HAEMATOLOGICAL CHANGES DUE TO DIETARY ASCORBIC ACID DEFICIENCY AT VARIOUS LEVELS OF CALCIUM AND PHOSPHORUS IN RAINBOW TROUT

A. M. AKAND*, M. SATO, R. YOSHINAKA and S. IKEDA†
Department of Fisheries, Faculty of Agriculture, Kyoto University, Kyoto 606, Japan.
**Present address: Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, Bangladesh.*
†*Present address: Department of Fisheries, Faculty of Agriculture, Kinki University, Kowakae, Higashi Osaka – 577, Japan.*

ABSTRACT

Haematological changes of some parameters were investigated in rainbow trout fed diet, containing different levels of calcium and phosphorus with or without ascorbic acid. Serum calcium and phosphorus contents did not vary between ascorbic acid supplemented and deficient groups. Similarly, serum albumin, GPT and haematocrit per cent also did not show any relationship with dietary ascorbic acid, calcium and phosphorus. Dietary deficiency of ascorbic acid decreased the serum haemoglobin contents and increased the serum LDH activities among the various groups.

INTRODUCTION

MANY reports demonstrate the dietary need of ascorbic acid (AsA) for salmonid[1–5]. A review of literature reveals that most of the studies are concerned with the growth and AsA deficiency syndromes such as lordosis, scoliosis, broken back etc. There are only a few reports available about the impact of AsA on blood of fishes [6–8]. These studies are limited to the parameters of haemoglobin, haematocrit and erythrocyte. Agrawal and Mahajan[9] tried to assess the nutritional status of fish, *Channa punctatus*, from haematological parameters. In the present experiment, efforts are made to see the changes in some blood parameters due to dietary AsA deficiency at various levels of calcium and phosphorus.

MATERIALS AND METHODS

Experimental fish

Five-month-old rainbow trouts, *Salmo gairdneri*, weighing 6.3 g on an average were used in the experiment. The number of fish used in one group was 30. All the fishes used in the experiments were hatched out in the laboratory and were maintained on commercial diets until used in the experiment. During the experiment, the fish were stocked in 50 l plastic aquaria provided with continuous supply of well-water flowing at a rate of 2.5 l/min. The well-water contained 10 ppm Ca and 0.36 ppm P.

Experimental diet

The compositions of the diets are given in table 1. An AsA-supplemented (200 mg/100g diet) and a deficient diet were prepared for each composition. The diets were given in semi-moist pellet which were always kept in refrigerator at −20°C. Dietary calcium level was adjusted by calcium lactate and phosphorus level by a mixture of sodium phosphate and potassium phosphate. Five mineral mixtures i.e. mineral mixture 1–5 were prepared to adjust the various levels of dietary calcium and phosphorus.

Composition of the mineral mixture

1. Mineral mixture-1 was McCollum Salt No. 185 which contained: sodium chloride, 4.35 g; magnesium sulphate, 13.70 g; sodium biphosphate, 8.72 g; potassium phosphate (dibasic), 23.98 g; calcium biphosphate, 13.58 g; ferric citrate, 2.97 g; calcium lactate, 32.70 g.
2. Mineral mixture-2 contained: sodium chloride, 4.35 g; magnesium sulphate, 13.70 g; sodium biphosphate, 17.12 g; potassium phosphate (dibasic) 31.30 g; ferric citrate, 2.97 g.
3. Mineral mixture-3 contained: sodium chloride, 4.35 g; magnesium sulphate, 13.70 g; ferric citrate, 2.97 g; calcium lactate, 49.29 g.
4. Mineral mixture-4 contained: sodium chloride, 4.35 g; magnesium sulphate, 13.70 g and ferric citrate, 2.97 g.
5. Mineral mixture-5 contained: sodium biphosphate, 15.51 g and potassium phosphate, 30.77 g.

