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Effect of Zinc-L-selenomethionine and Chromium-L-methionine on Growth Performance, Blood Glucose and Digestive Tract pH in Nile Tilapia (*Oreochromis niloticus*)

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Abstract

Dietary effects of Zinc-L-selenomethionine (Zn-L-Se) and Chromium-L-methionine (Cr-L-met) on growth performance, blood glucose and digestive tract pH were investigated in juvenile fingerling Nile Tilapia (*Oreochromis niloticus*). The trial was a CRD with 6 treatments and 20 replications. Fish were fed isonitrogenous and isocaloric diets with 36% crude protein and 6% lipid. The Control diet (T1) - was without Zn-L-Se or Cr-L-met addition. Remaining treatments were as follows: T2, - Control + Cr-L-met at 0.5 ppm; T3, - Control + Cr-L-met at 1.0 ppm; T4, - Control + Zn-L-Se at 0.3 ppm; T5, - Control + Zn-L-Se at 0.3 ppm + Cr-L-met at 0.5 ppm; and T6, - Control + Zn-L-Se at 0.3 ppm + Cr-L-met at 1.0 ppm. Nile tilapia with an average weight of 25 to 30 g were stocked in 100-L tanks at a density of 1 fish/tank/replicate, with 20 fish/treatment to reduce stress. Fish were fed experimental diets 2time/day at 3 to 4% body weight for 4 weeks. Growth performance, in terms of final weight, weight gain, average daily growth and feed conversion ratio differed by treatment ($P < 0.05$). Immunity, in terms of superoxide dismutase, glutathione peroxidase, lysozyme activity, and blood glucose showed no significant differences ($P > 0.05$). Digestive tract pH was not affected by treatment ($P > 0.05$). Adding Zinc-L-selenomethionine (Zn-L-Se) at 0.3 ppm and Chromium-L-methionine (Cr-L-met) at 0.5 to 1.0 ppm to the diet of fingerling Nile Tilapia improved growth performance.



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Introduction

The goal of aquaculture is to achieve efficient growth within the shortest possible time. During the culture period, many factors cause animal stress, such as stocking density, water quality, environment, temperature, disease, handling, etc. Mineral supplementation is one way to help enhance immunity, reducing energy allocation to stress compensation and subsequent losses in growth and muscle development, resulting in better growth and higher production efficiency. Chromium plays an important role in carbohydrate, protein, and lipid metabolism. It is a key component of glucose tolerance factor (GTF) that helps increase insulin efficiency by enhancing blood glucose uptake into cells for energy or conversion into reserves (Cheva-Isarakul et al., 2004; Hackney, 2018). Selenium plays an important role in growth, antioxidant, and reproductive functions. Selenium also acts in the conversion of thyroid hormones to their active form, a key to the control of metabolic rate. Cellular antioxidant processes require selenoproteins and glutathione peroxidase, which incorporate selenium. Working with vitamin E, selenium helps eliminate free radicals produced during the oxidation of unsaturated fats (McDowell, 2003). In this study, we investigated the effects of Chromium-L-methionine and Zinc-L-selenomethionine in the diets of juvenile fingerling Nile tilapia on growth performance, immunity, and digestive tract pH.

Objectives

To study the effect of chromium and selenium in the diet on growth performance, blood glucose and digestive tract pH in Nile Tilapia (*Oreochromis niloticus*).

Materials and Methods

Fish

Fingering Nile tilapia were obtained from a private fish farm in Phetchaburi Province, Thailand. Fish were acclimated for 2 weeks in a 1000-L tank, and fed a commercial diet two times daily. Following the acclimation phase, fish were graded and randomly assigned to experimental treatments.

