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Efficiency of Selenium Ion Inclusion into Common Carp (*Cyprinus carpio* L.) Diets

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Abstract: Efficiency has been investigated of the increase diets nutritional value by adding selenium as microelement into diets Common carp fingerlings were studied using aquarium and cement tanks systems, which allowed feeding and continues measurement of growth parameters. In experiment 150 fingerlings each weighing approximately 7.5±0.23 g was carried out in aquarium and fed either normal diet (A. group) or diets supplemented with 0.08, 0.16, 0.32 and 0.64 mg kg^{-1} of sodium selenite. In experiment 360 juvenile common carp, average weight 26.9±1.38 g was carried out in cement tanks and fed as in experiment. 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. There are 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 diet supplemented with 0.24 and 0.32 mg kg^{-1} $Na_2SeO_3.5H_2O$. The results of this study indicated that a diet supplemented, 0.16 and 0.32 mg kg^{-1} of sodium selenite is important for growth and survival of common carp.

Key words: Selenium, sodium selenite, common crap, efficiency, diets

INTRODUCTION

In recent years, the intensification of carp fingerlings production in Egypt 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 effect has been made to quantify the relative importance of dietary sources of trace elements in freshwater (Watanabe *et al*., 1997). Nevertheless, fresh-water fish depends on an adequate supply of minerals as there is continuous effluent of ions from the body (Cowey and Sargent, 1979) traditionally; Fish Meal (FM) has been main source of protein in diets for fish fry and fingerlings. However, the increasing cost of FM has restricted its use as a protein source for fry diets. The use of plant protein source in diets for fingerlings production leads to change trace elements balance of diets (NRC, 1993). Because FM contained adequate quantity of selenium and the use of plant protein lead to decreasing trace element supplements.

The biological availability of minerals from the diet is marked by the efficiency with which the body utilizes the dietary minerals. It varies depends 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 (Watanabe *et al*., 1997; Hilton, 1989; Steffens, 1989).

The purpose of this study was to evaluate the growth, feed utilization and economics value 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 (2003) contained 33.2% crude protein and gross energy 4.7 kcal g^{-1}.The energy values was calculated by using the gross energy values for the macronutrients (5.6 kcal g^{-1} protein, 9.5 kcal fat and 4.1 kcal carbohydrate). The proximate composition from the experimental basal diet is given in Table 1. The basal diet is considered control diets (A) and four diets (B, C, D and E) supplemented with different levels of sodium selenite as following 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. The experimental diets were pelleted; freeze dried and stored at -20°C until use. The mineral analyses of diets are given in Table 2.

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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
Vit. And min premix[2]	1.4
Vitamin C[3]	0.1
Dicalcium phosphate	3.0
Amino acid supplement	
Methionine	0.5
Lysine	0.5
Total	100.0
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 (2003), 2-Premix supplied according to Satoh (1991), 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 +%fat +% fiber+ %ash)

Table 2: Mineral content in the basal diet for common carp

	Diets				
Mineral	(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	0.2	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 (%) and Cu, Fe, Se, Zn and Mn as mg kg^{-1} dry matter

Table 3: Effect of dietary selenium in practical diets of common carp (initial weight 7.5g) on SGR, FCR, PER and Hemoglobin after 30 days of experiment, Values is means±SD

	Diets				
Parameters	(A)	(B)	(C)	(D)	(E)
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(gfish^{-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}

Value having the same superscript letter within the same row is not significantly different ($p \leq 0.05$).IBW: initial body weight; FBW: Final Body Weight; SGR: Specific Growth Rate; FCR: Feed Conversion Ratio; PER: Protein Efficiency Ratio. TPS = Total Serum Protein

Experimental design: To study the effect of dietary $Na_2SeO_3.5H_2O$ on growth, food intake and food conversion efficiency of common carp in fresh water. Two experiments were designed as shown in Table 3.

Experimental fish: In experiment 1: Common carp (*Cyprinus carpio* L.) were obtained from Fish Research Laboratory stock (Faculty of Agriculture, University of Menofiya, Egypt). At the beginning of the of the experiment, 15 glass aquaria (80 L) were each stocked with 10 fish with an average weight 7.5±0.23 g. The aquaria were supplied with fresh (chlorine free) at rate of 250 mL min^{-1} with supplemental aeration. The aquaria were illuminated using overhead fluorescent lights was 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 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 supplied with fresh at rate of 4 L min^{-1} with supplemental aeration.

