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Effect of Some Water Quality Parameters on Cholesterol and Triglycerides of Wild and Cultured Nile Tilapia *(Oreochromis Niloticus)*, Sudan

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ABSTRACT

The purpose of the current study was to evaluate fat profile (specifically, cholesterol and triglyceride), chemical composition and minerals of Nile tilapia (Oreochromis niloticus) in wild environment (general Nile) compared with aquaculture environment, specifically in different types of ponds (concrete, polyethylene and earthen Ponds) in fish farms in Khartoum State. Also this work was intended to determine and compare physic-chemical parameters of water in the studied fish environments. Moreover, the study was conducted to compare some parameters according to source of water (Nile or wells). A total of 60 samples of Nile tilapia were collected from Nile River and governmental and private fish farms around Khartoum State and the samples were subjected to cholesterol, triglycerides analysis. Total of 48 samples of water samples were collected for water quality parameters (Unionized ammonia NO3-N, total ammonia-nitrogen NH3-N and Hydrogen Sulphide H2S). The data was subjected to SPSS by using factorial arrangement. Results revealed that Nile tilapia from Nile environment had significantly ($P \leq 0.01$) lower levels of cholesterol and triglycerides than that from ponds. There were significant differences ($P \leq 0.05$) in all water quality parameters. Nile River and wells water had significant differences ($P \leq 0.05$) in H2S. Total ammonia-nitrogen (NH4) showed a highly significant difference ($P \leq 0.01$), and total ammonia showed a significant difference ($P \leq 0.05$). Accordingly, the study concluded that Nile river water has a high water quality than other sources of water, also, Nile River aquaculture environment is an immediate environment in relation to ponds environment.

Keywords: *Oreochromis niloticus, Concrete, Polyethylene, Earthen pond.*

INTRODUCTION

Fish has an important role in food security and poverty alleviation in both rural and urban areas of Sudan. However, a little is known about the nutritional value of the Nile fish that are normally utilized either fresh or preserved dried, salted or smoked (Waterman, 2000). Lipid is the major nutrient in fish tissue next to protein. Moreover, the taste, texture and characteristic flavour of the fish depends mainly on lipid profiles of the tissues. The nature and quantity of these lipids in fish vary according to species and habitat (Nanda and Sahu, 2005). Lipids are an important component in fish and human diets, both as energy and fatty acids (FA) sources (Sargent *et al*., 2002). Among the FA, particular emphasis has been placed on the n-3 and n-6 polyunsaturated fatty acids (PUFA). Polyunsaturated omega-3 (n-3) fatty acids, eicosapentaenoic acid (EPA, C-20:5) and docosahexaenoic acid (DHA, C-22:6), are of interest because they reduce the risk of cardiovascular diseases (Leaf and Weber, 1988).

In addition, fatty acid composition data are needed by food scientists and nutritionists to aid them in dietary formulation, processing and product development (Ackman, 1989). Since these fatty acids composition may vary among fish species, it is necessary to determine both the lipid content and the PUFA distribution. Lipids can be divided into two main classes, neutral lipids (NL) and polar lipids (PL). PL are important constituents of membranes and they function as precursors in eicosanoid metabolism (structural fat), whereas the NL serve mainly as a depot of lipids used as an energy source (depot fat) (Henderson and Tocher, 1987). Lipid composition in fish tissues can be affected by diet quality and environmental factors, such as salinity, temperature, seasonality and geographic location. Fish are a healthy source of protein, providing omega-3 (n-3) the fatty acids that reduce cholesterol levels, and reduce the incidence of heart disease and stroke (Michel *et al*., 2009). Fish meat contains biologically active protein which is characterized by a very favourable composition of amino acids, a high omega-3 polyunsaturated fatty acid content and fat-soluble vitamins as well as it represents a good source of micro- and macro-elements (Fuczynska *et al*., 2006; Maqsood and Benjakul, 2010). The fat content in fish meat are not constant and depends on: diet, size, age, reproductive cycle, salinity and temperature of water, season of catch and geographical location **(**Guler *et al*., 2008; Kalyoncu *et al*., 2009; Luzia *et al*., 2003; and Luzzana *et al*., 1996). Knowledge on the fat concentration and fatty acid profile in the meat of many common fish species is desirable. Metwally (2009) figured-out that, triglycerides and cholesterol in blood serum of Tilapia nilotica (*O. niloticus*) fed on garlic were range from 56.33 to 74.55mg/dl triglycerides and 114 – 140mg/dl cholesterol. Fish lipid is regarded as quality lipid being rich in cholesterol and triglyceride. The changes in habitat and nutrition may have a significant impact on the quantity of different components of lipid profile.

