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Water quality and health status of the monosex Nile Tilapia, *Oreochromis niloticus* cultured in aquaponics system (ASTAF-PRO)

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ABSTRARCT

The present study is a part of a cooperative project to enable the efficient transfer of innovative ecotechnology ASTAF-PRO aquaponic from Germany to Egypt for sustainable aquaculture and food production. The present part was carried out to evaluate the impact of ASTAF-PRO aquaponic system on water quality parameters and the health status of the monosex Nile tilapia *Oreochromis niloticus*. Water quality analysis revealed an improvement of most parameters in an aquaponic system with a significant reduction in toxic ammonia. In aquaponic, the nitrite level in ASTAF-PRO was lower than the permissible limit, probably because of the double-sided action of nitrobacteria present in the biological filters and the grow beds of the plant in the system. No significant difference was observed in the values of nearly all the hematological parameters in the blood of fishes reared in the aquaponic system and those reared in POND systems, being always within the normal ranges for the healthy Nile tilapia. Most of the measured biochemical variables exhibited an improvement in the blood of fish reared in the ASTF-PRO system compared to that of the POND. This suggests that fish health improved when reared in aquaponic with better water quality parameters. Immunoglobulin (IgM) levels and lysozyme activity were the same in the blood of fish reared in both systems, confirming that the monosex Nile tilapia was still fit with a good immunological status under aquaponic condition. In conclusion, the health status of the monosex Nile tilapia reared in ASTAF-PRO aquaponic system was better than those reared in POND in terms of hematology, biochemistry and immunological status. Obviously, the difference in the health status of fish in different systems can be linked to water quality since the same feed was used.

INTRODUCTION

Aquaculture is a vital industry, which accounts for over 50 percent of global fish production, providing cheap and essential animal protein to the population (**Somerville *et al.*, 2016; Kaleem *et al.*, 2020).** Aquaculture sector in Egypt expanded rapidly over the past four decades. As a result, Egypt became the seventh-largest aquaculture producer in the world, and the largest in Africa (**Feidi, 2003; FAO, 2016**). The traditional aquaculture system is always contaminated by metabolites from the fish and/or by feedstock's residues leading to eutrophication of water bodies. In addition, the water consumption of such systems is very high. In order to minimize these disadvantages, closed recirculation aquaculture systems (RAS) has been developed, where the used or the wastewater is reprocessed through combined mechanical-biological water purification, and the outcome is then returned to the fish culture (**Setiadi *et al.*, 2019**). In a closed circulation system, the nitrification process causes nitrate accumulation in the treated water. This nitrate can be readily used for supplying nutrients to plants. For this reason, attempts have been made in the past for a combined fish and plant production with the goal of an improved nutrient utilization and water purification. The so-called aquaponic facilities were created, where a hydroponic culture (or hydro-culture) was integrated in a closed-loop aquaculture system (**Tyson *et al.*, 2011; Zou *et al.*, 2016**). Aquaponics is one of the most efficient and environmentally sustainable farming methods of the twenty-first century (**Somerville *et al.*, 2014; Oladimeji *et al.*, 2020**). It produces both fish and plants in a complementary system (**Endut *et al.*, 2010; Goddek *et al.*, 2019**). Considerably, it is a developing agricultural technology that is rapidly gaining worldwide popularity, as a bio-integrated food production system, mainly for small-scale production systems (**Kloas *et al.*, 2015**). Moreover, aquaponic is the solution to many of nowadays global problems, having a huge potential to become the future farming method that provides year-round production of high quality fish and vegetable in a sustainable way, and at a relatively low cost (**Baganz *et al.*, 2020**).

