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COMBINED EFFECT OF TOTAL AMMONIA AND TEMPERATURE ON GROWTH, HEMATOLOGICAL, BIOCHEMICAL PARAMETERS AND HISTOLOGICAL INDICES FOR RED TILAPIA (*Oreochromis* sp.)

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ABSTRACT

This study aims to evaluate different levels of total ammonia, temperature and their combination on growth, feed utilization, hematological, biochemical parameters and histological indices in red tilapia (*Oreochromis* sp). Nine treatment which is combination of three levels of total ammonia (1.5, 3.0 and 4.5 ppm) and three different temperature degrees (24, 28 and 32°C) in a 3×3 factorial trial was designed for 56 days. Ten fish with an average initial body weight 11.1±0.27 g and an average initial body length 6.98±0.30 cm were distributed with two replicates. Analysis of variance showed significant differenc ($P<0.05$) of hematological parameters for RBCs, WBCs, Hb, Hct, PLT, MCH and MCHC among treatments. Additionally, biochemical parameters; glucose, uric acid, urea, albumin and globulin, and liver function activity were significantly different (*P<0.05*). The optimal activities of alanine and aspartate aminotransferase were obtained by fish of T5 and T1, respectively. For all treatments, the results of gill investigations, showed moderate degeneration of filaments with moderate deed of lining cells, shortening focal autolytic changes and focal congestion. The results of kidney histology showed marked interstitial edema, congestion with focal inflammatory infiltrate, mild vacuolar degeneration, focal edema and focal shrinkage for few glomeruli. The present findings suggest that interaction between total ammonia and temperature affect greatly fish health, physiological and histological status.

INTRODUCTION

Egypt's fish farming faces a number of challenges. Water pollution by inorganic and organic chemicals is one of these issues, and it is a major threat to aquatic organisms' survival **(Saeed and Shaker, 2008).** The majority of tropical species perish as a result of poor water quality caused by ammonia contamination. A number of aquatic creatures, including fish, are poisoned by ammonia **(Harris *et al.*, 1998).**

Under normal body conditions total ammonia will exist mainly in ionic form (>95%) but under conditions of increased temperature or pH, this equilibrium shifts towards formation of NH_3 **(Wood, 1993).** Toxic compounds in the aquatic environment might have a negative impact on fish reproduction and growth performance **(Kim and Kang, 2015).** Ammonia is one of the most poisonous compounds that can induce growth inhibition in fish farming, and could be a major cause of fish mortality **(El-Shafai *et al.*, 2004).** Ammonia is partially ionized when dissolved in water, depending on the pH and temperature. Ammonium is the name for ionized ammonia, which is not hazardous to fish. Ionization and ammonium rise when the pH declines and the temperature reduce, lowering the toxicity **(Norm, 1996)**.

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The health of aquatic organisms is influenced by the temperature of the water. Every specie can only survive in a given temperature range. The natural temperature range encountered in certain places of the sea is limited compared to freshwater, particularly impounded surface water **(Anzecc and Armcanz, 2000)**. Environmental factors, individual fish history, and genetic factors all influence the lowest lethal temperature **(Cnaani *et al.*, 2000)**. The most tolerant specie is *Tilapia sparmanii* (7°C) which is not commonly used in aquaculture, followed by *O. aureus* (8 to 9°C) however, *O. niloticus* is one of the least tolerant to low temperatures (11 to 12°C Most tilapias do not eat or grow at water temperatures below 15°C **(Baradach *et al.*, 1972)**. Many fish show seasonal changes in growth rate, and water temperature is one of the primary factors driving these changes. Albeit the exact effect of cultivation temperature varies by species and season **(Johnston, 1999)**. The rate of growth slows dramatically as the temperature drops, and at 20 to 22°C, growth is only approximately 30% of optimum **(Teichert-Coddington *et al.*, 1997)**.

Tilak *et al.* (2007) also discovered a significant drop in haemoglobin in common carp, *Cyprinus carpio*, exposed to ammonia, which they attribute to an increase in oxygen intake and an increase in methemoglobin due to gill damage. **Shin *et al.* (2016)** reported that temperature, as well as ammonia content, had a significant impact on *Sebastes schlegelii's* hematological characteristics. **Shin *et al.* (2016)** reported that *S. schlegelii* serum total protein was1.0 mg/l at 19°C and above 0.5 mgl^{-1} at 24°C which is significantly lower, whereas GOT, GPT, and glucose levels were significantly higher.

The objective of this study is to assess the combined effect of different total ammonia and temperature on growth performance, feed utilization, survival rate, hematological and biochemical parameters and histological investigations for red tilapia, *Oreochromis* sp.

MATERIALS AND METHODS

Experimental Design and Fish

This study was carried out at mariculture research Center (MRC), Faculty of Environmental Agricultural Science, Arish University, North Sinai, Egypt. Red tilapia fingerlings were obtained from the MRC. Fish were acclimated to the conditions of the experiment prior to start in the laboratory of fish. Fish were fed commercial feed (30% crude protein) during acclimation period of 15-day at a rate of 5% of their total biomass, which provided of equal rations at 09:00 am and 3:00 pm (6 days per a week). After this period, ten fish with an average initial body weight (11.1±0.27 g) and average body length (6.98 ± 0.30 cm) were distributed randomly into aquaria (58.5 × 38.40 × 26.27 cm; 60 l for each), representing the nine treatments with two replicates. The fish were stocked at three different concentrations of total ammonia 1.5, 3.0 and 4.5 ppm with three different temperature degrees 24, 28 and 32°C. Experimental design of this study was showed in Table 1.

The aquaria were supplied with running and continuously aerated water from an underground source with salinity (35 ppt). The concentrations of total ammonia-N and dissolved oxygen, pH, and water temperature were maintained at the same values as the prescribed experimental conditions and measured every day in each aquarium. Temperature was maintained at 24, 28 and 32°C using aquarium heater (300 W). The pH was kept constant at 7.86±0.41 by adjusting with 10% sulfuric acid or 10% sodium hydroxide **(Miron *et al.*, 2008).** Ammonia-N concentrations were reached by adding concentrated NH_4Cl solution. Approximately 10 l of the water in the tanks were replaced daily by water with previously adjusted ammonia-N concentration. The photoperiod was maintained at 12 L h: 12D h. The concentrations of ammonia-N

Table 1. Experimental design of the present study

Water temperature	Ammonia concentration (ppm)	Treatment
24°C	1.5	T1
	3.0	T2
	4.5	T3
28°C	1.5	T4
	3.0	T5
	4.5	T6
32°C	1.5	T7
	3.0	T8
	4.5	T9

were determined using the Nesslerization method as described by **Benli *et al*. (2008).** Fish fed at a rate of 3% of their total biomass for 56 days. The daily amount feed was divided into three equal amounts and offered three times a day at 09:00, 12:00 and 15:00 h. The numbers of dead fish were recorded daily (dead fish in all treatment groups for the survival rate), and feces were removed from the aquaria after feeding every day during the. Biweekly fish were taken from each aquaria, weighed and the amount of feed was adjusted according to the changes in body weight through the experimental period. Commercial diet 30% crude protein, 5% lipid and total energy 3830 kcal /kg was used in this study from Al-Etihad Group company for feed manufacturing in Kafr El-Sheikh Governorate.

