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The Effect of Source and Level of Dietary Chromium Supplementation on Performance, Chemical Composition and Some Metabolic Aspects in Hybrid Tilapia Fish (*Oreochromis niloticus* x *O. aureus*)

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Abstract: This study was undertaken to determine the effect of dietary Chromium (Cr) supplementation on performance, body chemical composition, plasma glucose, Hepatosomatic Index (HSI) and liver glycogen in hybrid tilapia (Oreochromis niloticus x O. aureus). Cr-yeast or Chromic Oxide (Cr₂O₃) were added to fish feeds at the rates of 1 or 2 mg kg⁻¹ while control fish received an unsupplemented diet. About 1400 fish (avg. weight 18.6 g) were randomly assigned to 5 feeding regimens, each consisting of four replicates. Each replicate was reared separately. Body Weight (BW), Feed Intake (FI), Growth Rate (GR) and Feed Efficiency Ratio (FER) were measured at days 14, 28, 42, 56, 70 and 84. Chemical composition, HSI and liver glycogen were determined at days 0, 30 and 60. Plasma glucose was measured after starving the fish for 24 h and at 1, 2, 3, 4, 6, 8 h after feeding. The results showed increased BW, FI, GR, FER, total protein, ether extract, gross energy, plasma glucose and liver glycogen and decreased ash percent in all groups. Comparison of the overall means of these variables in different feeding groups showed that BW, FI, GR and FER were significantly lower in fish receiving 2 mg kg⁻¹ Cr-yeast while overall mean values of ether extraction and gross energy were significantly higher in fish receiving 1 mg kg⁻¹ Cr₂O₃. On the other hand the highest overall mean value for liver glycogen was recorded in fish receiving 2 mg kg⁻¹ Cr. No significant changes were observed in moisture, ash, total protein, plasma glucose or HSI. These findings indicate that the inclusion of Cr in fish feeds can lead to improvement of tilapia chemical composition and physiology, namely ether extract, total energy and liver glycogen.

Key words: Chromium supplementation, tilapia, performance, chemical composition, plasma glucose, liver glycogen, hepatosomatic index

INTRODUCTION

Chromium (Cr) is a transition element which is widely spread in nature and occurs in different oxidation states. Earlier studies have focused on the role of Cr as an essential micro-nutrient for humans and animals (Anderson, 1987; Mertz, 1993) in which it appears to influence many aspects of metabolism and production (Burton, 1995; Mordenti *et al.*, 1997). According to Burton (1995) the most stable form of Cr is the trivalent state which is the one involved in Glucose Tolerance Factor (GTF).

The ability of fish to utilize carbohydrate is lower than from animals (NRC, 1981, 1983). This has been attributed primarily to poor glucose tolerance resulting from lack of adequate insulin response (Palmer and Ryan, 1972; Furuichi and Yone, 1981) or low insulin receptor

activity (Plisetskaya et al., 1986) in fish. Cr potentiates the action of insulin (Mertz et al., 1974) and is considered to be a co-factor for insulin activity and part of an organic GTF (Anderson, 1981). Being an active GTF component, Cr is needed for insulin to move glucose from the circulation into peripheral tissues (Anderson and Mertz, 1977) and is essential for normal metabolism of carbohydrates, protein and lipids (Mertz, 1992). It is not surprising, therefore that Cr deficient animals show poor growth, reduced life-span and decreased tolerance to glucose (Mallard and Borgs, 1997).

Interest in Cr supplementation in fish feeds has derived from information on human and farm animal nutrition. Previous studies on farm animals have shown that dietary supplementation with organic Cr complexes resulted in beneficial effects on growth, reproduction and immune responses (Burton *et al.*, 1996). On the other

hand, most of the studies dealing with the effect of Cr in fish have been related to its role in metabolism (Hertz *et al.*, 1989; Shiau and Lin, 1993; Ng and Wilson, 1997; Shiau and Shy, 1998; Fernandez *et al.*, 1999), growth (Tacon and Beveridge, 1982; Jain *et al.*, 1994) and toxicity (Calamari and Solbe, 1994) and have been largely limited to Cr supplementation from inorganic sources (Hertz *et al.*, 1989; Shiau and Lin, 1993; Shiau and Chen, 1993; Shiau and Liang, 1995).

