World Journal of Fish and Marine Sciences 3 (5): 396-402, 2011
ISSN 2078-4589

Effects of Sodium Selenite (Se Ion) on Feed Utilization and Some Hematological Parameters Factors of *Cyprinus carpio* Fish

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Abstract: Efficiency of increasing the nutritional value of the diets by adding selenium as microelement into diets of common carp fingerlings were studied using aquarium and cement tanks systems, which allowed feeding and continues measurement of growth parameters. In experiment (I) one hundred and fifty fingerlings each weighing approximately 7.5 ± 0.23 g was carried out, in aquaria and fed either normal diet (group A) or diets supplemented with 0.08, 0.16, 0.32 and 0.64 mg kg^{-1} of sodium selenite. In experiment (II) three hundred and sixty juvenile common carp, average weight 26.9 ± 1.38 g was carried out in cement tanks and fed as in experiment (I). When selenium added to the diets and fed to the fish caused a significant increase in weight gain and carp fingerlings growth rate was accelerated by 18- 22% feed expenditure out by 17-18% as compared to the control diet. Significant differences in survival rate were found between treatments, indicating that diets are likely to be responsible for increased survival rate, observed in fish fed diets supplemented with 0.24 and 0.32 mg kg^{-1} sodium selenite (0.12 and 0.15 mg kg^{-1} selenium). The results of this study indicated that a diet supplement, 0.24 and 0.32 mg kg^{-1} of sodium selenite (0.12 and 0.15 mg kg^{-1} selenium), is important for growth and survival of common carp.

Key words: Growth · Common carp · Mineral contents · Selenium

INTRODUCTION

In recent years, the intensification of carp fingerlings production in Iran has made it essential to develop complete and supplemental diets for use in hatcheries and nursery ponds. The quantitative dietary requirement for trace elements depends upon the amounts required for growth and reproduction and that which is unavoidably lost by the animal through gut, kidney and by passive diffusion across the gills and generally body surface. Little effort has been made to quantify the relative importance of dietary sources of trace elements in freshwater [1]. Nevertheless, freshwater fish depends on an adequate supply of minerals, as there is continuous effluent of ions from the water [2].

Selenium is required in the diet for normal growth and physiological function of fish. Deficiencies are associated with low glutathione peroxidase (GSHPx) activity, slow growth and oxidative diathesis in rainbow trout [3,4]. The dietary requirement for selenium (supplied as sodium selenite) for normal growth has been calculated as 0.38 mg / kg for rainbow trout while levels of 13 mg/kg have been found to be toxic. Selenium concentrations in different fish species support this notion [5,6]. Lobanove *et al.* [7] reported that evolutionary change from fish to mammals was accompanied by reduced use of protein containing selenium. They added, selenium-containing protein evolved in preventing heart disorder and mongolism disease, which attributed to deficiencies in selenium.

The biological availability of minerals from the diet is marked by the efficiency with which the body utilizes the dietary minerals. It varies depending on the feedstuffs and the form of the nutrient, nutrient interaction which may be either synergistic of antigenic, physiological and pathological condition of the fish, waterborne mineral concentration and the species under consideration [8,9]. Selenium supplementation is necessary for both aquaculture species fed diets based on grain and oilseed

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products such as catfish and tilapia and for species fed fishmeal based diets that do not contain adequate amounts of selenium. The most commonly used forms of selenium supplements to date have been inorganic compounds, sodium selenite and sodium selenate. Selenium from these supplements is passively absorbed from the gut and then reduced in the liver where it is incorporated into cystine to form selenocysteine.

The purpose of this study was to evaluate the growth and feed utilization of common carp fed diets supplemented with selenium.

MATERIALS AND METHODS

Feed: Five experimental diets were formulated from a basal or control diet according to El-Saidy and Gaber [10]; they contained 33.2% crude protein and gross energy 4.7 kcalg^{-1}. The energy values were calculated by using the gross energy values for the macronutrients (5.6 kcalg^{-1} protein, 9.5 k cal g^{-1} fat and 4.1 kcal g^{-1} carbohydrates). The proximate composition from the experimental basal diet is given in Table 1. The basal diet is considered as control treatment (A) and four treatments (B, C, D and E) supplemented with different levels of sodium selenite as follows: 0.0, 0.16, 0.24, 0.32 and 0.64 mg kg^{-1} were added to diets, respectively. The sodium selenite was first dissolved in water and mixed through with the basal diets.