Water Quality

Water quality was monitoring as follow: Water temperature was recorded daily at 1.00 pm using a mercury thermometer. Dissolved oxygen (DO) was measured at 07.00 am using YSI model 56 oxygen meters (YSI Company, Yellow Springs Instrument, Yellow Springs, Ohio, USA). Total ammonia and nitrite were measured twice a week using a DREL, 2000 spectrophotometer (Hash Company, Loveland, CO, USA). pH was measured daily morning by using a pH meter (Orion pH meter, Abilene, Texas, USA).

Growth Performance and Feed Utilization

Growth performance and feed utilization were measured using the following equations: Weight gain (WG) = final weight (g) – initial weight (w1); Gain%=(WG/W1)x100; Condition factor (K) = $(W/L^3) \times 100$, where, W is weight of fish in grams and L is total length of fish in cm; specific growth rate (SGR) = (LnW2 – LnW1)/ t X 100, Where, Ln is the natural log; W1 is initial body weight and W2 is the final body weight in grams and "t" is the experimental period in days; feed conversion ratio (FCR) = Feed intake (g)/ Weight gain (g); Feed efficiency (FE %) = gain in weight (g)/feed intake (g) and protein efficiency ratio (PER) = weight gain (g)/ protein ingested (g).

Blood Sampling for Hematological and Biochemical Parameters

At the end of the experiment the blood was collected form the heart of each fish in each treatment. The blood samples for hematological parameters were taken into clean tubes containing sodium salt of ethylene diamine tetra acetic acid

(Na_2EDTA) as anticoagulant or without coagulant and the blood samples were taken in dry clean centrifuge tubes. Serum was separated at 3000 rpm for15 minutes using the centrifuge. All tests were performed immediately after blood samples were withdrawn without keeping the sample.

Hematological Parameters

Red blood cells (RBCs) count and white blood cells (WBCs) count were determined according to the method of **Dacie and Lewis (1991);** Hemoglobin concentration (g/dl^{-1}) was determined according to **Drabkin and Austin (1932);** Hematocrit value was measured according to **Sorrell-Raschi and Tomasic (1998);** thrombocytes count was determined according to the method of **Brecher *et al.* (1953);** mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated according to **Seiverd (1964)**.

Biochemical Parameters

Glucose was determined according to the method of **Tietz (1986),** serum uric acid was measured according to **Young (1995),** serum urea enzymatic was determined according to **Patton and Crouch (1977),** total protein and albumin were determined according to **Doumas (1975)**, serum creatinine was measured according to the method of **Henry (1974),** aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined calorimetrically according to **Reitman and Frankel (1957).**

Histological Investigations

At the end of the experiment gills and kidneys were collected form 3-5 fish of each treatment, they were fixed in Bouin's solution for about 24 hours. The specimens were then preserved in 70% ethyl and then, were dehydrated through ascending gradient of ethanol solution. Cleared in xylene and embedded in paraffin wax as usual. Sometimes, tyrpinol was used for clearing and showed best results. Section of 4-6µ thickness were mounted on chemically clean glass slides. The sections were prepared then stained with Harri's Haematoxylin and Eosin (Hx and E) according to **Pearse (1972)**.

Statistical Analysis

All data were analyzed using SAS **(Statistical Analysis System, 1996)**, version 6.03**.** Two ways ANOVA was used for analysis the individual effects of factors and the interaction between there, Duncan multiple range test was conducted for individual means only if there was a significant interaction (ANOVA: $P<0.05$). All differences were considered signification at ($P<0.05$) and the results are presented as means with pooled standard error (SE).

RESULTS

Growth Performance and Survival Rate

The results of growth performance and survival rate of red tilapia (*Oreochromis* sp) treated with different concentrations of total ammonia and temperatures are presented in Table 2. The initial body weight (IBW) among different treatments exhibited insignificant differences ($P=0.48$). No significant differences were found in all indices of growth affected by the interaction between total ammonia (1.5, 3.0 and 4.5 ppm) and temperature (24, 28 and 32°C). Also, survival rate (SR) among different treatments were insignificant differences ($P>0.05$). Furthermore, SR of T1, T8 and T9 (100 %) was higher than other treatments (Table 2). Condition factor(k) was significantly different ($P<0.05$) between treatments among the highest K (2.29) was recorded for treatment T5 and T2, while the lowest one was recorded for each of T6 , T7and T8.

Table 2. Growth response of red tilapia is affected by total ammonia and temperature for 56 days

Treatments	Temperature °C	Total ammonia (ppm)	Growth Performance							
			IBW (g fish^{-1})	FBW (g fish^{-1})	WG (g fish^{-1})	ADWG	SGR (% day^{-1})	Survival rate %(SR)	GL (cm fish^{-1})	Condition factor (K)
Individual treatment mean*										
T1	**24**	**1.5**	11.05	26.95	15.90	0.29	1.59	100.00	3.85	2.08^{ab}
T2	**24**	**3.0**	11.05	25.55	14.49	0.26	1.49	95.00	3.15	2.27^{a}
T3	**24**	**4.5**	10.90	25.49	14.59	0.26	1.52	85.00	4.05	1.94^{ab}
T4	**28**	**1.5**	11.00	24.64	13.64	0.24	1.44	95.00	3.45	2.07^{ab}
T5	**28**	**3.0**	11.05	26.62	15.57	0.28	1.57	90.00	3.65	2.29^{a}
T6	**28**	**4.5**	10.95	21.93	10.98	0.20	1.24	90.00	4.00	1.87^{b}
T7	**32**	**1.5**	11.45	25.09	13.64	0.24	1.40	95.00	3.90	1.86^{b}
T8	**32**	**3.0**	10.95	22.60	11.65	0.21	1.29	100.00	3.65	1.87^{b}
T9	**32**	**4.5**	11.30	26.75	15.45	0.28	1.54	100.00	3.75	2.18^{ab}
Pooled SE			0.18	2.13	1.87	0.04	0.16	4.86	0.45	0.10
Means of the main effect**										
	24		11	25.99	14.99	0.27	1.53	93.33	3.68	2.09
	28		11	24.39	13.39	0.24	1.41	91.66	3.70	2.07
	32		11.23	24.81	13.67	0.24	1.41	98.33	3.76	1.97
		1.5	11.16	25.56	14.39	0.25	1.47	96.66	3.73	2.00
		3	11.01	24.92	13.90	0.25	1.45	95.00	3.48	2.14
		4.5	11.05	24.72	13.67	0.24	1.4 3	91.66	3.93	1.99
ANOVA (p-value)										
Temperature			0.5785	0.8831	0.9419	0.9113	0.9624	0.4729	0.5045	0.195
Total ammonia			0.2399	0.6493	0.5351	0.5994	0.4855	0.2739	0.9720	0.212
Temperature ×Total ammonia			0.4858	0.6887	0.7163	0.7023	0.6851	0.4119	0.8951	0.063

*Treatments means represent the average values of two glass aquaria per treatment. Duncan multiple range test was conducted for individual means only if there was a significant interaction (ANOVA: $P < 0.05$).

