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EFFECT OF DIETARY PHOSPHORUS AND ZINC LEVELS ON HEMATOCRIT VALUE, PLASMA MINERAL CONTENT AND PLASMA ALKALINE PHOSPHATASE ACTIVITY OF FINGERLING RAINBOW TROUT, *Oncorhynchus mykiss*

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ABSTRACT

A laboratory based 2 × 2 factorial experiment was conducted to investigate the effects of dietary phosphorus and zinc levels on hematocrit value, plasma alkaline phosphatase activity and plasma mineral contents of rainbow trout fingerling for 21 weeks. Two levels of phosphorus (19 and 30 mg/g) and two levels of zinc (55 and 100 µg/g) in the dry diets were tested. Duplicate tanks of 30 rainbow trout (average weight 1.56 ± 0.24g) per 60L glass tank were fed experimental diets three times a day to satiation level in 15 to 24°C water temperature. Zinc supplementation in the practical diets significantly increased the hematocrit value, plasma alkaline phosphatase activity and plasma zinc contents in rainbow trout fingerling. On the other hand, addition of phosphorus did not show any significant difference among the treatments. The result of the present study demonstrated that additional zinc significantly ($P<0.05$) influenced the hematocrit value, plasma alkaline phosphatase activity and plasma zinc contents in rainbow trout. Hence, it can be concluded that zinc supplementation is necessary in fingerling rainbow trout feed. Further studies in this area with different size and age of rainbow trout are needed broadly.

Key words : Hematocrit, Plasma mineral, Plasma alkaline phosphatase, Rainbow trout

INTRODUCTION

Rainbow trout, which belongs to the family Salmonidae, is endemic to western North American and eastern Asia and exists in a number of distinctive ecological forms and sub forms, which differ in morphology, behavior and life history traits. This species was originally taxonomically linked with the Atlantic or Eurasian trouts of the genus *Salmo*. The natural geographical distribution of rainbow trout includes freshwater systems along the eastern Pacific Ocean, mainly west of the Rocky Mountains, from the northwest Mexico (including extreme northern Baja California) to the Kuskokwim River in

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southwestern Alaska. In Asia, rainbow trout are most abundant on the Kamchatka Peninsula. Rainbow trout have been introduced widely outside their natural range in suitable habit throughout North America and other parts of the world, such as South America, Europe, Southern Asia, Japan, Africa and Oceania (Groot, 1996; Scott and Crossman, 1975).

In the natural environment rainbow trout feed on various invertebrates including plankton, larger crustaceans, fish insects, snails, and leeches. Nutritional requirements of rainbow trout have been well studied (NRC, 1993). Rainbow trout is by far the most widely farmed trout in the world and one of the few species of fish that may be regarded as truly domesticated.

Like in other animals, P is an essential nutrient for fish, being a major constituent of skeletal tissues, nucleic acids, DNA and RNA, energy transport compounds like ATP, and of phospholipids in cell membranes (Lall, 1991). Dietary phosphorus (P) is essential for optimal growth and metabolism of fish. It is the most important mineral needed by fish, since its requirement and functions are superior to that of any other mineral element (NRC, 1993; Satoh *et al*., 2002).

Information on the metabolism, excretion and utilization of dietary P in fish is limited (Lall, 1991). However, the necessity of P supplementation in a fish meal-based diet has been reported for the post juvenile stage (Masumoto, 2002). Fish meal is the source of most dietary P in fish diets, wherein it exists as hydroxyapatite and/or tricalcium phosphate (TCP). Due to its structural complexity, P and Ca from TCP have been reported to be less available to some fish species (Takamtus *et al*., 1975; Shitanda *et al*., 1979; Watanabe *et al*., 1980) as a result of which, large amount of P are excreted in feces, leading to wastage, and environmental pollution. On the other hand, inorganic phosphorus from sodium phosphate is highly available to all fish.

