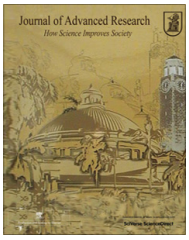
Journal of Advanced Research (2014) 5, 303–310

Cairo University

Journal of Advanced Research



ORIGINAL ARTICLE

Physiological, biochemical and histometric responses of Nile tilapia (*Oreochromis niloticus* L.) by dietary organic chromium (chromium picolinate) supplementation

Ahmed I. Mehrim *

Animal Production Department, Faculty of Agriculture, Al-Mansoura University, Al-Mansoura 35516, Egypt

ARTICLE INFO

Article history:
Received 15 October 2012
Received in revised form 2 April 2013
Accepted 4 April 2013
Available online 13 April 2013

Keywords:
Nile tilapia
Chromium
Safety
Blood parameters
Fish physiology

ABSTRACT

Chromium has been recognized as a new and important micro-nutrient, essential for both human and animal nutrition. This study was conducted to evaluate the appropriateness and/or the use of safety level of dietary chromium picolinate (Cr-Pic), and its effects on the physiological responses, the histometric characteristics, and the chemical analysis of dorsal muscles of mono-sex Nile tilapia, *Oreochromis niloticus*. niloticus. A total of 420 fingerlings (28.00 ± 0.96 g) were randomly distributed into 21 fiberglass tanks representing seven treatments at a rate of 20 fish m^{-3}. The control fish group (T_1) was fed a Cr-Pic free basal diet. Other fish groups were fed the basal diet supplemented with 200 (T_2), 400 (T_3), 600 (T_4), 800 (T_5), 1000 (T_6) and 1200 μg Cr-Pic kg^{-1} diet (T_7). Diets were offered to fish at a feeding rate of 3% of life body weight for 12 weeks. Results revealed that blood hematological parameters (hemoglobin, red blood cells, packed cell volume, mean corpuscular hemoglobin concentration, blood platelets, and white blood cells lymphocytes); serum biochemical measurements (total testosterone, high density lipoprotein, total protein, albumin, and globulin); and the dry matter and crude protein of the fish dorsal muscles all have significantly increased ($P \leqslant 0.05$) in the T_3 treatment compared with the other treatments. Meanwhile, no significant differences were found among all treatments with regard to the histometric characteristics. It can be concluded that Cr-Pic at 400 μg kg^{-1} diet (T_3) seems to be the most appropriate level for *O. niloticus* fingerlings.

Introduction

Chromium (Cr) is an essential micro-mineral that plays important roles in nutritional and physiological responses in fish [1,2]. It is found in the environment commonly in trivalent (Cr^{+3}) and hexavalent (Cr^{+6}) forms [3]. Chromium exists in several states of oxidation, ranging from +2 to +6, and its forms +3 and +6 are the most stable in the environment, they are also the most biologically important [4]. Various trivalent chromate compounds have been used as food additives in fish diet due to its participation in carbohydrate, protein, and fat metabolism [2,5]. Chromium picolinate (Cr-Pic) is the most popular form of Cr^{+3}, and its chemical formula is $Cr(C_6H_4NO_2)_3$ [6]. It is generally accepted that organic chromium sources such as chromium picolinate, chelated Cr, chromium–

* Tel.: +20 1002915069; fax: +20 502221688.
E-mail address: amehrim2002@yahoo.com
Peer review under responsibility of Cairo University.

2090-1232 © 2013 Production and hosting by Elsevier B.V. on behalf of Cairo University.
http://dx.doi.org/10.1016/j.jare.2013.04.002

amino acid complexes, and yeast-incorporated Cr have more bioavailability than inorganic sources [7]. However, dietary Cr^{+3} is often lost during animal feed handling and processing [4].

Several studies were performed to investigate the effect of Cr on growth performance of hybrid tilapia (*Oreochromis niloticus* × *Oreochromis aureus*) [8], grass carp (*Ctenopharyngodon idellus*) [2], and rainbow trout (*Oncorhynchus mykiss*) [9], its roles in metabolism in channel catfish (*Ictalurus punctatus*) [10], and gilthead sea bream (*Sparus aurata*) [11], as well as on carbohydrate utilization in hybrid tilapia (*O. niloticus* × *O. aureus*) [5,8], striped bass (*Morone saxatilis*) and sunshine bass (*Morone* chrysops × *M. saxatilis*) [12], and on immune status of *Oreochromis* mossambicus [13] and rainbow trout (*O. mykiss*) [14]. Moreover, its toxicity effects on Chinook salmon (*Oncorhynchus tshawytscha*) [15], largemouth bass (*Micropterus salmoides*) [16] and goldfish (*Carassius auratus*) [17] were reported too. However, few attempts had been made to determine the appropriateness level of dietary Cr-Pic for tilapia fish. To date, most of the existing studies related to Cr-Pic use safety levels are focused on human [18], but very few studies tackled use Cr-Pic safety levels and its physiological effects on fish. Therefore, the present study was carried out to determine the appropriateness and use of safety level of dietary Cr-Pic as an organic Cr^{+3} and its effects on the blood parameters, the histometric characteristics, and the chemical analysis of dorsal muscles of all-male mono-sex Nile tilapia (*O. niloticus*) fingerlings throughout a 12-week period.

Table 1 Ingredients and chemical composition (%, DM basis) of the basal diet.