Justifications

- The importance of Nile tilapia (*Oreochromis niloticus*) as a source of unsaturated fats which is low in cholesterol and triglycerides and a source of animal protein.
- The noticeable attention that has been paid at Tilapias culture in Sudan so that there is need for characterization of water quality for Nile and Fish farms.

The Specific Objectives

- To evaluate fat profile (cholesterol and triglycerides) of Nile tilapia (Oreochromis niloticus) according to their environment (wild and Ponds-cultured) in Khartoum State.
- To study the effect of some water quality parameters (physic-chemical parameters) on Nile tilapia environments (general nile and fish ponds) in Khartoum State.

Materials and Methods

Area of Study

In the present investigation, four sampling sites were selected in Nile River and Khartoum State fish farms. For easy interpretation of results, samples were analyzed depending on general experimental strategy as follows:

- The similarities and differences in fat profile specifically cholesterol and triglycerides of Nile tilapia (*Oreochromis niloticus*) were investigated among: wild fish, concrete fish ponds, polyethylene fish ponds and earthen fish ponds via laboratory analysis through this study.
- Physic-chemical characteristics of water were compared among Nile river water and ponds fish farms water in Khartoum State.
- Source of water for the fish farms where: surface and groundwater (well water, or spring water).
- The similarities and differences between sources of water also were compared.

Experimental Design

The study was carried out in four areas identified as treatments at Khartoum State, Sudan:

Treatment (1) wild tilapia fish was carried-out using four areas for sampling in Nile river where fish were captured;

Treatment (2) concrete ponds culture tilapia fish was carried out using four farms for sampling, feed types: sunken, manufactured normally by local feed plant and protein levels is ranged from 25 – 27%, water source is Nile;

Treatment (3) polyethylene ponds culture tilapia fish was carried out using four farms for sampling; feed types was afloat and manufactured by specialized manufacture and protein levels is up-to 35%, source of water is groundwater (wells).

Treatment (4) earthen ponds culture tilapia fish was carried out using four farms for sampling feed types: afloat and manufactured by specialized manufacture; and Protein levels is 27 – 35%, source of water is Nile.

Preparation of Fish Samples

Collected fish were cut into three parts horizontally and in the middle. Each one was gutted, scaled; fins removed and washed with clean, cold potable water, after that, 30 grams were taken from this part and transferred to sterilized container (60 ml size).

Preservation of Samples

All collected samples were put into sterilized containers and preserved immediately in minced ice preservative container by means of layers (first minced ice layer then samples layer and ice layer and so on).

Water Samples

A total of 48 samples of water were collected from Nile River and governmental and private fish farms around Khartoum State, 12 representative samples were randomly collected from each treatment. The samples were collected in sterilized water containers (300 ml) and transferred immediately to the laboratory.

Total Lipid Extraction

Sixty samples were selected from each treatment (a total of 240 samples for all treatments). Total lipids were extracted from dorsal muscle tissue according to Folch *et al*. (1957) method in the administration of laboratories and veterinary researches, department of chemistry, Khartoum.

