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Effect of salty feeding on sodium and potassium ions concentrations and water content of common carp (*Cyprinus carpio*) muscles

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Abstract
The present study was conducted to investigate the effect of salty feeding on sodium and potassium ions concentration and water content of common carp (*Cyprinus carpio*) muscles. Eighty four (n=84) *Cyprinus carpio* fingerlings of 20-40 g body weight were randomly distributed into four equal groups, with three replicates per each group. The fingerlings were distributed into 12 glass pool (7 fingerlings per pool of 30 ± 10 g average body weight). Thee experiment was conducting during the period from November 2016 to February 2017. Results revealed that the muscle's sodium concentrations were 42.98, 58.18 and 71.34 mM/Kg tissue in 1, 3 and 5% dietary NaCl treatments respectively, as compared with the control group (37.62 mM/Kg tissue). The corresponding muscle's potassium concentrations were 108.62, 78.06, 96.33, and 72.11 mM/Kg. In conclusion, this study confirmed that the dietary NaCl of common carp fish led to elevating of muscle's sodium, potassium and water contents as compared with unfed fish.

Keywords: Salty feeding, water content, Ions, Muscles*, Common carp*

1. Introduction

In aquaculture, it is well-known that the quality and quantity of feed are not the only factors that could affect the growth of fish as the adequate environmental conditions is another important factor. Salinity represents the most important abiotic environmental factors that could also affect the fish growth [1].

Vertebrates have 9 g/L salt in their blood. Of this salt the sodium and chloride ions represent 77%, while the remainder (23%) consist of bicarbonate, potassium and calcium ions. The sodium and potassium have an important role in heart, nerve and muscle functions [2].

Some researchers reported that that fishes grew better in brackish water than fresh water and sea water [3, 4, 5].

The transport of some fishes from sweet to salty waters required some changes in cardiovascular, digestion and nerve systems, and consequently, hormonal and physiological changes as well as in loss of energy, which may finally affect the growth of the fish [6]. The alteration in water salinity concentrations is regarded as the main factor for fish environmental hemostasis, thus, it requires many physiological responses for returning to the stability state before subjected to the stress factor [7].The first effect of salinity appears through fish's osmotic pressure, which influence the uptake and loss processes of ions according to increasing and decreasing of water salinity [8].

It is believed that the homeostasis of water and salts balance in sweet water depends on the osmotic sensing requirements that in turn detected the osmotic organization activity for stability point [9]. Due to continuous increases in salts concentrations in many regions of Iraqi internal waters [10], and this may threat presence, growth and productivity of fish, especially the common Carp fish that considered as the first nursing fish in Iraq.Accordingly, the aim of this study was to explore the effects of dietary NaCl feeding on ions concentrations and moisture content of muscles for common Carp (*Cyprinus carpio*).

2. Materials and Methods

2.1 Fish acclimation

Thee experiment was conducting during the period from November 2016 to February 2017. Eighty four (n=84) common carp fingerlings of 20-40 g body weight were put in potassium permanganate (1.5 ppm for 1.5 hours) sterilized plastic pool filled with 500 liters. Acclimation period was continued for 15 days, and the feeding schedule was done using 30.50% protein diet, and fed as 3% of wet body weight. Fish were randomly distributed into four equal groups, with three replicates per each group. The fingerlings were distributed into 12 glass pool (7 fingerlings per pool of 30 ± 10 g average body weight). The pools dimensions were 30 × 40 × 60 cm. The pools were filled with 48 liters and ventilated using air pump for 24 hour. The thermometer was also used to control the changes in water temperature during the experimental period. Cleaning of pools was done using syphon method with maintaining the water temperature inside the pools for suitable growth rate of *C. carpio*.

2.2 Preparation of salty diets

According to Al-Saadi and Al-Kashali [10] the salty diets were prepared at the laboratory by grinding quantities of a commercial diet. The protein percentage was adjusted to 30.05 by adding a percent of fish powder. NaCl was added to the experimental diets with different percentages (1, 3 and 5%) following mixed together. The water was added at 400 ml/kg for making a homogeneous paste. Thereafter, the paste was sliced using 3 mm-specific machine and dried under direct sunlight followed by chopped into tablets with suitable length. Each diet represented salty treatment by itself in addition to the salt control one. The fish were fed with the four experimental diets as 5% of the fresh body weight 90 days experimental period. Adjustment of food quantity in each replicate was done continuously according to fish weighs fortnightly.

