EFFECT OF AMMONIA ON NILE TILAPIA (*O. niloticus*) PERFORMANCE AND SOME HEMATOLOGICAL AND HISTOLOGICAL MEASURES

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Abstract

Nile tilapia (O. niloticus) fingerlings averaging 19.0± 1.0 g in weight where reared at four different concentrations of UIA-N (0.01, 0.05, 0.1 and 0.15 mg/l) besides the control using 15 glass aquaria (40 ×70 ×60 cm). Tilapia were stocked for 75 days and fed with diet containing 26.58 % protein. The physico-chemical analysis of water in aquaria were recorded. Water was changed twice daily with 100% of water size. Different growth measurements of tilapia were recorded every 15 days interval, blood parameter and histo-pathological changes in kidney, liver and gills were also recorded. The results showed that growth performance was significantly (P≤ 0.05) decreased with increasing concentration of UIA-N. The feed conversion ratio (FCR) increased with increasing concentrations of UIA-N, the differences were significant (P ≤ 0.05) among the high concentrations. Histopathological changes showed different signs in liver, gill and kidney among the four treatments. Increasing UIA-N concentrations resulted in decreasing of hematocrit and hemoglobin parameters, while differences were not significant (P≥ 0.05) from control in UIA-N concentration (0.01mg/l). Also, results showed that the 48 hr-LC_{50} for tilapia fingerlings was 7.1 mg/l NH_3-N.

Keywords: Ammonia - Nile tilapia (*O. niloticus*)- Performance- Hematology- Histology.

INTRODUCTION

Many species of tilapia have been cultured in developing countries where animal protein is lacking. Nile tilapia is by far the most important farmed tilapia species in the world. Tilapia are the most familiar and popular fishes in Egypt, as well as, in the middle east and warm climate countries (Philippart and Ruwet, 1982). Fish production should be increased in Egypt to meet the demand of the increasing population. Several problems face fish production in Egypt. Among these problems are the most tropical species die via low water quality because of pollution with ammonia. Ammonia is toxic to a variety of aquatic organisms including fish (Harris *et al.*, 1998) . Un-ionized form of ammonia is the most toxic form to aquatic organisms as it can readily diffuse through cell membranes and is highly soluble in liquids. It can cause impairment of cerebral energy metabolism, damage to gill, liver, kidney, spleen and thyroid tissue in fish, crustaceans and mollusks (Smart, 1978). Moreover, it is a common aquatic pollutant. It enters natural waters with municipal, agricultural, fish-

cultural, industrial wastes, and from energy- development processes such as oil-shale retorting, coal gasification, and coal liquefaction, it is also, a natural degradation product of nitrogenous organic matter (Randall and Tsui, 2002). Ammonia is the principal nitrogenous waste product of fishes and is normally oxidized first to nitrite then to nitrate. It is also, the main nitrogenous waste material excreted by gills beside urea and amines. Moreover, creatine, creatinine, and uric acid are being excreted through the kidneys (De Croux *et al., 2004*). Chronic un-ionized ammonia exposure may affect fish and other organisms in several ways, e.g. gill hyperplasia, muscle depolarization, hyper excitability ,convulsions and finally death (Ip *et al.*,2001). Therefore the present study was planed to investigate the effect of different sub-lethal concentrations of ammonia (NH_3-N) on growth performance and some physiological parameters of Nile tilapia fingerlings (*Oreochromis niloticus*).

MATERIALS AND METHODS

This study was carried out from June1st to 29 Dec., 2005, at the indoor wet lab of the Department of Animal Production and Fish Resources, Faculty of Agriculture, Suez Canal University , Ismailia, Egypt.

Experimental design:

1) This experiment was devoted to study the median lethal concentration LC_{50} of un-ionized ammonia (NH_3-N) to Nile tilapia (*O.niloticus*) averaging (19.0 ±1.0 g) at 48 hr static bioassay with fish mortality as the end point .The bioassay included 5 concentrations ranging from 0.18, 0.16, 0.12, 0.075 and 0.04 g NH_4Cl/l with duplicate of each concentration and control containing fresh water with no adding of ammonia . The experiment was conducted in 14 glass aquaria (40 ×70 ×60 cm). Ammonia concentration was determined on the first and last day of each treatment. The concentration of ammonia in the water were measured by ammonia ion specific meter (HI93715). Ten fishes were chosen randomly from the stocked and were used in each aquarium. The fish were acclimated to the test conditions for 48 hrs before each treatment and they were fed with fish pellets at a ration of 0.03 of the biomass twice daily .The water in the aquaria was replaced daily and aerated continuously during the acclimation period. The required level of ammonia was obtained by the addition of ammonium chloride at the beginning of the test. Feeding was stopped 48 h prior to the experiment and no food was given during the test. The fish observed twice daily to detect, count and remove dead fish.

2) This experiment was devoted to study the effect of different sub lethal concentrations of un-ionized ammonia (NH_3-N) on growth performance, environmental conditions ,survival rate , some physiological parameter (histopathological changes in

kidney , liver and gills) and some blood parameters of (*O.niloticus*). Fifteen glass aquaria (40 × 70 × 60 cm) were used and stocked with Nile tilapia (*O.niloticus*) averaging 19.0 ±1.0 g in weight for 75 days. Tilapia fish were stocked at a rate of 14 fish per aquarium (60 l water) at four different concentrations of un-ionized ammonia (NH_3-N), i.e.0.01, 0.05 , 0.10 and 0.15 ppm besides the control (no addition of ammonia) (Randall , 1976) . Ammonia stress was induced by adding ammonium chloride to each aquarium (Xu *et al.*,2005) and the percentage of un-ionized ammonia added in water was calculated using the equation NH_3-N (total ammonia ×percentage of ammonia in the pH ,temperature , ammonia relationship tables) / 100, (Emerson et al.,1975) . Each treatment had three replicates.

Histopathology:

Histopathological examination was carried out in 3 samples from each aquarium of fish organs taken before exposed to ammonia from freshly killed fish , preserved in Bouin's solution for 24 to 48 hrs, and stored in 65% ethanol then paraffin sections were cut to 5 micrometer thickness and stained with hematoxylin and eosin(Robert *et al .,* 1984). The slides were examined and photo micrographs were taken using light research microscope for histopathological examination . After 75 days exposure to ammonia three fishes from each aquarium in all treatments of the experiment were removed, killed by a sharp blow on the head and there gill arches , kidney and liver were obtained and processed as mentioned before to be examined histologically.