Cholesterol Analysis

The extract of total lipids was used for the determination of cholesterol. Cholesterol analysis was done according to manufacturer of reagent (MDSS GmbH Germany, 2007) were 10 mL of sample (lipid), reagent and standard were mixed together. Then 10 µL of each sample and standard was added to the mixture. The mix was incubated for 5 minutes at 37ºC after that, absorbance of cholesterol and standard was calculated against reagent blank within 30 minutes using the following equation:

$$\text{Cholesterol (mg/dL)} = \frac{\text{Cholesterol}}{\text{Standard}} \times 200$$

Triglyceride Analysis

The extract of total lipids was used for the determination of triglyceride. Triglyceride analysis was done according to manufacturer of reagent (MDSS GmbH Germany, 2007) were 10 mL of sample (lipid), reagent and standard were mixed together. Then 10 µL of each sample and standard was added to the mixture. The mix was incubated for 5 minutes at 37ºC after that, absorbance of triglyceride and standard was calculated against reagent blank within 30 minutes using the following equation:

$$\text{Triglyceride (mg/dL)} = \frac{\text{Triglyceride}}{\text{Standard}} \times 200$$

Water Quality Analysis

Water Quality Parameters (Physic-chemical parameters of water) were investigated and analysed **include**:

Determinations of NH_3-N, NO_3-N and H_2S

Ammonia-Nitrogen (NH3-N mg/l), Nitrate-nitrogen (NO3-N mg/l) and hydrogen sulphide (H2S mg/l), were analysed in Institution of Environmental Studied laboratory, University of Khartoum, by DR Spectrophotometer Apparatus (version DR 3900).

Statistical Analysis

The data was analysed by using statistical package for Social Studies (SPSS version 17.0). A factorial Completely Randomized Design (CRD) arrangement was used for means separation among types of farms (treatments). One way analysis of variance (ANOVA) was used for means separation between sources of water. A P-value of $\leq$ 0.05 was considered indicative of a statistically significant difference.

Results and Discussion

This study was conducted to evaluate of Nile tilapia (*Oreochromis niloticus*) in wild environment (Nile River) compared with aquaculture environment, specifically in different types of ponds (concrete, polyethylene and earthen Ponds) in fish farms in Khartoum State, mainly in fat profile (specifically, cholesterol and triglyceride). This work was also, intended to determine and compare physical and chemical parameters of water which include: Hydrogen Sulphide (H_2S mg/l) and Ammonia {total ammonia-Nitrogen (NH_3-N mg/l), nitrate-nitrogen (NO_3^-N mg/l)} in the same environments which were mentioned above. Moreover, the study was assessed and compared all measured parameters according to source of water (Nile or wells).

The findings of the present study showed some fact on the manifesto of the popular cultured fish emphasizing on fat profile among wild and farmed *Oreochromis niloticus* (concrete, polyethylene and earthen ponds) and between water sources (Nile and wells) which serves as the principle basis in evaluating the nutritional and economical value of the fish as well as water quality parameters.

Table1. *Profile of cholesterol and triglycerides (mg/dL) in fish according to the rearing sites*

Site	Cholesterol (mg/dL)	Triglyceride (mg/dL)
Nile	$39.10^c \pm 0.63$	68.70 ± 1.90
Concrete Pond Farms	$48.50^b \pm 0.63$	79.41 ± 1.90
Polyethylene Pond Farms	$49.30^b \pm 0.63$	81.56 ± 1.90
Earthen Pond Farms	$51.55^a \pm 0.63$	73.83 ± 1.90
Overall	47.10 ± 0.31	78.90 ± 2.90
Sig.	**	NS

**Means with similar superscripts within the same column are not significant different.*

*NS ≡ not significant, **≡ significant at ($P \leq 0.01$)*

Table2. *Profile of cholesterol and triglycerides (mg/dL) in fish according to the source of water*

