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Growth Rate and Chemical Muscle Composition of *Oreochromis niloticus* Fish Cultured in Drainage Water and Fed Antioxidant Supplemented Diet

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Abstract

Irrigation of fish farms with agricultural drainage water may affect cultured fish species. So, the present study utilizes antioxidants supplemented diet to overcome deterioration of drainage water and its negative effect on fish. The studied groups are fish cultured in dechlorinated tap water as control group or drainage water and fed commercial basal diet. While, the other studied groups represented by *Oreochromis niloticus* fish cultured in drainage water and fed either commercial diet supplemented with vitamin C (5 g/kg diet) or fennel (5 g/kg diet) for 12 weeks. Results of the water physico-chemical parameters of all studied treatments revealed deterioration of the drainage water with a decrease in dissolved oxygen and an increase in pH, total hardness, total alkalinity, salinity, ammonia, nitrite and heavy metals (Cu, Pb and Cd) with significant differences ($P \leq 0.01$) in comparison to that of the control dechlorinated tap water group. Data clarified also that *Oreochromis niloticus* cultured in drainage water showed a decrease in growth rate accompanied by deterioration of fish meat quality. However, fish reared in the same drainage water for the same exposure period and fed vitamin C or fennel supplemented basal diet (5 g/kg diet) recorded values of the studied parameters more or less similar to that of control group fish. Data of the present study, empowered aquaculturist to supplement fish rations with fennel or vitamin C as antioxidants to improve fish growth rate, meat quality as well as protect fish against heavy metals toxicity that could threat Human Being.

Keywords

Drainage Water, *Oreochromis niloticus*, Vitamin C, Fennel, Growth Indices

and Meat Quality

1. Introduction

Fish farms play an important role as an alternative solution for increasing fish yield. But, due to the regulation rules for use of water resources, fish farms which mainly established around El-Bats and El-Wadi drainage canals at Fayoum governorate, Egypt are allowed only to use water from the drainage network around [1]. Thus, these fish farms using agricultural drainage water may face the danger of negative effects on their cultured fish species [2]-[7].

This drainage water is loaded with salts, nutrients, wastes, heavy metals and pesticides [5] [8] [9] [10] [11] and this may affect fish production, either directly or indirectly through their damaging effect on the vital organs of the body which in turn affect growth and meat quality [5] [6] [7] [12] [13].

Hence application of an external source of antioxidants may offer some protection against oxidative stress. The term antioxidant refers to a wide spectrum of compounds, which are able to donate electrons and neutralize free radicals, resulting in the prevention of cell injuries [14] [15] [16]. In consequence, the search for effective, nontoxic, natural compounds with antioxidant activity has been intensified in recent years [17] [18] [19] [20] [21].

Since, deterioration of drainage water used for fish culture without prior treatment is considered as a serious threat to aquatic life and hence become threaten to human being. So, the aim of present study is to follow up possible ameliorative effect of fish food additive antioxidants, fennel and vit C, for fish culture in drainage water. The main goal of the present investigation could be achieved by investigating quality of the drainage water and determine the concentrations of the most abundant heavy metals. Moreover, stand on the biological status including growth rate of *Oreochromis niloticus* fish fed normal commercial diet and that supplemented with the studied antioxidant (vit C or fennel). Furthermore, the study concerned with estimating meat quality of fish reared in the different studied treatments.

2. Materials and Methods

Experimental Design:

One hundred and eighty healthy Nile tilapia, *Oreochromis niloticus*, weighing about 50 g each, were retrieved from a fish farm in the Egyptian governorate of Fayoum. Fish were transported to the wet lab at the institute of fisheries and Oceanography, El-Fayoum station. Fish were distributed in twelve concrete aquaria with a 1000-liter capacity at a rate of fifteen fish per aquarium, divided into four groups of 45 fish each. To prevent metabolite buildup, water was replaced twice weekly. Fish were exposed to the following treatments for 12 weeks in order to monitor the effects of the drainage water on fish culture and possible ameliorative effect of diet supplementation with vitamin C or fennel as antioxidants.

Studied treatments:

Group I: Control group, fish reared in dechlorinated tap water and fed on commercial basal diet

Group II: Fish reared in drainage water and fed commercial diet.

Group III: Fish reared in drainage water and fed vitamin c supplemented commercial basal diet (5 g/kg diet)

Group IV: Fish reared in drainage water and fed fennel supplemented commercial basal diet (5 g/kg diet).

Diet formulation:

The experimental fish ration was formulated according to Abdel-Tawwab and Abbass [18]. The main constituent of fish ration is crude protein, all diets contained 32% crude protein. The diet supplemented with 5 g vitamin C or 5 g fennel obtained from a local market for each kilogram diet.