However, the classical aquaponic system is not feasible to create optimum rearing conditions neither for fish nor for plants, which reduces productivity seriously. In order to overcome these problems, the aquaculture group at Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB) in Germany developed and validated an innovative ecotechnology for aquaponic (Aquaponic System for a (nearly) emission free Tomato and Fish production in greenhouses (ASTAF-PRO)). This system was designed to allow optimum production for both, aquaculture and hydroponic, to reduce emissions into environment (nutrients, CO2), and save the resource freshwater (**Kloas *et al.*, 2015**). The striking innovations introduced by ASTAF-PRO is the use of a one-way-valve to separate aquaculture within a classical closed aquaculture recirculation system (RAS) from a classical hydroponic system, and thus allowing both systems to run under optimum conditions (**Kloas *et al.*, 2015**). This sustainable farming technology received growing attention during the last years (**Goddek *et al.*, 2016; Junge *et al.*, 2017; Monsees *et al.*, 2017; Baganz *et al.*, 2020**). The present work was built upon this technology to enable the efficient transfer of ASTAF-PRO to Egypt. Despite of that, Egypt has a good global rank in aquaculture, but there is no documented study on the aquaponic until now. The high cost of aquaponics poses a major challenge to spread this type of unconventional agriculture in Egypt.

Therefore, a practical experiment was required to determine which types of aquaponics can be most suitable to contribute to solving the problem of food as an alternative to traditional farming methods in Egypt. As far as known, the installed ASTAF-PRO unit in Faculty of Science, Al-Azhar University, Assiut is the first university unit in Egypt. Hence, the present study is the first documented study in Egypt conducted to evaluate the effects of ASTAF-PRO units on water quality, hematology, blood biochemistry and immune status of fingerlings monosex Nile tilapia (*O. niloticus*) considering a small open fish farm as a positive control.

MATERIALS AND METHODS

Systems design

The current experiment consists of two parallel units:

1- ASTAF-PRO unit: Aquaponic system with one-way valve.

2- PUND unit:

ASTAF-PRO unit consisted of three rearing tanks (each 1 m^3), a sedimentation tank (1 m^3), bio-filter (1 m^3), three hydroponic rins, two separate pump units, and one one-way valve (Fig. 1) in a wooden greenhouse. This design followed the method of **Kloas *et al.* (2015)** with minor changes. In brief, the water flow from the aquaculture to the hydroponic unit was unidirectionally connected by a one-way valve, and thus in the ASTAF-PRO system the water did not directly go back into the fish tanks. The whole system was set up in a greenhouse. The effluent of fish tanks was reared of solid particles by a lamellae clarifier, and the solid-free water was launched into the sump of the biological filter unit set up as a trickling filter. In the sump, 1 pump (capacity 10 m3 h−1) lifted the water on top of the trickling filter to a rotating irrigation sprinkler, and another pump (capacity 30 m3 h−1) launched the water into the fish tanks to recirculate the process water in the RAS. The trickling filter consisted of a polyethylene cylinder with a width of 1.45 m and a total height of 2.70 m. The water passing through the trickling filter for nitrification returned degassed and saturated with oxygen. Water flow was regulated to exchange the volume of fish tanks twice per hour. In addition, all fish tanks were aerated via an air blower to maintain the oxygen supply for the fish. The hydroponic unit was based on a nutrient film technique (NFT) to supply water and nutrients via a thin film to the roots and consisted of 3 parallel plastic troughs with lengths of 6 m. The troughs were covered by black-and-white foil to prevent light reaching the roots and to inhibit algal growth and evaporation from the water surface. The tomato plants grew soilless on mineral wool cubes placed in the NFT troughs. The fertilizer consisted of the clarified fish water from the sump of the trickling filter.

POND unit consists of small open fishponds in Al-Azhar University (Assiut Branch), Assiut, Egypt to rear the Nile tilapia during the time of the experiment, and serve as a positive control for commercial conventional aquaculture (Fig. 2).