Zinc (Zn) is required in the diet as it is more efficiently absorbed than the waterborne Zn (NRC, 1993). It is essential for growth, development and maintenance of healthy bones (Yamaguchi, 1998), and functions as a cofactor of several enzymes and an integral part of about 20 metalloenzymes such as alkaline phosphates (ALP), alcohol dehydrogenase and carbonic anhydrase (Hambidge *et al*., 1986; Watanabe *et al*., 1997). Moreover, Zn deficiency affects the digestibility of protein and carbohydrate because of the reduced activity of carboxypeptidase (Ogino and Yang, 1978). There may be interaction effect of Zn and P on hematocrit value, plasma alkaline phosphates activity and plasma mineral contents of fingerling rainbow trout.

Therefore, the present study aimed to investigate the effects of dietary phosphorus and zinc levels and their interaction on hematocrit value, plasma alkaline phosphates activity and plasma mineral contents of fingerling rainbow trout.

MATERIALS AND METHODS

Experimental diets and design

Practical diets were formulated to contain 19 and 30 g/kg P employing monocalcium phosphate, and 54 and 100 g/kg Zn using inorganic zinc sulphate (Table 1). The diets were labeled according to factors (P and Zn) and as P0Z0, P0Zl, PlZ0 and PlZl. The experimental diets were formulated to be isocaloric and isonitrogenous. The carbohydrate sources and binders were wheat flour and pregelatinized starch, and the lipid source was pollock liver oil and soybean oil. The formulation and composition of experimental diets are presented in Table 1. The experiment was conducted in a 2 × 2 factorial design with the factors 'dietary phosphorus level' and 'supplemental Zn level'.

Table 1. Formulation and composition of the experimental diets

Ingredients (%)	Diets			
	P0Z0	P0Zl	PlZ0	PlZl
Jack mackerel meal	57	57	57	57
Wheat flour	20	20	20	20
Pregelatinized starch	5	5	5	5
Pollock liver oil	4	4	4	4
Soybean oil	5	5	5	5
Mineral premixutre[a]	0	1	0	1
Zn free mineral mixture[b]	0	1	0	1
Ca $(H_2PO_4)_2$	0	0	4	4
Vitamin premizture[c]	1.5	1.5	1.5	1.5
Choline chloride	0.5	0.5	0.5	0.5
Vitamin E (50%)	0.1	0.1	0.1	0.1
Cellulose	5.9	5.9	5.9	5.9

[a] Mineral premixtur (%) - NaCl 5.0, Mg $SO_4.7H_2O$ 74.5, $FeC_6H_5O_7.7H_2O$ 12.5, Trace element mix.[a*] 5.0, Cellulose 3.0

[a*] (%) - $ZnSO_4.7H_2O$ 553, $MnSO_4.5H_2O$ 162, $CuSO_4.5H_2O$ 31, $AlCl_3.6H_2O$ 10, $CoCl.6H_2O$ 1, KIO_3 3, Cellulose 986

[C] Vitamin mix (%)- Thiamine hydrochloride 6, Riboflavin 10, Pyridoxine hydrochloride 4, Cyanocobalamin 0.01, Ascorbic acid 500, Niacin 40, Ca-pantothenate, 10, Inositol 200, Biotin 0.6, Folic acid 1.5, *p*-aminobenzoic, Vitamin K_3 5, Vitamin A acetate 4000 IU, Vitamin D_3 4000 IU

The minerals mixture used in this study was the modified form of Ogino salt mixture (Ogino *et al*., 1980). The diets were pelleted using the labortory pelletizer (AEZ12M, Higaga-Seikakusho, Kobe, Japan), dried in a vacuum freeze-drier (RLE-206, Kyowa Vacuum Tech., Saitama, Japan), and stored at 4°C until used. The proximate composition and minerals contents of the experimental diets used in this study are shown in Table 2 and Table 3, respectively. The diets ware prepared with 57% fish meal (FM) as the sole

protein source. Ingredients used in the test diets were selected taking into consideration the amino acid balance of the whole protein sources (Watanabe *et al*., 1993).