**Within each column, Means followed by different letters are significantly different.

Feed Utilization Parameters

Feed intake (FI) and feed utilization; feed conversion ratio (FCR), protein efficiency ratio (PER) and feed efficiency (FE%) significantly affected by temperature, total ammonia and their interaction. The highest FI was recorded for each of T1 and T9 treatment, (42.19 and 42.52, respectively), while the lowest one was recorded for T6.

The lowest (best) FCR (2.49) and the highest PER (1.34) were obtained by treatment T5. The highest FCR (3.44) and the lowest PER (0.97) were obtained by treatment T6. The highest FE% (40.14) was recorded for treatment T5, while the lowest one was recorded for treatment T6 (Table 3).

Hematological Parameters

Analysis of variance showed significant different ($P<0.05$) among treatments for red blood cells count (RBCs), white blood cells count (WBCs), hemoglobin (Hb), hematocrit (Hct), thrombocytes count (PLT), mean corpuscular hemoglobin MCH (pg) and mean corpuscular hemoglobin concentration MCHC (%) of red tilapia (*Oreochromis* sp) treated with different concentration of total ammonia and temperatures and their interaction (presented in Table 4).

The highest RBCs (2.85 and 2.80) x 10^6 were recorded for treatment T3 and T4, while the lowest one(1.45) was recorded for T8. Highest WBCs values were recorded for treatment T1, T2, T4,T7 and T9, while the lowest one was recorded for T7. Value of Hb (g dl^{-1}) (8.80 g dl^{-1}) was significantly the highest in fish of treatment T3, while the lowest value was obtained by each of T2 and T8. The highest Hct % was recorded for fish of treatment T3, while the lowest one was recorded for T8. Fish exposed to temperature 32°C and total ammonia 4.5 ppm (T9) showed increased in thrombocytes 1857.00), while fish exposed to temperature 32°C and total ammonia 1.5 ppm (T7) showed decreased in thrombocytes.

No significant differences were found in MCV among different treatments.

Highest MCH values were recorded for all treatments except T2 which was the lowest one. MCHC was significantly different ($P<0.05$). Highest MCHC values were ($P=0.0154$) was recorded for all treatments except T2.

Biochemical Parameters

Results of glucose (mg dl^{-1}), uric acid (mg dl^{-1}), urea (mg dl^{-1}), albumin (g dl^{-1}), globulin (g dl^{-1}), A/G ratio and creatinine (mg dl^{-1}) of red tilapia (*Oreochromis* sp) treated with different concentration of total ammonia, temperatures and their interaction are presented in Table 5.

With respect to temperature effect, values of glucose did not affected. While total ammonia significantly affected glucose values regardless the effect of temperature.

Interaction between temperature and total ammonia significantly affected glucose values ($P=0.0048$) Glucose (mg dl^{-1}) recorded high values under T1,T2,T3,T4,T5 and T9 treatment, while low valued of glucose were obtained in T6,T7and T8 treatments. Uric acid (mg dl^{-1}) was significantly different (*$P<0.05$*). Regardless the effect of total ammonia, values of uric acid significantly affected by different level of temperature. Also, total ammonia significantly affected uric acid of red tilapia values regardless the effect of temperature. The highest uric acid value was recorded for fish of treatments T3 and T7, while the lowest one was recorded for T8. Urea (mg dl^{-1}) exhibited insignificant differences ($P=0.4692$) among different treatments.

Albumin and globulin values among different treatments were significantly different ($P<0.05$), the highest value of albumin was obtained by T1, while the lowest one was recorded for T8. The highest value of globulin was obtained by T7 compared with all treatments. A/G ratio ($P=0.4692$) and creatinine ($P=0.4125$) among different treatments was not affected by interaction between total ammonia (1.5, 3.0 and 4.5 ppm) and temperature (24, 28 and 32°C).

Table 3. Feed intake (FI), feed conversion ratio (FCR), protein efficiency ratio (PER) and feed efficiency (FE) of red tilapia during experimental period 56 days

Treatments	Temperature °C	Total ammonia (ppm)	Feed utilization			
			FI	FCR	PER	FE%
Individual treatment means*						
T1	**24**	**1.5**	42.19	2.65	1.26	37.68
T2	**24**	**3.0**	40.38	2.79	1.19	35.88
T3	**24**	**4.5**	39.47	2.71	1.23	36.96
T4	**28**	**1.5**	39.77	2.92	1.14	34.29
T5	**28**	**3.0**	38.79	2.49	1.34	40.14
T6	**28**	**4.5**	37.78	3.44	0.97	29.06
T7	**32**	**1.5**	41.29	3.03	1.00	33.03
T8	**32**	**3.0**	38.05	3.27	1.02	30.62
T9	**32**	**4.5**	42.52	2.75	1.21	36.34
Pooled SE			2.88	0.41	0.12	3.49
Means of the main effect						
	24		40.68	2.71	1.23	36.84
	28		38.78	2.59	1.15	34.49
	32		40.62	3.01	1.07	33.33
		1.5	41.08	2.86	1.13	35.00
		3.0	39.07	2.85	1.18	35.54
		4.5	39.92	2.96	1.13	34.12
AOVA (p-value)						
Temperature			0.7021	0.9964	0.8980	0.8987
Total ammonia			0.6695	0.2924	0.3700	0.3675
Temperature ×total ammonia			0.9179	0.4620	0.4100	0.4045

*Treatments means represent the average values of two glass aquaria per treatment. Duncan multiple range test was conducted for individual means only if there was a significant interaction (ANOVA: $P<0.05$).