Item	%
Fish meal	12.0
Soybean meal	31.0
Yellow corn	20.0
Wheat bran	25.0
Corn oil	5.0
Vitamins and minerals premix[1]	2.0
Molasses	5.0
Total	100
Chemical composition (%, DM)	
Dry matter (DM)	89.19
Crude protein (CP)	27.24
Ether extract (EE)	6.42
Ash	10.91
Total carbohydrates	55.43
Gross energy (GE) (Kcal/100 g DM)[2]	439.94
Protein/energy (P/E) ratio (mg CP/Kcal GE)[3]	61.91

[1] Each 3 kg premix contains: Vit. A, 12,000,000 IU; Vit. D_3, 3,000,000 IU; Vit. E, 10,000 mg; Vit. K_3, 3000 mg; Vit. B_1 200 mg; Vit. B_2, 5000 mg; Vit. B_6, 3000 mg; Vit. B_{12}, 15 mg; Biotin, 50 mg; Folic acid 1000 mg; Nicotinic acid 35,000 mg; Pantothenic acid 10,000 mg; Mn 80 g; Cu 8.8 g; Zn 70 g; Fe 35 g; I 1 g; Co 0.15 g and Se 0.3 g.

[2] GE = CP × 5.64 + EE × 9.44 + Total carbohydrates × 4.11.

[3] P/E ratio = CP/GE × 1000.

Material and methods

Experimental diets

Formulation and chemical composition of the basal diet are shown in Table 1. The dietary ingredients and Cr-Pic [Hi-Chrome® tablet a product of Amoun Pharmaceutical Company, El-Obour City, Cairo, Egypt, where each tablet contains 200 μg chromium as Cr-Pic] supplements were bought from the local market. Food ingredients were ground and mixed manually with warm water and molasses. Then, graded levels of Cr-Pic (0, 200, 400, 600, 800, 1000 or 1200 μg kg^{-1} diet) were added to the basal diet for preparing the experimental diets. Thereafter, the experimental diets were pressed by manufacturing machine to form pellets (1 mm diameter).

Experimental procedures and treatments

This study was conducted in the Fish Research Unit, Faculty of Agriculture, Al-Mansoura University, Al-Dakahlia Governorate, Egypt and all Institutional and National Guidelines for the care and use of fisheries were followed. All-male mono-sex *O. niloticus* fingerlings were obtained from a private hatchery, Kafr El-Sheikh Governorate, Egypt. Fish were stocked into a rearing fiberglass tank for two weeks as an adaptation period, during which they were fed a basal experimental diet. Each tank (1 m^3 volume), was supplied with an air stone connected to electric compressor. Dechlorinated tap water was used to change one third of the water in each tank every day. Wastes were removed from tanks by siphoning. Thereafter, a total of 420 apparently-healthy fish with an average body weight (28.00 ± 0.96 g) were randomly divided into seven groups (treatments; at three replications per treatment) at a stocking density of 20 fish m^{-3}. The control fish group (T_1) was fed the basal diet free of supplemented Cr-Pic. Other fish groups were fed the basal diet supplemented with Cr-Pic, at levels of 200 (T_2), 400 (T_3), 600 (T_4), 800 (T_5), 1000 (T_6), and 1200 μg kg^{-1} diet (T_7). During the 12-week experimental period, fish were daily fed the experimental diet at a rate of 3% of the live body weight for 6 days a week. Every two weeks, all fish in each tank were weighed and the amount of food was adjusted based on the actual body weight changes. Experimental diet was introduced manually twice a day, at 08:00 and 14:00 h.

Water quality parameters in each tank were measured weekly, including temperature (via a thermometer), pH-value (using Jenway Ltd., Model 350-pH-meter, Staffordshire ST15 0SA, UK) and dissolved oxygen (using Jenway Ltd., Model 970-dissolved oxygen meter, Staffordshire ST15 0SA, UK). Average values of water temperature were 26.0 ± 0.8 C, pH 8.19 ± 0.2 and dissolved oxygen 7.21 ± 0.3 mg L^{-1}, which were suitable for *O. niloticus* fingerlings rearing [19]. Light was controlled by a timer to provide 14 h light: and 10 h darkness as an immaculate imitation to actual light-darkness durations.

Sampling procedure

At the end of the experiment, five fish from each tank in all treatments were anaesthetized by putting them in a small plastic tank containing 10 L water supplemented with 3 mL pure clove oil (solved in 10 mL absolute ethanol) as a natural anesthetic material, where five fish dorsal muscles per tank were taken and kept frozen for chemical analysis. The chemical analyses of the basal diet and dorsal muscles were carried out according to the methods of AOAC [20].

At the end of the experiment, other ten fish from each tank were anaesthetized by the same anesthetic solution, then blood

samples were collected from the fish caudal peduncle of the different treatments by a plastic syringe (3 mL), which contained trisodium citrate (4%) as an anticoagulant to avoid the clotting of the blood sample during the collecting process before transferring it to dried small plastic vials for determination of the blood hematological parameters. Adequate amounts of whole blood were used for the determination of hemoglobin (Hb) using commercial colorimetric kits (Diamond Diagnostic, Egypt), and the hematocrit (packed cell volume, PCV %) was measured according to Stoskopf [21]. Also, red blood cells (RBCs), blood platelets and white blood cells (WBCs) were counted according to Dacie and Lewis [22] on an Ao Bright-Line Hämocytometer (Neubauer improved, Precicolor HBG, Germany). Other blood samples were collected and centrifuged at 3500 rpm for 20 min. to obtain blood serum for the determination of glucose according to Henry [23], total lipids according to Tietz [24], triglycerides according to MGowan et al. [25], cortisol and total testosterone hormones using commercial ELISA test kits Catalog number, M-1850 (Alpha Diagnostic International, USA), and BC-1115 (BioCheck, Inc., USA), respectively according to Tietz methods [26]. Serum total cholesterol was measured according to Ellefson and Caraway methods [27], high density lipoprotein (HDL) and low density lipoprotein (LDL) according to NCEP [28], total protein, albumin according to Gornall et al. [29] and globulin according to Doumas and Biggs [30].