Fish were fed twice daily at a rate of 3% of live body weight. Every two weeks, fish were weighed in order to monitor their rate of growth and alter their feed intake. At the end of experimental period (12 weeks), after which fish were sacrificed to acquire organ samples, and their muscles were chemically analyzed to determine their protein, lipid, ash, and moisture contents.

1) Physicochemical analysis of water

The water samples of the different studied treatments were subjected to a number of physicochemical analyses as mentioned later:

*Water pH and dissolved oxygen were measured in the field using Corning Checkmate II multi-parameter meter.

- Salinity was measured by using a salinity-conductivity meter (model, YSI 58).
- Total hardness and total alkalinity were measured by titration method according to the American Public Health Association standard methods [22].
- Ammonia (NH_3) was determined colorimetrically using Nessler's solution as described by Sauter and Stoup [23].
- Nitrite concentrations in water samples were measured by ion chromatography (IC) (model DX-600, USA) according to APHA [22].

Figure 1. Experimental fish, Nile tilapia *Oreochromis niloticus.*

- Heavy metal (Copper, Lead, and Cadmium) concentrations in water were determined by atomic absorption spectrophotometer (Model, Perkin Elmer-2280) according to APHA [22].

2) Growth indices

To optimize the artificial feed rate, fish body weights were measured to the nearest gram and total body length to the nearest 0.1 cm for the various study groups. The following growth indices were established at the end of exposure period (12 weeks).

***Specific growth rate:**

Specific growth rate (growth rate/day) was determined according to the equation postulated by Allen and Wooton [24]:

$$S.G.R. = \frac{LnWf - LnWo}{Tf - To} \times 100$$

where:

Wf: The final weight of fish in g.

Wo: The initial weight of fish in g.

(Tf − To): Time between the final and initial weight in days.

***Condition factor (*k*):**

K factor was calculated for individual fish from the formula recommended by Schreck and Moyle [25]:

$$K = \frac{W}{L^3} \times 100$$

where:

W: is the wet weight in g., *L*: is the total length in cm.

3) Chemical muscle composition

a) Muscle water content

Sidwell *et al.*, [26] method for determining muscle water content was used.

b) Muscle total protein

According to Josyln [27], the semi-microkjeldahl method was used to assess muscle total protein.

c) Muscle total lipids

The common method described in A.O.A.C. [28] was used to determine the total lipid content of fish muscle.

d) Muscle ash

The samples were burned for 16 hours at 650˚C in a muffle furnace to estimate the amount of muscle ash [26].

4) Statistical analysis

The results were statistically analyzed using analysis of variance (F-test) followed by Duncan's multiple range test to determine differences in means using Statistical Analysis Systems, Version 6.2 [29].

3. Results and Discussion

Current population growth rates call into question humanity's ability to continue to ensure food security. With catches from natural fisheries stabilizing or even declining, aquaculture development projects are being initiated as part of the solution to meet growing protein demand. Fish farms play an important role as an alternative solution to increase fish production. Due to regulation of water use in El-Fayoum province, fish farms can only use water from the surrounding drainage network [1]. Therefore, these fish farms using agricultural drainage may be at risk of negative impacts on their farmed fish [2] [3] [4]. High concentrations of organic compounds, inorganic salts, and heavy metals from agricultural emissions may be responsible for the decreased dissolved oxygen content in the effluent in this study (**Table 1**). Hypoxia impairs fish survival and leads to fish death [30] [31] [32]. Spillage of organic matter decomposition into the water column can lead to severe dissolved oxygen depletion. In addition, microbial overgrowth in polluted water consumes more oxygen during respiration and may be caused by phytoplankton blooms [1].

High ranges of drainage water ammonia and nitrite determined inside the current evaluation of water samples are in step with the ones of Lim *et al.* [33], who observed extended ammonia and nitrite concentrations due to the release of

Table 1. Quality of water of the different studied treatments.

