infected with *E. tarda*. Zhou et al. (2010) showed an increase in lysozyme activity and head kidney macrophage respiratory burst activity in fish infected with *O. niloticus*; whereas Welker et al. (2012) reported a reduction in plasma lysozyme activity in nitrite toxicity in fish infected with *S. iniae*. In this study, plasma lysozyme activity values were found increased between days according to groups and in the head kidney macrophage respiratory burst activity groups compared to the days ($P<0.05$). Our findings were parallel to the findings of Möck and Peters (1990), Gi-Hong et al. (2006), Martins et al. (2009), Yu et al. (2010) and Zhou et al. (2010).

Changes in the plasma lactate between days were not statistically significant whereas changes in plasma glucose levels between days were found to be significant ($P<0.05$). Changes in the hematocrit and plasma glucose levels. especially in NB and B. were evaluated as indicative of stress in fish.

Khattab et al. (2007) report the amount of plasma lactate decreased in *O. niloticus*. when probiotic bacteria i.p. ejected. It was determined that there was an increase in the plasma lactate among groups between days ($P<0.05$). Findings in the study indicate that the pathogenic strain is an

enhancing effect of plasma lactate.

Plasma glucose levels in *E. tarda* infected fish were increase between the experimental groups ($P<0.05$). Yildiz et al. (2006) found that increase in plasma nitrite in *O. niloticus* was not related to the nitrite in the environment. Martins et al. (2008) reported the increase in plasma glucose in the Nile tilapia infected with *Enterococcus* sp. and Benli and Gulen (2009) found as a result, acute toxicity test plasma glucose levels of *O. niloticus* was increased. Yu et al. (2010) reported that plasma glucose decreased in *Silurus asotus* experimentally infected with *E. tarda*. Akinrotimi et al. (2013) claimed there was a significant increase in blood glucose concentration values due to toxicant concentration in *Tilapia guineensis* and Kpundeh et al. (2013) reported without water exchange. stock density increased plasma glucose in *O. niloticus*'s. Results of plasma glucose in the study were consistent with findings of previous studies.

It was determined at the end of the short period of the experiment period that there was a statistically significant change between the bacterial infected groups. according to the other groups and the control group.

Conclusion

In this study, some changes in the haematological and immunological were investigated in *O. niloticus*, exposed to sublethal nitrite which were experimentally infected with *E. tarda*.

The parameters (erythrocyte sedimentation rate, haematocrit value, haemoglobin level, plasma glucose, plasma lactate, plasma lysozyme activity, head kidney respiratory burst activity) had been determined which are important indicators of fish health and welfare, and which also characterize the stress physiology response.

Our results clearly show that tilapia which exposure sublethal ammonia and nitrite levels with the *E. tarda* infection, in semi-static water condition procedures could affect the blood parameters negatively.

References

Akinrotimi, O.A., Aranyo, A.A. & Ibemere, I.F. 2012. Studies in plasma glucose as biomarkers for stress-response in *Tilapia Guinessis*. Advances in Agriculture, Sciences and Engineering Research, 2(4): 147-152

Akinrotimi, O.A., Orlu, E.E. & Gabriel, U.U. 2013. Haematological responses of *Tilapia guineensis* treated with industrial effluents applied ecology and environmental sciences, 1(1): 10-13.

Amandi, A., Hiu, S.F., Rohovec, J.S. & Fryer, J.L. 1982. Isolation and characterization of *Edwardsiella tarda* from fall Chinook salmon (*Oncorhynchus tshawytscha*); Appl. Environ. Microbiol., 43, 1380-1384.

Anonymous 2000. A.O.A.C Official method 17th edition 2000, 973.31 Nitrites in cured meats - Colorimetric method, Adopted as Codex Reference method (Type II)

Arda, M. 1971. Hastalık etkenlerinin titrasyon ve nötralizasyon testlerinde uygulanan laboratuar metodları. Ankara Üniversitesi Veteriner Fakültesi, Yayın 273, Tatbikat Klavuzu 175, pp. 105.

Azevedo, M., Souza, M.M. & Freire, C.A. 2004. Reversibility of deleterious effects of the pisciculture byproduct nitrite on cultured Nile tilapia (*Oreochromis niloticus*) Aquat. Living Resour. 17, 19

-23.