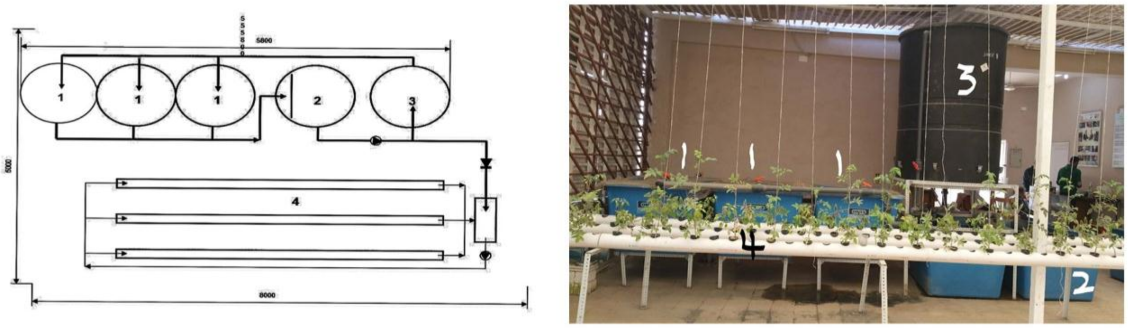

Fig. 1: The design of the basic ASTAF-PRO aquaponic unit connecting RAS and hydroponic by a one-way valve. 1 Rearing tanks, 2 Sedimentation tank, 3 bio-filter, 4 NFT-grounds.

Fig. 2: The open fish farm in the Faculty of Science, Al-Azhar University, Assiut, Egypt.

Fish culture

Fingerlings of mono-sex Nile tilapia from the same ages (TL= 3.0±0.2) were used in ASTAF-PRO and POND units. The densities of fingerlings were 50 kg/m3 of rearing tank in each ASTAF-PRO and POND units. The fish were manually fed 5% of their body weight six days a week in two portions per day, at 8:00 h and 16:00 h during the time of the experiment. The diet containing 30% protein used in the experiment was formulated to cover all nutrients required for the tilapia as recommended by the **NRC (1993)** (Table 1).

Table 1: The composition of the experimental diets.

ITEMS	PERCENTAGE
Fish meal (65%)	70
Soybean meal	250
Corn gluten	80
Yellow corn	100
Wheat bran	150
Rice bran	300
Fish oil	20
Premix[1]	30
Total	1000

The reduction in number of fish per tank between sampling events was considered, then the feeding levels were adjusted at each sampling event for the following days. All fingerlings were fed on the main experimental diet for 6 months extending from May to October, 2020.

Water quality

The functionality of the two systems was daily checked. Behavior of fishes and mortalities were monitored. Temperature, pH, dissolved oxygen (DO), salinity and electro-conductivity (EC) were monitored twice a day (at 08:00 and 16:00) before feeding events, and floc volume (FV) was monitored once a day prior to the 8:00 h feeding. Temperature and DO were measured using an oxygen probe (YSI, Pro ODO, Yellow Springs, USA), pH was measured using a portable pH meter (Hach, sension 1, Loveland, USA). Salinity and EC were measured using a combo pH/conductivity/ salinity/DO meter (IP67 Water Quality Meter). The FV in mg/L was measured using an Imhoff cone by letting 1 L of culture water settle for 15 min and recording the settled volume. Total ammonia (TA), nitrite (N02-), nitrate (N03-), water hardness and orthophosphate (P043-) were monitored weekly using a colorimeter (Hach, DR/850, Loveland; USA) with random repeat measurements from two systems. TA and N02- were periodically measured more frequently when high levels were detected or suspected using the salicylate and diazotization methods, respectively. N03- was measured using the cadmium reduction method. P043- was measured using the ascorbic acid method as described by **El-Shafai *et al.* (2004).** Sludge was drained once a week from the rearing tanks

Health Status

Hematological parameters

For hematological analysis, blood samples of each fish were collected from cardiac puncture in small plastic tubes containing heparin solution (0.2 mL/mL blood) as anticoagulant. Haemoglobin concentration (Hb), haematocrit (Hct), red blood cell (RBC) count, and white blood cell (WBC) were estimated using an automated technical analyser

(Celltac α MEK- 6400J/K). The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were calculated according to the methods of **Dacie and Lewis (2002).**

Biochemical parameters

For biochemical analysis, blood samples were allowed to coagulate for 15–20 minutes at 4°C prior to centrifugation for 20 minutes at 3000 rpm to separate serum (**Osman *et al.*, 2011**). The serum was stored at -20°C until use for biochemical and immunological analyses. Serum total protein, cholesterol, triglyceride, glucose, albumin (ALb), urea, creatinine, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were calculated according to the methods of **Henry (1964)**, **Trinder(1969)**; **Friedewald *et al.* (1972)**, **Reitman and Frankel (1975)** and **Thomas (1992**).