2.3 Determination of Ions concentrations

Ion concentrations were determined according to Eddy method [9]. Sample of fish muscle was collected, dried and grind by ceramic mortar. Thereafter, 0.1gm sample was weighted using sensitive balance and put in suitable test tube and 5 ml of nitric acid was added. The treated sample was left for 24 hours at room temperature with shaking tubes for quick extraction. The tubes were centrifuged (3500 rpm for 15 minutes).One ml of the extracted liquid was taken and distilled water was added to reach 15 ml volume. The samples were stored at -12ºC in plastic bottles until assay in unit mmol Kg^{-1} tissue water.

2.4 Water content calculation

A piece of tissue (after removing skin and scales) from the region under the Dorsal fin was taken, washed using distilled water and dried by filter paper. The tissue fresh weight was recorded using sensitive balance and dried by electrical oven gradually at the rate of 40, 60 and 80ºC until reached constant dry weight. The percentage of muscle's water was estimated using the following equation.

Muscle's water content (%) = Fresh weight (gm) -dry weight (gm) / Fresh weight (gm) × 100

2.5 The environmental factors

The temperatures ranged during the experimental period between 18-22ºC while the pH values ranged between 7.3-7.8. The soluble oxygen content ranged between 6.6-7.5 mg L^{-1}.

2.6 Statistical analysis

Statistical analysis were performed using a general liner model (GLM) procedure in the SAS program [11] to investigate the effect of salty feeding on sodium and potassium ions concentration and water content of common carp muscles. The statistical model for analysis of variance was:

$Y_{ij} = \mu + T_i + e_{ij}$

Where:

Y_{ij} = Dependent variable (Sodium and potassium concentrations and water contents).

μ = Overall mean.

T_i = Effect of salty feeding.

e_{ij} = Error term.

Differences among means were computed using the Duncan multiple range test [12].

3. Results and Discussion

Table (1) revealed increasing of sodium ion concentrations in fish tissues with increasing of salty feeding in treated groups of 1%, 3 and 5% as compared with the control group. The results showed significant increasing ($P < 0.05$) in 5% group in comparison with either the control or 1% and 3% treated groups. Moreover, both of 1 and 3% treated groups showed significantly ($P < 0.05$) higher values as compared with the control group (Table 1).

Potassium concentration recorded higher ($P<0.05$) values with increasing salty feeding from 1 to 5% concentrations. Greater ($P<0.05$) potassium concentrations were observed in 5% treated group as compared with control and the remaining groups. The 3%treated group recorded higher ($P<0.05$) potassium concentrations in comparison with the control group (Table 1). The differences between 1% treated group and control group in potassium concentrations lacked significance (Table 1). There was slowly numerical decreasing in water percentage of tissues with increasing salty feeding concentrations. However, the differences among groups in this character lacked significance (Table 1).

Table 1: Sodium and potassium concentrations (mmol Kg^{-1} tissues) and percentage of water in fish tissues for salty feeding and control groups (Mean ± SE).

Traits / Groups	Sodium conc. (mmol Kg^{-1} tissues)	potassium conc. (mmol Kg^{-1} tissues)	Water content in tissue (%)
Control	37.62±1.15 D	72.11±1.15 C	77.30±.15 A
1% Salty feeding	42.98±1.15 C	78.06±1.73 C	76.63±0.57 A
3% Salty feeding	58.18±1.73 B	96.33±2.30 B	75.24±0.13 A
5% Salty feeding	71.34±1.15 A	108.62±2.30 A	74.36±0.57 A

Means with different superscripts within each column differ significantly ($P<0.05$).