In addition, at the end of the experiment, the remained five fish in each tank were sacrificed, and fish dorsal muscles (from the middle part) were taken for histometric examination. This means that the number of samples examined per treatment was 75 muscular fibers, where 5 muscular fibers × 3 replications × 5 examined fields in each slide were counted for the muscular fibers and measured for their sizes. Samples were fixed in 10% neutralized formalin solution followed by washing with tap water, then dehydrated using different grades of alcohol (70%, 85%, 96% and 99%). Samples were cleared by xylene and embedded in paraffin wax. The wax blocks were sectioned to six microns and stained with hematoxyline and eosin (H & E) for preparing the histological slides according to Roberts [31] and then subjected to histometric examination according to Radu-Rusu et al. [32].

Statistical analysis

Data was reported as mean values of all treatments (T_1–T_7). Replications ($n = 3$) and standard errors of mean (±SEM) are based on tank values (pooled values). Data was subjected to the General Linear Model procedure (GLM) using SAS software package [33]. Ratio and percent data were arcsine-transformed prior to statistical analyses and evaluated by using the following model:

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

where, Y_{ij} is an observation of blood hematological and biochemical parameters ($n = 30$), histometric characteristics ($n = 75$), or chemical analysis of fish dorsal muscles ($n = 15$); μ is the overall mean; A_i is the fixed effect of dietary Cr-Pic levels (T_1–T_7); and e_{ij} is the random error. Significant differences among mean (at $P \leqslant 0.05$) were determined with Tukey's studentized range test, which was described by Bailey [34].

Results

Blood hematological parameters

Data of blood hematological parameters was illustrated in Tables 2 and 3. Dietary supplementation with Cr-Pic at levels of 400 (T_3) and 600 μg kg^{-1} diet (T_4) led to significant ($P \leqslant 0.05$) increase of Hb concentration, RBCs count, PCV percentage, mean corpuscular hemoglobin concentration (MCHC), blood platelets count, WBCs count, and the percentage of lymphocytes compared to the other treatments. However, mean corpuscular volume (MCV) and neutrophils percentage were significantly decreased compared with the other graded levels of dietary Cr-Pic. Meanwhile, mean corpuscular hemoglobin (MCH), monocytes and eosinophils percentages were not affected in all treatments. Generally, increasing the other graded levels of dietary Cr-Pic had significantly ($P \leqslant 0.05$) decreased most of blood hematological parameters (Hb, RBCs, PCV, MCHC and WBCs) compared with the control treatment.

Blood biochemical parameters

Dietary supplementation with Cr-Pic at 400 μg kg^{-1} diet (T_3) led to significant ($P \leqslant 0.05$) decrease in serum glucose, total lipids, total cholesterol, and LDL concentrations Table 4. However, serum total testosterone, HDL Table 4, total protein, albumin, and globulin concentrations Table 5 were significantly increased ($P \leqslant 0.05$) compared with the other levels of Cr-Pic or the control treatment. Meanwhile, in the same treatment (T_3), the levels of triglyceride, cortisol Table 4, and albumin/globulin ratio Table 5 did not differ significantly ($P \geqslant 0.05$) compared with the other Cr-Pic treatments, but were significantly decreased ($P \leqslant 0.05$) compared with the control treatment.

Histometric examination and chemical analysis of fish dorsal muscles

Fish fed diet supplemented with Cr-Pic at 400 μg kg^{-1} (T_3) showed significant ($P \leqslant 0.05$) increases of smallest, largest and mean diameters compared with the other Cr-Pic treatments Table 6. However, other histometric characteristics (smallest/largest ratio; intensity of muscular bundles mm^{-2}; the percentage of muscular bundles area mm^{-2}, and the percentage of connective tissue mm^{-2}) had no significant ($P \geqslant 0.05$) differences among all treatments Table 6. On the other hand, diet supplemented with Cr-Pic at 400 μg kg^{-1} (T_3) led to significant ($P \leqslant 0.05$) increase in the dry matter and crude protein contents Table 6. However, ether extract and ash of fish dorsal muscles were significantly decreased as compared with other Cr-Pic treatments Table 6. Generally, it could be noted that the other dietary supplemented levels of Cr-Pic had neutral effects on histometric characteristics and chemical composition of fish dorsal muscles compared with the control treatment (T_1).

Discussion

In recent years, more attention was given to hematological studies as an integral part of the health conditions, and the productivity and the physiological state of fish. Hence, the

Table 2 Effect of graded levels of dietary chromium picolinate supplementation on blood hematological parameters of *Oreochromis niloticus*.

Treat.	Hb (g dL^{-1})	RBCs ($\times 10^6$ mm^{-3})	PCV (%)	Blood indices			Platelets ($\times 10^3$ mm^{-3})
				MCV (μ^3)	MCH (pg)	MCHC (%)	
T_1	$5.95^{a,b,c}$	$1.75^{b,c}$	16.0^{b}	$91.6^{c,d}$	33.9	37.2^{a}	612.7^{b}
T_2	$5.85^{b,c}$	$1.65^{d,e}$	16.1^{b}	$97.4^{a,b,c}$	35.5	$36.5^{a,b}$	620.0^{b}
T_3	6.30^{a}	1.85^{a}	16.6^{a}	89.7^{d}	34.1	37.9^{a}	672.7^{a}
T_4	$6.15^{a,b}$	$1.80^{a,b}$	16.5^{a}	$91.4^{c,d}$	34.2	37.4^{a}	662.7^{a}
T_5	$5.60^{c,d}$	$1.70^{c,d}$	15.9^{b}	$93.8^{b,c,d}$	32.9	$35.1^{a,b,c}$	605.0^{b}
T_6	$5.40^{d,e}$	$1.60^{e,f}$	$15.9^{b,c}$	$99.5^{a,b}$	33.9	$34.1^{b,c}$	607.7^{b}
T_7	5.20^{e}	1.55^{f}	15.6^{c}	100.7^{a}	33.6	33.3^{c}	600.0^{b}
SEM	±0.12	±0.03	±0.09	±2.02	±0.79	±0.86	±7.23
P-value	0.002	0.001	0.001	0.009	0.50	0.01	0.001

Hb, hemoglobin; RBCs, red blood cells; PCV, packed cell volume; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; platelets, blood platelets; SEM, standard error of means, as pooled values, $n = 30$.
Means in the same column having different superscripts were significantly different ($P \leqslant 0.05$).