A set of 360 juvenile common carp, with an average weight 26.9±1.38 g were collected from Fish Research Laboratory stock and 24 fish were randomly placed into each concrete tanks at stocking density 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.

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 (1985) as following: SGR (Specific Growth Rate %) = 100 (Ln final weight - Ln initial weight)/days. 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 experimental period. Four fish/group were randomly chosen and anesthetized with tricane Methanesulfonate (MS-222, Argent Chemical Redmond, WA) at 125 mg L^{-1}. Blood samples were collected from the caudal vein using heparinized 27-gauge needles and tuberculin syringes (20 units mL^{-1}) for determination of Hemoglobin (Hb) and total serum protein.

Hemoglobin was determined by the total hemoglobin kit (Sigma Diagnostics, Sigma, St. Louis, MO) which is 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 (AOAC, 1995) and gross energy (Ballistic bomb calorimeter, Gallenkamp, England).

The minerals analyses were determined 1n the diets and whole fish in second experiment. Samples were prepared for analyses using a dry-wet-dry ashing procedure (AOAC, 1995). 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. Ashed samples were solubilized in 6 N HCL and brought to volume with distilled water to a ratio of 1:5 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 (Perkin-Elmer, Uberlingen, Germany.

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.* (1978) 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 (1985). 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 (Zar, 1986).

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 0.3 to 0.33 mg L^{-1}. There were no significant differences in the water quality parameters

Table 4: Effect of dietary selenium in practical diets of common carp (initial weight 26.9g) on SGR, FCR, PER and survival percentage after 120 days of experiment, Values is means±SD

	Diets				
Parameters	(A)	(B)	(C)	(D)	(E)
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(gfish 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±0.07^{a}	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}	6666±4.17^{b}

Value having the same superscript letter within the same row is not significantly different ($p\leq0.05$).IBW: Initial Body Weight; FBW: Final Body Weight; SGR: Specific Growth Rate; FCR: Feed Conversion Ratio; PER: Protein Efficiency Ratio

Table 5: Effect of dietary selenium in practical diets of common carp (initial weight 26.9g) on, Values is means±SD.

	Diets				
Minerals	(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±0.02d	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±0.02d	1.23±0.02^{c}	1.29±0.02^{b}	1.43±0.02^{a}
Zn	102.4±1.01^{e}	112.3±1.1d	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 ($p\leq0.05$).IBW: P, K, Ca, Na and Mg expressed as percentage and Fe, Ma, Cu, Se and Zn as mg kg^{-1} of dry matter

among the treatments during the whole two experiments period. The water quality parameters were found to be within the acceptable range for carp growth (Stickney, 1979).

Growth performance: In experiment 1: 30 days growth experiment (Table 3) indicated that fish fed on diets C and D supplemented with 0.24 and 0.32 mg kg^{-1} $Na_2SeO_3.5H_2O$ gained more weight than those fed on control diet and diet E (Table 4). Numerically higher growth rate when fish fed on diet D, supplemented with 0.32 mg kg^{-1} $Na_2SeO_3.5H_2O$, 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 4). 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, although it was not significantly different from the control. Differences in Specific Growth Rate (SGR) were found to be significant ($p\leq0.05$) between control diets and those fed on 0.24 and 0.32 mg kg^{-1} $Na_2SeO_3.5H_2O$ (Table 4).

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

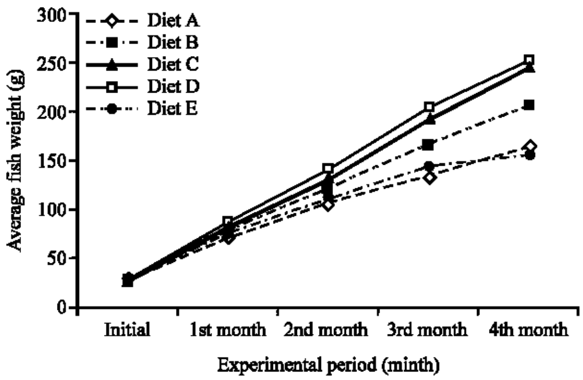

Fig. 1: Change in average body weight (g/fish) of common carp, *Cprinus carpio* fed five different diets (Table 4)

on diet (C) and (D). Statistical analysis (Table 4) showed that there was a significant ($p \leq 0.05$) difference in feed intake between 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} $Na_2SeO_3.5H_2O$ seemed to stimulate fish growth.