Table 1: Feed formulation and proximate composition of basal diet.

Ingredients (%)	Diet
PPM (40.6 % C.P.)[1]	74.0
Wheat brane	14.5
Fish oil	4.0
Molasses	2.0
Vitamin & mineral premix[2]	1.4
Vitamin C[3]	0.1
Dicalcium phosphate	3.0
Amino acid supplement	
L-Methionine	0.5
L-Lysine	0.5
Proximate analysis (%)[4]	
Moisture	8.28
Crude protein	33.2
Crude fat	14.77
Ash	9.56
Crude fiber	7.47
NFE[5]	26.72
Gross energy (g kg^{-1})	4.7

1-PPM- plant protein mixture was a mixture of equal proportion of soybean meal, cottonseed meal, sunflower meal and linseed meal according to El-Saidy and Gaber [10].
2-Premix supplied according to Satoh [11].
3-Phosphiten=Mg (ascorpyl- phosphate) (Showa, Denkok, k. Tokyo, Japan).
4-Values represent the mean of three sample replicates.
5-NFE (nitrogen free extract)=100-(% moisture+ % protein + % fa + %fiber+ %ash).

Table 2: Minerals content in the basal diet for common carp.

Mineral	Diets				
	(A)	(B)	(C)	(D)	(E)
Ca	0.92	1.14	1.16	1.19	1.23
P	1.38	1.39	1.4	1.43	1.48
K	1.41	1.43	1.45	1.47	1.51
Mg	0.55	0.56	0.57	0.59	0.61
Na	0.40	0.41	0.43	0.46	0.49
Cu	4.62	4.65	4.71	4.76	4.83
Fe	160.47	161.53	163.8	167.5	174.12
Se	0.04	0.08	0.12	0.20	0.36
Zn	19.5	19.6	19.8	20.2	20.5
Mn	14.27	14.31	14.37	14.51	14.68

Ca, P, K, Mg and Na expressed as percentage and Cu, Fe, Se, Zn,and Mn as mg kg^{-1} dry matter.

The experimental diets were pelleted; frozen-dried and stored at -20°C until use. The mineral analyses of diets are given in Table 2.

Experimental Design: To study the effect of different levels of dietary sodium selenite on growth, food intake and food conversion efficiency of common carp in fresh water, two experiments were designed as shown in Table 3.

In experiment 1, common carp (*Cyprinus carpio)* was obtained from private fish farm. At the beginning of the experiment, 15 glass aquaria (80 L) were each stocked with 10 fish with an average weight of 7.5 ± 0.23 g. The aquaria were supplied with fresh water (chlorine free) at rate 250 ml/min^{-1} with supplemental aeration. The aquaria were illuminated using overhead fluorescent lights set on a 14 h light: 10 h dark cycle. Each group of fish was weighed at the beginning and every week throughout the experimental period.

In experiment 2, the experiment was conducted in 15 experimental concrete tanks. Each of the tanks was 2 m long, 2 m wide and 1.25 m high. Water levels in concrete tanks were kept at 1 m deep to maintain water volume of 4 m^3. The concrete tanks were supplied with freshwater at rate of 4 L/min^{-1} with supplemental aeration.

A set of 360 juvenile common carp, with an average weight of 26.9±1.4 g were collected from Fish Research Laboratory stock and twenty-four fish were randomly placed into each concrete tanks at stocking density of 6 fish / m^3. Each group of fish was weighed at the beginning and every week throughout the two experimental periods. The fish were fed 3% of body weight and fed 6 days /week.

Table 3: Effect of dietary selenium in practical diets of common carp (initial weight 7.5 g) on SGR, FCR, PER and Hemoglobin after 28 and 120 days of experiments I and II, Values is means ±SD.