Immunological status

Lysozyme activity was measured spectrophotometrically according to the method of **Ellis (1990)**. Immunoglobulin M (IgM) levels were determined using an ELISA kit (Catalogue No. CSB-E12045Fh (96k test), Cusabio Biotech Co., Ltd.).

Statistical analysis

Data were presented as Mean±SD. Data were analyzed using **SPSS (1997)** program, version 16. Differences between means were compared using t- tests at $p < 0.01$ level.

RESULTS AND DISCUSSION

The interest in aquaponics has been growing as an important part of the solution of increasing food shortages and needs for healthy food (**Savidov *et al.*, 2005**). Egypt is facing a steady increase in water scarcity; population growth and climate change are all factors that seriously threaten the Egyptian food security and stimulate approaching unconventional solutions to meet the challenge of providing food for future generations. Aquaponics was a worthy alternative solution that presents itself strongly. Considerably, Egypt can benefit from aquaponics, as aquaponics can make a big difference by saving large quantities of water and solving the problem of food quality. Hence, water quality and health status of monosex Nile tilapia reared in ASTAF-PRO were investigated to examine the efficient transfer of ASTAF-PRO technology to Egypt.

Water Quality

Water quality parameters are crucial for fish production and exert an immense influence on the maintenance of a healthy aquatic environment (**Sunny *et al.*, 2017**). Remarkably, growth performance and health status of fish are normally governed by the water quality variables, such as temperature, pH, oxygen, nitrate, nitrite and ammonia (**Islam *et al.*, 2018**). In the current study, the measured parameters were water temperature, pH, dissolved oxygen, salinity, conductivity, floc volume, total ammonia nitrogen, nitrite, nitrate, orthophosphate and hardness. The water quality of both, ASTAF-PRO and POND systems are presented in Table (2). Temperature ranged from 19 to 27.8˚C in ASTAF-PRO system, while the temperature ranged from 21 to 29.5 ˚C in the POND system. The recorded temperature in the present study for both systems were still within the permissible limit (**Kohinoor, 2000; Bhatnagar & Devi, 2013**). The previous authors found that water temperature that ranges from 18.5 to 32.9°C and 15-30°C, respectively is suitable for fish culture, while at water temperature <12 >35 °C fish would face stess. The pH is the most interactive parameter with the other water quality parameters (**Essa & Sayed, 2015**), therefore it was measured daily and showed optimum values in the aquaponic system (7 – 7.2) compared to the POND (7 to 7.7) (Table 2). "Dissolved Oxygen is an important parameter for identification of different water masses" **(Ibrahim & Ramzy, 2013)**. The dissolved oxygen ranged from 4.4 to 5.7 for ASTAF-PRO and from 4.5 to 6.6 for the POND system (Table 2). The values observed were within the tolerant range of *O. niloticus* (≥ 3, **(de Holanda Cavalcante *et al.*, 2014)**. **Boyd (1982**) opined that dissolved oxygen should be around 5 mg/L to support the survival and the development of aquatic life in any culture system. However, the Nile tilapia was reported to tolerate much lower. The salinity and conductivity were higher in aquaponic system, and almost twice the value of salinity in the POND system (Table 2), but they are still within the permissible rate for tilapia culture. The Floc volume was very high in the water of the POND; ranging from 2- 5.9 ml L. While it was very low in ASTAF-PRO; ranging from 0.2 to 2 ml L (Table 2). It could be attributed to the larger volume of POND water compared to the aquaponic tanks.