Table 4. Hematological indices of red tilapia after experimental period 56 days

Treatment	Temperature °C	Total ammonia (ppm)	Hematological indices							
			RBCs $\times 10^6$	WBCs $\times 10^3$	Hb (gdl^{-1})	Hct %	PLT	MCV (fl)	MCH (pg)	MCHC (%)
Individual treatment mean*										
T1	**24**	1.5	2.30^{ab}	502.80^{a}	5.45^{bc}	16.00^{bcd}	884.00^{bc}	69.57	23.96^{ab}	34.06^{a}
T2	**24**	3.0	2.45^{ab}	480.00^{a}	3.80^{c}	23.00^{abc}	1345.50^{ab}	93.88	15.51^{b}	16.52^{b}
T3	**24**	4.5	2.85^{a}	225.50^{b}	8.80^{a}	26.50^{a}	735.00^{bc}	92.98	30.88^{a}	33.21^{a}
T4	**28**	1.5	2.80^{a}	371.50^{a}	6.85^{bac}	21.00^{abcd}	830.00^{bc}	75.00	24.46^{ba}	32.62^{a}
T5	**28**	3.0	1.60^{ab}	88.00^{bc}	4.85^{bc}	14.50^{cd}	890.00^{bc}	90.36	30.31^{a}	33.45^{a}
T6	**28**	4.5	1.95^{ab}	144.00^{bc}	6.20^{bac}	23.50^{ab}	864.50^{bc}	120.51	31.79^{a}	26.58^{a}
T7	**32**	1.5	2.20^{ab}	570.00^{c}	6.00^{bac}	18.00^{bdac}	472.50^{c}	81.82	27.27^{a}	33.33^{a}
T8	**32**	3.0	1.45^{b}	175.00^{bc}	4.55^{c}	14.00^{d}	1024.00^{bc}	96.55	31.38^{a}	32.50^{a}
T9	**32**	4.5	2.70^{ab}	470.5^{a}	8.00^{ab}	24.00^{ab}	1857.00^{a}	88.89	29.63^{a}	33.33^{a}
Pooled SE			0.37	43.94	0.93	2.44	226.46	18.60	2.98	2.53
Means of the main effect**										
	24		2.53	402.7666	6.01	21.83	988.16	85.47	23.45	27.93
	28		2.11	201.1666	5.96	19.66	861.50	95.38	28.85	30.88
	32		2.11	234.1666	6.18	18.66	1117.83	89.08	29.42	33.05
		1.5	2.43	310.4333	6.10	18.33	728.83	75.46	25.23	33.33
		3.0	1.83	247.6666	4.40	17.16	1086.50	93.68	25.73	27.49
		4.5	2.50	280.0000	7.66	24.66	1152.16	100.79	30.76	31.04
ANOVA (p-value)										
Temperature			0.1086	0.2736	0.0083	0.0116	0.1044	0.2169	0.0930	0.0577
Total ammonia			0.3308	0.0011	0.9567	0.3201	0.4226	0.6007	0.0679	0.0981
Temperature × total ammonia			0.1930	0.0003	0.0678	0.0480	0.0647	0.5016	0.0607	0.0154

*Treatments means represent the average values of two glass aquaria per treatment. Duncan multiple range test was conducted for individual means only if there was a significant interaction (ANOVA: $P<0.05$).
** Within each column, Means followed by different letters are significantly different.

Table 5. Biochemical parameters of red tilapia during experimental period 56 days

Treatment	Temperature °C	Total ammonia (ppm)	Biochemical parameters						
			Glucose (mg dl^{-1})	Uric acid (mg dl^{-1})	Urea (mg dl^{-1})	Albumin (g dl^{-1})	Globulin (g dl^{-1})	A/G ratio	Creatinine (mg dl^{-1})
Individual treatment mean*									
T1	**24**	**1.5**	277.50^{a}	7.20^{b}	14.55	3.45^{a}	5.60^{ab}	0.62	0.50
T2	**24**	**3.0**	268.50^{a}	8.35^{b}	13.25	2.80^{ab}	3.60^{ab}	0.78	0.55
T3	**24**	**4.5**	272.00^{a}	14.60^{a}	13.50	1.70^{ab}	4.15^{ab}	0.41	0.60
T4	**28**	**1.5**	286.50^{a}	3.60^{c}	12.25	2.40^{ab}	2.50^{ab}	0.96	0.65
T5	**28**	**3.0**	286.50^{a}	2.60^{c}	12.75	1.25^{ab}	2.30^{ab}	0.54	0.45
T6	**28**	**4.5**	136.50^{b}	3.20^{c}	13.00	1.50^{ab}	3.90^{ab}	0.39	0.60
T7	**32**	**1.5**	160.50^{b}	12.20^{a}	8.30	1.75^{ab}	8.20^{a}	0.21	0.10
T8	**32**	**3.0**	181.00^{b}	2.15^{c}	10.50	1.08^{b}	6.57^{ab}	0.16	0.35
T9	**32**	**4.5**	288.50^{a}	2.85^{c}	12.25	1.50^{ab}	1.50^{b}	1.00	0.40
Pooled SE			22.41	0.77	2.58	0.64	1.73	0.44	0.20
Means of the main effect**									
	24		272.66	10.05	13.76	2.65	4.45	0.60	0.55
	28		236.50	3.13	12.66	1.71	2.90	0.63	0.56
	32		210.00	5.73	10.35	1.44	5.42	0.45	0.28
		1.5	241.50	7.66	11.70	2.53	5.43	0.59	0.41
		3.0	245.33	4.36	12.16	1.71	4.15	0.49	0.45
		4.5	232.33	6.88	12.91	1.56	3.18	0.60	0.53
ANOVA (p-value)									
Temperature			0.7725	0.0020	0.8462	0.1987	0.3312	0.8141	0.9162
Total ammonia			0.0265	<.0001	0.3063	0.1111	0.2568	0.9721	0.3353
Temperature×total ammonia			0.0048	<.0001	0.8162	0.02892	0.02679	0.4692	0.4125

*Treatments means represent the average values of two glass aquaria per treatment. Duncan multiple range test was conducted for individual means only if there was a significant interaction (ANOVA: $P < 0.05$).
** Within each column, Means followed by different letters are significantly different

Liver Function

Results of total protein (g dl^{-1}), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) values were affected by total ammonia, temperature and their interactions and are presented in Table 6.

No significant differences (P>0.05) were found in total protein (g dl^{-1}) among all treatments. Analysis of variance results showed that AST and ALT significantly (P<0.05) affected by total ammonia, temperature and their combination. The lower (best) AST(P=0.0025) and ALT (P=0.0014) were obtained by fish of T5, T8 and T1, T4, respectively.

Histological Examination

The Gill

Examination of gill tissues from T1 and T2 after 56 days showed moderate degeneration of filaments with moderate deeding of lining cells, shortening focal autolytic changes and focal congestion (Figure 1). When examining gill tissues from T3 and T4 the results show moderate congestion and focal degenerative changes. The gill tissues in T5 and T6 show mild shortening of lamellae, moderate degeneration of lamellae with fragmentation and mild degenerative changes in lamellae lining cells with focal congestion. Gill tissues from T7 and T8 show normal filaments, lamellae with few focal and moderate congestion. The gill tissues from fish exposed to the highest level of TAN and temperature (T9) show moderate focal shortening and blunting of lamellar with mild degenerative changes of lining cells (Fig. 1).

The Kidney

Examination of kidney tissues from T1 and T2 showed marked interstitial edema, congestion with focal inflammatory infiltrate, mild vacuolar degeneration and focal edema. Histological examination of kidney tissues from T3 and T4 showed moderate vacuolar degeneration of tubular epithelial cells. The kidney tissues in T5 and T6 showed moderate vacuolar degeneration of tubular epithelial cells with moderate edemas, marked congestion and few glomeruli showed focal shrinkage. Kidney tissues from T7 and T8 showed mild congestion, mild vacuolar tubular, degeneration focal mild interstitial edema and focal minimal vacuolar degeneration. The kidney tissues from fish exposed to the highest level of TAN and temperature (T9) showed mild focal vacuolar degeneration of tubules and moderate vacuolar degeneration of tubular epithelial cells (Fig. 2).