Table 3 Effect of graded levels of dietary chromium picolinate supplementation on white blood cells count and its differentiation of *Oreochromis niloticus*.

Treat.	WBCs ($\times 10^3$ mm^{-3})	Lymphocytes (%)	Monocytes (%)	Neutrophils (%)	Eosinophils (%)
T_1	$855.0^{b,c}$	$91.9^{c,d}$	2.00	5.32^{a}	0.82
T_2	865.0^{b}	$92.7^{b,c}$	2.00	4.30^{b}	1.00
T_3	935.0^{a}	94.7^{a}	1.00	3.31^{c}	0.93
T_4	935.0^{a}	$93.9^{a,b}$	1.00	$4.33^{b,c}$	0.81
T_5	840.0^{c}	91.0^{d}	1.70	6.32^{a}	1.00
T_6	815.0^{d}	90.8^{d}	2.00	6.31^{a}	0.92
T_7	805.0^{d}	90.7^{d}	2.00	6.30^{a}	1.00
SEM	±7.07	±0.48	±0.28	±0.30	±0.01
P-value	0.001	0.002	0.10	0.001	0.10

WBCs, white blood cells; SEM, standard error of means, as pooled values; $n = 30$.
Means in the same column having different superscripts were significantly different ($P \leqslant 0.05$).

Table 4 Effect of graded levels of dietary chromium picolinate supplementation on some blood serum biochemical parameters of *Oreochromis niloticus*.

Item	Treatment							SEM	*P*-value
	T_1	T_2	T_3	T_4	T_5	T_6	T_7		
Glucose (mg dL^{-1})	252.5^{a}	$177.8^{b,c}$	127.2^{d}	160.3^{c}	$176.5^{b,c}$	186.8^{b}	$176.7^{b,c}$	±7.21	0.001
Total lipids (mg dL^{-1})	3015^{a}	2913^{a}	1945^{d}	$2010^{c,d}$	$2166^{b,c}$	$2162^{b,c}$	2254^{b}	±58.63	0.001
Triglyceride (mg dL^{-1})	208.8^{a}	$205.1^{a,b}$	172.5^{d}	$174.7^{c,d}$	$191.3^{b,c}$	$187.8^{c,d}$	$184.6^{c,d}$	±5.12	0.001
Cortisol (μg dL^{-1})	2.95^{a}	2.25^{b}	1.75^{c}	$1.90^{b,c}$	$2.10^{b,c}$	2.05^{bc}	$2.05^{b,c}$	±0.11	0.001
Total testosterone (ng mL^{-1})	331.0^{d}	359.5^{c}	472.5^{a}	460.0^{a}	392.5^{b}	400.0^{b}	397.5^{b}	±7.01	0.001
Total cholesterol (mg dL^{-1})	282.5^{a}	$255.7^{c,d}$	242.0^{e}	$249.6^{d,e}$	$263.8^{b,c}$	268.6^{b}	268.7^{b}	±3.92	0.001
HDL (mg dL^{-1})	92.0^{e}	101.8^{d}	132.8^{a}	126.4^{b}	116.4^{c}	112.8^{c}	115.6^{c}	±2.02	0.001
LDL (mg dL^{-1})	148.8^{a}	113.0^{b}	74.7^{c}	88.4^{c}	109.1^{b}	118.2^{b}	116.2^{b}	±4.86	0.001

HDL, high density lipoprotein; LDL, low density lipoprotein, SEM, standard error of means, as pooled values; $n = 30$.
Means in the same row having different superscripts were significantly different ($P \leqslant 0.05$).

obtained results revealed that dietary supplementation with Cr-Pic at levels of 400 μg kg^{-1} diet (T_3) and 600 μg kg^{-1} diet (T_4) led to significant ($P \leqslant 0.05$) increases of Hb concentration, RBCs count, PCV percentage, MCHC, blood platelets count, WBCs count, and the percentage of lymphocytes. However, they caused significant decreases of MCV and neutrophils percentage compared with the other levels of Cr-Pic. Increasing WBCs count can be correlated with an increase in antibody production, that helps in survival and recovery of fish exposed to toxicants [35]. This confirms the role of chromium in enhancement of the immune responses of the hybrid tilapia (*O. niloticus* × *O. aureus*) [36]. The present results are nearly similar to those reported by Askar et al. [37] who reported that Hb and PCV values significantly increased ($P \leqslant 0.01$) due to

Table 5 Effect of graded levels of dietary chromium picolinate supplementation on blood serum total protein of *Oreochromis niloticus*.