Total ammonia in the water consists of unionized ammonia (NH_3) and ionized ammonia (NH_4) (**Van Rijn *et al.*, 2006**). The major source of ammonia in the aquaculture is feed (**Hargreaves & Tucker, 2004**). According to **Ebeling *et al.* (2006),** from 80% of nitrogen excreted, 90% contained as ammonia and 10% as urea. It was noticed that the total ammonia in the water of the ASTAF-PRO was 0.11 mg L at the beginning of the experiments, while it decreased to be very low (0.08 mg L) at the end of the experiment. Whereas the level of total ammonia in the POND was much higher compared to ASTAF-PRO; starting with 0.37 mg L and increased toward the end of the experiment recoding 1.09 mg L in the sixth month (Table 2). The high value of ammonia recorded in the POND system could be linked to nonfrequent water renewal as done in some other closed systems (**Oladimeji *et al.*, 2020**). This could be also, associated with the results of pH and temperature, shifting the equilibrium of total ammonia nitrogen into ammonia. This imply that ASTAF-PRO system is great efficient for reducing the waste, especially total ammonia, providing one of the best water quality control in the industry (**Savidov & Brooks, 2004**). Such result is in accordance with other studies who reported the same trend of reducing total ammonia in aquaponic systems (**Endut *et al*., 2009; Li & Li, 2009; Eissa *et al.*, 2015**). It is worthy to mention that, nitrite is the intermediate product of nitrification process, which occurs by the action of highly aerobic, gram-negative bacteria (**Lawson, 1995**). In aquaponic, the conversion rate is quickly so that the nitrite level in ASTAF-PRO by the end of the present experiment was recorded to be 0.2 mg L, which is lower than the permissible limit (0.5 mg/L) (**Swann, 1997**). This happened probably because of the double-sided action of nitrobacteria present in the biological filters and the grow beds for the plant in the system. According to **Syafiqah *et al.* (2015**), plants in the aquaponics system act as biological filters, thereby absorbing nutrients such as nitrate and NH_3 from the system. This, therefore, explains the low levels observed in the aquaponics system in our study. In contrast, the POND system exhibited very high level of nitrate by the end of the current experiment (1.09 mg L). Nitrate is relatively non-toxic to tilapias (**Ibrahim & Ramzy, 2013; Salam *et al.*, 2013**). Noticeably, the observed pattern of nitrate level during the whole experiment was nearly located within the permissible limit (**Piper, 1982**). According to **Stone and Thomforde (2004**), nitrate does not cause any health hazard except at exceedingly high levels (above 90 mg/ L), which was not recorded here for both systems. The result of the present work exhibited higher nitrate level compared to nitrite in both systems due to the fast conversion of NO2-to NO3-ions by nitrifying bacteria (**Abdel-Satar *et al.*, 2010**). This goes to the fact that nitrate could provide sufficient source of nitrogen for plant (**Savidov & Brooks, 2004**). The present results are in accordance with those of other **Hambrey *et al.* (2013)** and **Wahyuningsih *et al.* (2015**), who observed that the plant in the aquaponic system significantly decrease nitrogen waste for up to 92%.

Furthermore, the value of orthophosphate ranged from 1.03 to 6.7 mg/ L in aquaponic, and from 0,03 to 5.1 mg/ L in the POND which are still within the permissible limit for both systems. Hardness represents the overall concentration of divalent salts (calcium, magnesium, iron, etc.). Aquatic animals, in general, can tolerate a broad range of hardness. A desirable range would lie between 75 and 200 mg/L CaCO3 (**Wurts & Durborow, 1992**). Moreover, the hardness in the present experiment was higher in ASTAF-PRO water compared to that of the POND (Table 1), but still very low in both systems compared to the tolerant level of hardness of fish in aquaculture, which is usually desirable to have alkalinity above 50 mg/l.

Table 2: Water quality parameters (mean±SD) in ASTAF-PRO aquaponic and POND systems during six months of rearing experiment.