Diet preparation

Experimental diets included a Control diet without Zn-L-Se or Cr-L-met addition (T1), Control diet + Cr-L-met at 0.5 ppm (T2), Control diet + Cr-L-met at 1.0 ppm (T3), Control diet



+ Zn-L-Se at 0.3 ppm (T4), Control diet + Zn-L-Se at 0.3 ppm + Cr-L-met at 0.5 ppm (T5), and Control diet + Zn-L-Se at 0.3 ppm + Cr-L-met at 1.0 ppm (T6). All diets were produced by a mincer machine (small, low-pressure extruder). Ingredient composition of the diets (Table 1) were ground through a 500 micron screen, mixed, then blended with 25% water. Mash feed was then passed through a mincer machine, dried at 75°C for 6 hr, then cooled and stored in plastic bags at room temperature. Test diets were a sinking feed formulation produced in a 3.0 mm diameter cylindrical shape.

Table 1: Ingredient composition of experimental diets.

Raw material (%)	T1	T2	T3	T4	T5	T6
Soybean meal	40.0	40.0	40.0	40.0	40.0	40.0
Wheat flour	25.0	25.0	25.0	25.0	25.0	25.0
Wheat gluten	8.0	8.0	8.0	8.0	8.0	8.0
De-oiled rice bran	6.6	6.6	6.6	6.6	6.6	6.6
Dried brewer's yeast	5.0	5.0	5.0	5.0	5.0	5.0
Poultry meal (65% CP)	3.0	3.0	3.0	3.0	3.0	3.0
Poultry meat meal (Hydrolyse; 70% CP)	2.0	2.0	2.0	2.0	2.0	2.0
Fish oil	4.0	4.0	4.0	4.0	4.0	4.0
Soybean oil	0.5	0.5	0.5	0.5	0.5	0.5
L-Lysine	0.5	0.5	0.5	0.5	0.5	0.5
DL-Methionine	0.4	0.4	0.4	0.4	0.4	0.4
L-Threonine	0.1	0.1	0.1	0.1	0.1	0.1
Salt	0.5	0.5	0.5	0.5	0.5	0.5
Monocalcium phosphate	3.0	3.0	3.0	3.0	3.0	3.0
Calcium carbonate	0.7	0.7	0.7	0.7	0.7	0.7
M-vitamins	0.4	0.4	0.4	0.4	0.4	0.4
M-minerals	0.8	0.8	0.8	0.8	0.8	0.8
Cr (Cr-L-methionine; 1000 ppm)	0.0	0.5	1.0	0.0	0.5	1.0
Se (Zn-L-Selenomethionine; 1000 ppm)	0.0	0.0	0.0	0.3	0.3	0.3

Remark: M-minerals is the commercial mix of trace mineral including zinc, manganese and copper.

Experimental design

This study was a Completely Randomized Design (CRD) with 6 treatments and 20 replicates per treatment. Fish were fed experimental diets two times daily at 3 to 4% body



weight for 4 weeks. At the end of the experiment all fish in each tank were anesthetized to evaluate growth performance and immune response.

Data collection

At the end of the 4-week experiment, all fish from each treatment were collected to study the following parameters:

Growth performance

Recorded initial weight, final weight, and feed consumption for use in calculation of weight gain, average daily growth, feed intake, feed conversion ratio, and survival rate.

Immune response

Following the morning feeding at the end of the 4-week study, blood samples were drawn at 15-, 30-, 60-, and 90-minutes. Blood was used to determine blood glucose by glucose meter, while serum was collected to evaluate superoxide dismutase activity, glutathione peroxidase, and lysozyme activity by Sigma kit.

Digestive tract pH

After the 15-, 30-, 60-, and 90-minute postprandial blood and serum collections were complete, all fish gastrointestinal tracts were dissected into 4 parts. Segments of the tracts were separated into stomach, foregut, midgut, and hindgut. The digestive tract pH was determined by pH paper.

Statistical analysis

Results were presented as means \pm standard deviation. A Completely Randomized Design (CRD) was utilized, as all data was analyzed by one-way ANOVA (analysis of variance). Tukey's procedure was used for multiple comparison of differences between treatment means. Differences were regarded as significant when $P < 0.05$. Alphabetical notation was used to identify significant treatment differences at an alpha 0.05.