Only a few studies are available on the use of organic Cr as a fish feed supplement (Bureau et al., 1995; Pan et al., 2003; Liu et al., 2009). On the other hand, no comparative studies on the effect of organic versus inorganic Cr supplementation in fish diets are found in the literature, apart from the study comparing the effect of these two forms of Cr on blood constituents and humoral antibody response in tilapia (Al-Batshan et al., 2009). The following study was undertaken to investigate the effect of two different sources and two concentration levels of Cr on performance, body chemical composition, plasma glucose, hepatosomatic index and liver glycogen of tilapia.

MATERIALS AND METHODS

Fish and husbandry: About 1400 fish fingerlings (Average weight 18.55±0.22 g) were obtained from the fish hatchery of King Abdul Aziz City for Science and Technology in Riyadh, Saudi Arabia and reared at the experimental farm of the Department of Animal Production, as previously described (Al-Batshan et al., 2009), briefly the fish were reared in stock tanks for 2 weeks for acclimatization then distributed randomly into five feeding groups, each comprising four replicates of 70 fish/replicate. Each replicate was kept separately in a fiberglass tank (0.5 m³) supplied with filtered freshwater with flow-through of 3 L min⁻¹ to eliminate water waste and aerated with air stone to maintain adequate oxygen Water temperature was maintained approximately 28°C using electric thermostat and cooling devises. Tank water was changed every 48 h. The photoperiod was adjusted to 12 h light and 12 h dark while water quality was monitored regularly for temperature, pH and dissolved O2, HCO3-, Cl, NH3, NO3- and NO2concentration to ensure that they remained within tolerance limits for tilapia (Balarin and Hatton, 1979).

Experimental diets: A commercial tilapia feed (Arabian Agricultural Services Co., Riyadh) supplemented with either Cr₂O₃ (Sigma-Aldrich Chemie GMBH, Germany) or Cr-yeast (Alltec Co., UK) was used. The supplementary Cr

Table 1: Feed formulation, composition and chemical analysis of experimental diets

Formulation	CONT	CRO1	CRO2	CRY1	CRY2
Treatments1 (g kg-1)					
Corn	82.50	82.50	82.50	82.50	82.50
Soybean	382.87	382.87	382.87	382.87	382.87
Wheat flour	180.00	180.00	180.00	180.00	180.00
Fish meal	300.00	300.00	300.00	300.00	300.00
Limo stone	15.30	15.30	15.30	15.30	15.30
Palm oil	29.00	29.00	29.00	29.00	29.00
KEMIN	2.00	2.00	2.00	2.00	2.00
Premix	3.33	3.33	3.33	3.33	3.33
Chromium mixture 2	5.00	5.00	5.00	5.00	5.00
Total	1000.00	1000.00	1000.00	1000.00	1000.00
Chemical analysis laborate	ory (calcula	ited)			
Dry matter (%)	90.58	90.58	90.58	90.58	90.58
Salt (%)	1.02	1.02	1.02	1.02	1.02
A. Phosphorus (%)	0.68	0.68	0.68	0.68	0.68
Sodium (%)	0.38	0.38	0.38	0.38	0.38
Calcium (%) ^(1.75)	1.80	1.80	1.80	1.80	1.80
T. Phosphorus (%) (0 90)	1.04	1.04	1.04	1.04	1.04
Chromium (mg kg ⁻¹)	0.73	1.33	2.27	1.27	2.18
Ether extract (%) (7.00)	7.04	7.04	7.04	7.04	7.04
Crude (%) (40.00)	40.49	40.49	40.49	40.49	40.49
Ash (%) (9.00)	9.07	9.07	9.07	9.07	9.07
Crude fiber (%) (3.00)	2.18	2.18	2.18	2.18	2.18
Total energy (kg cal kg ⁻¹)	3603.00	3603.00	3603.00	3603.00	3603.00

¹Treatments are described as CONT: without chromium addition, CRO1: 1 ppm chromic oxide addition, CRO2: 2 ppm chromic oxide addition, CRY1: 1 ppm chromium yeast addition, CRY2: 2 ppm chromium yeast addition. ²Chromium in each formula mixed with corn at concentrations 0, 1.433, 2.866, 1000 and 2000 mg Cr kg¹ feed as CONT, CRO1, CRO2, CRY1 and CRY2, respectively

was mixed with corn and added to the feed at the rate of 1 or 2 mg kg $^{-1}$ feed of Cr_2O_3 (groups CRO1 and CRO2) or Cr-yeast (groups CRY1 and CRY2). An unsupplemented diet served as control (CONT). The fish were fed their prescribed diets 3 times daily at the rate of 5% of their average body weight. Details of Cr supplementation and feed proximate analysis (AOAC, 1992) are shown in Table 1.