Treat.	Total protein (g dL^{-1})	Albumin (g dL^{-1})	Globulin (g dL^{-1})	AL/GL ratio
T_1	3.37^{d}	1.95^{b}	1.42^{d}	1.38^{a}
T_2	$3.90^{b,c}$	2.20^{a}	1.70^{c}	1.29^{a}
T_3	4.27^{a}	2.23^{a}	2.04^{a}	1.09^{b}
T_4	4.05^{b}	2.15^{a}	1.90^{b}	1.13^{b}
T_5	3.83^{c}	2.00^{b}	1.83^{b}	1.09^{b}
T_6	3.85^{c}	2.00^{b}	1.84^{b}	1.08^{b}
T_7	3.81^{c}	1.95^{b}	1.86^{b}	1.05^{b}
SEM	±0.05	±0.04	±0.03	±0.04
P-value	0.001	0.001	0.001	0.004

AL/GL ratio = albumin/globulin ratio; SEM, standard error of means, as pooled values; $n = 30$.
Means in the same column having different superscripts were significantly different ($P \leqslant 0.05$).

Table 6 Effect of graded levels of dietary chromium picolinate supplementation on histometric characteristics and chemical analysis of *Oreochromis niloticus* dorsal muscles.

Treat.	T_1	T_2	T_3	T_4	T_5	T_6	T_7	SEM	*P*-value
Smallest diameter (μm)	$44.4^{a,b}$	$40.0^{a,b}$	47.2^{a}	$38.0^{a,b,c}$	$34.4^{b,c}$	32.0^{c}	$33.2^{b,c}$	±3.51	0.03
Largest diameter (μm)	$55.6^{a,b}$	$52.4^{a,b,c}$	60.8^{a}	$53.2^{a,b,c}$	$47.6^{b,c}$	$47.6^{b,c}$	42.0^{c}	±3.71	0.03
Mean diameter (μm)	$50.0^{a,b}$	$46.2^{a,b,c}$	54.0^{a}	$45.6^{a,b,c}$	$41.0^{b,c}$	39.8^{c}	37.6^{c}	±3.10	0.009
Smallest/largest ratio	0.802	0.764	0.782	0.736	0.720	0.676	0.798	±0.06	0.70
Intensity of muscular bundles (mm^{-2})	350.0	350.0	350.0	422.0	422.0	494.0	515.0	±81.38	0.60
% of muscular bundles area (mm^{-2})	74.1	70.1	77.4	75.2	61.9	66.4	60.2	±8.07	0.60
% of connective tissue (mm^{-2})	25.9	29.9	22.6	24.8	38.2	33.6	39.8	±8.07	0.60
Chemical analysis of dorsal muscles (%, dry matter basis)									
Dry matter	$15.9^{d,e}$	$16.5^{c,d}$	18.3^{a}	$17.6^{a,b}$	$17.2^{b,c}$	$16.3^{c,d}$	15.2^{e}	±0.29	0.001
Crude protein	89.9^{c}	$90.4^{b,c}$	92.9^{a}	90.2^{c}	91.4^{b}	88.4^{d}	$90.4^{b,c}$	±0.36	0.001
Ether extract	$4.74^{a,b}$	$3.82^{c,d}$	2.71^{d}	$4.40^{b,c}$	$3.23^{c,d}$	5.62^{a}	2.94^{d}	±0.44	0.001
Ash	5.44^{d}	5.91^{b}	4.63^{c}	5.42^{b}	5.44^{b}	5.93^{b}	6.72^{a}	±0.23	0.001

SEM, standard error of means, as pooled values; % of muscular bundles area, $mm^{-2} = ([3.14 \times (\text{mean diameter}/2)^2] \times \text{Intensity of muscular bundles } mm^{-2}) \times 100$, whereas: the muscular bundles were appeared as circularity shape approximately; % of connective tissue, $mm^{-2} = (1 - \text{muscular bundles area}, mm^{-2}) \times 100$; $n = 75$ for histometric characteristics; $n = 15$ for chemical analysis of fish dorsal muscles.
Means in the same row having different superscripts were significantly different ($P \leqslant 0.05$).

the effect of dietary Cr-Pic supplementation. This may be due to the role of chromium in stabilizing the red blood cells against cellular changes caused by peroxidation [38].

Meanwhile, increasing the level of Cr-Pic in fish diet had significantly ($P \leqslant 0.05$) decreased most of blood hematological parameters (Hb, RBCs, PCV, MCHC and WBCs) compared with the control treatment, confirming the negative effect of Cr-Pic with levels higher than 600 μg kg^{-1} diet on *O. niloticus*. In this perspective, present findings show that high values of MCV were obtained in fish fed diet supplemented with Cr-Pic at levels up to 600 μg kg^{-1} may be attributed to reduction of RBCs Table 2 or related to the swelling of RBCs as reported by Murad and Mustafa [39]. The reduction of RBCs count and Hb content observed in fish groups treated with Cr-Pic up to 600 μg kg^{-1} diet may be due to the disruptive action on the erythropoietic tissue, which in turn affects the cell viability. Also, chromium has been shown to impair iron metabolism and storage, leading to significant reduction in serum iron, total iron-binding capacity, ferritin and hemoglobin [40]. However, reduction of WBCs count Table 3 in fish exposed to high levels of Cr-Pic may be a consequence of a sharp decline in number of lymphocytes Table 3 and blood platelets Table 2. Similar results were reported in rainbow trout (*O. mykiss*) fed the high level (2340 μg kg^{-1} diet) of dietary chromium yeast [14] and in *Cyprinus carpio* exposed to 500 μg kg^{-1} diet of chromium(VI) [41]. Generally, in the present study, the negative effects on hematological parameters of fish treated with levels of Cr-Pic higher than 600 μg kg^{-1} diet may be reflected on the general health status and productivity of fish.