Table 2. Proximate composition of the experimental diets (dry matter basis)

Parameters	Diets			
	P0Z0	P0Z1	P1Z0	P1Z1
Moisture (%)	4.5	4.5	3.0	4.5
Crude ash (%)	11.1	10.8	13.7	13.6
Crude protein (%)	44.9	44.7	45.5	45.2
Crude lipid (%)	16.6	16.7	16.6	16.9
Gross energy (kcal/g)	5.2	5.2	5.0	5.0

Table 3. Mineral contents of the experimental diets (dry matter basis)

Macro elements	Diets			
	P0Z0	P0Z1	P1Z0	P1Z1
P (mg/g)	19.14	19.49	30.30	30.09
Ca (mg/g)	28.03	30.19	34.76	35.20
Mg (mg/g)	3.00	3.05	3.00	2.97
Na (mg/g)	3.86	3.41	3.58	3.82
K (mg/g)	4.71	3.75	4.32	4.50
Trace elements				
Zn (µg/g)	54.54	103.24	55.85	96.07
Mn (µg/g)	40.44	40.71	41.18	40.62
Fe (µg/g)	345.54	341.90	352.17	348.24
Cu (µg/g)	7.04	7.05	10.63	8.35

Fish rearing and feeding methods

Eyed egg of rainbow trout were obtained from Fuji Trout Farm of Shizuoka Prefecture Fisheries Experiment Station and hatched under laboratory conditions at the Tokyo University of Marine Science and Technology in the year 2005. Fish with an average body weight of 1.56 ± 0.24g were randomly selected from stock and distributed into 60 L tanks at a density of 30 fish per tank. Triplicate groups were assigned to each experimental diet and the feeding was conducted for 21 weeks. The fish were hand fed three times per day, six days a week to apparent satiation level. The tanks had a continuous water supply at a rate of 0.6 – 1.0 l/min and the temperature was 15 to 24°C.

Sampling and analytical methods

The fish were starved for 24 h before being individually weighed at the initial day and every 21 days of the experimental period after being anesthetized with ethylene glycol

monophenyl ether (300 ppm). At the same time 5 fish were randomly sampled from each tank and stored at -20°C for analyses.

Proximate composition and chemical analyses of the diets and fish whole body samples were made in three replicates. Proximate analysis of the samples were performed as follows: moisture contents was measured gravimetrically, crude ash contents was determined by incinerating a known amount of sample in an electric muffle furnace (Yamato, FA-21) at 600°C for 8 hours, crude protein was analyzed using the Kjeltec Auto Sampler System 1035/38 (Netherland), and crude lipid was measured by following the method of Folch *et al*. (1957). Samples for minerals were digested in nitric acid using the MLS-1200 Mega Microwave Digestion System (Italy), cooled in flowing water for 30 minutes, and diluted with de-ionized water to the required volume. Concentration of each element was measured by a Polarized Zeeman Atomic Absorption Spectrophotometer (Hitachi Z-5010, Tokyo, Japan) except for phosphorus which was analyzed by a visible light spectrophotometry (Shimadzu, UV 265 FW, Kyoto, Japan) at 750 nm.

Hematocrit (Hct)

Hct level was determined by centrifugation of the heparinized blood samples in microhematocrit tubes at 5000 rpm for 5 min using a High-Speed Micro centrifuge MC-150 (Tomy, Japan).

Enzyme assay (Plasma alkaline phosphates)

Plasma sample were obtained from heparinized blood samples of the fish at the end of the experiment. The alkaline phosphate (ALP) activity of the antocoagulated blood plasma was determined using the test pack by the Abbott Vision System (Abbott Laboratories, IL, USA). The ALP of the sample catalyses the magnesium-activated base hydrolysis of p-nitro-phenyl phosphate producing nitrophenolate and the reaction is measured based on the increase in absorbance at 418 nm. The rate of hydrolysis is directly proportional to the activity of the enzyme (Abbott Laboratories 1989).