Parameter	Aquaponic System							POND System						
	1st Month	2 nd Month	3 rd Month	4 th Month	5 th Month	6 th Month	Ref.	1st Month	2 nd Month	3 rd Month	4 th Month	5 th Month	6 th Month	Ref
(Temperature) (C)	27±0	27.8±0	26.4±0	24±0	21.5±0	19±0	30–18 FAO (2016)	29.5±0	28.6±0	27.5±0	26±0	24.2±0.1	21±0	25C – 30C FAO (2006)
(PH)	7.2±0	7±0	7.1±0	7.2±0.1	7±0	7.1±0	7–6 FAO (2016)	7.4±0	7±0.1	7.4±0	7.7±0	7.7±0	7.5±0	6.0 – 9.0 (fresh) Davis (1993)
(Dissolved Oxygen) (mg/L)	5.3±0	5.7±0.1	4.8±0.1	5.4±0.1	4.4±0.1	5.2±0.1	>5 FAO (2016)	5.9±0.1	5.1±0.1	4.5±0.1	5.8±0.1	6.4±0.1	6.6±0.1	3 – 20 mg/l Boyd (2003)
(Salinity) (P.P.M)	581±6.41	609±3.01	643±1.84	593±2.03	631±3.67	636±3.69	less than 800 p.p.m FAO (2016)	247±1	247±0.51	265±1.26	234±1.35	313±1.64	348±0.84	0.13 mg/l Davis (1993)
(Electro-Conductivity) (µS/cm)	968±10.59	1010±3.69	1077±8.53	935±10.33	1051±6.69	1051±10.05	less 1500 mS/cm FAO (2016)	411±1.33	408±0.19	414±0.51	401±0.33	448±0.69	519±0.51	100-2,000 mS/cm Stone & Thomforde (2004)
(Floc Volume) (ml/L)	0.2±0	0.6±0	0.8±0.1	1±0	1.7±0.1	2±0	----	2±0	2.5±0	2.7±0	3.8±0.1	5.2±0.1	5.9±0	---
(Total Ammonia) (mg/L)	0.11±0.03	0.12±0.02	0.7±0.03	0.43±0.05	0.12±0.03	0.08±0	1> FAO (2016)	0.37±0.02	0.79±0.01	0.64±0.02	0.44±0.04	1.72±0.03	1.49±0	0.1 mg L Santhosh and Singh, 2007
(Nitrite NO2--) (mg/L)	0.8±0.01	0.22±0.02	0.2±0.04	0.05±0.02	0.55±0.02	0.2±0.03	1> FAO (2016)	0.87±0.02	0.77±0.02	0.06±0.02	0.04±0.01	0.87±0.05	1.09±0.04	Less 0.2 mg L OATA (2008)
(Nitrate NO3---) (mg/L)	5.73±0.02	8.27±0.41	29.67±0.51	15.47±0.4	8.47±0.36	7.9±0.22	5-150 FAO (2016)	5.17±0.08	0.1±0.03	0.5±0.03	2.23±0.05	2.47±0.05	2.67±0.02	1 to 4.0 mg L Santhosh and Singh (2007)
(Orthophosphate Po4---) (mg/L)	1.03±0.02	2.97±0.86	3.07±0.73	6.7±0.23	4.57±0.02	4.87±0.04	3.2−16.3 mgL Kloas *et al.* (2015)	0.03±0.02	0.67±0.02	0.1±0.03	0.3±0.03	5.13±0.12	4.57±0.1	0.06 mg L Stone and Thomforde (2004)
(Hardness) (mg/L)	16.4±0.12	17.5±0.17	17±0	14.67±0.1	15.67±0.1	17.17±0.1	60 – 140 mgL FAO (2016)	7.83±0.1	7.83±0.35	12±0.33	7.67±0.1	8.67±0.1	9.67±0.38	50 – 100 mgL WHO (2003)