Parameters	Diets				
	(A)	(B)	(C)	(D)	(E)
Experiment 1					
IBW(g)	7.5±0.3	7.5±0.25	7.5±0.31	7.4±0.12	7.6±0.2
FBW(g)	11.8±0.25^{c}	12.4±0.25^{b}	13.4±0.3^{a}	13.6±0.25^{a}	10.0±0.25^{d}
FI (g fish^{-1})	13.1±0.2^{b}	13.5±0.2^{b}	15.1±0.1^{a}	15.3±0.1^{a}	12.4±0.4^{c}
SGR (%day)	1.6±0.1^{b}	1.77±0.06^{b}	2.06±0.08^{a}	2.11±0.04^{a}	1.31±0.18^{c}
FCR(F/WG)	3.06±0.5^{b}	2.8±0.46^{a}	2.59±0.31^{a}	2.52±0.21^{a}	3.31±0.42^{b}
FER	32.7±0.6^{c}	35.7±0.6^{b}	38.7±0.5^{a}	39.6±0.2^{a}	27.1±2.9^{d}
PER	0.99±0.02^{b}	1.08±0.02^{b}	1.16±0.01^{a}	1.18±0.04^{a}	0.81±0.1^{c}
Hemoglobin (g%)	8.87±0.2^{ab}	8.97±0.2^{a}	9.2±0.1^{a}	9.3±0.1^{a}	8.5±0.4^{b}
TSP (%)	2.9±0.1^{bc}	3.0±0.1^{b}	3.3±0.2^{a}	2.7±0.1^{c}	2.7±0.1^{c}
Experiment 2					
IBW(g)	27.3±1.45	26.6±1.37	27.7±0.9	27.8±1.4	27.0±1.96
FBW(g)	162.5±4.^{2}d	207.1±5.6^{c}	240.7±1.91^{b}	251.3±6.78^{a}	155.3±5.06^{d}
FI (g fish^{-1})	271.4±0.02^{c}	315.7±5.59^{b}	351.5±12.05^{a}	350.1±11.76^{a}	256.5±12.8^{c}
SGR (%day)	1.62±0.02^{c}	1.84±0.08^{b}	1.93±0.03^{b}	2.0±0.07^{a}	1.56±0.06^{c}
FCR(F/WG)	2.0±0.1^{c}	1.75±0.05^{b}	1.65±0.05^{ab}	1.56±0.06^{a}	2.0±0.1^{c}
FER	50.0±2.5^{c}	57.17±1.64^{b}	60.4±2.06^{ab}	64.24±2.43^{a}	50.02±2.51^{c}
PER	1.45±0.04	1.72±0.08	1.82±0.03^{a}	1.93±0.0^{7a}	1.48±0.7^{c}
Survival (%)	77.76±2.41^{b}	77.8±2.42^{b}	97.2±4.82^{a}	98.6±2.42^{a}	66.7±4.17

Value having the same superscript letter within the same row is not significantly different (P=0.05).IBW: initial body weight; FBW: final body weight; SGR: specific growth rate; FCR: feed conversion ratio; PER: protein efficiency ratio. TPS=total serum protein.

Growth Study: At the beginning of the growth study, 25 fish were sampled and stored at -20°C for analysis of whole body minerals. At the end of the growth study, three fish from each tank were withdrawn for analyses and frozen at -20°C.

Growth performances were determined according to Cho and Kaushik [12] as follows:

- SGR (specific growth rate %) = 100 (Ln final weight - Ln initial weight) / day,
- FCR (feed conversation ratio) = dry feed intake (g) / wet weight gain (g),
- FER (feed efficiency ratio) =wet weight gain (g)/dry feed intake (g),
- PER (protein efficiency ratio) = weight gain (g) / protein intake (g).

Hematological Assay: Blood samples were obtained from fish at the end of the experimental period. Four fish/group were randomly chosen and anesthetized with tricane methanesulfonate (MS-222, (Argent Chemical Redmond, WA) at 125 mg/l. Blood samples were collected from the caudal vein using heparinized 27- gauge needles and tuberculin syringes (20 units/ml) for determination of hemoglobin (Hb) and total serum protein (TSP). Hemoglobin was determined by the total hemoglobin kit (Sigma Diagnostics, Sigma, St. Louis, MO) which is a standardized procedure of cyanomethemoglobin method.