Dietary Cr-Pic at 400 μg kg^{-1} (T_3) led to significant ($P \leqslant 0.05$) decrease of serum glucose, total lipid, triglyceride and cortisol levels compared with the other levels of Cr-Pic and the control treatment, which may be related to the stimulatory role of Cr^{+3} on the physiological glucose metabolism and the positive effects of dietary Cr-Pic at 400 or 600 μg kg^{-1} diet on blood hematological parameters Tables 2 and 3. This may be supported by the finding that dietary Cr has been associated with changes in circulating cortisol concentrations [42], and that Cr is an integral structural component of glucose tolerance factor (GTF), where; GTF (tentatively identified as a chromium–nicotinic acid complex) may be the biological active form of Cr^{+3}. In addition, Cr^{+3} is thought to potentate the action of insulin through the increase of insulin binding, insulin receptor number and function through lowering glucose and lipids, thereby regulating the uptake of glucose into cells [43]. Moreover, the present physiological findings are in agreement

with those reported by Kücükbay et al. [1] who revealed that serum glucose decreased ($P \leqslant 0.001$) by increasing the level of Cr-Pic (800 or 1600 μg kg^{-1} diet) in rainbow trout (*O. mykiss*), and hybrid tilapia (*O. niloticus* × *O. aureus*) fed dietary Cr-yeast (200 μg kg^{-1} diet) [36], as well as *O. niloticus* fed Cr-Pic up to 1200 μg kg^{-1} diet which also significantly decreased serum triglyceride [44].

In particular, Cr^{+3} has shown a positive influence on the reproductive efficiency of pigs and cattle [45]. However, it appears that there were no attempts done related to its reproductive effects in fish. Thus, serum total testosterone was measured in the present study because of its relation to the total cholesterol, HDL, LDL and also as an indicator to the reproductive effect of Cr-Pic on tilapia fish. In the present study, significant ($P \leqslant 0.05$) increases of serum testosterone and HDL was enhanced by dietary Cr-Pic especially at 400 μg kg^{-1}, which is related to the finding by Mehrim [46] who reported a significant increase of the gonado–somatic index (GSI %) in *O. niloticus* treated with 400 μg Cr-Pic kg^{-1} diet among all treatments. Similarly, Liu et al. [2] mentioned that grass carp (*C. idellus*) fed a diet supplemented with 800 μg Cr-Pic kg^{-1} had higher serum HDL concentration, but less serum cholesterol concentration.

In the present study, significant positive effects of 400 μg Cr-Pic kg^{-1} diet (T_3) on serum total protein, albumin and globulin concentrations were detected compared with the other levels of Cr-Pic or the control group. Hence, the superiority of T_3 over the other treatments was confirmed by its positive effects on WBCs and lymphocytes Table 3, that reflects the role of Cr-Pic at 400 μg kg^{-1} diet in enhancement of fish immune responses. These results are related to those reported on the role of chromium for improving the physiological and immune responses in *O. mossambicus* [13]; hybrid tilapia (*O. niloticus* × *O. aureus*) [36], and rainbow trout (*O. mykiss*) [1]. Meanwhile, decreasing of serum total protein content in fish exposed to levels of Cr-Pic higher than 400 μg kg^{-1} diet Table 5 may be due to decreased rate of protein synthesis, utilization of energy or secreted mucous proteins could alter the protein levels under metallic stress [47]. Generally, it could be stated that Cr-Pic at 400 μg kg^{-1} diet is safe and advantageous to the health of *O. niloticus* while its level higher than 400 μg kg^{-1} diet has drastic effects on *O. niloticus*. This is in agreement with the findings of El-Sayed et al. [44] who found that dietary Cr-Pic (up to 1200 μg kg^{-1} diet) has significantly ($P < 0.05$) decreased serum cholesterol, total protein, albumin, and globulin concentrations of *O. niloticus*.

The graded levels of dietary Cr-Pic had neutral effects on histometric characteristics of fish dorsal muscles compared with the control treatment, which is in line with the findings of Mehrim [46] who found that Cr-Pic had no significant effects on *O. niloticus* growth performance in a complementary study related to the present work. However, dietary Cr-Pic at level of 400 μg kg^{-1} diet (T_3) has significantly ($P \leqslant 0.05$) increased the smallest, largest and mean diameters of fish dorsal muscles compared with the other levels of Cr-Pic. The superiority effect of T_3 on fish dorsal muscles may be related to the positive effects of T_3 that increased the dry matter, crude protein and decreased ether extract contents in fish muscles than other levels of Cr-Pic, which reflected the effects of Cr-Pic in increasing the lean muscles mass of treated fish. In this trend, Zimmerman and Lowery [48] revealed that muscle growth is a dynamic process in White Sea bass (*Atractoscion nobilis*) that begins early in their development and continues throughout much if not all of their life span. Furthermore, Mommsen [49] demonstrated that muscle growth in fish differed from other vertebrates because it occurs indeterminately, due to continuous growth through life.

The present findings of chemical composition of dorsal muscles of fish treated with dietary supplementation of 400 μg Cr-Pic kg^{-1} diet (T_3) represented significant ($P \leqslant 0.05$) increase in dry matter and crude protein contents compared with the other levels of dietary Cr-Pic, which may be due to the significant ($P \leqslant 0.05$) increase of serum protein by the same treatment (T_3, Table 5). Meanwhile, there is a negative relation between dry matter and ether extract among all treatments, which may be due to the increasing of crude protein content in dorsal muscles. Also, it may be related to the role of Cr in reducing the lipid retention by decreasing the activities of lipogenesis [50], and/or Cr roles to make insulin function more efficiently by enhancing the uptake of glucose from the blood into the cell [43].