DISCUSSION

Growth Performance and Feed Utilization Parameters

In this study, the combined effect of total ammonia and temperature exposure did not affect growth performance of red tilapia (*Oreochromis* sp). However, survival rates (SR) among different treatments were insignificant differences. Although, the high-water temperature increases the toxicity of ammonia which in turn affects growth and reduces the fish appetite of red tilapia fish, but fish in this experiment not affected. When stressed, fish will utilize more feed or glycolysis energy for swimming, regulation, and respiration rather than growth, reproduction, and storage **(Klein and Sheridan, 2008). Morrow (2009)** showed that under aquaculture conditions, sublethal amounts of total ammonia have a negative effect on the growth of young Nile tilapia (*Oreochromis niloticus*) by suppressing weight and length gain in comparison to controls.

Nguyen *et al.* (2014) reported that the survival rate observed in the 24°C treatment was lower than all other treatments. There was no significant difference in fish survival rate among all other treatments except that survival in the 36°C treatment was the lowest when compared with 27°C.

Table 6. Total protein, aspartate aminotransferase and alanine aminotransferase of red tilapia during experimental period 56 days

Treatment	Temperature °C	Total ammonia (ppm)	Biochemical parameter		
			T. protein (g dl^{-1})	AST	ALT
Individual treatment mean*					
T1	**24**	**1.5**	9.05	80.00^{bc}	10.00^{d}
T2	**24**	**3.0**	6.40	98.00^{ab}	14.00^{cd}
T3	**24**	**4.5**	5.85	89.00^{b}	12.00^{d}
T4	**28**	**1.5**	4.90	59.00^{cd}	10.00^{d}
T5	**28**	**3.0**	3.55	52.00^{d}	15.50^{cd}
T6	**28**	**4.5**	5.40	81.50^{bc}	22.00^{bc}
T7	**32**	**1.5**	9.95	80.50^{bc}	37.00^{a}
T8	**32**	**3.0**	7.65	52.50^{d}	25.50^{b}
T9	**32**	**4.5**	3.00	112.00^{a}	17.00^{bcd}
Pooled SE			2.14	6.76	2.66
Means of the main effect**					
	24		7.10	89.00	12.00
	28		4.61	64.16	15.83
	32		6.86	81.66	26.50
		1.5	7.96	73.16	19.00
		3.0	5.86	67.50	18.33
		4.5	4.75	94.16	17.00
ANOVA (p-value)					
Temperature			0.24	0.0031	0.66
Total ammonia			0.34	0.0055	0.0004
Temperature ×total ammonia			0.4015	0.0025	0.0014

*Treatments means represent the average values of two glass aquaria per treatment. Duncan multiple range test was conducted for individual means only if there was a significant interaction (ANOVA: $P<0.05$).

** Within each column, means followed by the same letter are not significantly different.

[1]AST = Aspartate amino transferees

[2]ALT = Alanine amino transferase

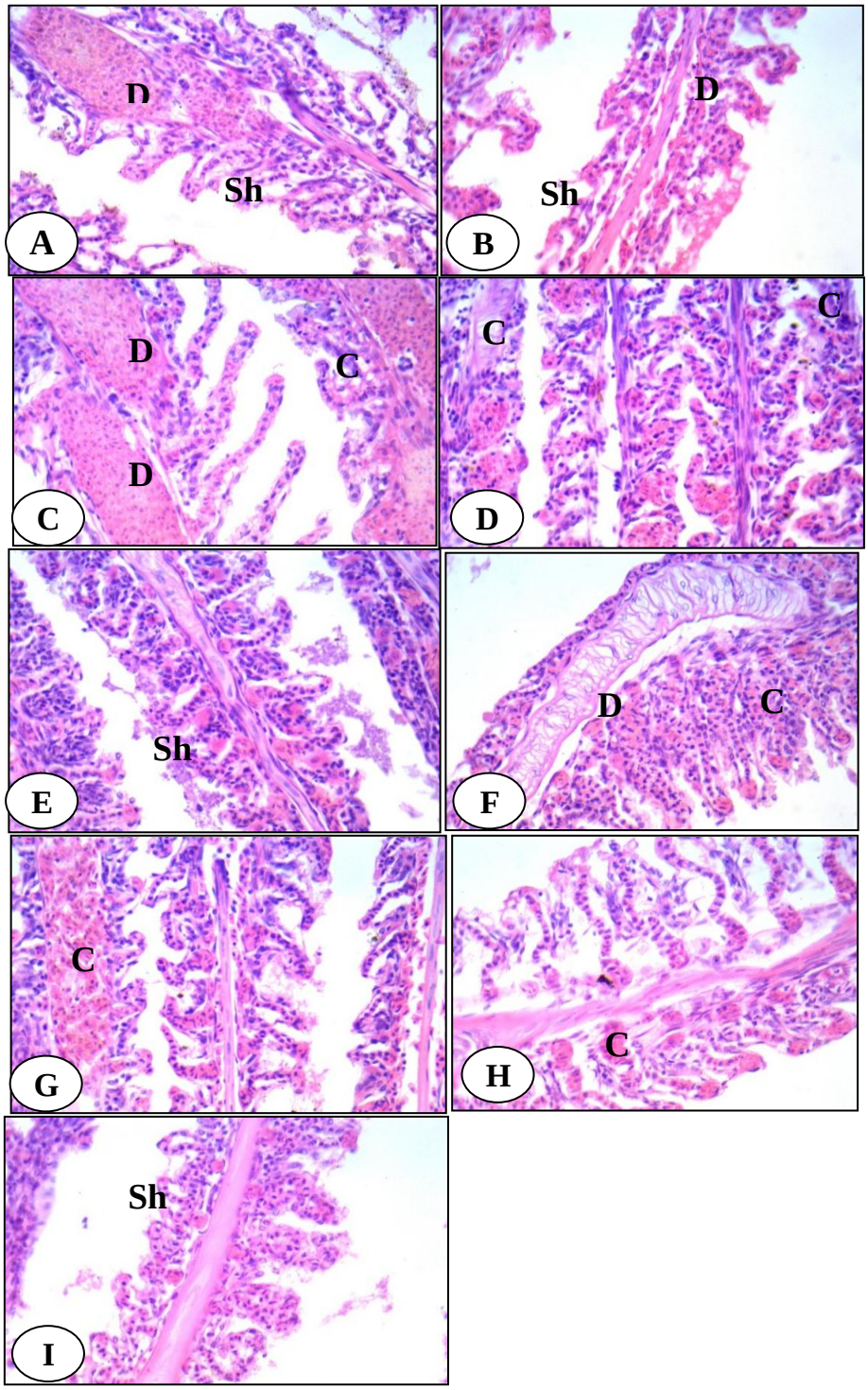

Fig. 1. Transverse sections of gill for red tilapia (*Oreochromis* sp) affected by total ammonia and temperature for 56 days (X400, H & E stain). Gills from the fish show moderate degeneration (D) of filaments with moderate dead of lining cells, shortening (sh) focal autolytic changes of lamellae and focal congestion (C); treatment 1 T1 (A), treatment 2 T2 (B), treatment 3 T3(C), treatment 4 T4 (D), treatment 5 T5 (E), treatment 6 T6 (F), treatment 7 T7 (G), treatment 8 T8 (H) and treatment 9 T9 (I).

