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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*)

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Abstract: This study was undertaken to determine the effect of dietary chromium supplementation on humoral antibody response and some blood constituents in hybrid tilapia fish (*Oreohromis niloticus* × *O. aureus*). Cr-yeast or Chromic Oxide (Cr_2O_3) were added to fish feed at the rates of 1 or 2 mg kg^{-1}, while control fish received unsupplemented diet. Three hundred and forty fish (Avg. weight 37.8 g) were randomly assigned to 5 feeding regimens with two replicates each. Each replicate was reared separately. The fish were immunized with Sheep Red Blood Cells (SRBCs) at 0 day and re-immunized at 30 days of the experiment to determine primary and secondary humoral antibody responses, respectively. Blood samples were collected from 15 fish/replicate at 0 and 25 days to determine blood plasma constituents and at 5 days intervals during primary and secondary immunization up to 50 days to determine anti-SRBC antibody titers. The results shown decreased plasma glucose and increased plasma cholesterol concentrations at 25 days compared to 0 days in all groups. Increased triglyceride levels was noted in fish receiving Cr_2O_3 (1 mg kg^{-1}) and decreased albumin: globulin ratio in those receiving Cr yeast (2 mg kg^{-1}) at 25 days ($p<0.05$). No significant changes were observed in total protein, albumin and total globulin concentrations due to either Cr treatment or feeding duration. Anti-SRBC antibody titers were significantly higher in Cr-supplemented groups versus control, with highest titers in fish receiving 2 mg kg^{-1} Cr-yeast supplement during secondary immunization. These findings indicate that the inclusion of Cr in the feed significantly augments humoral antibody response of tilapia.

Key words: Chromium supplementation, tilapia fish, blood parameters, immune response, SRBC

INTRODUCTION

Diseases are a major source of economic concern in intensive fish aquaculture. Increasing attention is paid to preventing aquaculture fish diseases and developing vaccines to control them (Midtlyng, 1997). Attention is also focused on the role of nutrition in promoting fish health and reducing disease incidence (Blazer, 1992). Several micro-nutrients such as vitamins, retinol, tocopherol and pyridoxine have been shown to enhance fish immunity (Pulsford *et al*., 1995). On the other hand, although, many trace elements are known to augment immunological responses in mammals and birds, only limited information is available on their role in fish immunology (Anderson, 1996; Sealey *et al*., 1997). Nutrient mineral supplementation is fundamental to cost effective and sustainable fish production. Recent studies have shown that organic forms of nutritional minerals are better absorbed and constitute a lesser environmental risk than inorganic forms (Evans, 1982; Anderson, 1987). Chromium (Cr) is an essential trace element of wide distribution in nature. It occurs in different oxidation states, the most stable of which is the trivalent state, which has long been identified as the active ingredient of glucose tolerance factor in man and animals (Mertz, 1992, 1993; Burton, 1995). Previous studies have confirmed the benefits of Cr as a micronutrient in animal production (Anderson, 1987). Its incorporation as a feed supplement appears to influence metabolic processes, resulting in improved growth, reproductive efficiency and carcass composition of animals, whereas its deficiency has been shown to decrease growth, expected life-span and glucose tolerance (Mallard and Borgs, 1997). Cr has also been shown to enhance or modulate immune responses in various animals (Mordenti *et al*., 1997; Mallard *et al*., 1994).

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Fish have a limited ability to utilize carbohydrates. This is primarily due to their relatively poor glucose tolerance associated with lack of adequate insulin response (Furuichi and Yone, 1981) or low insulin receptor activity (Plisetskaya *et al*., 1986). Cr re-enforces the action of insulin by serving as a co-factor of insulin activity (Mertz, 1992). It has been reported that dietary Cr_2O_3 supplementation increased weight gain, feed intake and protein and energy metabolism and decreased plasma glucose concentration in tilapia (Shiau and Chen, 1993; Shiau and Liang, 1995). Similar results have also been obtained, when organic Cr was included in a high glucose diet of tilapia (Pan *et al*., 2003).