Chemical Analyses: Analysis of samples were made as follows: dry matter after desiccation in an oven (105°C for 24 h), ash (incineration at 550°C for 12 h), crude protein (micro kjeldahl, N_6.25), crude lipid (ether extract by Soxlhet method), crude fiber [13] and gross energy (Ballistic_bomb calorimeter, Gallenkamp, England).

The minerals analyses were determined in the diets and whole fish in second experiment. Samples were prepared for analyses using a dry-wet-dry ashing procedure [13]. In brief, samples weighed in dry porcelain crucible were dried in an oven. Dry matter was determined and crucibles with dry samples were placed in an ashing oven (400°C) for 24 h, removed and allowed to cool. Samples were moistened with distilled water, then nitric acid (9M) was added and were gently heated on an electric hotplate to evaporate the liquid until only residual remained. Crucible were placed in the ashing oven for additional 1 h, then removed and allowed to cool. Ashes samples were solubilized in 6 N HCL and brought to volume with distilled water to a ratio of 1:5 of acid water. Appropriate dilutions were prepared to bring mineral

concentration within standard reading range. Ionic composition of diets and whole fish were measured by Atomic Absorption spectrophotometer.

Water Quality: Water temperature and dissolved oxygen were measured every other day using titration method. Total ammonia, nitrite and nitrate were measured using spectrophotometer (Spectronic 601, USA). Alkalinity was monitored twice-weekly using titration method of Golterman *et al.* [14]. pH was monitored daily using an electronic pH meter (pH pen, Fish Scientific Cinicinneti, Ohio, USA).

Calculations and Statistical Analysis: Calculations of growth parameters were conducted according to Cho and Kaushik [12]. Statistical analyses were carried by one–way ANOVA and the comparison between the treatments was made using the Duncan's multiple range tests (Statistical for Windows, release 12.21 Minitab, 1998 edition). The significance of differences was tested at $P<0.05$. All percentage and ratio were transformed to arcsine values prior to analysis [15].

RESULTS

Water Quality: In experiments 1 and 2, water temperature ranged from 27.5 to 28.3°C, DO from 4.5 to 5.1 mg L^{-1}, pH from 7.7 to 8.1, alkalinity from 170 to 184 mg L^{-1} and total ammonia from 0.02 to 0.03 mg L^{-1}. There were no significant differences in the water quality parameters among the treatments during the whole two experiments period. The water quality parameters were found to be within the acceptable range for carp growth [16].

Growth Performance: In experiment 1, growth experiment period was 28 days (Table 3), which indicated that fish fed on diets C and D supplemented with 0.24 and 0.32 mg kg^{-1} sodium selenite gained more weight than those fed control diet and other diets. Numerically higher growth rate was obtained when fish fed on diet D, supplemented with 0.32 mg kg^{-1} sodium selenite, although it was not significantly different from that achieved by, the fish fed on diet B contained 0.24 mg kg^{-1} sodium selenite (Table 3). Poorest growth was recorded for fish fed on diet E, supplemented with 0.64 mg kg^{-1} sodium selenite, which was significantly different from diet D (15 mg kg^{-1} selenium) although it was not significantly different from the control. Differences in specific growth rate (SGR) were found to be significant (P=0.05) between control diets and those fed on 0.24 and 0.32 mg kg^{-1} sodium selenite (0.12 and 0.15 mg kg^{-1} selenium) (Table 3).

A great deal of variation in feed intake (FI) was found in all treatments. The fish fed the control diet and diet (E) showed lower FI than those recorded in fish fed on diet (C) and (D). Statistical analysis (Table 3) showed that there were a significant (P=0.05) differences in feed intake among all dietary treatments. It appeared that fish intake was affected by the addition of sodium selenite to the diets and in fact, 0.24-0.32 mg kg^{-1} sodium selenite seemed to stimulate fish growth.