Measurements: Body Weight (BW) and Feed Intake (FI) were measured bimonthly from day 0 up to day 84 and their Growth Rate (GR) and Feed Efficiency Ratio (FER) were calculated. Body chemical composition, namely moisture, ash, ether extract, total protein and total energy of the fish was determined according to the AOAC (1992). Relative weight of the liver (hepatosomatic index, HSI%) was calculated according to Berhaut method (Berhaut, 1973) while liver glycogen concentration was determined according to the method described by Murat and Serfaty (1974).

Samples for chemical composition, HSI and liver glycogen were taken at the onset and after 30 and 60 days of the experiment. Blood samples were collected from 160 fish (8 fish/replicate) by cardiac puncture using a 3 mL, $22~{\rm g}\times10.7\times38$ mm syringe coated with EDTA-K₂ and the plasma was separated by centrifugation at 3000 rpm for 10 min, dispensed into clean glass vials and stored

at -20°C. Samples for plasma glucose determination were collected after fasting the fish for 24 h and at 1, 2, 3, 4, 6 and 8 h after feeding. The plasma glucose concentration was measured spectrophotometrically using commercial reagent kits (Randox, UK).

Statistical analysis: Data were statistically analyzed using a general linear model procedure in SAS (Goodnight *et al.*, 1986). Duncan's test was used to determine significant differences between means. Data expressed as percentages were converted to arcsin values prior to statistical analysis (Steel *et al.*, 1996).

RESULTS AND DISCUSSION

Performance parameters: Highly significant (p<0.0001) increases in BW, FI, GR and FER were recorded in all groups, including the controls, with increasing duration of the experiment (Table 2). However, the overall mean values of these parameters were significantly lower (p<0.01) in group CRY2 as compared to the remaining groups.

Fish chemical composition: Highly significant increases in fish total protein, ether extraction and gross energy, with corresponding decreases in ash were recorded in all groups with the increasing duration of the experiment

(Table 3). The overall means of gross energy and ether extraction % were significantly higher in group CRO1 compared to the remaining groups (p<0.005). On the other hand the overall mean ether extraction % for CRO2 and CRY1 were significantly higher than the controls. By contrast, no significant differences in moisture content were observed between groups.

Blood glucose: A highly significant increase in plasma glucose concentration occurred with increasing duration of the experiment in all groups (Table 4). On the other hand, no effect on plasma glucose concentration that could be attributed to the treatment was observed among the groups (Table 4).

Liver glycogen and hepatosomatic index: A highly significant increase in liver glycogen was recorded in all groups with increasing duration of the experiment the highest overall mean value being recorded in group CRY2. On the other hand, neither treatment nor treatment period were found to have a significant effect on the percentage HSI.

Significant treatment×duration interactions were observed only in ether extraction % (p<0.02) and plasma glucose concentration (p<0.05). Interest in Cr supplementation of fish feeds has evolved over the past decade in part due to studies indicating that the addition

Table 2: Effect of source and level of chromium supplementation on some performance values of tilapia