Source	Cholesterol (mg/dL)	Triglyceride (mg/dL)
Nile	$46.34^b \pm 0.48$	77.90 ± 1.10
Wells	$49.26^a \pm 0.94$	81.56 ± 0.64
Overall	47.10 ± 0.44	78.90 ± 1.90
Sig.	**	NS

**Means with similar superscripts within the same column are not significant different.*

*NS ≡ not significant, **≡ significant at ($P \leq 0.01$)*

Fat Profile

Lipid profile is the major nutrient in fish tissue next to protein. Moreover, the taste, texture and flavour characteristic of the fish depends mainly on lipid profiles of the tissues. The nature and quantity of these lipids in fish vary according to species and habitat (Nanda and N. P. Sahu, 2005). Fish are a healthy source of protein, providing omega-3 (n-3) the fatty acids that reduce cholesterol levels, and reduce the incidence of heart disease and stroke (Michel *et al*., 2009). Today, one of the major concerns about food quality and nutrition in developed countries is the cholesterol content (United Health care, 2011). According to the World Health Organization, the maximum cholesterol amount should be 300 mg/ day.

Cholesterol

Table1 showed that, cholesterol (mg/dL) of *Oreochromis niloticus* from nile river site and ponds cultured farms (concrete, polyethylene and earthen ponds) was 39.10 mg/dL, 48.50 mg/dL, 49.30 mg/dL and 51.55 mg/dL, respectively. There was a highly significant difference ($P \leq 0.01$) in cholesterol among *Oreochromis niloticus* from Nile River, concrete ponds, polyethylene ponds and earthen ponds. And there was no difference ($P > 0.05$) between concrete and polyethylene ponds. So, the higher cholesterol was observed in *O. niloticus* from earthen ponds and the lower cholesterol was found in *O. niloticus* from Nile River. However, the level of cholesterol in this study were agreed with Kamal *et al*., (2012) studied nutrients composition of tilapia , they were figured-out that, cholesterol levels in tilapia 50mg/100g. Whereas, Guler *et al*. (2008) and Kalyoncu *et al*. (2009) mentioned that the cholesterol content in fish meat doesn’t correlate with the fat percentage concentration**.** Also, different sources of water (Nile River and wells) as in table 2 showed that, the cholesterol was 46.34 mg/dL and 49.26 for *O. niloticus* according to source of water (Nile river and wells), respectively.

There was a highly significant difference ($P \leq 0.01$) in cholesterol between fish according to water source. However, wells were recorded a higher percentage than nile river. The differences probably might be due to the differences in feeding because, wild fish was fed selectively on natural nutrients (planktons, algae and water plants), while earthen ponds cultured fish is always was fed on natural feed and manufactured feed (supplementary diets) and this differ according to ingredients (inputs) used to formulate the supplementary feed, specifically these difference may be returned to the oil cake and ingredients used in processed feed.

Triglycerides

Table 1 showed that, triglycerides (mg/dL) of *O. niloticus* from Nile river site and ponds cultured farms (concrete, polyethylene and earthen ponds) were 68.70 mg/dL, 79.41 mg/dL, 81.56 mg/dL and 73.83 mg/dL, respectively. There was no significant difference (P>0.05) in triglycerides among *O. niloticus* from Nile river, concrete ponds, polyethylene ponds and earthen ponds. However, the higher triglycerides were recorded in *O. niloticus* from polyethylene ponds and the lower triglycerides were found in *O. niloticus* from Nile river. Also, table 2 showed that, the triglycerides was 77.90 mg/dL and 81.56 for *O. niloticus* according to source of water, respectively. There was no significant difference (P>0.05) in triglycerides between fish according to water source. However, wells were recorded a higher triglycerides than Nile river. However, the findings were in agreement with Metwally (2009) who was figured-out that, triglycerides in blood serum of Tilapia nilotica (*O. niloticus*) were range from 56.33 – 74.55mg/dl. Triglycerides are fatty substance in the blood; high levels can thicken human artery walls and put them at a higher risk for stroke and heart attack. Goal: Less than 150 mg/dL in human blood (United Healthcare, 2011).