Results and Discussions

Growth performance

Growth performance and feed utilization of Nile tilapia fed experimental diets were presented in Table 2. Results show growth performance in terms of final weight, weight gain,



average daily growth, and feed conversion ratio are significantly different ($P < 0.05$), whereas feed intake and survival rate show no significant difference among treatment groups ($P > 0.05$). Treatment 5, Cr-L-met at 0.5 ppm and Zn-L-Se at 0.3 ppm, showed the highest potential for improved growth performance. From the collaboration of chromium and selenium. Chromium plays a key role in glucose metabolism by acting as a co-factor for insulin and its role in glucose metabolism. That helps increase insulin efficiency by enhancing blood glucose uptake into cells for energy or conversion into reserves. While selenium helps reduce stress by neutralizing excess free radicals and protecting cells from damage caused by oxidative stress. Shiao and Lin (1993) reported 2 ppm chromium chloride could improve body weight. Likewise, Lee et al. (2016) reported 1 to 2 ppm selenomethionine could improve weight gain and feed conversion ratio.

Table 2: Growth performance of Nile tilapia fed experimental diets for 4 weeks (Mean \pm SD)

Treatment	IBW (g/fish)	FBW (g/fish)	WG (g/fish)	ADG (g/fish/d)	FI (%/fish/d)	FCR	SR (%)
T1	26.75 \pm 2.20	48.78 \pm 3.94 ^a	22.03 \pm 3.40 ^a	0.79 \pm 0.12 ^a	1.67 \pm 0.13	1.05 \pm 0.15 ^b	100.00 \pm 0.00
T2	26.75 \pm 2.05	52.68 \pm 3.49 ^b	25.93 \pm 3.75 ^b	0.93 \pm 0.13 ^b	1.56 \pm 0.11	0.90 \pm 0.11 ^a	100.00 \pm 0.00
T3	26.75 \pm 2.31	52.39 \pm 3.88 ^{ab}	25.64 \pm 3.69 ^b	0.92 \pm 0.13 ^b	1.60 \pm 0.15	0.93 \pm 0.15 ^a	100.00 \pm 0.00
T4	26.75 \pm 2.05	52.20 \pm 4.20 ^{ab}	25.45 \pm 3.61 ^{ab}	0.91 \pm 0.13 ^{ab}	1.61 \pm 0.16	0.93 \pm 0.14 ^{ab}	100.00 \pm 0.00
T5	26.75 \pm 2.12	54.02 \pm 3.86 ^b	27.27 \pm 3.60 ^b	0.97 \pm 0.13 ^b	1.62 \pm 0.16	0.90 \pm 0.12 ^a	100.00 \pm 0.00
T6	26.75 \pm 1.92	52.88 \pm 5.45 ^b	26.13 \pm 4.95 ^b	0.93 \pm 0.18 ^b	1.64 \pm 0.11	0.95 \pm 0.14 ^{ab}	100.00 \pm 0.00
P-value	1.000	0.005	0.002	0.002	0.201	0.006	1.000

Note: Data without superscript letters in the same row indicates no significantly difference ($P > 0.05$).

IBW, Initial body weight; FBW, Final body weight; WG, Weight gain; ADG, Average daily growth; FCR, Feed conversion ratio; SR, Survival rate.

Immune response

Examination of Nile tilapia immunity (Table 3) by evaluating superoxide dismutase, glutathione peroxidase, and lysozyme activity showed no significant differences among treatment groups ($P > 0.05$). Treatment groups T2 through T6 had numerically higher glutathione peroxidase activity than the Control group, showing similar results as Pacini et al. (2013), who reported selenium cysteine effects at 1.25 to 20 ppm in Siberian sturgeon. Ilham et al. (2016) reported 2 ppm selenomethionine in Barramundi and Xia et al. (2019) reported 5 to 20 ppm chitosan-selenium nanoparticle in Zebrafish could promote glutathione activity.