Statistical analysis:

The data obtained in this study were analyzed by one-way ANOVA procedure of Statistical Analysis System (SAS, 1988). Mean were compared by Duncan's new multiple range test (Zar, 1996).

RESULTS AND DISCUSSION

The mean individual body weights:

Table (1) illustrates the mean individual weight of Nile tilapia (*O. niloticus*) fingerlings reared in aquaria for 75 days at different concentrations of UIA-N (i.e. 0.01, 0.05, 0.1, 0.15 and 0.004 mg/l). No mortality occurred in any of the experimental groups through out the experimental period. At the end of the experimental period (after 75 days from the stocking) the final average body weights (FBW) of Nile tilapia (*O. niloticus*) fingerlings were 37.2, 36.4, 32.5, 26.9 and 37.7 g for UIA-N concentrations of 0.01,0.05, 0.1, 0.15 and 0.004 mg/l, respectively. The statistical analysis of mean results indicated that the mean individual weight of Nile tilapia (*O. niloticus*) fingerlings showed no significant ($P \leq 0.05$) differences between FBW of tilapia in the control (0.004 mg/l UIA-N) and FBW of those exposed to (0.01 and 0.05

mg/l UIA-N). While, FBW of tilapia exposed to 0.1 and 0.15 mg/l UIA-N was significantly ($P \leq 0.05$) reduced from the control. The results showed that the lowest-observable effect concentration on the FBW was 0.1 mg/l UIA-N . Such result was in agreement with Saber *et al.*(2004) who showed that the lowest-observable effect concentration on the growth performance of Nile tilapia is 0.144 mg/l UIA-N and there was no significant differences between the mean individual weight of fish exposed to 0.068 mg/l UIA-N and control0.004 mg/l UIA-N. On the other hand, in marine fish Atle *et al.*(2003 and 2004) , Sten *et al.* (2004) and Lemarie *et al.*(2004) reported that fish weight decreased when concentrations of UIA-N/l increased. It was attributed to a decrease in daily food intake, daily feed consumption and decrease in food conversion efficiency.

Table 1. Mean individual body weight (g) of Nile tilapia **(***O.niloticus*) fingerlings reared in aquaria for 75 days under different concentrations of ammonia (NH_3-N mg/l) (Mean ± SE).

Period (days)	Concentration of ammonia NH_3-N(mg/l)				
	0.01	0.05	0.1	0.15	Control (0.004)
0	19.0 ±1.0 [a]	19.0 ±1.0 [a]	19.0 ±1.0 [a]	19.0 ±1.0 [a]	19.0 ±1.0 [a]
15	21.0 ±0.35 [a]	21.0 ±0.89 [a]	20.9± 0.71[b]	19.9 ±0.71 [c]	21.0 ±0.21[a]
30	23.3 ±0.42 [a]	23.3 ±0.86 [a]	23.0 ±0.77 [b]	21.0 ±0.76 [c]	23.4 ±0.33[a]
45	26.7 ±0.49 [a]	26.5 ±0.86 [a]	25.5 ±0.8 [b]	22.4 ±0.84[c]	26.9 ±0.47[a]
60	31.3 ±0.60 [a]	30.8 ±0.89 [a]	28.6 ±0.93[b]	24.3 ±0.91[c]	31.7 ±0.64[a]
75	37.2 ±0.72 [a]	36.4 ±0.92 [a]	32.5±1.02 [b]	26.9 ±1.02 [c]	37.7 ±0.81[a]

Means with the same letter in each row are not significantly different ($P \leq 0.05$).

The mean body weight gain:

Table (2) indicated that body weight gain at the end of the experimental period was 5.9, 5.6, 3.9, 2.6 and 6.0 g/ fish/ 15 days for UIA-N concentrations of 0.01, 0.05, 0.1, 0.15 and 0.004 mg/l, respectively. From the presented data in Table (2) it can be shown that mean body weight gain per fish in the various treatment groups were significantly influenced by UIA-N concentrations and decrease with increasing levels of UIA-N. Similar results were obtained by Foss *et al.*(2002), Atle *et al.*(2003 and 2004), Lemarie *et al.*(2004) and Saber *et al.*(2004). Generally, mean body weight gain was significantly reduced in concentrations of 0.1 and 0.15 mg/l UIA-N compared to the control ones. This was attributed to a decrease in daily food consumption.

Table 2. Average body weight gain (g /individual fish) of Nile tilapia (*O.niloticus*) fingerlings reared in aquaria for 75 days under different concentrations of ammonia (NH_3-N mg/l) (Mean ± SE) .

Period per (day)	Concentration of ammonia NH_3-N(mg/l).				
	0.01	0.05	0.1	0.15	Control (0.004)
15	2.0 ±0.09 [a]	2.0 ±0.12 [a]	1.9 ±0.15 [b]	0.9 ±0.15 [c]	2.0 ±0.17 [a]
30	2.3 ±0.12 [a]	2.3 ±0.17 [a]	2.1 ±0.12 [b]	1.1 ±0.17 [c]	2.4 ±0.15 [a]
45	3.4 ±0.12 [a]	3.2 ±0.17 [a]	2.5 ±0.12 [b]	1.4 ±0.17 [c]	3.5 ±0.15 [a]
60	4.6 ±0.15 [a]	4.3 ±0.17[a]	3.1 ±0.12 [b]	1.9 ±0.12 [c]	4.8 ±0.17 [a]
75	5.9 ±0.15 [a]	5.6 ±0.15[a]	3.9 ±0.12 [b]	2.6 ±0.15 [c]	6.0 ±0.17 [a]

Means with the same letter in each row are not significantly different (P ≤ 0.05).

The average daily body weight gain (ADG):

It can be concluded from the Table (3) that the average daily body weight gain at the end of the experimental period were 0.39, 0.37, 0.26, 0.17 and 0.4 g/ fish/ day for UIA-N concentrations 0.01, 0.05, 0.1, 0.15 and 0.004 mg/l respectively. It can be shown from the statistical analysis that there was no significant (P ≥ 0.05) differences between the average daily body weight gain of (*O. niloticus*) fingerlings in the control (0.004 mg/l UIA-N) and of those exposed to (0.01 and 0.05 mg/l UIA-N). While, there was significant differences (P ≤ 0.05) between (ADG) in the control and of those exposed to (0.1 and 0.15 mg/l UIA-N). The gain per fish per day decrease as the UIA-N concentrations increase. Similar results were obtained by Atle *et al.*(2004), Lemarie *et al.*(2004) and Saber *et al.* (2004). Wang and Walsh (2000) reported that the reduction in average daily body weight gain was attributed to physiological disturbances.