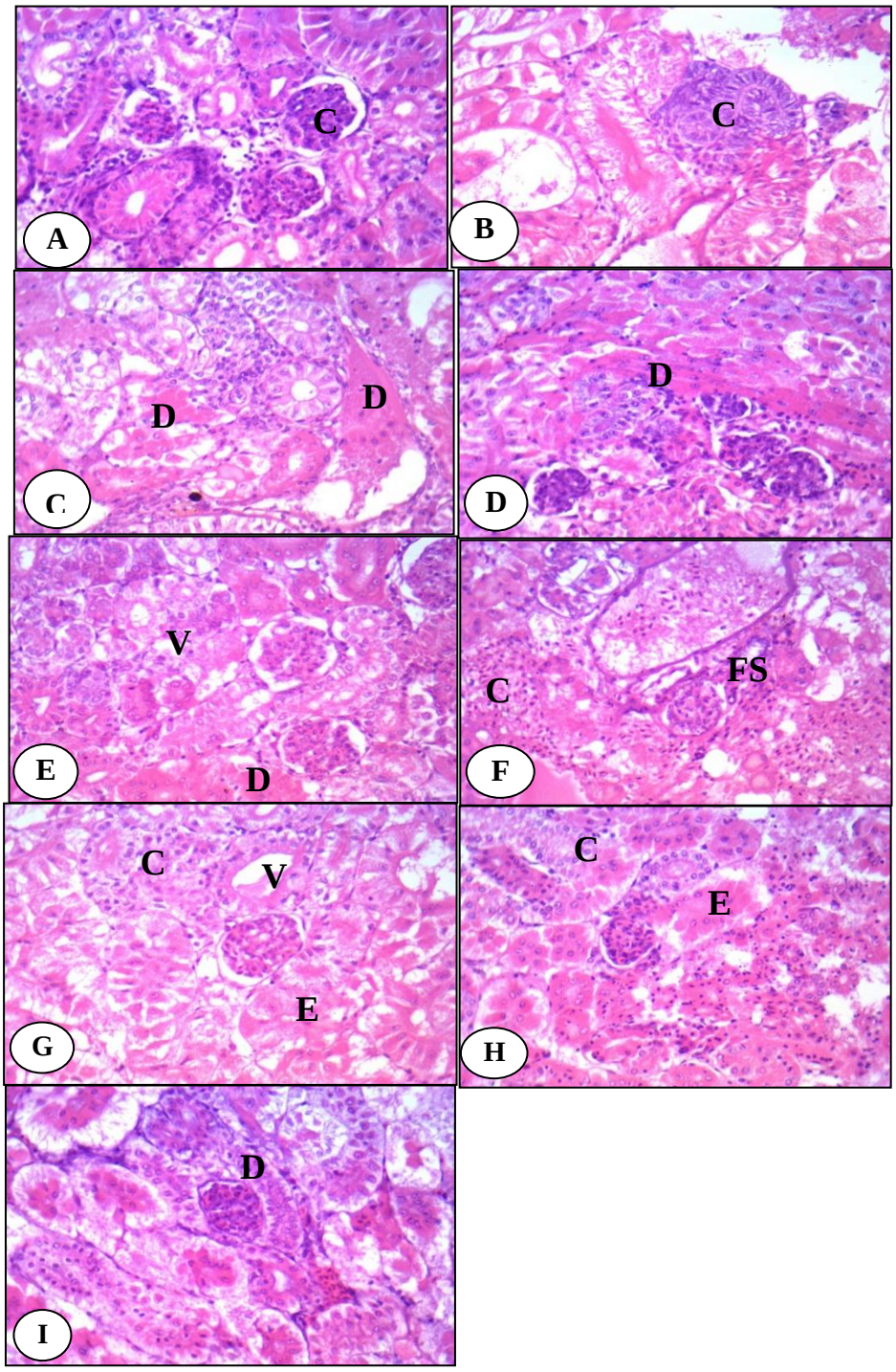

Fig. 2. Transverse sections of kidney for red tilapia (*Oreochromis* sp) affected by total ammonia and temperature for 56 days (X400, H & E stain). kidneys from the fish show congestion(C) with focal inflammatory infiltrate, moderate vacuolar degeneration (D), tubular epithelial cells with moderate edemas (E), few glomeruli showed focal shrinkage (FS) and mild focal vacuolar (V) with degeneration; treatment 1 T1 (A), treatment 2 T2 (B), treatment 3 T3 (C), treatment 4 T4 (D), treatment 5 T5 (E), treatment 6 T6 (F), treatment 7 T7 (G), treatment 8 T8 (H) and treatment 9 T9 (I).

Hegazi and Hasanein (2010) found that increase the ammonia level reduced the (DGR), and specific growth rate (SGR) in tilapia. **De Silva *et al*. (2013)** noted that significantly lower final body weight, SGR and yield of fish than acidic and neutral tanks. As explained that tilapia growth was lower in the neutral tanks when the NH_4Cl application rate was increased to 0.50g/tank. This was due probably to nitrite toxicity, which has increased in neutral tanks when more NH_4Cl was applied. Reduction in the growth performance was noted in Nile tilapia exposed to 5, 7.5, and 10 mg $TANL^{-1}$ and blue tilapia exposed to 5 mgl^{-1} TAN.

The obtained results are similar to the ones obtained by **Shin *et al.* (2016)** who showed that after two weeks, there was a significant reduction in length gain growth of over 0.5 mgl^{-1} of total ammonia at 19 and 24°C. The daily length increase was considerably reduced over 0.5 mgl^{-1} at 19°C and over control at 24°C after 4 weeks. After two weeks, the condition factor concentration of 1.0 mgl^{-1} at 19 and 24 °C declined, and after four weeks, the concentration of 1.0 mgl^{-1} at 19°C and above 0.5 mgl^{-1} at 24°C decreased significantly.

Nguyen *et al*. (2014) found that water temperature of 27°C significantly increased growth performance of fish compared with reared in 24°C. Also **Suja *et al.* (2009)** showed that the highest weight gain and best FCR were recorded in fish reared at 27°C. **Meeuwig *et al*. (2004)** reported that growth and feed intake generally increase with increasing temperature up to an optimal temperature and then decrease in cutthroat trout (*Oncorhynchus clarki henshawi*). In contrast with **Cotton *et al.* (2003)** who showed that FCR was the lowest in the 25°C treatment. This is consistent with the results of another temperature experiment with black sea bass. **Lahav and Ra'anan (1997)** found that growth performance and feed utilization of a red tilapia with *O. mossambicus* significantly decreased when water temperature dropped below 25°C and stopped at water temperatures below 21°C. As a result, temperature has a little to major impact on the nutritional requirements of fish for optimal performance **(NRC, 1993)**. In general, the connection between temperature and growth in fish is positive, growth and food intake increased as temperature rises to an optimum level, then decreased **(Meeuwig *et al*., 2004)**.