Table3. *Water quality parameters according to rearing sites*

Rearing Site/Parameters	Nile	Concrete Pond Farms	Polyethylene pond farms	Earthen pond farms	Overall	Sig.
Unionized ammonia NO_3^-N (mg/l)	1.67^{b} ± 0.02	1.29^{c} ± 0.02	1.82^{a} ± 0.02	0.44^{d} ± 0.02	1.31± 0.01	**
Total ammonia NH_3^- N (mg/l)	0.00^{d} ± 0.00	4.95^{a} ± 0.00	0.43^{c} ± 0.00	0.96^{b} ± 0.00	1.58 ± 0.00	**
Hydrogen sulphide H_2S (mg/l)	0.03^{d} ± 0.00	0.05^{c} ± 0.00	0.18^{b} ± 0.00	1.03^{a} ± 0.00	0.32 ± 0.00	**

**Means with similar superscripts within the same row are not significant different.*

*NS ≡ not significant, **≡ significant at ($P \leq 0.01$)*

Table4. *Water quality parameters according to source of water*

Water Source	Nile	Wells	Overall	Sig.
Unionized ammonia NO_3^-N (mg/l)	1.16^{b} ± 0.08	1.82^{a} ± 0.03	1.34 ± 0.07	**
Total ammonia NH_3^-N (mg/l)	1.99^{a} ± 0.37	0.43^{b} ± 0.00	1.57 ± 0.28	*
Hydrogen sulphide H_2S (mg/l)	0.35 ± 0.07	0.18 ± 0.00	0.30 ± 0.05	NS

**Means with similar superscripts within the same row are not significant different.*

*NS ≡ not significant, *≡ significant at ($P \leq 0.05$), **≡ significant at ($P \leq 0.01$)*

Physic-Chemical Parameters of Water

Because water is an essential requirement for fish farming, any properly prepared business plan for aquaculture must describes the quality and quantity of water available for the proposed enterprise or feasibility. An experienced aquaculturist can judge whether the water is adequate for the proposed fish farm. The physic-chemical parameters analyzed during study period at different chosen sites of Nile River, and fish rearing sites are presented into table 3 **as follows:**

Ammonia

The major source of ammonia in a water of a heavily stocked culture pond or in the effluent of a raceway is from excretion of fish, mostly via their gills. Ammonia is produced by animals as a byproduct of protein metabolism (Boyd, 1990).

Nitrate-Nitrogen (NO_3-N)

Table 4, showed that, nitrate-nitrogen (NO_3^-N) mg/L variations in studied water sites. The mean values of water from Nile river, concrete, polyethylene and earthen ponds was 1.67 mg/L, 1.29 mg/L, 1.82 mg/L and 0.44 mg/L, respectively. There was a highly significant difference ($P \leq 0.01$) in nitrate-nitrogen (NO_3^-N) among water sites. The higher NO_3-N was found in polyethylene ponds water and the lower NO_3-N was recorded in earthen ponds water. Also NO_3-N according to the sources of water (nile river and wells) as showed in table 4 Nile river and wells was 1.16 mg/L and 1.82 mg/L, respectively. There was a highly significant difference ($P \leq 0.01$) in NO_3-N between Nile river and wells water. Wells water was recorded the higher NO_3-N than nile river water.