Table 3. Average daily body weight gain(g /individual fish) of Nile tilapia (*O.niloticus*) fingerlings reared in aquaria for 75 days under different concentrations of ammonia (NH_3-N mg/l) (Mean ± SE).

Period per (day)	Concentration of ammonia NH_3-N(mg/l).				
	0.01	0.05	0.1	0.15	Control (0.004)
15	0.13 ±0.01[a]	0.13 ±0.01[a]	0.12 ±0.01[b]	0.06 ±0.01[c]	0.13 ±0.01[a]
30	0.15 ±0.01[a]	0.15 ±0.01[a]	0.14 ±0.01[b]	0.07 ±0.01[c]	0.16 ±0.01[a]
45	0.22 ±0.01[a]	0.21 ±0.01[a]	0.16 ±0.01[b]	0.09 ±0.01[c]	0.23 ±0.01[a]
60	0.30 ±0.01[a]	0.28 ±0.01[a]	0.20 ±0.01[b]	0.12 ±0.01[c]	0.32 ±0.01[a]
75	0.39 ±0.01 [a]	0.37 ±0.01[a]	0.26 ±0.01[b]	0.17 ±0.01[c]	0.4 ±0.01[a]

Means with the same letter in each row are not significantly different (P ≤ 0.05).

Average food consumption:

Results in Table (4) indicated that food consumption at the end of the experimental period was 16.7, 16.3, 14.6, 12.1 and 16.96 g food /fish for the UIA-N concentrations of 0.01, 0.05, 0.1, 0.15 and 0.004 mg/l, respectively. From the presented data in Table (4) it can be showed that the average food consumption in the experimental groups was decreased with increasing concentrations of UIA-N. This is in full agreement with that found by Foss *et al.*(2002), Atle *et al.*(2003 and 2004) and Saber *et al.* (2004). Generally, significant differences were found between UIA-N concentrations (0.1and 0.15 mg/l) and control (0.004 mg/l ,$P \leq 0.05$). While, the differences were not significant ($P \geq 0.05$) between food consumption in the control and of those exposed to UIA-N concentrations (0.01 and 0.05 mg/l).

Table 4. Average food consumption(g /individual fish) Nile tilapia (*O.niloticus*) fingerlings reared in aquaria for 75 days under different concentrations of ammonia (NH_3-N mg/l) (Mean ± SE).

Period per (day)	Concentration of ammonia NH_3-N(mg/l).				
	0.01	0.05	0.1	0.15	Control (0.004)
15	9.5 ±0.16 [a]	9.5 ±0.40 [a]	9.4 ±0.32 [b]	8.9 ±0.32 [c]	9.5 ±0.09 [a]
30	10.4 ±0.19 [a]	10.40 ±0.39 [a]	10.3 ±0.35 [b]	9.4 ±0.34 [c]	10.5 ±0.15 [a]
45	12.0 ±0.22 [a]	11.9 ±0.39 [a]	11.4 ±0.38 [b]	10.1 ±0.38 [c]	12.1 ±0.21[a]
60	14.08 ±0.27 [a]	13.86 ±0.39 [a]	12.8 ±0.42[b]	10.9 ±0.41[c]	14.26 ±0.29 [a]
75	16.7 ±0.32 [a]	16.3 ±0.41 [a]	14.6 ±0.46 [b]	12.1 ±0.46 [c]	16.96 ±0.37 [a]

Means with the same letter in the same row are not significantly different ($P \leq 0.05$).

Specific growth rate "SGR":

The calculation of this parameter (SGR) is useful for comparing growth of fish of different sizes (Jauncey and Ross, 1982). Changes in SGR value of (*O. niloticus*) fingerlings reared in aquaria at different UIA-N concentrations were illustrated in Table (5). It can be noticed from the tabulated results that SGR of tilapia at the end of the experimental period were 1.15, 1.11, 0.85, 0.68 and 1.16% of UIA-N concentrations (0.01, 0.05, 0.1, 0.15 and 0.004 mg/l) respectively. The statistical analysis showed no significant differences ($P \geq 0.05$) between the control group (0.004 mg/l UIA-N) and groups exposed to (0.01 and0.05 mg/l UIA-N). The SGR of treatment 3 (0.1 mg/l UIA-N) and 4 (0.15 mg/l UIA-N) were significantly lower ($P \leq 0.05$) than the control and treatments 1 and 2. There was a significant difference ($P \leq 0.05$) between the SGR of groups exposed to 0.1 and 0.15 mg/l UIA-N but no difference was detected between the SGR in group 1 and those in group 5. This is in agreement with the findings of Saber *et al.* (2004). Also, Harris *et al.* (1998) and

Atle *et al.*(2003 and 2004) who found that SGR decrease with increasing the concentration of UIA-N and it was attributed to a decrease in food intake.

Table 5. Average specific growth rate (%/day) of Nile tilapia (*O.niloticus*) fingerlings reared in aquaria for 75 days under different concentrations of ammonia (NH_3-N mg/l) (Mean ± SE).

Period per (day)	Concentration of ammonia NH_3-N(mg/l)				
	0.01	0.05	0.1	0.15	Control (0.004)
15	0.7 ±0.017 [a]	0.7 ±0.027 [a]	0.6 ±0.025[b]	0.31 ±0.026 [c]	0.7 ±0.032 [a]
30	0.6 ±0.021[a]	0.6 ±0.036 [a]	0.63 ±0.015 [b]	0.35 ±0.029 [c]	0.72 ±0.023 [a]
45	0.9 ±0.018 [a]	0.8 ±0.031[a]	0.68 ±0.015 [b]	0.43 ±0.026 [c]	0.93 ±0.018 [a]
60	1.05 ±0.019 [a]	1.0 ±0.025 [a]	0.76 ±0.015 [b]	0.54 ±0.018 [c]	1.09 ±0.017 [a]
75	1.15 ±0.015 [a]	1.11 ±0.023 [a]	0.85 ±0.015 [b]	0.68 ±0.020 [c]	1.16 ±0.015 [a]

Means with the same letter in the same row are not significantly different ($P \leq 0.05$).