Balfry, S.K., Shariff, M. & Iwama G.K. 1997. Strain differences in non-specific immunity of tilapia *Oreochromis niloticus* following challenge with Vibrio parahaemolyticus Diseases of Aquatic Organisms, 30, 77-80.

Baya, A. M., J. L. Romalde, D. E. Green, R. B. Navarro, J. Evans, E. B. May, & A. E. Toranzo. 1997. Edwardsiellosis in wild striped bass from the Chesapeake Bay. Journal of Wildlife Diseases 33, 517-525.

Benli, Ç.K. & Gülen Z. 2009. Fenitrothion'un etkisinde bırakılan Tilapia'da (*Oreochromis niloticus* L.) sekonder stres indikatörleri hematokrit ve plazma glikoz seviyesinin değişimi. YYÜ Tarım Bilimleri Dergisi, 19(1): 19-22

Blaxhall P.C. & Daisley K.W. 1973. Routine haematological methods for use with fish blood. Journal of Fish Biology 5, 771-781.

Çetinkaya, O. & Şahin, A. 2005. Balıklarda anestezi uygulamaları ve başlıca anestezikler, pp. 237-273. Balık Biyolojisi Araştırma Yöntemleri. Editör: Karataş, M., Nobel Yayın Dağıtım no772. pp. 498, Ankara

El-Sherif, M.S. & El-Feky, A.M. 2008. Effect of ammonia on Nile Tilapia (*O. niloticus*) performance and some hematological and histological measures. 8th International Symposium on Tilapia in Aquaculture.

Francis-Floyd, R., P. Reed, B. Bolon, J. Estes, & S. McKinney. 1993. An epizootic of *Edwardsiella tarda* in largemouth bass (*Micropterus salmoides*). Journal of Wildlife Diseases, 29, 334-336.

Gi-Hong, K., Se-Ryeon, K., Yun-Kwon, N., Eun-Hye, L. & Min-Sun, K. 2006. Respiratory burst activity of tilapia (*Oreochromis mosambicus*) phagocytes to *Edwardsiella tarda* Ghosts. Journal of fish pathology, 19(3): 285-288.

Khattab, Y. A., Shalaby, A. M., & Abdel-Rhman, A. A. 2007. Use of probiotic bacteria as growth promoters, anti-bacterial and their effects on physiological parameters of *Oreochromis niloticus*. Aquaculture, 28, 74-81.

Kpundeh MD, Xu P, Yang H, Qiang J & He J 2013. Stocking densities and chronic zero culture water exchange stress' effects on biological performances, hematological and serum biochemical indices of GIFT Tilapia Juveniles (*Oreochromis niloticus*). J Aquac Res Development, 4, 189

Lan, J., Zhang, X.-H., Wang, Y., Chen, J. & Han, Y., 2008. Isolation of an unusual strain of Edwardsiella tarda from turbot and establish a PCR detection technique with the gyrB gene. J. Appl. Microbiol., 105(3): 644-651.

Lawson T.B. 1995. Fundamentals of aquacultural engineering. Chapman-Holl, An International Thompson Publishing Company, pp. 355, USA.

Martins, M.L., Mouriño, J.L.P., Amaral, G.V., Vieira, F.N, Dotta, G, Jatobá, A.M.B, Pedrotti, F.S, Jerônimo, G.T., Buglione-Neto, C.C. and Pereira-Jr., G. 2008. Haematological changes in Nile tilapia experimentally infected with Enterococcus sp. Braz. J. Biol., 68(3): 657-661,

Martins, M.L., Vieira, F.N., Jerônimo, G.T., Mouriño, J.L., Dotta, G., Speck, G.M., Bezerra, A.J., Pedrotti, F.S., Buglione-Neto, C.C., & Pereira, G.Jr. 2009. Leukocyte response and phagocytic activity in Nile tilapia experimentally infected with *Enterococcus* sp. Fish hysiology and Biochemistry, 35(1): 219.

Möck, A. & Peters, G., 1990. Lysozyme activity in rainbow trout, *Oncorhyncus mykiss* (Walbaum), stressed by handling, transport and water pollution. Journal of Fish Biology. 37, 873-885.