Health status

Hematological parameters

Blood characteristics (RBCs, Hg, Ht, WBC) are closely related to individual responses to the surrounding environment. Complete blood cell count (CBC) is an important component of a minimum database, which can be used to monitor the health status of fish in response to changes related to water quality parameters (**Fazio, 2019**). It is worthy to consider that, **Docan *et al.* (2011**) mentioned that there is growing interest in the study of hematological parameters for aquaculture purposes. In fact, blood is a perfect mirror apparently reflects what occurs inside fish body. In addition to the easiness of blood analyses for determination, they can also help to evaluate the health status of the fish (**Shehata & Shehata, 1994; De Pedro *et al.*, 2005**). Consequently, blood parameters such as number of erythrocytes counts, hemoglobin content and hematocrit percentage are used as warning alarm of adverse water quality (**Shalaby, 1997; Fernandes, 2003**). The average values of Hb, RBCs, WBCs, MCH, MCHC, Hct, and MCV of the monosex *O. niloticus* in both ASTAF-PRO and POND systems are presented in Table (3).

Non-significant differences were observed in the values of nearly all the hematological parameters in the blood of fishes reared in aquaponic system and those reared in the POND systems (excluded Hb, WBC, and Platelet) (Table 3). RBCs count was nearly the same in the blood of fish collected from both systems. Leucocytes exhibited a remarkable elevation in the blood of the monosex Nile tilapia collected from aquaponic compared to the POND, although the values of leucocytes and their differentials were within the normal ranges (Table 3). This finding seems to confirm that the monosex Nile tilapia was not negatively influenced by the rearing under aquaponic condition. The observed differences in the hematological parameters may not indicate a serious problem, because they were within the normal ranges for the healthy Nile tilapia, and all were not far from values given by other authors for the same fish in different localities (**Al-Zahaby *et al.*, 2017**). The non-significant differences in the evaluated parameters between the two systems implies that ASTAF-PRO system could be successfully used in Egypt instead of the open fish farm. Some hematological values observed here appeared to be slightly different from those reported for the Nile tilapia by some authors but were within the acceptable range. Notably, the variations in hematological values within species can be influenced by environmental conditions, sex, age, origin, breeding system, and feeding, among other factors (**Osman *et al.*, 2019**).

Table 3: Hematological parameters (Mean ±SD) of *O. niloticus* reared in ASTAF-PRO aquaponic and POND systems during six months of rearing experiment.

	ASTAF-PRO Aquaponic	POND
(Hb) (g/dl)	8.2±0.5*	9.7±.0.2*
(R.B.Cs)(x10^6/ µL)	1.5±0.04NS	1.5±0.1NS
(WBCs) (x10^3 / µL)	75.5±27.9**	58.2±3.0**
(MCH) (pg)	51.9±2.3NS	62.7±8.0NS
(MCHC) (g/dl)	30.9±2.1NS	34.6±4.4NS
(HCT) (%)	26.6±3.0NS	28.5±3.3NS
(MCV) (fL)	169.3±17.6NS	183.7±23.2NS
(Lymphocytes) (%)	87.6±9.4NS	85.5±7.7NS
(Neutrophil) (%)	4.9±4.0NS	7.9±4.3NS
(Eosinophils) (%)	6.7±5.2NS	5.5±2.6NS
(Monocytes) (%)	0.7±0.3NS	1.2±0.7NS
(Platelet) (x10^3/ µL)	126.3±11.6**	370±12.1**