The feed conversion ratio "FCR":

A careful study to Table (6) indicates that the feed conversion ratio of tilapia at the end of the experimental period were 2.8, 2.9, 3.7, 4.6 and 2.8 for UIA-N concentrations (0.01, 0.05, 0.1, 0.15 and 0.004 mg/l), respectively. Mean feed conversion ratio of tilapia increased as UIA-N concentrations increased, since the FCR achieved in the fourth UIA-N concentrations was significantly higher than achieved in the first ones (4.6 and 2.8, respectively). These results are in agreement with that obtained by Atle *et al.*(2004) who found that mean feed conversion ratio decreased as UIA-N concentrations increased. Also, Saber *et al.*(2004) found that, the feed conversion was affected by ammonia concentrations over 0.068 mg/l UIA-N and there was no difference between the FCR of the control (0.004 mg/l UIA-N) and those exposed to (0.068 mg/l UIA-N) as FCR was (1.5 and 1.6 ,respectively), FCR was 3.9 and 5.6 at those exposed to 0.14 and 0.26 mg/l UIA-N, respectively. While, John and Semra (2001) reported that at 0.91 mg/l UIA-N there was no effect on growth or feed conversion ratio of channel catfish and blue tilapia.

Table 6. Average feed conversion ratio (g food /g weight gain) of Nile tilapia (*O.niloticus*) fingerlings reared in aquaria for 75 days under different concentrations of ammonia(NH_3-N mg/l)(Mean ± SE).

Period per (day)	Concentration of ammonia NH_3-N(mg/l)				
	0.01	0.05	0.1	0.15	Control (0.004)
15	4.7 ±0.25 [c]	4.7 ±0.51[c]	4.9 ±0.44 [b]	9.8 ±0.55 [a]	4.7 ±0.44 [c]
30	4.5 ±0.32 [c]	4.5 ±0.60 [c]	4.9 ±0.38 [b]	8.5 ±0.92 [a]	4.3 ±0.30 [c]
45	3.5 ±0.23 [c]	3.7 ±0.48 [c]	4.5 ±0.53 [b]	7.2 ±1.24 [a]	3.4 ±0.22 [c]
60	3.0 ±0.22 [c]	3.2 ±0.39 [c]	4.1 ±0.69 [b]	5.7 ±1.26 [a]	2.9 ±0.21[c]
75	2.8 ±0.18 [c]	2.9 ±0.29 [c]	3.7 ±1.09 [b]	4.6 ±3.56 [a]	2.8 ±0.14 [c]

Means with the same letter in each row are not significantly different ($P \leq 0.05$).

Blood measurements:

Hematocrit value (PCV%): Results in Table (7) indicated that hematocrit value at the end of the experimental period was 26.0, 22.5, 22.0, 21.3 and 26.0% for the UIA-N concentrations of 0.01, 0.05, 0.1, 0.15 and 0.004 mg/l, respectively. It can be concluded that the average PCV(%) in the experimental groups was decreased with the increase of UIA-N concentrations. Similar results were obtained by Atle *et al* .(2004). It was evident that these fishes were anemic. Generally, significant differences were found between the UIA-N concentrations 0.05, 0.1, 0.15 and 0.004 mg/l ($P \leq 0.05$). But the differences were not significant ($P \geq 0.05$) between UIA-N (0.01 mg/l) and control ones (0.004 mg/l).

Table 7. Average PCV (%) values of Nile tilapia (*O.niloticus*) fingerlings reared in aquaria for 75 days under different concentrations of ammonia (NH_3-N mg/l)(Mean ± SE).

Period per (day)	Concentration of ammonia NH_3-N (mg/l)				
	0.01	0.05	0.1	0.15	Control (0.004)
0	24.0 ±0.8 [a]	23.0±0.13 [a]	23.5 ±1.01 [a]	24.0 ±0.11 [a]	24.0 ±0.8 [a]
60	26.0 ±0.3 [a]	22.5±0.26 [b]	22.0 ±0.32 [c]	21.3 ±1.5 [d]	26.0±1.04 [a]

Means with the same letter in each row are not significantly different ($P \leq 0.05$).

Hemoglobin concentration (Hb):

It can be shown from the Table (8) that the average hemoglobin concentration at the end of the experimental period was 10.0, 6.5, 6.0, 5.8 and 10.3 $g100ml^{-1}$ for UIA-N concentrations of 0.01, 0.05, 0.1, 0.15 and 0.004 mg/l , respectively. It can be concluded that there were differences in the average Hb concentration of (*O. niloticus*) fingerlings at the UIA-N concentrations (0.05, 0.1, 0.15 and 0.004 mg/l). While, there were no significant differences between UIA-N concentration (0.01 mg/l) and control (0.004 mg/l).The average Hb value decreased as the UIA-N concentrations increased. Similar results were obtained by Pratap *et al.*(2004a and b). Otherwise, Hrubinko *et al.* (1996) found that Hb increase when exposed to ammonia (0.1mg/l). It was evident that these fishes were anemic.

Table 8. Average Hb (g 100 ml^{-1}) concentrations of Nile tilapia (*O.niloticus*) fingerlings reared in aquaria for 75 days under different concentrations of ammonia (NH_3-N mg/l) (Mean ± SE).

Period per (day)	Concentration of ammonia NH_3-N (mg/l)				
	0.01	0.05	0.1	0.15	Control (0.004)
0	7.5 ±0.07 [a]	7.5 ±0.12 [a]	7.4 ±0.05 [a]	7.0 ±0.07 [a]	7.6 ±0.09 [a]
60	10.0 ±0.05 [a]	6.5 ±0.13 [b]	6.0±0.01[b]	5.8 ±0.14[c]	10.3±0.15[a]

Means with the same letter in each row are not significantly different ($P \leq 0.05$).