Statistical analyses

Statistical analyses were performed using one-way and two-way ANOVA with SYSTAT 8.0 software (SPSS Inc., 1998). Differences between treatments were evaluated by Tukey's test. The level of significance was set at $P<0.05$ for all tests.

RESULTS AND DISCUSSION

Weight gain of the fish did not show any significant difference among treatments of both P and Zn throughout the culture period. Also SGR, FCR and TGC were not significantly affected by the treatment (Table 4). The results of growth performance and feed utilization suggest that different levels of both P and Zn had no influence on the feed intake and growth performance of fish. In addition, the results represent the stated parameters not to ie potential and appropriate indices to assess P and Zn level

differences. Similar results were obtained by Hardy and Shearer (1985); Li and Robinson (1996); Apines *et al*. (2001).

Table 4. Growth and feed performance of the experimental diets for 21 weeks

Diet group	Weight gain	SGR[1] (%/day)	FCR[2]	TGC[3]	Condition factor
P0Z0	73.62[a]	2.62[a]	0.97[a]	0.001125[a]	1.161[a]
P0Zl	72.61[a]	2.62[a]	0.96[a]	0.001118[a]	1.153[a]
PlZ0	62.88[b]	2.52[b]	1.01[b]	0.001047[b]	1.162[a]
PlZl	59.54[b]	2.48[b]	1.02[b]	0.001021[b]	1.125[a]
P	<0.05	<0.05	<0.05	<0.05	NS
Z	NS	NS	NS	NS	NS
P × Z	NS	NS	<0.05	NS	NS

[1] Specific growth rate; [2]Feed conversion ratio. [3]Thermal-unit growth coefficient

* Values in the same column not sharing a common superscript letter are significantly different (P<0.05)

Feed conversion in the present study also did not differ among the treatments, which indicates that the tested sources had no influence on feed intake and growth performance of the fish. Conversely, growth and feed consumption were significantly influenced by the dietary Zn levels in abalone (Tan and Mai, 2001).

Carcass proximate composition of rainbow trout at start and end of the experiment presented in the Table 5 revealed influence of Zn supplementation on crude ash though not remarkable while P supplementation showed significant difference on whole body crude ash contents. This is in agreement in our previous study (Sarker et al. 2009).

Table 5. Proximate carcass composition of fish at start (n = 30) and end (n = 12) of the experiment fed experimental diets

Initial/Diet group	Moisture (%)	Crude ash (%)	Crude protein (%)	Crude lipid (%)
Initial	76.36	2.63	15.36	5.88
P0Z0	67.37	1.97b	16.46	14.35
P0Zl	67.33	2.03[ab]	16.26	14.33
PlZ0	68.58	2.36[a]	16.28	12.71
PlZl	68.58	2.17[ab]	16.31	13.22
P level	NS	<0.05	NS	NS
Zn level	NS	NS	NS	NS
P level × Zn level	NS	NS	NS	NS

Values in the same column not sharing a common superscript letter are significantly different (P<0.05)

Hematocrit level of the fish at the end of the experiment is presented in Table 6. It was in the control group and it increased slightly (P>0.05) with the additional Zn. On the other

hand, the increase was not very prominent in the P supplement group. Higher fish hematocrit level in the Zn supplemental groups compared to the control group in this study indicates better health condition achieved by Zn supplemented groups as well as influencing the availability of trace minerals. This is in agreement with the earlier findings of rainbow trout and red sea bream (Apines-Amar *et al*., 2004; Sarker *et al*., 2005, 2007).