Oliveira, S.R., Souza, R.T.Y.B., Brasil, E.M., Andrade, J.I.A., Nunes, É.S.S., Ono, E.A. & Affonso E.G. 2011. LD_{50} of the bacteria *Aeromonas hydrophila* to matrinxã Brycon amazonicus. Acta Amazonica, 41(2): 321-326

Öztürk, T. & Egemen A. 2003. Birinci Basamakta Bir Laboratuvar Testi: Eritrosit Sedimentasyon Hızı. Sürekli Tıp Eğitimi Dergisi (STED), 12(10): 383-385.

Plumb J.A. (1999) Edwardsiella septicemias. In: Fish Diseases and Disorders III, Viral, bacterial and fungal infections, P.T.K. Woo & D.W. Bruno (Eds.), pp. 479-521. CABI Publishing, NY.

Pressley, M.E., Phelan, P.E., Witten, P.E., Mellon, M.T. & Kim, C.H. 2005. Pathogenesis and inflammatory response to *Edwardsiella tarda* infection in the zebrafi sh; Dev. Comp. Immunol., 29, 501-513

Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: a laboratory manual. 2nd ed. New York, Cold Spring Harbor Press, 1989.

Sebastião, F. A., Nomura, D., Sakabe, R. & Pilarski F. 2011. Hematology and productive performance of nile tilapia (*Oreochromis niloticus*) naturally infected with *Flavobacterium columnare*. Brazilian Journal of Microbiology, 42, 282-289

Secombes C.J. (1990) Isolation of salmonid macrophages and analysis of their killing activity. In: Techniques in fish immunology, J.S. Stolen,T.C. Fletcher, D.P. Anderson, B.S. Robertson & W.Buiswinkel (Eds.), pp.137-154. SOS Publications, New Jersey, USA.

Siwicki, A.K. & Anderson, D.P. 1993. Immunostimulation in fish: Measuring the effects of stimulants by serological and immunological methods. The Nordic Symposium on Fish Immunology, 19-22 May, Sweden.

Svobodova Z., Vykusova, B. & Machova, J. 1994. The Effects of pollutants on selected haematological and biochemical parameters in fish sublethal and chronic effects of pollutants on freshwater fish. Müller, R. and Lloyd, R. (Eds.) 4, 39-52, FAO USA

Uhland, F.C., Helie, P. & Higgins, R. 2000. Infections of *Edwardsiella tarda* among brook trout in Quebec. J. Aquat. Anim. Health, 12, 74-77.

Walters, G.R., & Plumb, J.A. 1980. Environmental stress and bacterial infection in channel catfish, *Ictalurus punctatus* Rafinesque. Journal of Fish Biology, 17, 177-185.

Yildiz H.Y. 2009. Reference biochemical values for three cultured sparid fish: Striped Sea Bream, lithognathus mormyrus; Common Dentex, Dentex Dentex; and Gilthead Sea Bream, Sparus Aurata Comp Clin Pathol., 18, 23-27

Yildiz, H.Y., Köksal, G., Borazan, G. & Benli, C.K. 2006. Nitrite-induced methemoglobinemia in Nile tilapia, *Oreochromis niloticus*. J. Appl. Ichthyol., 22, 427-429.

Yu, J.H., Han, J.J. & Park, S.W. 2010. Haematological and biochemical alterations in Korean catfish, *Silurus asotus*, experimentally infected with *Edwardsiella tarda*. Aquaculture Research, 41, 295-302.

Zheng D, Mai K, Liu S, Limin C, Liufu Z, Xu W, Tan B & Zhang W 2004. Effect of temperature and salinity on virulence of *Edwardsiella tarda* to Japanese flounder, *Paralichthys olivaceus*. Aqua Res., 35, 494-500.

Zhou, X., Tian, Z., Wang, Y. & Li, W. 2010. Effect of treatment with probiotics as water additives on tilapia (*Oreochromis niloticus*) growth performance and immune response. Fish Physiol Biochem., 36, 501-509.

Correspondence: F. Sertel Secer. email: sertelsecer@yahoo.com