Chronic ammonia exposure can have an effects on teleost biology, such as lowering growth rate performance **(Hegazi and Hasanein, 2010),** causes gill hyperplasia **(Benli *et al.*, 2008)**, and induces hyperexcitability, coma, convulsions and finally death **(Ip *et al.*, 2001).** It is speculated that high levels of NH_4^+ in water could also impair fish growth. *Sebastes schlegelii's* growth performance was significantly reduced by increased ammonia levels may be due to the need for energy to detoxicate the ammonia, resulting in a decrease in growth energy **(Clearwater *et al.*, 2002**). Also **Madison *et al.* (2009)** reported that after 42 days of exposure to concentrations between 75 and 150 μM total ammonia at 18°C, juvenile walleye showed faster growth rates, better protein turnover, and greater whole-body protein.

Hematological Indices

Results in Table 4 show that red blood cells count (RBC_s), white blood cells count (WBC_s), hemoglobin (Hb), hematocrit (Hct), platelets count (PLT), mean corpuscular hemoglobin MCH and mean corpuscular hemoglobin concentration MCHC were significantly ($P<0.05$) affected by interaction between total ammonia and temperature. Mean corpuscular volume MCV was insignificantly different ($P>0.05$). These results disagreed with **Knoph and Thorud (1996)** who noted that with water total ammonia level, there was a drop in RBCs and an increase in MCV, but not statistically significant except in one case possibly. The lack of an effect on Hct at lower ammonia levels matched with the results of **Fivelstad *et al.* (1995)**.

De *et al.* (2019) found that RBCs, WBC, PCV and MCH were shown to have substantially higher haematological indices at 30°C compared to 26, 22, and 34°C. Although MCV and MCHC were high at 30°C, they were not substantially different from those at 26°C in hybrid grouper juveniles; nevertheless, they were considerably different from those at 22 and 34°C. **Mali and Chavan (2014)** showed that at low temperatures (20°C) and high temperatures (40°C), the overall number of RBCs dropped. The fish *Oreochromis mossambicus* had a slightly higher RBC count at 32°C when compared to the control. **Cristea *et al.* (2013)** reported that the hematocrit (Hct) under stressing effect of the temperature, records increasing value at 20°C comparing with the 28°C in *O. niloticus*.

Metwally and Wafeek (2014) reported that *O. niloticus* hematological indices were negatively impacted by ammonia exposure in the form of elevated cortisol and glucose levels. The toxicity of ammonia, nitrate, and nitrite was discovered to impede carp fish oxygen absorption, posing a risk to red blood cell formation and components **(Tilak *et al.*, 2007)**. **Shokr (2015)** observed similar results in Nile tilapia exposed to increasing doses of ammonium nitrates.

Zeitoun *et al.* (2016) observed relative decrease in erythrocyte counts in fish exposed to ammonia. Hb concentration was lower in fish exposed to ammonia than concentration control group by about 18%. The ammonia toxicity in the medium may have caused harm to the vital organs (gills, liver, spleen and kidneys), as evidenced by the reductions in RBCs, PCV, and Hb concentrations. **Adeyemo *et al.* (2003)** reported that temperature causes a reduction in the amount and quality of erythrocytes and hemoglobin, resulting in a reduction in oxygen supply.

Biochemical Parameters

The obtained results in Table 5 show that glucose and uric acid were significantly different ($P<0.05$). But urea was insignificantly different ($P>0.05$). When fish are stressed, they consume more energy from food or glycolysis to swim, resulting in higher plasma glucose concentrations. The results are similar to that obtained by **Metwally and Wafeek (2014)** who reported vast increases of cortisol and glucose in fish exposed to ammonia. **Lermen *et al.* (2004)** reported that in a 21-day trial, plasma glucose levels in silver catfish increased after exposure to 31°C and reduced after exposure to 15°C. Silver catfish (*Rhamdia quelen*) glucose levels dropped at 15 °C and climbed at 31°C. **Metwally and Wafeek (2014)** noted that glucose levels in serum of Nile tilapia (*O. niloticus)* after treated with different concentration of NH_4C1 showed significant increase. Glycogen content in liver tissues of Nile tilapia (*O. niloticus)* groups showed significant decreased in fish after treated with different concentrations of NH_4C1. **Nguyen *et al.* (2014)** noted that the highest glucose concentration was seen in 34 and 36°C, and was significantly higher than in 24°C and 27°C at the first day. **Shin *et al.* (2016)** found that the concentration of glucose increased significantly from 1.0 mg/l at 19°C to over 0.5 mg/l at 24°C. they added that after 4 weeks, the glucose level had dropped by 0.5 mg/l at 19 and 24°C. **Abd Elnabi *et al.* (2018)** reported that fish exposed to high amounts of total ammonia nitrogen had a greate levels of serum creatinine, urea, and uric acid.

The obtained results in Table 5 show that albumin and globulin were significantly different. But A/G ratio and creatinine were insignificant differed. These results are similar to those obtained by **Farghaly *et al.* (1973)** who showed that a 4°C decrease in temperature caused who showed that a 4°C decrease in temperature cause alpha globulins raised dramatically, beta globulin dropped, and gamma globulin vanished. As a result, the drop in total serum proteins observed at low temperatures can be explained by the loss of gamma globulin and the decrease in beta globulin. As a result, the increase in

the albumin fraction may be responsible for the rise in total serum protein. At 30°C, the A/G ratio increased to more than double its typical amount. These results disagree with **Panase *et al.* (2019)** who reported that the kidney function, creatinine and blood urea nitrogen were not affected by rapid increases in temperature. Creatinine had an increased tendency when exposed to highe temperatures 37°C.

ALT and AST are liver enzymes that govern the transfer of amino group function from alpha-amino acids to alpha-keto acids. They are essential markers of liver health and function. When liver cells are damaged, large amounts of ALT and AST are released into the bloodstream of animals. **(Kumar *et al.*, 2010).** Increases in blood enzyme activity, such as ALT and AST, in response to exogenous substances are one of the indicators of liver disease **(Shi *et al.*, 2006)**. The obtained results in Table 6 show that no significant differences were found in total protein among all treatments. Analysis of variance results showed that AST and ALT significantly *(*P<0.05*)* affected by total ammonia, temperature and the combination between them. The concentration of total protein in red tilapia's serum was substantially higher, possibly as a result of exposure to high levels of TAN, such an increase in total protein suggests liver impairment **(Zaki *et al.*, 2010). Das *et al.* (2004)** kreported that as the ammonia concentration increased, the serum protein continued to decrease. Exposed fingerlings exhibited a substantial drop in protein levels compared to the control group at 8 mg L^{-1} TAN, with the maximum percentage loss (31.47%) occurring at 16 mg L^{-1} TAN.