Increased sodium and potassium concentrations in fish tissues during the present study may be considered as a part of the second stage of ionic and osmotic regulations, where increasing of tissue's content of ions (muscles in particular) were noticed [13]. The salty potential resulted from increasing the salty feeding does not equally distributed among the body tissues, but concentrated in cell contents, that may regard as an important from ionic and osmotic regulation point of view [14]. Increasing tissue's sodium and potassium concentrations may due to the osmotic potential that fish faced during salty feeding. The current results agreed with the other reports reviewed the muscle's content of ions of fish following higher salty feeding. Salman [15] found increasing of potassium and sodium concentrations in tissues of Al-Trwt Al-Kazahe (*Salmo gairdneri*) fed high salty-content diet. Furthermore, Smith *et al* [16] reported that feeding of Al-Trwt Al-Kazahe (*Oncorhynchus mykiss*) on salty diet caused increasing of sodium ion concentrations in tissues as compared with fish fed on ordinary control diets.

The potassium ion concentration was kept its stable level due to its high regulation and the continuous need of potassium by the cells for protein and amino acids production inside the living cells [16]. Sultan [17] found that exposure of Al-Kashne fish (*Liza abu*) to high salty feeding caused elevation of sodium concentrations during the first 24 hours of transport, whereas, the potassium concentration remained stable. Sultan [18] showed that exposure of golden Al-Sha'am fish (*Acanthopagrus latus*) to the salty concentrations of 7, 15, 23 and 30 gmL-1resulted in increasing of Na and K concentrations in muscles from the first till the 5th day of transportation.

Gafar [19] noticed that increase the salty feeding gradually to 5, 10 and 15 gm. L^{-1} resulted in increasing of Na and K concentrations in muscles in common Carp fish. This limited tolerance of salinity by sweet water fish might returns to the narrow tolerance determined by their ability to do the processes that regulate the body water suitable with water loss. This may explain the slight decreased in water content noticed currently. The salty potential exposed by fish may disturb the osmotic regulation process [20]. If the extracellular fluids were concentrated, the tissues would be under osmotic challenge effect. This effect may cause water loss and dryness [21]. The reason behind decreasing of muscle's water content in the current study may returns to the external loss of water by diffusion through the bronchia and water movement from the cell components to the external component of cell [13]. The high gradualism in osmotic pressure between blood and the external environment induces loss of water from fish bodies to the external medium causing decrease of muscle water content [13]. These results agreed with many related studies. Salman and Eddy [15] found that slight decreasing in tissue's water content of Al-Kazane Al-Trwat fish fed on high salty diets led to the water move from the cells to the extracellular fluids. Dimaggio *et al.* [22] noticed a decreasing of water content of Seminol Kill fish tissues subjected to gradual increasing in salinity to 32 gm. L^{-1} as compared with water content in their muscles in sweet water. Martine Z-Alvarez *et al* [23] found decreased in water content of *Acipensen naccarii* fish muscles subjected to increase in salinity to 25.15 gm. L^{-1} as compared with its level in sweet water. Luz *et al* [3] revealed the significant decreasing in moisture of *Carassius auratus* fish muscles due to increasing of salinity to 10.8 mg L^{-1}, Salman *et al* [24] noticed a decreasing of water content of brown *Barbus sharpayi* fish muscles due to increased salinity to 10.9 mg. L^{-1}, namely 72.12% for saline water and 76.06 % for sweet water.The sudden increasing in the salt concentrations for brown fish resulted a significant decline in water content of muscles. Sangaio-Alvarellos *et al* [25] observed an increasing of water content in muscles of *Gillthead seabream* when subjected to 15 mg.L^{-1} salinity for 14 days. Kally and woo [26] found increasing of water content in muscles of *Mylio macrocephalus* fish that transported from 33, 12, 6 and 55 gm. L^{-1} salinity to the sweet water. In contrast, Al-Kashali [27] noticed decline in water content percentage in muscles of herbal Carp fish with increasing salt concentrations to 8 and 12 gm. $L^{-1.}$

4. Conclusion
This study showed a significant increasing (P<0.05) in sodium and potassium concentrations in fish tissues associated with increasing the feed salty. This may be considered as a part of the second stage of ionic and osmotic regulations. Moreover the effect of different concentrations of sodium and potassium was not significant on the water content of the tissue.

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