Total Ammonia-Nitrogen (NH_3-N)

Table 3, showed that, total ammonia-nitrogen (NH_3^-N) mg/L of water from Nile river site and farms (concrete, polyethylene and earthen ponds) was 0.001 mg/L, 4.95 mg/L, 0.43 mg/L and 0.96 mg/L, respectively. There was a highly significant difference ($P \leq 0.01$) in ammonia-nitrogen (NH_3^-N) among water from nile river, concrete ponds, polyethylene ponds and earthen ponds. The higher NH_3-N was found in concrete ponds water and the lower NO_3-N was found in nile river water. Also NH_3-N according to the sources of water (nile river and wells) as showed in table 3 nile river and wells was 1.99 mg/L and 0.43 mg/L, respectively. There was a significant difference ($P \leq 0.05$) in NH_3-N between nile river and wells water. Nile river water was recorded the higher NH_3-N than wells water. However, these differences in all types of ammonia whether nitrate- nitrogen (NO_3-N) or total ammonia- nitrogen (NH_3-N) probably might be due to the difference in fish density, water change duration and quantity of supplementary feed in fish farms. Hence, the more dense ponds the more waste, also when water spend more time (+20 day for instance) without change this leads to more feces, as it is known that fish feces generate ammonia which is toxic to fish. Moreover, feed residues accumulate in ponds and this also generates ammonia. In addition, there was a strong correlation between temperature and pH with ammonia, the increase in temperature and pH leads to increase in ammonia, as mentioned **by Boyd (1990)** when temperature fall in 15 ºC and pH 7.0 the ammonia is 0.273, but when temperature increase to 30 ºC and pH 8.0 the ammonia increase to 7.45 accordingly. Ammonia production is directly related to feeding and depends on the quality of feed, feeding rate, fish size and temperature **(**Riche and Garling, 2003). However, the findings of this study in agreement with (Lloyd, 1992) he was stated out that, the recommended levels of ammonia for tilapia aquaculture is (total ammonia-nitrogen is >1.00 mg/L and nitrate-nitrogen is 1.00 – 100.00 mg/L).

Hydrogen Sulphide (H_2S)

Hydrogen sulfide (H_2S), rotten-egg gas, is present in some well waters but is so easily oxidizable that exposure to oxygen readily converts it to harmless form. Its toxicity depends on temperature, pH, and dissolved oxygen. Table 3, showed that, hydrogen sulphide (H_2S) mg/L of water from nile river site and farms (concrete, polyethylene and earthen ponds) was 0.03 mg/L, 0.05 mg/L, 0.18 mg/L and 1.03 mg/L, respectively. There was a highly significant difference ($P \leq 0.01$) in hydrogen sulphide (H_2S) among water from nile river, concrete ponds, polyethylene ponds and earthen ponds. The higher H_2S was found in earthen ponds water and the lower H_2S was found in nile river water. Also H_2S according to the sources of water (nile river and wells) as showed in table 4 nile river and wells was 0.35 mg/L and 0.18 mg/L, respectively. There was no significant difference ($P > 0.05$) in H_2S between nile river and wells water. However, nile river water was recorded the higher H_2S than wells water. The recorded differences in H_2S might be due to the anaerobic decomposition of organic matter by bacteria in mud, as figured-out by Rick (2012), any measurable amount after providing reasonable aeration could be considered to have potential to harm fish life. Hydrogen sulfide occurs in ponds as a result of the anaerobic decomposition of organic matter by bacteria in mud and it is toxicity is increased at higher temperatures and a pH less than 8 when the largest percentage of hydrogen sulfide is in the toxic un-ionized form. However, the findings of this study in agreement with (Lloyd, 1992) he was pointed out that, the recommended levels of H_2S for tilapia aquaculture is (total ammonia-nitrogen is >0.002 mg/L).

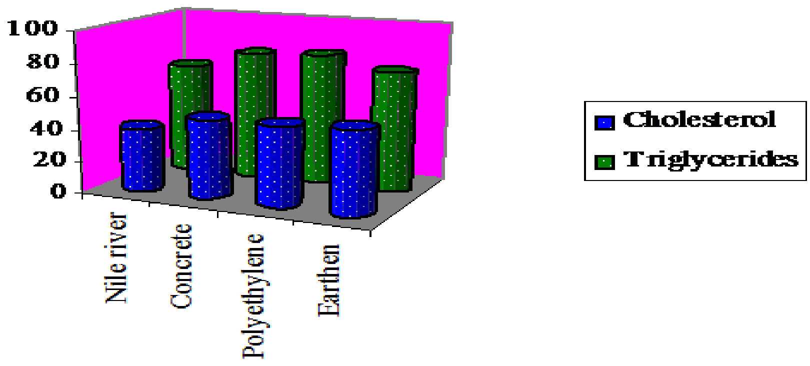

FIGURE1. *Profile of cholesterol and triglycerides (mg/dl) in fish according to the rearing sites*