Supplemental sodium selenite in the diets increased feed efficiency ratio (FER) so that weight gain produced per unit weight of food consumed was higher for diets supplemented with sodium selenite than control diet. Significantly, (P=0.05) higher value of 38.5 and 39.6% were recorded for fish fed on diets C and D, respectively compared with average value of 32.7% recorded for control diets. Feed conversion ratio (FCR) decreased progressively with increasing dietary sodium selenite levels and reach maximum at diet D then decreased at diet (E).

Protein efficiency ratio (PER) value, calculated to assess the effect of differences in protein intake among fish fed with the control diet or diets supplemented with sodium selenite, showed marked differences in their protein contents. Like FER value, differences in PER values among dietary treatments were significant (P=0.05) (Table 3), indicating that weight gain per unit of protein intake is different in all treatments. The differences in levels of sodium selenite supplement to diets are likely to be responsible for the increased feed efficiency ratio observed in fish fed diets C and D supplemented with 0.24 and 0.32 mg kg^{-1} sodium selenite.

Hemoglobin content increased with increasing dietary sodium selenite levels and significant (P=0.05) differences was found between 0.12 and 0.30 mg kg^{-1} selenium). Total serum protein increased with increasing sodium selenite levels and reach maximum at diet C (contained 0.24 mg kg^{-1} sodium selenite or 0.12 kg^{-1} selenium) which is significantly different from diet D.

In experiment 2, 120 days growth experiment (Table 3 and Figure 1) indicated that fish fed on diet (C) and (D) supplemented with sodium selenite, showed the same trend as in experiment 1, for final fish weight, SGR, FER and PER. There are significant (P=0.05) differences of FBW, SGR, FER and PER that, fish fed on diet (D) showed maximum growth rate. Significant (P=0.05) differences in survival rate were found between treatments (Table 3),

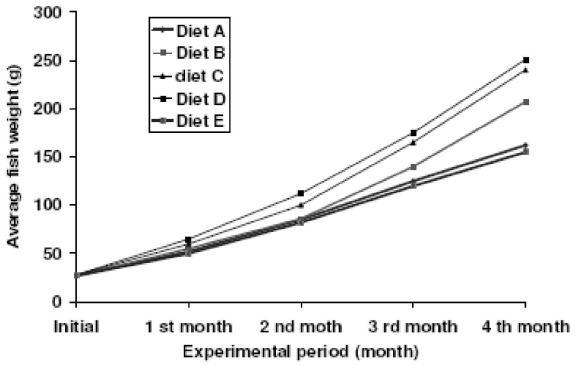

Fig. 1: Change in average body weight (g/fish) of common carp, *Cprinus carpio* fed five different diets (see Table 4).

indicating that diets are likely to be responsible for increased survival rate, observed in fish fed diets supplemented with 0.24 and 0.32 mg kg^{-1} sodium selenite (0.12 and 0.15 mg kg^{-1} selenium).

Comparison of the growth common carp in the two experiments showed that fish fed on diets supplement with 0.24 and 0.32 mg kg^{-1} sodium selenite (0.12 and 0.15 mg kg^{-1} selenium) had a determinable effect on fish growth of experiment 2 because culture fish in intensified culture requires more trace elements.

DISCUSSION

We demonstrated that selenium had an additive effect on the growth of common carp fingerlings and juvenile. A plant protein mixture was used in the present study, on least cost-nutrient basis according to El-Saidy and Gaber [10] without a detrimental effect on growth. Significant differences in growth rate of common carp occurred, when fish fed on diets supplemented with 0.24 and 0.32 mg kg^{-1} sodium selenite (0.12 and 0.15 mg kg^{-1} selenium). Reduction in growth rate associated with lower and higher selenium supplemented with 0.16 and 0.64mg kg^{-1} sodium selenite (0.12 and 0.15 mg kg^{-1} selenium) in the diets. These agree with Wang *et al.* [17] who reported that, there was significant difference in relative gain rate (RGR) of trial supplemented with sodium selenite with control (basal diet) because diets rich in selenium may slow the rate of calories burns.