Relatively few studies have been published on Cr supplementation in fish feed, especially in the common carp (Hertz *et al*., 1989), tilapia (Shiau and Lin, 1993; Shiau and Chen, 1993; Shiau and Liang, 1995; Pan *et al*., 2003) and rainbow trout (Bureau *et al*., 1995; Gatta *et al*., 2001) feeds. Most of these studies were concerned with the role of Cr in fish 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; Arunkumar *et al*., 2000), while no studies are found on the effect of Cr source and level on immunity and blood composition of these animals. The following study was undertaken to determine the effect of two different sources and two concentration levels of Cr on blood parameters and immune response of tilapia.

MATERIALS AND METHODS

Fish and husbandry: Fish fingerlings (Avg. 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 in the experimental farm for 6 weeks. Thereafter, 340 fishes (Avg. weight 37.8 g) were collected from the stock tanks and divided randomly into five feeding groups, each comprising two replicates of 34 fish/replicate. Each replicate was kept separately in a glass aquarium (31×41×61 cm^3) supplied with filtered water and aerated with airstone to maintain adequate oxygen supply. The aquaria were fitted with a waste filtration devise, while water temperature was maintained at 28°C using an electric thermostat. Aquarium water was changed every 48 h. Photoperiod was adjusted to 12 h light and 12 h dark, while water quality was monitored regularly for temperature, pH and dissolved O_2, HCO_3^-, Cl^-, NH_3, NO_3^- and NO_2^- concentrations to ensure that they remained within tolerance limits for tilapia (Balarin and Hatton, 1979). The fish were fed their prescribed diets 3 times daily at the rate of 3% of their average body weight.

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

Formulation	CONT	CRO_1	CRO_2	CRY_1	CRY_2
Treatments[1] (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 laboratory(calculated)					
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

[1]Treatments are described as CONT control without chromium addition, CRO_1: 1 ppm chromic Oxide addition; CRO_2: 2 ppm chromic Oxide addition; CRY_1: Chromium yeast 1 ppm addition; CRY_2: 2 ppm chromium yeast addition; [2]Chromium concentrations in each formula mixed with corn; Chromium laboratory analyses are 0.73, 1.33, 2.27, 1.27 and 2.18 ppm as CONT, CRO_1, CRO_2, CRY_1 and CRY_2, respectively

A pre-immunization period of 7 days was allowed for acclimatization and collection of pre-immunization data. At the onset of the experiment (0 day), all groups of fish were immunized and 30 days later, re-immunized with a 25% suspension of Sheep Red Blood Cells (SRBCs) in alsevers solution. Each fish was inoculated intraperitoneally with 5 μL g^{-1} body weight using a tuberculin syringe (1 mL, 25 g) (Nakanishi, 1986).

Experimental diets: A commercial tilapia feed (Arabian Agricultural Services Co., Riyadh) supplemented with either Cr_2O_3 (Sigma-Aldrich Chemie GMBH, Germany) or Cr-yeast (Alltec Co., UK) was used. The supplementary Cr 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 CRO_1 and CRO_2) or Cr-yeast (groups CRY_1 or CRY_2). An un-supplemented diet served as control (CONT). Details of Cr supplementation and feed analysis are given in Table 1.

Measurements: Blood samples were collected from 150 fish (15 fish/replicate) by cardiac puncture using a 3 mL, 22 g ×1" 0.7×38 mm syringe coated with EDTA-K_2 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 chemical determinations were collected at 0 and 25 days of the experiment. The plasma

concentrations of glucose, cholesterol, triglycerides, total protein, albumin and total globulin were measured spectrophotometrically using commercial reagent kits (Randox, UK). To determine humoral immune responses, plasma samples were collected at 5, 10, 15, 20 and 25 days (primary immunization period) and 35, 40, 45 and 50 days (secondary immunization period) of the experiment. Anti-SRBC antibody titers were determined using a microtiter hemagglutination technique (Witlin, 1967).

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

Blood constituents: As shown in Table 2, a highly significant increase in total cholesterol was recorded at 25 days compared to 0 day of the experiment in all groups, including the controls ($p<0.0001$). On the other hand, glucose concentration tended to decrease at 25 days in all groups, with a statistically significant decrease being recorded in groups CRO_1 ($p<0.05$) and CRY_2 ($p<0.005$). A significant increase in albumin concentration and hence, total proteins concentration and albumin: globulin ratio, was observed in the CRY2 group ($p<0.05$). All other parameters were not significantly affected by dietary Cr supplementation.