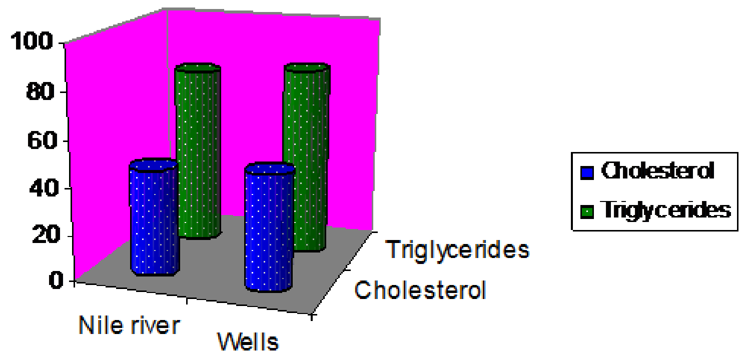

FIGURE2. *Profile of cholesterol and triglycerides (mg/dl) in fish according to the source of water*

CONCLUSION AND RECOMMENDATIONS

Conclusion

Fish has an important role in food security and poverty alleviation in both rural and urban areas of Sudan, but little is known about the nutritional value of the Nile fish that are normally utilized either fresh or preserved dried, salted or smoked. The study was conducted to evaluate fat profile (specifically, cholesterol and triglyceride) of nile tilapia (*Oreochromis niloticus*) in wild environment (general nile) compared with aquaculture environment, specifically in different types of ponds (concrete, polyethylene and earthen Ponds) in fish farms in Khartoum State. Also this work was intended to determine and compare physic-chemical parameters of water in the studied fish environments. Moreover, the study was conducted to compare some parameters according to source of water (Nile or wells). A total of 60 samples of nile tilapia (*Oreochromis niloticus*) were collected from Nile River and governmental and private fish farms around Khartoum State and the samples were subjected to cholesterol and triglycerides. Water samples were collected for water quality parameters (unionized ammonia NO_3^-N, total ammonia-nitrogen NH_3^-N, and hydrogen sulphide H_2S). The data was subjected to SPSS by using factorial design. The findings of this study revealed that, *Oreochromis niloticus* from Nile environment or from ponds with source of Nile water has lower level cholesterol and triglycerides, and there was highly significant difference ($P \leq 0.01$). 48 water samples were collected from Nile River and governmental and private fish farms around Khartoum State and subjected to physic-chemical analysis. There was a significant difference ($P \leq 0.05$) in all water quality parameters, between Nile River water and ponds water. However, when compared Nile River water and wells water and hydrogen sulphide showed no significant difference ($P \leq 0.05$), while total ammonia-nitrogen (NH_3^{-1}) showed a highly significant difference ($P \leq 0.01$), ammonia, showed a significant difference ($P \leq 0.05$). Accordingly, the study concluded that, Nile River water has a high water quality in relation to other sources of water, also, Nile river aquaculture environment is an immediate environment in relation to ponds environment.

Recommendations

According to the findings, we recommended that:

- Measurements of water quality parameters should be taken regularly in order to decide whether to change ponds water or not.
- Monitoring should be focused on unionized ammonia in fish farms because it is toxic to fish even in small amount.
- Aquaculture is so recent in Sudan and only few farmers are aware about water quality parameters, hence the facilities and equipment for physic-chemical parameters measurements should be facilitated to aquaculturists.

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