Studied Treatments	pH	Dissolved oxygen mg/l	Total Hardness as $CaCO_3$ mg/l	Total alkalinity as $CaCO_3$ mg/I	Salinity g/l	NH_3 mg/l	NO_2 mg/l	Cu^{+2} P.l. = 1.0 mg/l	Pb^{+2} P.l. = 0.05 mg/l	Cd^{+2} P.l. = 0.01 mg/l
Group I Control group (Fish reared in dechlorinated tap water and fed commercial diet)	7.70 ± 0.04 **B**	7.96 ± 0.08 **A**	158 ± 2.63 **B**	119 ±1.3 **B**	0.61 ±0.18 **B**	0.50 ±0.04 **B**	0.67 ±0.04 **B**	0.027 ± 0.01 **B**	0.014 ± 0.002 **B**	**N.D.**
Group II (Fish reared in drainage water and fed commercial diet)	8.32 ± 0.07 **A**	5.47 ± 0.16 **B**	339 ± 16.1 **A**	262 ± 9.9 **A**	5.0 ± 0.41 **A**	3.91 ± 0.54 **A**	5.43 ± 1.03 **A**	0.35 ± 0.02 **A**	0.18 ± 0.01 **A**	0.026 ± 0.05 **A**
Group III (Fish reared in drainage water and fed vitamin C supplemented diet, 5 g/kg diet)	8.20 ± 0.06 **A**	5.60 ± 0.14 **B**	333 ± 14 **A**	257 ± 11 **A**	4.5 ± 0.32 **A**	3.56 ± 0.45 **A**	5.28 ± 0.76 **A**	0.40 ± 0.017 **A**	0.15 ± 0.02 **A**	0.02 ± 0.004 A
Group IV (Fish reared in drainage water and fed fennel supplemented diet, 5 g/kg diet)	8.10 ± 0.09 **A**	5.73 ± 0.21 **B**	340 ± 18.9 **A**	260 ± 7.3 **A**	4.76 ± 0.39 **A**	3.77 ± 0.58 **A**	5.83 ± 1.14 **A**	0.337 ± 0.014 **A**	0.20 ± 0.02 **A**	0.023 ± 0.004 A
F-values	15**	58**	38*	71**	38**	13**	8.07**	118**	40.6**	16.1**

Data are represented as means of six samples ± S.E. **N.D.** = Not detectable. **P.l.** = Permissible level in water according to WHO (199). Means with the same letter for each parameter are not significantly different, otherwise they do, Duncan multiple range test (SAS, 2000). **Highly Significant difference ($P < 0.01$).

energetic pollutants from sewage, agricultural, and commercial effluents which have now not passed through enough and former remedy.

The ecological damage of the environment due to anthropogenic elements in addition to the presence of hazardous agents might also have an effect on fish subculture as well as its customers. Among the diverse poisonous pollutants, heavy metals represent a very thrilling group of elements because of their strong impact on balance of aquatic ecosystems, bioaccumulation in living organisms [34] [35], toxicity patience and tendency to build up in water and sediments[36].

Data concerning growth rates (fish weight gain percentage, specific growth rate and condition factor) of the studied Nile tilapia; *Oreochromis niloticus* revealed that fish fed commercial basal diet and cultured in the drainage water exhibited the lowest growth rate in comparison with the control group fish. However, the highest growth rates were recorded in fish fed 5 g fennel /kg diet followed by that of fish fed 5 g vit. C/kg diet.. Moreover, fish reared in drainage water and fed antioxidants supplemented diet revealed more or less growth rates similar to that of control group (**Table 2**).

The poisonous results of the various heavy metals in water samples may be the reason of the decrease values of the condition factor "k" of *Oreochromis niloticus* cultured within the drainage water and fed commercial basal diet. The same locating was previously made concerning fish that have been not able to thrive in infected aquatic environments [31] [32] [37]; and/or low densities of both phytoplankton and zooplankton that may have been related to the decline in water quality, previously mentioned by way of Shaaban *et al.* [38]. However, high k

Table 2. Growth parameters of the Nile tilapia; *Oreochromis niloticus* reared in drainage water fed commercial basal diet and diet supplemented with vitamin C or fennel for 12 weeks.

Studied Treatments	Body weight gain (g)			Specific growth rate (g/day)	Condition factor (k)
	Initial weight	Final weight	Weight Gain Percentage (%)		
Group I Control group (Fish reared in dechlorinated tap water and fed commercial diet)	56.10 ± 2.08 A	228.7 ± 4.25 A	307.7	1.97 ± 0.05 A	2.00 ± 0.024 B
Group II (Fish reared in drainage water and fed commercial diet)	54.00 ± 2.91 A	170.9 ± 4.36 B	216.5	1.64 ± 0.09 B	1.77 ± 0.03 C
Group III (Fish reared in drainage water and fed vitamin C supplemented diet, 5 g/kg diet)	53.50 ± 1.64 A	225.7 ± 9.31 A	321.9	1.98 ± 0.07 A	2.02 ± 0.03 B
Group IV (Fish reared in drainage water and fed fennel supplemented diet, 5 g/kg diet)	52.47 ± 2.17 A	239.4 ± 6.77 A	356.3	2.13 ± 0.05 A	2.17 ± 0.08 A
F-values	0.56	22.3**		10.0**	12.60**

Data are represented as means of fourty five fish ± S.E. Means within the same column, with the same letter for each parameter are not significantly different, otherwise they do (Duncan's multiple range test, SAS 2000). **Highly significant difference ($P < 0.01$).

values helps the findings of Kheir *et al.* [39], who connected the upward thrust in fish circumstance thing to more food intake introduced on with the aid of an expanded metabolism, reduced oxygen intake, and an increase in hormones.