Conclusions

In accordance with the obtained physiological and biochemical results, it could be concluded that Cr-Pic at 400 (T_3) followed by 600 μg Cr-Pic kg^{-1} diet (T_4) as dietary supplementation are the most appropriate and/or the safest levels for mono-sex Nile tilapia, *O. niloticus*, fingerlings. Thus, these levels of Cr-Pic may be recommended as food supplements for Nile tilapia diet in fish farms and fish food factories. Further scientific attempts are needed to study the role of Cr-Pic on the reproductive efficiency of *O. niloticus* in this stage and other stages of maturation.

Conflict of interest

The author has declared no conflict of interest.

Acknowledgements

The author would like to thank Dr. Abdelhamid M. Abdelhamid, Professor of Animal Nutrition, Dr. Abd-Elkhalek E. Abd-Elkhalek, Professor of Animal Physiology, Dr. Yasser M. Shabana, Professor of Plant Pathology, Faculty of Agriculture, Al-Mansoura University, Egypt and Dr. Mohsen Abdel-Tawwab Prof. of Aquaculture, Department of Fish Biology and Ecology, Central Laboratory for Aquaculture Research, Abbassa, Egypt for their critical reading of the manuscript and generous assistance. All Institutional and National Guidelines for the care and use of fisheries were followed.

References

[1] Kücükbay FZ, Yazlak H, Sahin N, Cakmak MN. Effects of dietary chromium picolinate supplementation on serum glucose, cholesterol and minerals of rainbow trout (*Oncorhynchus mykiss*). Aquacult Int 2006;14:259–66.

[2] Liu T, Yuan D, Gao P, Zhao Y. Effect of dietary chromium picolinate on growth performance and blood parameters in grass carp fingerling, *Ctenopharyngodon idellus*. Fish Physiol Biochem 2010;36:565–72.