Humoral immunity: Table 3 provides mean antibody titers in all groups of fish following immunization against SRBCs. Antibody production became evident following primary immunization with SRBCs and reached its peak by 10 days in the control and CRO_2 groups and 15 days in the CRY_1 group, then started to decline. In CRO_1 and CRY_2 groups, on the other hand, the titer continued to rise gradually until, 20 days before declining. The overall mean antibody titers during the primary immunization period were significantly higher in all Cr-supplemented groups when compared to the controls and in CRO_1 and CRY_2 groups, when compared to CRO_2 group (Table 3). Secondary immunization induced a more rapid and a higher rise in antibody titers and the titers remained

Table 2: Some blood parameters values in immunized fish against Sheep Red Blood Cells (SRBCs)

							Analysis of variances			
		Treatments							F[1]>Pr	
Measurements	Days	CONT	CRO_1	CRO_2	CRY_1	CRY_2	SEM[2]	p[3]	T[4]	(P×T)[5]
Glucose (mg dL^{-1})	0	77.46	72.24^{x}	73.59	71.19	76.56^{x}	2.766		0.907	0.5720
	25	64.33	56.63^{y}	58.89	64.05	52.22^{y}	2.599		0.349	
		2.544	2.483	2.968	2.788	2.555				
		0.0620	0.0240	0.1175	0.3738	0.0022		F>Pr		
Cholesterol (mg dL^{-1})	0	094.45^{y}	110.42^{y}	108.48^{y}	107.95^{y}	103.99^{y}	3.493		0.367	0.8930
	25	145.38^{x}	146.21^{x}	154.46^{x}	155.90^{x}	153.41^{x}	3.886		0.931	
		2.858	4.151	3.799	3.495	3.494				
		0.0001	0.0254	0.0013	0.0002	0.0001		F>Pr		
Triglyceride (mg dL^{-1})	0	152.85^{b}	182.61^{ab}	213.81^{ab}	243.61^{a}	208.84^{ab}	6.001		0.041	0.4750
	25	180.13	258.19	190.10	294.60	213.43	7.695		0.280	
		4.559	7.118	6.372	7.354	7.184				
		0.1856	0.1197	0.5176	0.3001	0.9202		F>Pr		
Total protein (g L^{-1})	0	2.99	3.09	3.20	3.03	2.82^{y}	0.400		0.216	0.2660
	25	3.12	3.34	3.06	3.12	3.14^{x}	0.385		0.389	
		0.335	0.423	0.443	0.365	0.368				
		0.2809	0.1799	0.4758	0.5387	0.0346		F>Pr		
Albumin (g L^{-1})	0	1.40^{a}	1.40^{a}	1.43^{a}	1.39^{a}	1.18^{b}	0.292		0.039	0.1033
	25	1.48	1.53	1.35	1.38	1.43^{x}	0.304		0.306	
		0.288	0.319	0.291	0.310	0.279				
		0.3885	0.2090	0.3837	0.9083	0.0066		F>Pr		
Globulins (g L^{-1})	0	1.59	1.69	1.78	1.65	1.64	0.330		0.499	0.7430
	25	1.64	1.81	1.71	1.74	1.72	0.322		0.594	
		0.335	0.305	0.385	0.286	0.285				
		0.6316	0.2188	0.6378	0.2576	0.3946		F>Pr		
Albumin:globulin ratio	0	0.89	0.83	0.83	0.85	0.72^{y}	0.243		0.070	0.6710
	25	0.93	0.84	0.80	0.80	0.80^{x}	0.276		0.397	
		0.320	0.211	0.252	0.260	0.215				
		0.6934	0.7624	0.6719	0.5061	0.0248		F>Pr		

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

Table 3: Haemagglutination titers in immunized fish against Sheep Red Blood Cells (SRBCs)