Additionally, fish might need to detoxicate the metal and consequently require more energy, which might be a reason for fish weight reduction [40]. Furthermore, Carvalho *et al.* [41] demonstrated a correlation between decreased fish weight and the activation of metal detoxing mechanisms, inclusive of an increase in metallothionien ranges. Lanno, *et al.* [42] claimed that rainbow trout exposed to sublethal concentrations of copper skilled lack of appetite, which resulted in stunted boom.

According to Bonga and Lock [43], water-born toxicants have an effect on the gills via making the gill epithelium extra permeable to water and ions and via inhibiting the chloride cells' potential to trade ions. Reduced growth will take place from the fish's compensatory responses, which might dramatically boom the strength had to hold water and ion balance.

The meat quality of fish cultured in agricultural drainage water deteriorated in the present investigation (**Table 3**). There were considerable increase inside the muscle's water content and ash, in addition to considerable declines in the total protein and lipid content of the muscle. These findings corroborated the ones recognized by Zaghloul [44] and Elghobashy, *et al.* [45], who recorded a decrease in muscle proteins and lipids of African catfish (*Clarias gariepinus*) and Nile tilapia (*Oreochromis niloticus*) subjected to high concentrations of heavy metals. Exposure of fish to high levels of heavy metals in the present study may be

Table 3. Meat quality of the Nile tilapia; *Oreochromis niloticus* reared in drainage water fed commercial basal diet and diet supplemented with vitamin C or fennel for 12 weeks.

Studied Treatments	Water contents (%)	Total protein (% of wet weight)	Total lipids (% of wet weight)	Ash (%)
Group I Control group (Fish reared in dechlorinated tap water and fed commercial diet)	76.0 ± 0.37 B	18.0 ± 0.18 A	3.23 ± 0.09 A	1.47 ± 0.02 C
Group II (Fish reared in drainage water and fed commercial diet)	82.0 ± 0.40 A	12.8 ± 0.09 B	1.97 ± 0.06 C	2.53 ± 0.05 A
Group III (Fish reared in drainage water and fed vitamin C supplemented diet, 5 g/kg diet)	76.7 ± 0.80 B	18.3 ± 0.42 A	2.97 ± 0.06 B	1.67 ± 0.06 B
Group IV (Fish reared in drainage water and fed fennel supplemented diet, 5 g/kg diet)	76.67 ± 0.40 B	18.0 ± 0.36 A	2.83 ± 0.08 B	1.60 ± 0.10 B/C
F-values	30.6**	80**	58.77**	58**

Data are represented as means of six samples ± S.E. Means within the same column with the same letter for each parameter are not significantly different, otherwise they do (Duncan's multiple range test, SAS 2000). **Highly Significant difference at $P < 0.01$.

responsible for the decrease in total protein and total lipids in the muscles of fish exposed to agricultural drainage water. According to earlier research by Reader *et al.*, [46] these metals induce damage to the gill structure and a decrease in oxygen consumption, both of which result in a dramatic decrease in metabolic rate.

In the present study, fish cultured in drainage water and fed commercial basal diet supplemented with vitamin C or fennel (5 g/kg diet) restore growth rate and meat quality of *Oreochromis niloticus* to values more or less similar to that of the control group fish that cultured in dechlorinated tap water.

Hence utility of an external supply of antioxidants may additionally offer a few protection in opposition to oxidative stress. The term antioxidant refers to a huge spectrum of compounds, that are capable of donate electrons and neutralize loose radicals, ensuing inside the prevention of cellular injuries [14] [47]. In consequence, the look for powerful, secure, natural compounds with antioxidant activity has been intensified in current years [17] [21] [48] [49].

In particular, fennel and vitamin c (Dietary supplemented antioxidants) have beneficial health effects that studied and established by the scientific community. However, there is little information about their protective roles against noxious effects caused by exposure to heavy metals, including those related to hepatic damage as well as other physiological status disturbances as previously reported [3] [5] [35]. The ameliorative effect of the studied antioxidants against heavy metals toxicity was very obvious in our study. This effect can be attributed to its scavenging ability of free radicals [50] [51] [52] [53], as well as the reductive metabolites produced [54].

Therefore, it may be concluded that Fish culture in drainage water induce reduction in growth rate and meat quality. Fennel or vitamin c individually improved and restore the biological status of fish, changes their ability to scavenge the toxic heavy metals and some reactive species, regenerate other antioxidants for protection.

Moreover, Data of the present study spot light on the role that should be taken by responsible authorities to follow up water quality irrigating fish farms. Moreover, empowered aquaculturist to supplement fish diet with antioxidants, fennel or vitamin C, that improve fish growth rate and meat quality as well as protect fish against heavy metals toxicity that could threat Human Being.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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