	Treatme	ents					Analysis of variances F1>Pr				
Measurements	Days	CONT	CRO1	CRO2	CRY1	CRY2	Mean	SEM^2	p ³	t ⁴	(pxt) ⁵
Body weight (g)	14	26.78	26.58	26.92	26.93	26.09	26.66 ^f	0.936	0.0001	0.0001	0.7567
	28	29.53	29.70	30.31	29.65	28.16	29.47⁰				
	42	31.73	32.89	33.57	32.00	29.89	32.02^{d}				
	56	34.29	36.35	36.17	34.83	31.61	34.65€				
	70	41.87	41.48	43.59	39.96	37.02	40.78^{b}				
	84	53.53	51.87	55.15	51.00	43.82	51.07ª				
Mean		36.29 ^a	36.48⁴	37.62ª	35.73ª	32.76^{b}					
Feed intake (g day ⁻¹)	14-28	1.39	1.37	1.39	1.40	1.34	1.38€	0.197	0.0001	0.0009	0.9569
	29-42	1.51	1.53	1.57	1.52	1.44	1.51^{d}				
	43-56	1.65	1.73	1.74	1.67	1.54	1.67€				
	57-70	1.82	1.88	1.92	1.80	1.65	$1.81^{\rm b}$				
	71-84	2.19	2.22	2.34	2.13	1.93	2.16^{a}				
Mean		1.71ª	1.74ª	1.79ª	1.70a	1.58^{b}					
Growth rate (g day ⁻¹)	14-28	0.195	0.223	0.245	0.195	0.148	0.201°	0.172	0.0001	0.0020	0.2459
	29-42	0.155	0.230	0.233	0.168	0.125	0.182°				
	43-56	0.183	0.248	0.185	0.205	0.125	0.189°				
	57-70	0.540	0.365	0.530	0.365	0.388	0.438 ^b				
	71-84	0.833	0.745	0.825	0.788	0.485	0.735a				
Mean		0.381ª	0.362^{a}	0.404ª	0.344ª	0.254^{b}					
Feed efficiency ratio (g g ⁻¹)	14-28	0.143	0.163	0.173	0.138	0.108	0.145°	0.124	0.0001	0.0173	0.4345
	29-42	0.105	0.148	0.145	0.110	0.085	0.119^{c}				
	43-56	0.108	0.140	0.103	0.118	0.080	0.110°				
	57-70	0.303	0.195	0.288	0.208	0.230	0.245^{b}				
	71-84	0.380	0.333	0.355	0.370	0.248	0.337^{a}				
Mean		0.208^a	0.196^{a}	0.213^{a}	$0.189^{\rm sb}$	0.150^{b}					

Means in the same row letters indicate significant difference between groups; means in the same column letters indicate significant difference within group, a>b> c>d>e>f; ¹Probability; ²Pooled standard error mean; ³Period; ⁴Treatments; ⁵Interaction between period and treatment

Table 3: Effect of source and level of chromium supplementation on chemical composition values of tilapia

	Treatme	nts		Analysis of variances F1>Pr							
Measurements	Days	CONT	CRO1	CRO2	CRY1	CRY2	Mean	SEM^2	p ³	t ⁴	(pxt) ⁵
Moisture (%)	0	72.57	72.57	72.57	72.57	72.57	72.57ª	0.011	0.5977	0.5530	0.4092
` '	30	73.17	72.74	72.39	72.37	73.14	72.76a				
	60	72.76	71.99	72.71	72.81	72.71	72.60a				
Mean		72.85ª	72.29ª	72.60^{a}	72.65ª	72.82ª					
Ash (%)	0	5.56	5.56	5.56	5.56	5.56	5.56ª	0.013	0.0001	0.3189	0.3099
, ,	30	4.95	4.67	5.14	4.98	5.14	4.98 ^b				
	60	4.86	4.83	4.86	4.89	4.86	4.86^{b}				
Mean		4.99⁴	4.89ª	5.04ª	5.01a	5.04ª					
Total protein (%)	0	16.38	16.38	16.38	16.38	16.38	16.38 ^b	0.015	0.0001	0.8289	0.8054
• , ,	30	16.48	16.27	16.47	15.95	16.12	16.26 ^b				
	60	17.25	17.65	17.26	17.22	17.52	17.38a				
Mean		16.91ª	17.07a	16.91ª	16.74ª	16.96a					
Ether extraction (%)	0	4.82	4.82	4.82	4.82	4.82	4.82°	0.015	0.0001	0.0049	0.0214
	30	4.66	5.36	5.29	5.44	4.76	5.10 ^b				
	60	5.29	5.98	5.42	5.31	5.30	5.46ª				
Mean		5.04°	5.64ª	5.30 ^b	5.28 ^b	5.08^{bc}					
Gross energy (kcal kg ⁻¹)	0	4928	4928	4928	4928	4928	4928 ^b	0.053	0.0001	0.0274	0.2906
3, \ 0 /	30	4987	5139	5070	5107	4949	5050°				
	60	5084	5178	5069	5064	5036	5086a				
Mean		5034 ^b	5131a	5049b	5057°	4996₺					