Shin *et al.* (2016) reported that after 2 and 4 weeks, AST levels at 19 and 24°C were substantially higher than 0.5 mg/l. After two weeks, there was a significant rise in ALT of >0.5 mg/l at 24°C, but no change at 19°C. The ALT concentration was significantly raised after 4 weeks, in the concentration of 1.0mg/l of ammonia at 19°C and over 0.5 mg/l at 24°C. **Hegazi *et al.* (2010)** found that fish exposed to 5 mgl^{-1} of ammonia had their ALT activity raised by 86%, while fish exposed to 10 mgl^{-1} of ammonia had their activity increased by more than 2-fold). AST activity was raised by 1.4-fold in fish exposed to 5 mgl^{-1} and by more than 2.4-fold in fish exposed to 10 mgl^{-1}, while ALT activity was lowered by 21.25% in fish exposed to 5 mgl^{-1} and by 25.4% in fish exposed to 10 mgl^{-1}. In fish subjected to 5 mgl^{-1} or 10 mgl^{-1}, however, the activity of AST did not alter appreciably.

Histological Examination

The Gill

Histological analysis detected that all treatments showed satisfactory results as a moderate degeneration of filaments with moderate deed of lining cells, shortening focal autolytic changes and focal congestion. The obtained results are similar to those obtained by **Saber (2011).** The fish gills revealed hyperplasia of epithelial cells in branchial secondary lamellae, congestion of blood vessels, and hypertrophy. Abundance of mucous substance and hemorrhage between the branchial secondary lamellae in comparison with control group of pillar cells following the exposure to a high temperature of 31°C. The fish gills showed hyperplasia of epithelial cells at the bases of branchial secondary lamellae, as well as shrinkage of branchial blood vessels, fusion of the ends of branchial secondary lamellae, atrophy of some epithelial cells and pillar cells of branchial secondary lamellae, damage of some branchial secondary lamellae, and lamellar disorganization. Some of the lamellar epithelial cells become rounded, causing bleeding between the secondary branchial lamellae. **Dong *et al.* (2013)** found that regardless of oxygen treatments, the secondary gill lamella in 93.3 mgl^{-1} TAN was significantly thicker than that in the ammonia control, but the gill lamella in normal oxygen was thicker than that in supersaturated oxygen when

fish were exposed to the same ammonia concentration (93.3 mgl^{-1} TAN), suggesting that supersaturated oxygen protects fish gills from ammonia damage.

Hanna *et al*. (2013) noted that the most significant changes between control and fish subjected to 2.5 mg/l total ammonia nitrogen (TAN) were found. Gills exhibited telangiectasis and hyperemia in branchial and lamellar blood vessels, as well as lamellar hyperplasia, which led to secondary lamellae fusion from the base to the apex, and degenerative alterations in the secondary lamellae's epithelial lining. **Chezhian *et al.* (2012)** reported that in *Cyprinus carpio*, ammonia at different pH levels caused secondary lamellar fusion, oedema, hyperplasia, and chloride cell proliferation in the gills. The quantity of ammonia in fish plasma and tissues rises in direct proportion to the amount of ammonia in the water.

Manissery and Madhyastha (1993) observed that a microscopic inspection of the gill revealed a few lesions caused by ammonia exposure. Secondary lamellae showed epithelial hyperplasia, oedematous enlargement, and moderate necrosis. Many of the gill lamellae's basement membranes were discovered to be ruptured. Because of the significant proliferation of epithelial cells and the subsequent fusing of lamellae, club-shaped lamellae were seen. With increasing exposure time and concentration, the deterioration signs were more common and intense**.**

The Kidney

Examination of kidney tissues showed marked interstitial edema, congestion with focal inflammatory infiltrate, mild vacuolar degeneration and focal edema and focal shrinkage in few glomeruli, the results were in agreement with **Abd Elnabi *et al*. (2018)** who reported that formation of more thrombus and higher infiltration of cells, majority of destroyed renal tubules, hemorrhage, hemolysis, and hemosiderin (a yellowish-brown granular intracellular pigment) were seen between renal tubules and in the renal parenchyma in the kidney tissues of fish exposed to the highest amount of TAN. The obtained results are similar to those obtained by **Benli *et al*. (2008)** who reported that after exposure to 2, 5, and 10 mg/l TAN, glomerulonephritis and hyperemia were seen in the kidney of Nile tilapia (*O. niloticus*).

Conclusion

Red tilapia can be reared in high total ammonia and temperature levels up to 3.0 ppm and 32°C, respectively, but this environmental stress will affect health and physiological status of fish. When maintaining the other environmental stressors at the safe level, a moderate production of fish will be obtained.

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الملخص العربي

التأثير المشترك للأمونيا الكلية ودرجة الحرارة على نمو، وهيماتولوجيا وكيمياء الدم والمؤشرات النسيجية لأسماك البلطي الأحمر

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تهدف هذه الدراسة إلى تقييم مستويات مختلفة من الأمونيا الكلية ودرجات الحرارة والتداخل بينهم على النمو، الاستفادة من الغذاء، هيماتولوجيا وكيمياء الدم والمؤشرات الهستولوجية لأسماك البلطي الأحمر. تسعة معاملات بثلاث مستويات من الأمونيا الكلية (1.5، 3 و4.5 جزء في المليون) وثلاث مستويات من درجات الحرارة (24، 28 و32 درجة مئوية) وتم تصميمها فى تجارب عامليه (3*3) لمدة 56 يوم. متوسط الوزن الابتدائي للأسماك في بداية التجربة 11.1 ± 0.27 جرام ومتوسط الطول الابتدائي للأسماك 6.98 ± 0.30 سم تم توزيعها بطريقة عشوائية بمكررتين لكل معاملة. يوضح تحليل التباين فروق معنوية في هيموتولوجيا الدم التى اشتملت على عدد كرات الدم الحمراء، عدد كرات الدم البيضاء، تركيز الهيموجلوبين، الهيماتوكريت، متوسط الهيموجلبين في كرات الدم الحمراء ومتوسط تركيز الهيموجلبين في كرات الدم الحمراء بين المعاملات المختلفة. بالإضافة إلى وجود فروق معنوية في عوامل كيمياء الدم من جلوكوز، حمض اليوريك، اليوريا، الألبومين، الجلوبيولين ونشاط وظائف الكبد بين المعاملات. حققت المعاملة الخامسة والمعاملة الأولي نشاط أمثل للألانين والاسبرتات أمينوترانسفيريز على التوالي. في كل المعاملات أظهرت نتائج الفحص النسيجي للخياشيم وجود تحطم متوسط للشعيرات الخيشومية وموت للخلايا المبطنة وقصر وتحلل موضعي واحتقان موضعي في الخلايا. أما نتائج الفحص النسيجي للكلية فأظهرت تورم بين خلوي ملحوظ، احتقان موضعي، وتحطم فجوي متوسط وانكماش لبعض الكبات. أوضحت نتائج الدراسة الحالية أن التفاعل بين الأمونيا الكلية ودرجات الحرارة يؤثر على الحالة الصحية والفسيولوجية والتركيب النسيجي للأسماك. كذلك أوضحت أن أسماك البلطي الأحمر تستطيع أن تواجه إلى حد ما التغيرات في العوامل البيئية عندما يتم تربيتها في الأماكن التي تعاني من تغيرات بيئية في مياه الاستزراع.

الكلمات الاسترشادية: البلطي الأحمر، الأمونيا الكلية، المعلمات الدموية، المعايير البيوكيميائية، المؤشرات النسيجية.