Table 1 *Composition of experimental diets used in the experiment*

Ingredients	Diet No.						
	1	2	3	4	5	6	7
Egg albumin[a]	40%	40%	40%	40%	40%	40%	40%
Dextrin	25	25	25	25	25	25	25
α-starch	20	20	20	20	20	20	20
Soybean oil	3	3	3	3	3	3	3
Cod liver oil	2	2	2	2	2	2	2
Mineral mixture-1	—	—	—	4	4	4	—
Mineral mixture-2	—	2.76	2.76	—	—	—	—
Mineral mixture-3	—	—	—	—	—	—	2.81
Mineral mixture-4	0.84	—	—	—	—	—	—
Mineral mixture-5	—	—	3	—	3	—	—
Calcium lactate	—	—	—	—	2	3	3
Vitamin mixture[b]	1	1	1	1	1	1	1
Cellulose	8.16	6.24	3.24	5.00	—	2.00	3.19
	Calculated Ca and P content						
Calcium (%)	0	0	0	0.25	0.51	0.64	0.64
Phosphorus (%)	0	0.31	0.89	0.38	0.96	0.38	0
	Estimated Ca and P content						
Calcium (%)	0.06	0.05	0.03	0.31	0.64	0.79	0.83
Phosphorus (%)	0.08	0.40	1.05	0.43	1.17	0.44	0.08

[a]Egg albumin was denatured by boiling with ethyl alcohol for 5 hr.
[b]Provided with following nutrients: Thiamine HCl, 5 mg; riboflavin, 20 mg; pyridoxin HCl, 5 mg; choline chloride, 500 mg; nicotinic acid, 75 mg; calcium panthothenate, 50 mg; inositol, 200 mg; biotin, 5 mg; folic acid, 1.5 mg; menadione, 4 mg; α-tocopherol acetate, 40 mg; and cyanocobalamin, 0.01 mg.

During the preparation of diet, trace elements mixture were added at the rate of 24 mg/100 g diet. Trace elements mixture contained: aluminium chloride, 18 mg; zinc sulphate, 357 mg; cuprous chloride, 11 mg; manganous sulphate, 80 mg; potassium iodide, 17 mg; and cobaltous chloride, 105 mg.

Feeding

Fish in all the groups were fed *ad libitum* twice a day during the feeding period of 16 weeks. At the end of feeding trial 10 fish from each group were used for collection of blood for chemical analyses.

Chemical analyses

Blood was collected in a test-tube by cutting caudal peduncle and was kept at room temperature for 1 hr. Then the blood was centrifuged at 650× g for 10 min to get serum which was then kept at 0°C and analyzed within 4 to 5 hr. The blood serum calcium, phosphorus, total protein, albumin, lactate dehydrogenase (LDH), glutamic pyruvic transaminase (GPT) and haemoglobin were estimated by a Rapid Blood Analyzer (RaBA) kit (Chugai Pharmaceutical Co., Japan, Type-3010) using their reagents and methods. Haematocrit per cent was estimated by the capillary method using a Kubota Haematocrit (model No. KH 120). Diets were digested with 5% perchloric acid to estimate calcium and phosphorus contents. Calcium was estimated by an atomic absorption spectrophotometer (Hitachi, model No. 208). Phosphorus was estimated according to the method of Nakamura[10].

RESULTS AND DISCUSSION

Table 2 shows the contents of total protein, albumin, LDH, GPT, haemoglobin and haematocrit per cent of serum of fish fed diets containing different levels of calcium and phosphorus with or without AsA. Serum total protein contents were low in fish fed diet 1 (calcium and phosphorus-free) and diet 7 (containing no phosphorus). Contents of serum albumin were found to be unaffected by dietary calcium, phosphorus and AsA. LDH activities of serum were comparatively high in all AsA deficient fish (table 2). Van Steirteghem *et al*[11] found variable responses of this enzyme activity following admini-

Table 2 *Effect of dietary AsA deficiency at different levels of dietary Ca and P on blood serum parameters of rainbow trout*