Blood biochemistry

The average concentrations of total protein, albumin, glucose, cholesterol, triglyceride, creatinine, urea, ALT, and AST in the blood serum of the monosex *O. niloticus* are presented in Table (4). Biochemical parameters vary among species and can be influenced by many biotic and abiotic factors, such as water quality parameters (**Osman *et al.*, 2019**). The measurements of total protein and albumin are of considerable diagnostic value in fish, as it relates to general health status of fish (**Ahmad *et al.*, 2015**). A significant elevation was observed in the total protein in the blood of the monosex Nile tilapia reared in ASTAF-PRO system compared to those reared in POND system. Such an increase in the plasma protein level in the fish collected from aquaponic system suggests that fish health improved when reared in aquaponic with better water quality parameters. Additionally, the observed improvement of albumin concentration may be attributed to the fact that better water quality coordinates the function of various endocrine glands in the fish body. Considerably, plasma glucose levels in fishes increase during stress, probably due to the action of catecholamine on stored glycogen in liver and other tissues (**Osman *et al.*, 2019**). It wasrecorded that, the level of glucose was significantly lower in the blood of fish reared in AFTAF-PRO than in the blood of fish reared in the POND, hence indicating that the POND fish was under stress, which reduced the appetite (**Talpur & Ikhwanuddin, 2013**). In stress conditions, the blood glucose would increase to keep homeostasys of the fish, resulting in the decline of insulin (**Royan *et al.*, 2014**).

Cholesterol recorded the same trend as being higher in the blood of fish reared in the POND (Table 4). In contrast, the triglyceride exhibited nonsignificant elevation in the blood of fish reared in ASTAF-PRO compared to that in the POND system.The triglyceride levels increased due to the increase in protein levels in the blood of fish in aquaponic, which may be because the muscle is a pivotal compartment that is directly linked to amino acid turnover (**Osman *et al.*, 2019**). Moreover, higher level of urea and creatinine were recorded in the fish reared in aquaponic compared to those in the POND system. Markedly, significant changes in the levels of ALT and AST were observed in the blood of aquaponic fish compared to POND ones. These results may be related to the immune-potentiating effect of the good water quality in aquaponic, changing the level of both enzymes to the range of normal levels.

Table 4: Biochemical parameters (Mean ±SD) of *O. niloticus* reared in ASTAF-PRO aquaponic and POND systems during six months of rearing experiment.

	ASTAF-PRO Aquaponic	POND
(Total Protien) (g/dl)	3.4±0.07*	2.9±0.05*
(Albumin)(g/dl)	1.7±0.04^{NS}	1.5±0.06^{NS}
(Glucose) (mg/dL)	136.3±6.6*	154±7**
(Cholestrol) (mg/dL)	168.3±3.0^{NS}	177±6.0^{NS}
(TriGlycride) (mg/dL)	221±12.7^{NS}	219.7±8.6^{NS}
(Urea) (mg/dL)	8.9±0.3^{NS}	7.4±1.2^{NS}
(Creatinine) (mg/dL)	0.4±0.02^{NS}	0.3±0.02^{NS}
(ALT (Alanine Aminotransferase)) (U/L)	112±3.0*	174.3±14**
(Aspartate Aminotransferase (AST)) (U/L)	106.7±3.0*	90.3±3.0*

Immunological status

The non-specific immune system of fish is considered to be the first line of defense against stress and invading pathogens (**Badrey *et al.*, 2019**). Since immunoglobulin (IgM) levels and lysozyme activity are important indices of non-specific immunity in fishes, thus they were analyzed here, and their results are presented in Table (5). Both IgM levels and lysozyme activity exhibited the same level in the blood of fish reared in both systems, confirming that the monosex Nile tilapia was still fit and had a good immunological status under aquaponic condition.

Table 5: Immune status (IgM levels and lysozyme activity) (Mean ±SD) of *O. niloticus* reared in ASTAF-PRO aquaponic and POND systems during six months of rearing experiment.

	ASTAF-PRO Aquaponic	POND
IgM (mg/dL)	0.1±0.0^{NS}	0.1±0.0^{NS}
(**lysozyme**) (U/L)	0.4±0.0^{NS}	0.4±0.00^{NS}

CONCLUSION

The results of the water quality parameters in ASTAF-PRO system exhibited improvement compared to the water quality of the POND system, especially the level of ammonia, nitrite, and nitrate. Health status of the monosex Nile tilapia reared in ASTAF-PRO aquaponics system was better than those reared in the POND system in terms of hematology, biochemistry and immunological status. Obviously, the difference of the health status in the fish in the different systems can be linked to water quality since the same feed were used.

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