Histopathological studies:

The microscopic structures of tissues from the control fish: Gills: The gills comprise two sets of four holobranches forming the side of the pharynx. Each holobranch consists of two hemibranches. The hemibranch consists of a row of long thin filaments, the primary lamellae. The area of the primary lamellae is increased by the formation of regular semi lunar folds across its dorsal and ventral surface, secondary lamellae. The primary lamellae are supported by a central core of cartilage containing supply and exchange blood vessels and are covered by epithelium that is continuous with that of the secondary lamellae. The secondary lamellae of the gills are covered with a squamous epithelium. The layers of the epithelium is separated by intercellular space that contains macrophages, piller cells, RBCs and some mucous cells (Figure 1). Examination of tissues from Nile tilapia (*O. niloticus*) fingerlings after 75 days of exposure to (0.01 mg/l) UIA-N concentration showed, slight pathological alteration. The gills secondary lamellae showed mild vacuolation (Figure 2), mild hyperplasia of epithelium. The same results were obtained by Smith and Piper (1975) who reported mild pathological changes in gills (hyperplasia of epithelium) of rainbow trout when exposed to 0.0125 mg/l NH_3-N. They also showed that the resulting gill lesions may cause reduced oxygen diffusion across membranes and predispose fishes to bacterial infections, fishes exposed to increase metabolic ammonia are known to be more susceptible to bacterial gill diseases. Examining tissues from (*O. niloticus*) after 75 days of exposure to 0.05mg/l UIA-N concentration revealed that : epithelial hyperplasia (Figure 3), congestion of central vein, secondary lamellae showed telangiectasis and mononuclear cells infiltration in primary and secondary lamellae with vacuolation of gill lamellae. These results were in agreement with Aysel and Gulten (2005). When examining tissues from Nile tilapia (*O. niloticus*) fingerlings after 75 days of exposure to 0.1 mg/l UIA-N concentration, the results showed vacuolation of epithelial cells in gill, telangiectasia of gill lamellae (Figure 4) and gill hyperplasia. The same results were obtained by Randall (1976) and Robert *et al*. (1984) who reported gill hyperplasia in channel catfish exposed to 0.12 mg/l NH_3-N for 27 days. Smith and Piper (1975) and Smart (1976) found that the most characteristic feature for chronic exposure of rainbow trout to ammonia was the appearance of swollen, rounded secondary gill lamellae or telangiectatic capillaries in the secondary lamellae. Also, Kirk and Lewis (1993) reported that the gills of rainbow trout exposed to0.1 mg/l ammonia for 2 h exhibited deformation of the lamellae. The filamental and lamellar epithelium was covered with shallow, circular depressions. The tissues exposed to the highest UIA-N concentration (0.15 mg/l) results in gill hyperplasia, degeneration of epithelium lining the secondary lamellae and other places showed proliferation of the

epithelium. Sloughing of the lamellar epithelium and telangiectasia of gill lamellae (Figure 5). These results were supported by Smith and Piper (1975), Smart (1976), Robert *et al.*(1984) and Aysel and Gulten (2005)who reported gill hyperplasia, degeneration of epithelium when tissues exposed to the highest UIA-N concentration.

Kidney:

The fish kidney consists of head and body kidneys. The head kidney is the anterior portion of the kidney and consists of lymphoid tissue. The body kidney is composed of nephron and renal tubules. The nephron is formed of renal corpuscle and Bowman's capsule. The capsular epithelium is continuous with the renal epithelium. The renal tubules begins with :- a) short neck portion lined by low cuboidal epithelium with long cilia, b) proximal convoluted tubule which has divided into segment I lined with acidophilic cuboidal to columnar epithelium with distinct brush border. The epithelial cells of the segment II are columnar and taller than those of segment I. The epithelium becomes lower and more cuboidal in the intermediate segment. The distal convoluted tubules have epithelium with lightly eosinophilia and have no brush border (Figure 6). Examination of tissues from Nile tilapia (*O. niloticus*) fingerlings after 75 days of exposure to (0.01 mg/l) UIA-N concentration showed marked hyaline droplet degeneration and swelling of renal tubules. The same results were obtained by Robert *et al.* (1984). Renal epithelium showed more basophilic cytoplasm, some cells are vacuolated, congestion of some blood vessels and melanomacrophages center exhibited some necrotic changes (Figure 7). The apical surface of the renal tubules showed strong PAS positive. Hyaline droplets in kidney tubule epithelium suggest re absorption of excessive amounts of proteins from glomerular filtrate (Robert and Rosemarie, 1983). Examining tissues from (*O. niloticus*) after 75 days of exposure to 0.05mg/l UIA-N concentration revealed : degeneration of blood vessels endothelium, majority of renal tubules showed vacuolation, necrotic epithelium and finally necrosis in melanomacrophages center (Figure 8). Smith and Piper (1975) and Thurston *et al.* (1984), reported that degeneration of renal tubule epithelia, hyaline droplet degeneration and in some instances, partially occluded tubule lumens invariably result in impaired glomerular blood flow and filtrations, and eventually may induce renal failure. When examining tissues from Nile tilapia (*O. niloticus*) fingerlings after 75 days of exposure to 0.1 mg/l UIA-N concentration, the results showed, thrombus of blood vessels, necrosis of the renal tubules, severe glomerulosclerosis, rupture of melanomacrophages center and dispersed the melanomacrophage cells between the renal tubules in the renal parenchyma (Figure 9)., the glomerular tuft showing some mononuclear cells infiltration. The tissues exposed to the highest UIA-N concentration

(0.15 mg/l) resulted in increase the thrombus formation and infiltration of melanomacrophage cells between the renal tubules (Figure 10).

Liver:

The liver is composed of hepatic lobule in which the central vein obscure. The parenchyma of the hepatic lobule is formed from hepatocytes which are arranged around the blood sinusoid in cord-like structure known as hepatic cell cord. There are bile ductile in between the cord of hepatic cells which are directed toward the periphery of the lobule to open in the bile duct (Figure 11). Examination of tissues from Nile tilapia (*O. niloticus*) fingerlings after 75 days of exposure to (0.01 mg/l) UIA-N concentration showed that some hepatic cells were vacuolated (Figure 12). Examining tissues from (*O. niloticus*) after 75 days of exposure to 0.05mg/l UIA-N concentration revealed : marked degeneration of hepatocyte, vacuolated cytoplasm, congestion of hepatic vessels and proliferation of melanomacrophage cells (Figure 13). This result was in agreement with Thurston *et al.*(1984) and Saber *et al.* (2004). When examining tissues from Nile tilapia (*O. niloticus*) fingerlings after 75 days of exposure to 0.1 mg/l UIA-N concentration, the results showed, vacuolation, congestion of blood vessels, degeneration of some cells, infiltration of melanomacrophage cells (Figure 14). These results were obtained by Saber *et al.*(2004). The tissues exposed to the highest UIA-N concentration (0.15 mg/l) resulted in diffuse vacuolar degeneration (Figure 15).This was in agreement with the findings of Saber *et al.* (2004). Also, Wajsbrot *et al.* (1993) referred the histopathological effects affecting gills and liver function may contribute to reduce fish growth through inducing tissue hypoxia.