Table 3: Immunological parameters of Nile tilapia fed experimental diets for 4 weeks (Mean±SD)

Treatment	Superoxide dismutase (% inhibition)	Glutathione peroxidase (nM)	lysozyme activity (units/ml)
T1	152.46±56.69	7.94±2.62	92.09±20.88
T2	132.00±35.35	8.49±2.68	80.46±19.94
T3	149.13±59.76	9.17±3.91	97.46±32.18
T4	126.25±41.02	8.16±2.06	93.33±26.51
T5	119.90±32.27	9.62±4.51	89.93±25.67
T6	146.88±67.23	9.85±3.33	100.66±24.15
P-value	0.384	0.447	0.343

Note: Data without superscript letters in the same row indicates no significantly difference ($P>0.05$).

Blood glucose of Nile tilapia fed experimental diets showed no significant differences among treatment groups ($P > 0.05$) in Table 4. Maximum blood glucose was achieved 30 minutes post-feeding, followed by glucose clearance nadir at 60 minutes post-feeding. Furthermore, at 90 minutes post-feeding, blood glucose began to return to normal maintenance levels. Castro *et al.* (2014) reported 18 ppm and 18 to 36 ppm chromium carbochelate could reduce blood glucose in Nile tilapia and Pacu fish, respectively.

Table 4: Blood glucose of Nile tilapia 15-, 30-, 60- and 90-minutes post-feeding of experimental diets (Mean±SD)

Treatment	0 minutes	15 minutes	30 minutes	60 minutes	90 minutes
T1	146.75±46.38	126.25±64.23	160.50±42.02	147.25±49.89	172.00±46.77
T2	148.25±23.94	134.25±32.17	126.75±22.02	105.00±11.22	151.25±35.98
T3	131.00±15.60	134.50±37.55	145.75±10.24	118.50±42.85	101.00±26.70
T4	145.25±37.73	138.25±32.42	149.00±33.54	117.75±30.66	127.00±29.52
T5	138.75±31.49	122.75±19.26	113.25±41.18	125.75±41.17	129.25±22.51
T6	189.25±25.59	119.00±43.52	132.25±33.95	100.75±28.61	139.75±57.15
P-value	0.200	0.980	0.391	0.542	0.219

Note: Data without superscript letters in the same row indicates no significantly difference ($P>0.05$).



Digestive tract pH

The pH of digestive tract segments: stomach, foregut, midgut, and hindgut showed no significant difference among treatment groups ($P > 0.05$) in Table 5, Table 6, Table 7, and Table 8, respectively. Nile tilapia had differences in stomach pH, depending on volume of feed in the stomach. When the stomach was full, pH was reduced to 1.4, possibly to digest blue-green algae, green algae, and diatoms (Moriarty, 1973). The foregut had a pH of 5.5 and continuously rose to 8.0. From this study, empty stomachs had pH levels from 2.0 to 4.0 and increased to 4.0 to 5.0 with feed inclusion. Following the stomach, foregut pH ranged from 6.0 to 7.0 with midgut increasing to 7.0 to 8.0, and approached a constant pH of 8.0 in the hindgut.

Table 5: Stomach pH of Nile tilapia 15-, 30-, 60-, and 90-minutes post-feeding of experimental diets (Mean±SD)

Treatment	0 minutes	15 minutes	30 minutes	60 minutes	90 minutes
T1	3.88±1.65	4.00±0.71	4.63±0.48	5.00±0.58	5.00±1.58
T2	4.13±0.85	5.38±0.63	5.50±0.41	4.75±0.96	4.38±1.11
T3	1.75±0.50	4.13±0.85	4.88±0.48	5.25±1.55	3.50±0.71
T4	3.50±2.35	4.63±0.63	5.38±0.75	4.38±0.85	4.25±0.65
T5	2.25±0.96	5.00±0.58	5.13±0.63	5.25±0.96	4.00±1.08
T6	3.13±2.02	5.25±0.87	5.13±0.63	5.13±1.03	4.50±1.08
P-value	0.247	0.063	0.330	0.815	0.521

Note: Data without superscript letters in the same row indicates no significantly difference ($P > 0.05$).