In our experiments, the optimum selenium levels were 0.24 and 0.32 mg kg^{-1} sodium selenite or 0.12 and 0.15 mg kg^{-1} selenium in diets for common carp in both experiments, where maximum growth was achieved and higher level of 0.64 mg kg^{-1} sodium selenite, or 0.30 mg kg^{-1} selenium growth was reduced. High levels of selenium in the diets have a toxic effects resulting in reduced growth, feed efficiency and increased mortality as reported by Hilton and Hodson [18] which showed that trout receiving over dose of selenium developed renal calcinosis.

In addition, Baines *et al.* [19] reported that, bioaccumulations selenium was almost exclusively through dietary uptake and fish weight was affected by selenium levels. Weight gains of common carp significantly affected levels of minerals in their body (Table 4). This was also partly the result of low levels of some essential elements in the diets, for instance, selenium and manganese. In the present study, selenium came rather close to the levels required for optimal physiological indicator [11]. Also Wang *et al.* [17] decided that fish fed basal showed lower selenium content in muscle compared to fish fed selenium

Table 4: Effect of dietary selenium on minerals content in common carp muscle (experiment 2). Values are means ±SD.

Minerals	Diets				
	(A)	(B)	(C)	(D)	(E)
Ca	4.74±0.05^{e}	5.05±0.03^{d}	5.57±0.04^{c}	5.94±0.04^{b}	6.05±0.02^{a}
P	2.21±0.06^{d}	2.29±0.06^{c}	2.41±0.05^{b}	2.57±0.04^{a}	2.67±0.07^{a}
K	1.14±0.02^{b}	1.15±0.06^{b}	1.16±0.08^{ab}	1.2±0.02^{a}	1.06±0.02^{c}
Mg	0.12±0.02^{d}	0.15±0.01^{b}	0.17±0.01^{a}	0.17±0.01^{a}	0.14±0.02^{c}
Na	0.62±0.02^{c}	0.67±0.01^{b}	0.7±0.02^{ab}	0.71±0.02^{a}	0.62±0.02^{c}
Cu	4.3±0.1^{d}	4.7±0.1^{c}	5.1±0.1^{b}	5.4±0.1^{a}	4.3±0.1^{d}
Fe	110.9±4.1^{c}	120.4±4.9^{bc}	125.3±5.3^{b}	130.4±4.8^{ab}	136.23±8.3^{a}
Se	1.04±0.03^{e}	1.13±0.02^{d}	1.23±0.02^{c}	1.29±0.02^{b}	1.43±0.02^{a}
Zn	102.4±1.01^{e}	112.3±1.1^{d}	127.6±1.86^{b}	136.7±2.2^{a}	122.3±0.85^{c}

Value having the same superscript letter within the same row is not significantly different (P= 0.05). IBW: P, K, Ca, Na and Mg expressed as percentage and Fe, Ma, Cu, Se and Zn as mg kg^{-1} of dry matter.

supplements. In fact moderate levels of selenium (0.24 and 0.32 mg/kg^{-1} sodium selenite or 0.12 and 0.15 mg/kg^{-1} selenium) seemed to have a beneficial effect on the growth of common carp and such an effect has been previously suggested by Poston and Combs [20] to promote growth Atlantic salmon. Also Pope *et al.* [21] reported that dietary selenium levels of 0.15 mg kg^{-1} are commonly used in salmon diet.

In view of the present study, extra selenium (0.64 mg kg^{-1} sodium selenite or 0.30 mg kg^{-1} selenium) incorporated in common carp diets could be compensated by decreasing the feeding level and the growth rate. There is similar observation by Gatlin *et al.* [22]. For Atlantic salmon, they reported, high levels of selenium (levels above 0.13-0.15 mg kg^{-1}) in the diet have toxic effect, resulting in increased mortality. These results are in agreement with our results in second experiment, where there was increased mortality.

From the above discussion, it may be concluded that the reduced growth performance of common carp fed diets supplemented with 0.64 mg kg^{-1} sodium selenite or 0.30 mg kg^{-1} selenium might be because it is compensated by decreasing the feeding consumption and growth rate. The significantly better growth of fish fed diets supplemented with 0.24 and 0.32 mg kg^{-1} sodium selenite or 0.12 and 0.15 mg kg^{-1} selenium makes it necessary to increase growth rate more than 18- 22% and feeding expenditure by 12-18% lead to increasing food utilization.

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