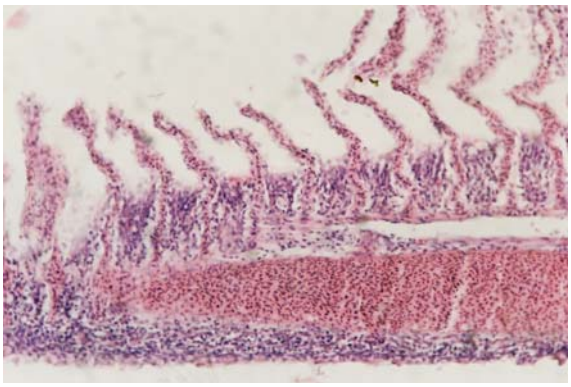

Figure 1. Gill from control group showing. H&E, X 250.

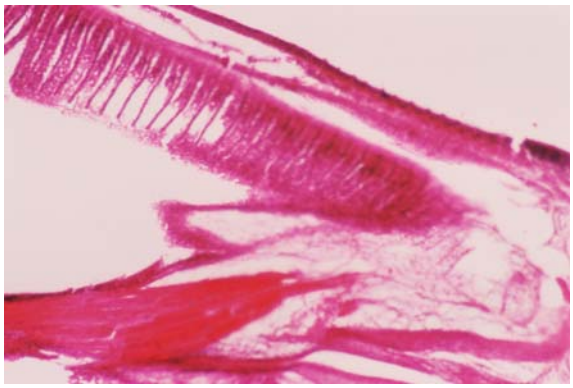

Figure 2. Slight pathological alteration consisting of mild vacuolation of secondary lamellae in gills of (*O. niloticus*) exposed to 0.01 mg/l UIA-N. H&E,X 40.

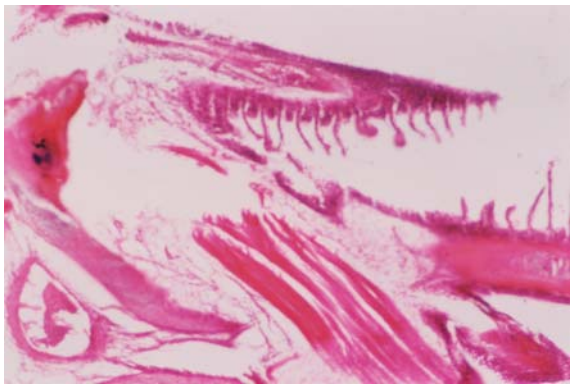

Figure 3. Congestion of central vein (a) and telangiectasis of secondary lamellae (b) in gills of (*O. niloticus*) exposed to 0.05 mg/l UIA-N. H&E, X 10.

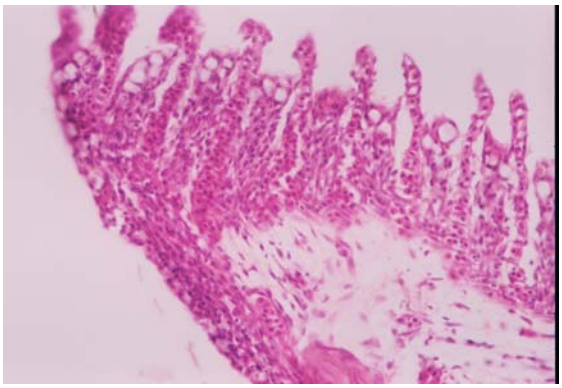

Figure 4. Vacuolar degeneration of the secondary lamellae along with telangictasis and mononuclear cell infiltration in gills of (*O. niloticus*) exposed to 0.1 mg/l UIA-N. H&E,X 40.

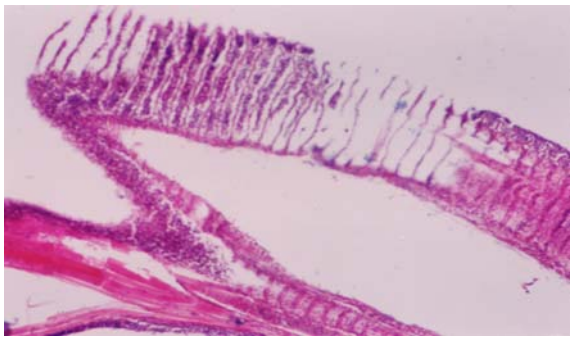

Figure 5. Degeneration, sloughing and necrosis of the lamellar epithelium in gills of (*O. niloticus*) exposed to 0.15 mg/l UIA-N. H&E,X 20.

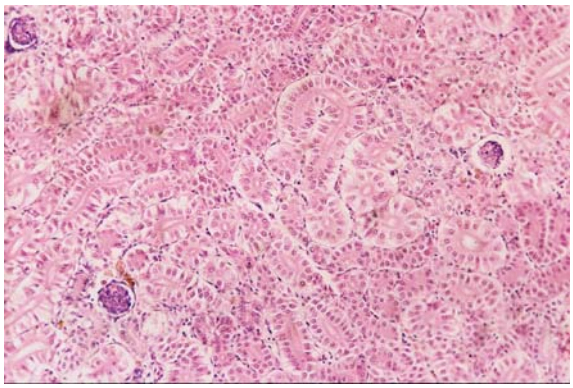

Figure 6. Kidney from control group showing normal. H&E, X 250.

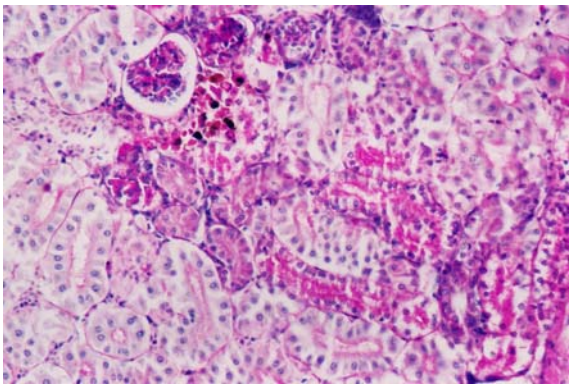

Figure 7. Degeneration and swelling of renal tubules (a) and congestion of peritubular capillaries (b) and strong PAS +ve of the apical surface of renal tubules (arrow) in kidney of (*O. niloticus*) exposed to 0.01 mg/l UIA-N. PAS,X 20.