		Treatments						Analysis of variances			
									F[1]>Pr		
Measurements	Days	CONT	CRO_1	CRO_2	CRY_1	CRY_2	Mean	SEM[2]	p[3]	T[4]	(P×T)[5]
Antibodies titers											
Primary dose	5	1.41	2.04	1.72	1.96	1.79	1.78[b]	0.869	0.0001	0.0001	0.5507
	10	2.19	2.81	2.67	2.52	2.62	2.56[a]				
	15	1.97	2.82	2.32	2.73	2.65	2.50[a]				
	20	1.95	2.85	2.30	2.38	2.87	2.47[a]				
	25	1.50	1.45	1.33	1.75	2.00	1.61[b]				
	Mean	1.82[c]	2.46[a]	2.11[b]	2.30[ab]	2.43[a]					
Secondary dose	35	3.17	3.38	2.67	2.00	3.71	2.98[a]	0.860	0.1131	0.0001	0.0017
	40	2.81	3.06	2.96	3.08	4.13	3.21[a]				
	45	2.79	3.04	3.17	3.21	4.13	3.27[a]				
	50	2.71	3.04	2.88	3.42	4.29	3.27[a]				
	Mean	2.87[b]	3.13[b]	2.92[b]	2.93[b]	4.06[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; [1]Probability; [2]Pooled standard error mean; [3]Period; [4]Treatments; [5]Interaction between period and treatment

relatively unchanged in all groups, until the termination of the experiment at 50 days. The highest antibody titers of all groups during secondary immunization occurred in the CRY_2 group receiving 2 mg kg^{-1} organic chromium supplement. Comparison of the overall mean antibody titers during secondary immunization shown significantly higher overall titer in that group compared to all other treatments.

DISCUSSION

Interest in Cr supplementation of fish feeds has evolved over the past decade in part due to studies indicating that addition of organic Cr to human and animal diets resulted in beneficial effects on growth, reproduction and immunological performance (Burton *et al*., 1996). Studies on laboratory animals (Gray and Bowman, 1992; Morris *et al*., 1992) calves (Kegley *et al*., 1996) and sheep (Al-Mufarrej *et al*., 2007) have also shown that dietary supplementation with organic Cr produced positive effects on glucose metabolism, insulin activity, growth and survival of the animals.

The aim of the present study was to delineate the effect of dietary Cr supplementation on humoral antibody response and certain blood biochemical parameters of tilapia. The occurrence of a statistically significant decrease in plasma glucose in two of the Cr-supplemented groups in the present study is in line with the findings of Shiau and Chen (1993), Shiau and Lin (1993), Shiau and Liang (1995), Shiau and Shy (1998) and Bureau *et al*. (1995). However in contrast to the findings in mammals (Mossop, 1983; Anderson, 1987), no reduction in cholesterol concentration was observed in tilapia receiving Cr supplemented diets in fact, cholesterol concentration in this fish tended to increase, though non-significantly, after 25 days of feeding.

Several studies previously have indicated that dietary Cr-supplementation improved cellular and humoral immune responses in mammals (Chang and Mowat, 1992; Burton *et al*., 1993; Mathison and Engstrom, 1995; Burton *et al*., 1996) and birds (Uyanik *et al*., 2002; Lee *et al*., 2003; JiYue *et al*., 2004; Bhagat *et al*., 2008). However, studies dealing with the effect of chromium on immunity in fish have been virtually limited to studying the effect of toxic levels of Cr, which suppressed immune responses of these animals (Khangarot *et al*., 1999; Arunkumar *et al*., 2000; Prabakaran *et al*., 2006; Saxena and Kapur-Ghai, 2008). A notable exception is the study of Gatta *et al*. (2001), who reported enhanced serum lysozyme activity and increased phagocytic response of macrophages in juvenile rainbow trout (*Oncorhynchus mykiss*) receiving organic Cr-supplemented diets. The present study is the first to shows that dietary Cr supplementation enhanced humoral immunity in tilapia. Comparison of the SRBC-immunized tilapia consistently showed that a significant increase in antibody titers was induced by organic as well as inorganic dietary Cr-supplementations, during both primary and secondary immunizations. The higher response following secondary immunization was expected since secondary immunization typically produces an anamnestic reaction resulting in more pronounced and longer lasting humoral and cell mediated immune responses (Tizard, 2000).

CONCLUSION

It is concluded from this study that the inclusion of Cr in the feed significantly enhances humoral antibody responses of tilapia fish, both during primary and secondary immunization and particularly when organic Cr is used. These results are encouraging and warrant further, more detailed studies into the effect of chromium on humoral and cellular immune responses of tilapia and, hence, their ability to resist infection.

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