Table 6. Hematocrit value (n = 12) and plasma alkaline phosphate activities (n = 10 of rainbow trout at the end of the experiment)

Diet group	Hematocrit value (%)	Plasma alkaline phosphate activities (U/L)
P0Z0	35[b]	114.93[b]
P0Zl	41[a]	122.67[a]
PlZ0	34[b]	116.52[b]
PlZl	40[a]	121.35[a]
P level	NS	NS
Zn level	<0.05	<0.05
P level × Zn level	NS	NS

Values in the same column not sharing a common superscript letter are significantly different (P<0.05). NS: Not significant

Supplementation of both P and Zn in the practical diet of rainbow trout did not show any significant difference on plasma P, Ca, Mg, Na, K, Fe, Cu and Mn (Table 7). On the country, addition of Zn in the practical diet significantly increased plasma Zn contents of rainbow trout (Fig. 1). This result indicates that additional Zn deposits in the plasma tissue and plasma is the indicator of Zn status of fish. David *et al*. 1982 reported that plasma Zn levels were positively correlated with dietary Zn intake.

Table 7. Plasma mineral contents of rainbow trout at the end of the experiment (n = 12)

Diet group	P (mg/g)	Ca (mg/g)	Mg (mg/g)	Na (mg/g)	K (mg/g)	Fe (µg/g)	Cu (µg/g)	Mn (µg/g)
P0Z0	0.52	0.10	0.04	2.71	0.08	1.50	0.74	0.07
P0Zl	0.50	0.10	0.04	2.71	0.08	1.46	0.70	0.09
PlZl	0.46	0.10	0.04	2.72	0.08	1.79	0.88	0.09
PlZl	0.46	0.10	0.04	2.71	0.07	1.41	0.66	0.14
P level	NS	NS	NS	NS	NS	NS	NS	NS
Zn level	NS	NS	NS	NS	NS	NS	NS	NS
P level × Zn level	NS	NS	NS	NS	NS	NS	NS	NS

NS : Not significant

The plasma alkaline phosphates activity was significantly higher in the Zn supplemented group compared to the control (Table 6). Several indicators such as serum levels (Turner *et al*. 1978), enzyme activities (Kfoury *et al*., 1968; Huber and Gershoff, 1973), whole body (Wekell *et al*., 1986) and bone levels (Huber and Gershoff, 1970) have been used to

quantify Zn status in animals. Alkaline phosphates (ALP), a Zn-requiring enzyme is considered as a sensitive indicator of Zn levels in animals. The ALP activity in plasma of rainbow trout in the present study increased with the elevation of dietary Zn indicating that its concentration in the diet affected the enzyme level. This is in agreement with previous studies (Apines *et al*., 2001; Tan and Mai, 2001).

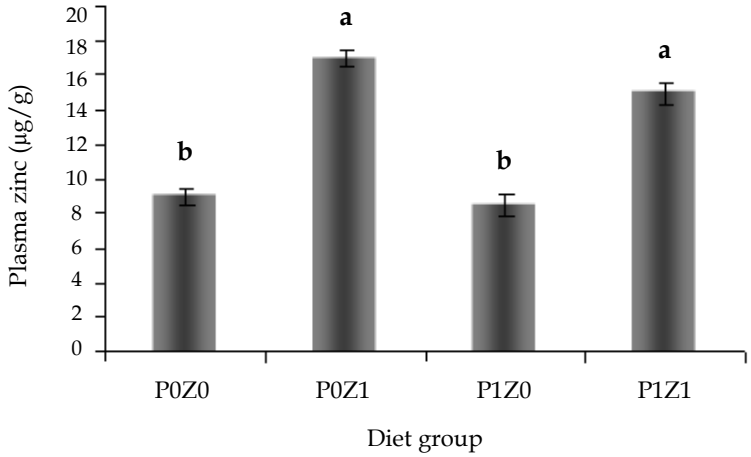

Fig. 1. Effect of dietary P and Zn levels on plazma zinc contents of rainbow trout at the end of the experiment (n = 12) fed the experimental diets for 21 weeks

The overall results of the present study demonstrated that additional zinc significantly influenced the hematocrit value, plasma alkaline phosphates activity and plasma zinc contents in rainbow trout. Hence it can be concluded form the study that zinc supplementation is necessary in fingerling rainbow trout feed and P supplementation is not needed. Thus further study in this area with different sized and aged rainbow trout will corroborate these findings.

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