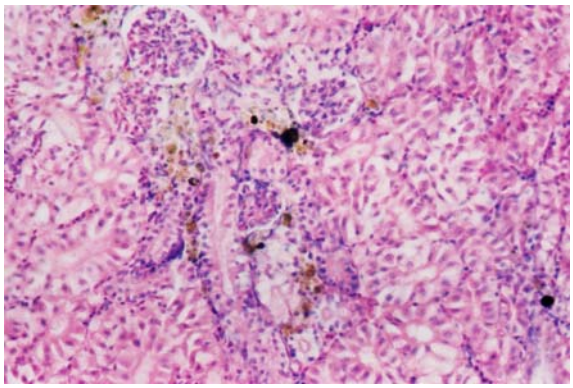

Figure 8. Degeneration of renal tubular epithelium (a), vacuolation and necrosis of renal tubules (b) along with necrosis of melanomacrophage center (arrow) in kidney of (*O. niloticus*) exposed to 0.05 mg/l UIA-N. H&E,X 10.

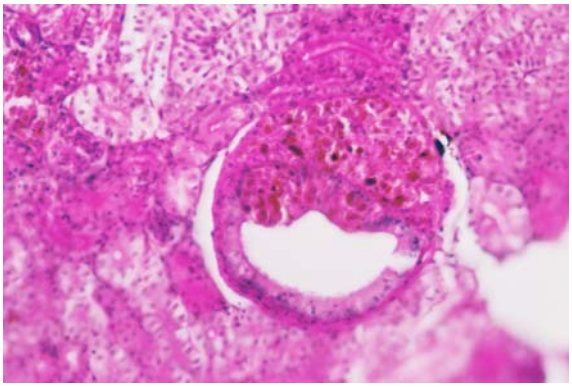

Figure 9. Necrosis of renal tubules (a) and thrombus formation of blood vessels (b) in kidney of (*O. niloticus*) exposed to 0.1 mg/l UIA-N. PAS,X 20.

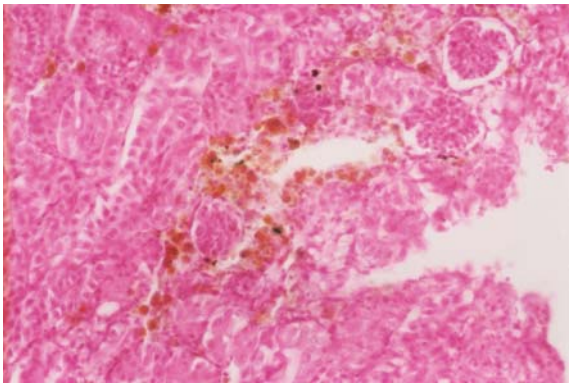

Figure 10. Infiltration of melanomacrophage center between the renal tubules in kidney (arrow) of (*O. niloticus*) exposed to 0.15 mg/l UIA-N. H&E,X 20.

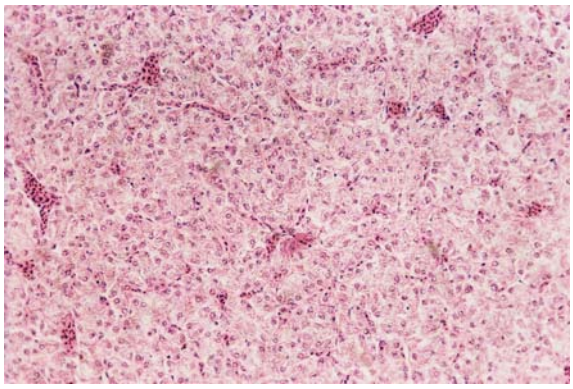

Figure 11. Liver from control group showing normal. H&E, X 250.

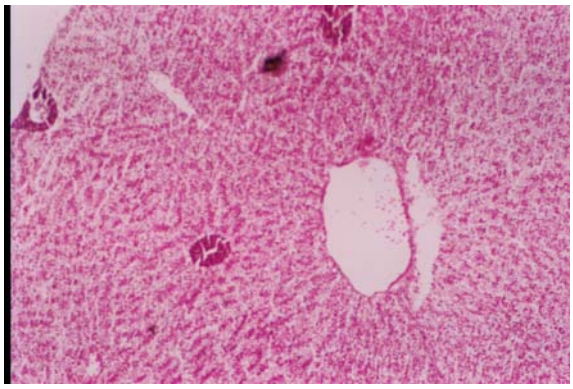

Figure 12. Slight vacuolation of some hepatic cells in liver of (*O. niloticus*) exposed to 0.01 mg/l UIA-N. H&E,X 20.

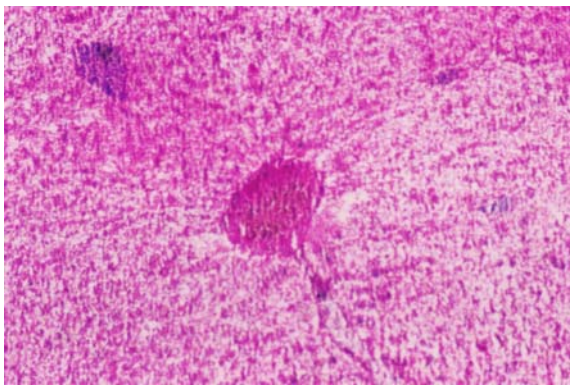

Figure 13. Congestion of blood vessels (a), activation of melanomacrophage center (b) and vacuolation of hepatocytes (c). some hepatocytes have pyknotic nuclei (d) in liver of (*O. niloticus*) exposed to 0.05 mg/l UIA-N. PAS,X 20.