Means in the same row letters indicate significant difference between groups; means in the same column letters indicate significant difference within group, a>b>c; ¹Probability; ²Pooled standard error mean; ³Period; ⁴Treatments; ⁵Interaction between period and treatment

Table 4: Effect of source and level of chromium supplementation on plasma glucose, lever glycogen and hepatosomatic index values of tilapia

		Treatments							Analysis of variances F1>Pr		
Measurements		CONT	CRO1	CRO2	CRY1	CRY2	Mean	SEM^2	p ³	t ⁴	(p×t) ⁵
Glucose (mg dL ⁻¹)	Hours										
. – .	0	34.64	26.42	42.38	36.36	32.36	34.26^{d}	1.933	0.0001	0.5495	0.0472
	1	44.14	35.42	44.82	55.70	47.02	45.73°				
	2	53.18	61.90	59.91	54.54	50.01	55.92 ^b				
	3	65.27	61.62	68.49	53.12	81.35	65.26a				
	4	64.65	62.22	56.53	53.17	69.03	61.34^{ab}				
	6	59.28	73.79	70.48	60.57	64.66	66.02ª				
	8	66.29	63.06	75.47	72.43	51.20	66.19 ^a				
Mean		54.04ª	55.85ª	59.99 ^a	54.65°	56.22ª					
Liver glycogen (mg g ⁻¹)	Days										
0, 0, 00,	0	31.52	31.52	31.52	31.52	31.52	31.52^{b}	1.589	0.0001	0.0163	0.2337
	30	24.51	25.94	27.87	26.52	33.36	27.62 ^b				
	60	35.25	39.80	50.64	42.88	58.25	45.36°				
Mean		29.26°	32.95^{bc}	36.42^{ab}	33.38 ^{bc}	42.08°					
Hepatosomatic index (%)	Days										
. , ,	0	1.83	1.83	1.83	1.83	1.83	1.83ª	0.263	0.2458	0.8171	0.5685
	30	1.60	1.56	1.86	1.99	1.87	1.77ª				
	60	1.91	2.00	1.97	1.80	1.93	1.92ª				
Mean		1.78ª	1.79ª	1.89ª	1.87ª	1.88^{a}					

Means in the same row letters indicate significant difference between groups; means in the same column letters indicate significant difference within group, a>b> c>d; ¹Probability; ²Pooled standard error mean; ³Period; ⁴Treatments; ⁵Interaction between period and treatment

of organic Cr to animal diets resulted in beneficial effects on growth, reproduction and performance (Burton et al., 1996). Studies on laboratory animals (Gray and Bowman, 1992; Morris et al., 1992), cattle (Kegley et al., 1996) and sheep (Mufarrej et al., 2007; Kraidees et al., 2009) have also shown that dietary supplementation with Cr had positive effects on glucose metabolism, insulin activity, growth, performance and resistance to stress.

The predominant physiological role of Cr is to potentiate the action of insulin through its role as a component of the Glucose Tolerance Factor (GTF), an essential dietary agent that regulates carbohydrate,

protein and lipid metabolism (Anderson and Mertz, 1977). Cr is needed as a co-factor for insulin to move circulatory glucose into peripheral tissues.

There are several reports on the beneficial effect of trivalent Cr supplementation primarily on glucose tolerance and related variables of hyperglycemic and diabetic mammals (Anderson *et al.*, 1990). Dietary supplementation with Cr bound to organic molecules was also shown to have positive effects on glucose metabolism and insulin activity and to increase glucose clearance rate and decrease glucose half-life (Gray and Bowman, 1992). It was also shown to increase the rate of

lean deposition and improve carcass characteristics in different species of livestock (Bunting *et al.*, 1994; Lindemann *et al.*, 1995; Kraidees *et al.*, 2009). The aim of this study was to delineate the effect of source and level of dietary Cr on performance, chemical composition, plasma glucose, hepatosomatic index and liver glycogen in hybrid tilapia. The results indicated that the inclusion of Cr in tilapia fish feed can lead to general improvement of body chemical composition and physiology with respect to ether extract, total energy and liver glycogen.