[3] Bagchi D, Stohs SJ, Downs BW, Bagchiand M, Preuss HG. Cytotoxicity and oxidative mechanisms of different forms of chromium. Toxicology 2002;180: 5–22.
[4] Lushchak VI. Environmentally induced oxidative stress in aquatic animals a review. Aquat Toxicol 2011;101:13–30.
[5] Pan Q, Bi YZ, Yan XL, Pu YY, Zheng C. Effect of organic chromium on carbohydrate utilization in hybrid tilapia (*Oreochromis niloticus* × *O. aureus*). Acta Hydrobiol Sin 2002;26:393–9.
[6] NBI. Nutrition Business International Supplement Business Report. NBJ. San Diego Ca, USA, 9(3); 2001.
[7] Shiau SY, Shy SM. Dietary chromic oxide inclusion level required to maximize glucose utilization in hybrid tilapia, *Oreochromis niloticus* × *O. aureus*. Aquaculture 1998;161:357–64.
[8] Pan Q, Liu S, Zheng C, Bi YZ. The effect of chromium nicotinic acid on growth, feed efficiency and tissue composition in hybrid tilapia (*Oreochromis niloticus* × *O. aureus*). Acta Hydrobiol Sin 2002;26:197–200.
[9] Selcuk Z, Tiril SU, Alagil F, Belen V, Salman M, Cenesiz S, et al. Effects of dietary L-carnitine and chromium picolinate supplementations on performance and some serum parameters in rainbow trout (*Oncorhynchus mykiss*). Aquacult Int 2010;18:213–21.
[10] Paripatananont T, Lovell RT. Comparative net absorption of chelated and inorganic trace minerals in channel catfish *Ictalurus punctatus* diets. J World Aquac Soc 1997;28:62–7.
[11] Gatta PP, Piva A, Paolini M, Testi S, Bonaldo A, Antelli A, et al. Effects of dietary organic chromium on gilthead sea bream (*Sparus aurata* L.) performances and liver microsomal metabolism. Aquacult Res 2001;32:60–9.
[12] Rawles SD, Gatlin III DM. Carbohydrate utilization in striped bass (Morone saxatilis) and sunshine bass (*M. chrysops* ♀ × *M. saxatilis* ♂). Aquaculture 1998;161:201–12.
[13] Arunkumar RI, Rajasekaran P, Michael RD. Differential effect of chromium compounds on the immune response of the African mouth breeder *Oreochromis mossambicus* (Peters). Fish Shellfish Immun 2000;10:667–76.
[14] Gatta PP, Thompson KD, Andrea RS, Testi PS, Adams A. Dietary organic chromium supplementation and its effect on the immune response of rainbow trout (*Oncorhynchus mykiss*). Fish Shellfish Immun 2001-b;11:371–82.
[15] Farag AM, Harper DD, Cleveland L, Brumbaugh WG, Little EE. The potential for chromium to affect the fertilization process of chinook salmon (*Oncorhynchus tshawytscha*) in the Hanford Reach of the Columbia River, Washington. USA. Arch Environ Contam Toxicol 2006;50:575–9.
[16] Kuykendall JR, Miller KL, Mellinger KN, Cain AV. Waterborne and dietary hexavalent chromium exposure causes DNA-protein crosslink (DPX) formation in erythrocytes of largemouth bass (*Micropterus salmoides*). Aquat Toxicol 2006;78:27–31.
[17] Kubrak OI, Lushchak OV, Lushchak JV, Torous IM, Storey JM, Storey KB, et al. Chromium effects on free radical processes in goldfish tissues: comparison of Cr(III) and Cr(VI) exposures on oxidative stress markers, glutathione status and antioxidant enzymes. Comp Biochem Physiol C 2010;152: 360–70.
[18] Eastmond DA, MacGregor JT, Slesinski RS. Trivalent chromium: assessing the genotoxic risk of an essential trace element and widely used human and animal nutritional supplement. Crit Rew Toxicol 2008;38:173–90.
[19] Lim C, Webster CD. Tilapia: biology, culture and nutrition. New York USA: Food Product Press; 2006.
[20] AOAC. Association of Official Analytical Chemists Animal Feed, Official methods of analysis, 17th ed. Washington DC USA; 2000.
[21] Stoskopf MK. Fish medicine. Saunders WB, Philadelphia USA; 1993.
[22] Dacie JV, Lewis SM. Practical haematology, 8th ed. Edinburgh Churchill Livingstone UK; 1995.
[23] Henry RJ. Clinical chemistry, principles and techniques. New York (USA): Harper and Row Publishers; 1964.
[24] Tietz NW. Fundamentals of clinical chemistry, 2nd ed. Saunders WB, Philadelphia (USA); 1976.
[25] MGowan MW, Artiss JD, Standbergh DR, Zak B. A peroxidase-coupled method for colorimetric determination of serum triglycerides. Clin Chem 1983;29:538–52.
[26] Tietz NW. Clinical guide to laboratory tests, 3rd ed. Saunders WB, Philadelphia (USA); 1995.
[27] Ellefson RD, Caraway WT. Fundamentals of clinical chemistry. In: Tietz NW, editor. Saunders WB, Philadelphia USA; 1976.
[28] NCEP. National cholesterol education program recommendation for measurement of high-density lipoprotein cholesterol: executive summary. Clin Chem 1995; 41: 1427–33.
[29] Gornall AG, Bardawill GJ, Parid MM. Method of determination protein in serum blood. J Biol Chem 1949;177:751.
[30] Doumas BT, Biggs HG. Determination of serum albumin. In: Cooper GR, editor. Standard method of clinical chemistry. New York (USA): Academic Press; 1972.
[31] Roberts RJ. Fish pathology. 3rd ed. Philadelphia USA: Elsevier Health Sciences; 2001.
[32] Radu-Rusu RM, Teuşan V, Vacaru-Opriş I. Aspects concerning the histological stucture of the biceps brachialis muscles in chicken broilers. Luc Ştiinţif Ser Zoot 2009;52:266–70.
[33] SAS. SAS/STAT Procedure User's Guide, Version 8.0 SAS In: Campus Drive, Cary NC; 2001.
[34] Bailey NTJ. Statistical methods in biology. 3rd ed. Cambridge (UK): The press syndicate of the University of Cambridge; 1995.
[35] Ramesh M, Saravanan M. Haematological and biochemical responses in a freshwater fish Cyprinus carpio exposed to chorpyrifos. Int J Integrative Bio 2008;3(1):80–3.
[36] Magzoub MB, Al-Batshan HA, Hussein MF, Al-Mufarrej SI, Al-Saiady MY. The effect of source and level of dietary chromium supplementation on humoral antibody response and blood chemical parameters in hybrid tilapia fish (*Oreochromis niloticus* × *O. aureus*). Res J Biol Sci 2009;4:821–7.
[37] Askar AA, El-Hindawy MM, Sonbol SM, El-Kholy MS. Effect of ambient temperature and some dietary supplementations on some physiological traits of laying Japanese quail. J Agric Sci Mansoura Univ 2008;33:75–87.
[38] Linder MC. Nutrition and metabolism of the trace elements. In: Linder MC, editor. Nutritional biochemistry and metabolism with clinical applications. New York USA: Elsevier; 1991.
[39] Murad A, Mustafa S. Ethological and haematological responces of catfish *Heteropneustes fossilis*, exposed to exogenous urea. Jpn J Icthyol 1989;36:75–81.
[40] Ani M, Moshtaguie AA. The effect of chromium on parameters related to iron metabolism. Biol Trace Elem Res 1992;32:57–64.
[41] Çiftçi N, Günalp C, Cicik B, Erdem C, Ay Ö. Effects of chromium on the hematocrit levels and erythrocyte numbers of *Cyprinus carpio*. E-J New World Sci Acad (NWSA) 2010;5:82–8.
[42] Borgs P, Mallard BA. Immune-endocrine interactions in agricultural species: chromium and its effect on health and performance. Domest Anim Endocrinol 1998;15:431–8.
[43] Mertz W. Chromium occurrence and function in biological systems. Phys Rev 1969;49:163.
[44] El-Sayed EH, Hassanein, EI, Soliman, MH, El-khatib, NR. The effect of dietary chromium picolinate on growth performance, blood parameters and immune status in Nile tilapia, *Oreochromis niloticus*. In: Proceeding of the 3rd global fisheries & aquaculture research conference 2010; 29th November–1st December foreign agricultural relations (FAR), Egypt; 2010.
[45] Mallard BA, Borgs P. Effects on supplemental trivalent chromium on hormone and immune responses of cattle. In: Biotechnology in the feed industry, proceedings of Alltech's

thirteenth annual symposium 1997. Nottingham, UK: Nottingham University Press.
[46] Mehrim AI. Effect of dietary chromium picolinate supplementation on growth performance, carcass composition and organs indices of Nile tilapia (*Oreochromis niloticus* L.) fingerlings. JFAS 2012;7(3):224–32.
[47] Vutukuru SS. Chromium induced alterations in some biochemical profiles of the Indian major carp, *Labeo rohita* (Hamilton) Bull. Environ Contam Toxicol 2003;70:118–23.
[48] Zimmerman AM, Lowery MS. Hyperplastic development and hypertrophic growth of muscle fibers in the White Sea bass (*Atractoscion nobilis*). J Exp Zool 1999;284:299–308.
[49] Mommsen TP. Paradigms of growth in fish. Comp Biochem Physiol B Biochem Mol Biol 2001;129:207–19.
[50] Gang X, Zirong X, Si HW. Effects of chromium picolinate on growth performance, carcass characteristics, serum metabolites and metabolism of lipid in pigs. Asian Aust J Anim 2001;14:258–62.