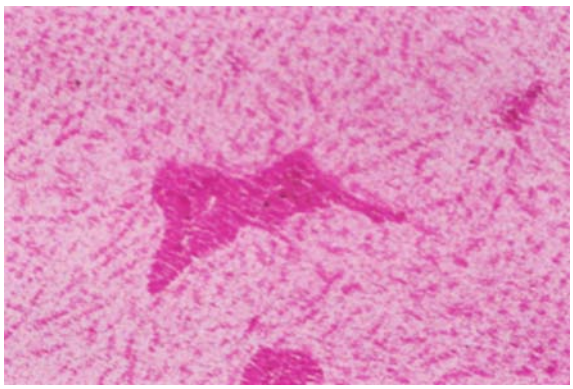

Figure 14. Diffuse vacuolar degeneration of hepatocytes (a) along with congestion of blood vessels (b) in liver of (*O. niloticus*) exposed to 0.1 mg/l UIA-N. H&E,X 20.

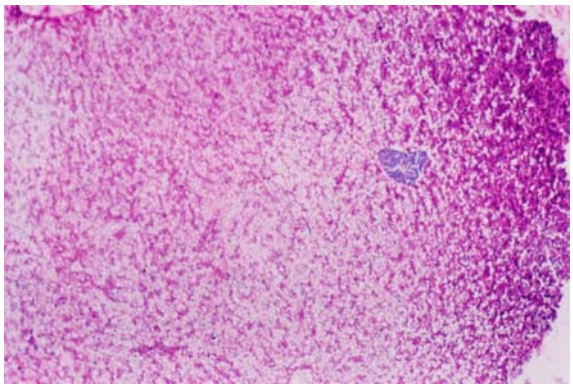

Figure 15. Diffuse vacuolar degeneration (a) and pyknotic of nuclei (b) in liver of (*O. niloticus*) exposed to 0.15 mg/l UIA-N. PAS,X 10.

LC 50 values:

From the tabulated data (9) it can be showed that the unionized ammonia concentrations were 2.3, 5.17, 7.1, 8.5 and 11.0 mg/l in the aquaria of fingerlings corresponding to 0.04, 0.075, 0.12, 0.16 and 0.18 g NH_4CL/ l), respectively. The acute toxicity of ammonia UIA-N (48 h) of Nile tilapia (*O.niloticus*) fingerlings were found to be 7.1 mg/l. These results were in full agreement with Aysel and Gulten (2005). Also, Daud *et al.*(1988) reported 6.6 mg/l 48-h LC_{50} in hybrid tilapia species (*O. mossambicus × O. niloticus*). The differences could be attributed to differences in the average weight of fish besides. On the other hand, Barry and Robert (1979) and Redner and Stickney (1979) found that he 48-h LC_{50} for *Tilapia aurea* was 2.4 mg/l NH_3-N.

Table 9. Lethal concentrations of NH_3-N for Nile tilapia (*O.niloticus*) fingerlings throughout 48 h of the experimental period.

Period (h)	Toxicity				
	LC_{20}	LC_{40}	LC_{50}	LC_{70}	LC_{90}
48 h	2.3 mg/l NH_3-N	5.1 mg/l NH_3-N	7.1 mg/l NH_3-N	8.5 mg/l NH_3-N	11 mg/l NH_3-N

CONCLUSION

It could be concluded that Nile tilapia(*O. niloticus*) fingerlings with average weight 19.0± 1.0 g, were more suitable to culture at water UIA-N concentration of 0.01- 0.004 mg/l for optimum growth performance and survival rate than other water conditions. Therefore, it can be recommended to be carried out under the similar experimental conditions.

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تأثير الأمونيا على أداء البلطى النيلى وبعض القياسات الدموية والنسيجية

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يعتبر تركيز الأمونيا غير المتأينة في المياه و العوامل البيئية المختلفة من أهم العوامل تأثيرا على معدل النمو و معدل البقاء لأسماك البلطي النيلى المستزرعة. وقد استهدفت هذه الدراسة تأثير كلا من التركيزات المختلفة لكل من الامونيا الغير متأينة الأقل من المميتة على كلا من معدلات النمو و البقاء و أيضا بعض القياسات الفسيولوجية لاصبعيات اسماك البلطي النيلى. و قد أجريت هذه الدراسة بمعمل الثروة السمكية- قسم الإنتاج الحيواني و الثروة السمكية- بكلية الزراعة بالإسماعيلية – جامعة قناة السويس.

تمت تربية اصبعيات اسماك البلطي النيلى (بمتوسط وزن ١٩ ± ١,٠ جم) في أربعة تركيزات مختلفة من الامونيا غير المتأينة (٠,٠١ ، ٠,٠٥ ، ٠,١ ، ٠,١٥ ، ملجم/لتر) بالإضافة إلى مجموعة المقارنة(٠,٠٠٤ ملجم/لتر) باستخدام ١٥ حوض زجاجي (٤٠×٧٠×٦٠ سم) و ذلك لمدة ٧٥ يوما مع التغذية على عليقة متوازنة تحتوى على ٢٦,٥٨% بروتين. سجلت التحاليل الفيزيوكيميائية لمياه الأحواض مع تغييرها ١٠٠% مرتين يوميا. تم تقدير قياسات النمو المختلفة كل أسبوعين خلال فترة التجربة كما تم اخذ بعض القياسات في الدم مع دراسة بعض التغيرات الهستوباثولوجية في أنسجة الخياشيم و الكلية و الكبد. و قد أوضحت النتائج أن معدل النمو انخفض معنويا ($P\leq0.05$) بزيادة تركيز الامونيا غير المتاينة و لم تظهر اى نسبة نفوق في اى معاملة خلال مدة التجربة، و كانت هناك فروقآ معنوية عالية بين التركيزات المرتفعة من الامونيا غير المتأينة. و قد زاد معامل تحويل الغذاء(FCR) بزيادة تركيز الامونيا غير المتاينة و كانت الفروق معنوية بين التركيزات المرتفعة. ظهرت تغيرات هستوباثولوجية مختلفة في الخياشيم و الكلية و الكبد، كما أن زيادة الامونيا غير المتأينة أدت إلى نقص الهيموجلوبين و الهيماتوكريت، و كانت التغيرات غير معنوية بين المعاملة الأولى ومجموعة المقارنة . كما أوضحت النتائج أن التركيز القاتل ل٥٠% من الأسماك كان ٧,١ ملجم/لترمن الامونيا غير المتأينة.

وعلى ذلك يمكن التوصية بأن أنسب تركيز للأمونيا (NH_3-N) هو من ٠,٠١ إلى ٠,٠٠٤ ملجم/لتر لإستزراع أصباعيات البلطى النيلي بوزن ١٩,٠٠±١,٠جم، وذلك للحصول على أعلى معدلات للنمو والبقاء.