Table 6: Foregut pH of Nile tilapia 15-, 30-, 60-, and 90-minutes post-feeding of experimental diets (Mean±SD)

Treatment	0 minutes	15 minutes	30 minutes	60 minutes	90 minutes
T1	7.13±0.75	7.13±0.25	7.00±0.58	7.13±0.25	6.50±0.71
T2	7.00±0.71	7.13±0.25	6.88±0.75	7.25±0.50	6.50±0.48
T3	7.50±0.41	7.13±0.25	7.00±0.58	7.13±0.25	6.38±0.48
T4	6.25±0.87	7.13±0.25	7.00±0.71	7.38±0.48	6.63±0.25
T5	7.38±0.48	7.25±0.29	7.00±0.71	7.25±0.29	6.50±0.41
T6	6.88±1.31	7.13±0.25	6.50±0.71	7.50±0.41	6.88±0.75
P-value	0.350	0.975	0.874	0.701	0.827

Note: Data without superscript letters in the same row indicates no significantly difference ($P > 0.05$).



Table 7: Midgut pH of Nile tilapia 15-, 30-, 60-, and 90-minutes post-feeding of experimental diets (Mean+SD)

Treatment	0 minutes	15 minutes	30 minutes	60 minutes	90 minutes
T1	7.50±0.41	7.75±0.29	8.00±0.00	7.75±0.29	6.25±1.85
T2	7.38±1.31	7.50±0.00	7.50±0.41	8.00±0.71	7.00±0.41
T3	7.88±0.25	7.63±0.63	7.88±0.25	7.63±0.48	7.50±0.41
T4	7.50±0.41	7.63±0.25	7.63±0.48	7.75±0.29	7.63±0.48
T5	7.88±0.25	7.75±0.29	7.13±0.63	7.50±0.00	7.25±0.29
T6	7.63±0.25	7.88±0.25	7.38±0.48	7.75±0.29	6.75±0.29
P-value	0.796	0.701	0.087	0.647	0.236

Note: Data without superscript letters in the same row indicates no significantly difference ($P>0.05$).

Table 8: Hindgut pH of Nile tilapia 15-, 30-, 60-, and 90-minutes post-feeding of experimental diets (Mean+SD)

Treatment	0 minutes	15 minutes	30 minutes	60 minutes	90 minutes
T1	8.25±0.29	8.25±0.29	7.88±0.85	7.88±0.25	8.00±0.41
T2	7.75±0.50	7.88±0.25	7.75±0.50	8.38±0.48	7.75±0.50
T3	8.13±0.63	7.75±0.29	7.88±0.48	7.75±0.65	8.00±0.41
T4	7.75±0.29	7.88±0.25	7.25±0.65	8.00±0.00	7.75±0.29
T5	8.25±0.29	8.25±0.65	7.50±0.00	7.88±0.25	8.00±0.00
T6	7.75±0.29	8.00±0.00	7.88±0.25	8.13±0.48	7.75±0.29
P-value	0.212	0.244	0.470	0.349	0.701

Note: Data without superscript letters in the same row indicates no significantly difference ($P>0.05$).

Conclusions

Juvenile Nile tilapia supplemented with solely 0.3 ppm Zinc-L-selenomethionine (Zn-L-Se) or 0.5 to 1.0 ppm Chromium-L-methionine (Cr-L-met), or in combination, showed enhanced growth performance in terms of weight gain, average daily growth, and feed conversion ratio, including improved immunity in terms of glutathione peroxidase activity for reduced oxidative potential. Chromium-L-methionine in tilapia diets has a tendency to reduce fluctuation of blood glucose. Digestive tract pH showed no treatment differences in stomach, foregut, midgut and hindgut. Therefore, under normal conditions, with low-stress, the recommended dosages



for improving growth performance of Nile tilapia is solely 0.5 to 1.0 ppm Chromium-L-methionine and the combination of 0.3 ppm Zinc-L-selenomethionine with 0.5 ppm Chromium-L-methionine.

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11th International Academic Conference

"Global Goals, Local Actions: Looking Back and Moving Forward 2020"

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