Previous studies by Shiau and Chen (1993) indicated that tilapia fish fed on glucose diets supplemented with any type of Cr attained higher weight gain than those fed the glucose diet without Cr supplementation. According to these researchers, fish receiving a glucose diet supplemented with Cr₂O₃ had greater weight gain, food intake, protein retention, energy retention and body lipid concentration than those fed an unsupplemented glucose diet or glucose diet supplemented with CrCl₃.6H Q or Na₂CrO₄.4H₂O.

Shiau and Lin (1993) also reported that Cr supplementation of glucose diets significantly increased weight gain, energy deposition and liver glycogen content in tilapia fish fed the glucose diet. Comparing 0.5 and 2% levels of Cr_2O_3 as supplements for tilapia fish diets containing glucose or starch, Shiau and Liang (1995) concluded that the level of Cr_2O_3 in the diet affected glucose utilization and nutrient digestibility by tilapia, whereas the time of Cr_2O_3 inclusion had no effect on carbohydrate utilization and digestibility.

On the other hand, Bureau *et al.* (1995) observed no increase in performance parameters in rainbow fish as a result of dietary supplementation with 0.5 mg kg⁻¹ of chelated Cr at different levels of protein in the feed ingredient.

The occurrence of statistically significant increase in ether extraction and gross energy at 1 mg kg⁻¹ Cr₂O₃ in the present fish is in line with the findings of previous researchers (Shiau and Lin, 1993; Shiau and Chen, 1993; Shiau and Liang, 1995). Similarly, the occurrence of statistically significant increased in liver glycogen as result of Cr supplementation is in line with Shiau and Lin (1993), Shiau and Chen (1993), Shiau and Liang (1995) and Pan *et al.* (2003). However, studies dealing with Cr effect on HSI in fish are extremely meager. The present findings agree with Bureau *et al.* (1995) in that Cr-supplementation had no significant effect on HSI.

The specific mechanisms by which Cr enhances the utilization of dietary glucose in hybrid tilapia remain to be elucidated. It is evident, however that supplementation of Cr_2O_3 to a diet containing glucose improved glucose utilization by hybrid tilapia and was much more effective

than other sources of inorganic chromium (Shiau and Liang, 1995). Experiments carried out so far provided clear evidence that Cr is able to exert some effect on fish metabolism (Tacon and Beveridge, 1982; Sastry and Sunita, 1982; Hertz *et al.*, 1989; Shiau and Lin, 1993; Shiau and Chen, 1993; Shiau and Liang, 1995; Shiau and Shy, 1998; Bureau *et al.*, 1995; Pan *et al.*, 2003). On the other hand, some studies have shown that dietary trivalent Cr did not improve growth performance or feed efficiency. Ng and Wilson (1997) and Jain *et al.* (1994) reported growth enhancement in carp fed 10 mg Cr kg⁻¹ of diets and stated that carcass Cr increased with Cr intake. However, growth reduction was recorded when the carp were fed 20 and 40 mg Cr kg⁻¹

Improvement of glucose utilization by dietary Cr_2O_3 supplementation has significant implications in aquatic animals nutrition because Cr_2O_3 is an important and widely used indirect marker for measuring nutrient digestibility values. It has been recently reported that the level of Cr_2O_3 in the diet alters glucose utilization and affects nutrient digestibility by tilapia (Shiau and Liang, 1995).

When these researchers added 0.5 and 2% of Cr_2O_3 to the diet of glucose-fed fish, higher weight gain was observed in the 0.5 than in the 2% Cr_2O_3 supplemented group while digestibility estimates decreased with increasing dietary Cr_2O_3 levels.

On the other hand, fish glucose diets without supplemental Cr produced significantly less weight gain, more rapid plasma glucose peak time and elevated glucose-6-phosphate activity, compared with fish fed glucose diets with supplemental Cr, regardless of source.

Currently, there are a few studies demonstrating the influence of dietary Cr supplementation on tilapia nutrition. The overall results of these studies, including the present study are encouraging and warrant further more detailed investigations into the effect of the source and level of Cr on fish performance and metabolism. It is also imperative to understand the mechanisms underlying those effects.

CONCLUSION

It is observed that the inclusion of Cr in the feed significantly enhances ether extract, total energy and liver glycogen of tilapia fish when organic and inorganic Cr are used while 2 mg kg⁻¹ of organic Cr supplementation might lead to decrease in performance parameters. Further, more detailed studies into the effect of Cr on the performance of fish and hence, their ability to increase tilapia production per meter, should be carried out.

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