Supplemental $Na_2SeO_3.5H_2O$ 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 $Na_2SeO_3.5H_2O$ than control diet. Significantly ($p \leq 0.05$) higher value of 38.5and 39.6% were recorded for fish fed on diets C and D, respectively. Compared with average value of 32.65% 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 were calculated to assess the effect of differences in protein intake among fish fed the control diet or diets supplemented with $Na_2SeO_3.5H_2O$, showed a marked differences in their protein contents. Like FER value, differences in PER values between dietary treatments were significant ($p \leq 0.05$) (Table 4) indicating that weight gain per unit of protein intake is different in all treatments. The differences in levels of $Na_2SeO_3.5H_2O$ 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} $Na_2SeO_3.5H_2O$,

Hemoglobin content increased with increasing dietary sodium selenite levels and significant ($p \leq 0.05$) differences was found between 0.08-0.12 and 0.0-0.4 mg kg^{-1} sodium selenite. Total serum protein increased with increasing sodium selenite levels and reach maximum at diet C (contained 0.08 mg kg^{-1} sodium selenite) which significantly different from diet D.

In experiment 2: 112 days growth experiment (Table 5 and Fig. 1) indicating that fish fed on diet (C) and (D) supplemented with $Na_2SeO_3.5H_2O$, showed the same trend as in experiment É, for final fish weight, SGR, FER and PER.

There are significant ($p \leq 0.05$) differences in survival % were found between treatments (Table 4), indicating that diets are likely to be responsible for increased survival %, observed in fish fed diet s supplemented with 0.24 and 0.32 mg kg^{-1} $Na_2SeO_3.5H_2O$.

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} $Na_2SeO_3.5H_2O$.had a determinable effects on fish growth of experiment two because culture fish in intensifies culture require more trace elements

DISCUSSION

We demonstrated that selenium had an additive effect on growth 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 (2003) 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} $Na_2SeO_3.5H_2O$. Reduction in growth rate associated with lower and higher selenium supplemented with 0.16 and 0.64 mg kg^{-1} $Na_2SeO_3.5H_2O$.in diets Common carp during feeding trials (Table 3 and 4) were pronounced in comparison with studies carried out with (Poston and Combs, 1979) reported that, when Atlantic salmon were fed a selenium deficient diet for 26 weeks the deficiency signs recorded were lethargy, loss appetite, reduced muscle tone and increased mortality. In addition, they recorded that the best growth was achieved at selenium level of 0.15 mg kg^{-1}. In our experiments, the optimum selenium levels were 0.24 and 0.32 mg kg^{-1} $Na_2SeO_3.5H_2O$ in diets for common carp, when maximum growth was achieved and higher level of 0.64 mg kg^{-1} $Na_2SeO_3.5H_2O$, growth was reduced. High levels of selenium in the diets have a toxic effects resulting in reduced growth, feed efficiency and increased mortality. Also Hilton and Hodson (1983) reported that trout receiving over dose of selenium developed renal calcinosis.

The selenium level in plant meal-based diet was particularly low (Table 3). Because, there was indication of a decrease in whole-body selenium was found (Table 5). Long-term feeding with deficient in trace elements specially selenium lead to cataracts and short body dwarfism in juvenile fish (Watanabe *et al.*, 1980).

Weight gains of common carp significantly affected levels of minerals in their body (Table 5). 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 (Satoh, 1991). In fact moderate levels of selenium (0.24 and 0.32 mg kg^{-1} $Na_2SeO_3.5H_2O$ or 0.12 and 0.15 mg kg^{-1} selenium) seemed to be have a beneficial effect on growth common carp and such an effect has been previously suggested by Poston and Combs (1979) to promote growth Atlantic salmon. Also Pope *et al*. (1986) 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} $Na_2SeO_3.5H_2O$ or 0.3 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*. (1986) 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 increased mortality. These results are agree with our results in second experiment, where increased mortality.

Overall, the results indicated that fish fed diet supplemented with0.24 and 0.32 mg kg^{-1} $Na_2SeO_3.5H_2O$ or 0.12 and 0.15 mg kg^{-1} selenium would be responsible to increase growth rate more than 18-22% and feed expenditure out by 12-18% as compared to control diet.

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