Diet No	1	2	3	4	5	6	7
Total protein	3.7	4.8	4 3	5.1	4 9	4 6	3.4
(g 100 ml)	(3.4)*	(4.5)	(4.5)	(4.4)	(4 9)	(4.7)	(3.7)
Albumin	1 4	1.4	1.0	1.1	1.5	1.0	1.7
(g 100 ml)	(0.7)	(1.0)	(1.0)	(0.9)	(1.7)	(1.1)	(1.7)
LDH	13355	—	11840	13010	11175	8930	9410
(Wroblewski unit)	(17865)	(10200)	(12360)	(15157)	(17430)	(10360)	(19080)
GPT	177.5	68.5	67.3	81.5	90.8	51.0	201.3
(Karmen unit)	(95.5)	(51.0)	(57.8)	(87.0)	(80 0)	—	(199.0)
Haematocrit (%)	26.4	33.5	30.9	24.7	34.7	29.2	29.3
	(31.1)	(35.5)	(34.9)	(26 1)	(32.2)	(31 6)	(26.4)
Haemoglobin	5.7	6.5	9.0	14.4	9.2	8.4	9.2
(g 100 ml)	(5.4)	(4.3)	(8.2)	(8.5)	(8.9)	(8 9)	(6 0)

*Figures in the parentheses are the values for pair-fed AsA-deficient groups.
Each determination was made from the pooled blood sample of 8 to 10 fish.

Table 3 *Effect of dietary AsA deficienty at different levels of dietary Ca and P on blood serum parameters of Ca and P in rainbow trout*

	Diet No.						
	1	2	3	4	5	6	7
Calcium	15 0	16.5	14.9	17.5	15.5	16 2	16.9
(mg/100 ml)	(17.2)*	(12 9)	(18.4)	(17.6)	(18.2)	(18.6)	(15.9)
Phosphorus	10.0	20.1	19.0	24 0	23.2	18.8	8.7
(mg/100 ml)	(6.9)	(20.1)	(23.1)	(21 5)	(22 6)	(23.9)	(10.3)
Ca/P ratio	1.5	0.8	0 8	0.7	0.7	0.9	1.9
	(2 5)	(0.6)	(0.8)	(0.8)	(0.8)	(0.8)	(1.5)

*Figures in the parentheses are the values for AsA-deficient groups. Each determination was made from pooled blood sample of 8 to 10 fish.

stering 3 g of AsA per day per head to 10 adults for 18 days. GPT activities were high among the fish fed diet 1 (calcium and phosphorus-free) and diet 7 (phosphorus-free). This may be due to the abnormal physiological conditions of fish due to the absence of dietary phosphorus or both calcium and phosphorus. Haematocrit per cent also did not vary markedly due to dietary deficiency of AsA at various levels of calcium and phosphorus. This is in conformity with the results of Elliott[12] who reported that the haematocrit per cent did not vary in men fed voluntarily with 1 g of sodium ascorbate per day for 6 weeks. Irrespective of dietary calcium and phosphorus contents, the contents of haemoglobin were comparatively low in all AsA deficient groups except the group fed diet 6. Blood haemoglobin content was reported to be significantly low in AsA-deficient fish, *Channa punctatus*, than control fish[8].

The serum calcium and phosphorus contents did not vary between AsA-supplemented and AsA-deficient groups (table 3). Low serum phosphorus contents in fish fed diet 1 and 7 may be due to the absence of phosphorus in these diets. There were no comparable data to evaluate these results.

The above results indicate that the dietary deficiency of AsA decreases the serum haemoglobin contents and increases the serum LDH activities.

1 August 1986

1. Kitamura, S., Suwa, T. and Nakagawa, K., *Bull. Jpn. Soc. Sci. Fish.*, 1965, **31**, 818.
2. Kitamura, S., *Fish. Pathol.*, 1969, **3**, 73.
3. Halver, J. E., Smith, R. R., Tolbert, B. M. and Baker, E. M., *Ann. N.Y. Acad. Sci.*, 1975, **258**, 81.
4. Sato, M., Yoshinaka, R. and Ikeda, S., *Bull. Jpn. Soc. Sci. Fish.*, 1978, **44**, 1029.
5. Yamamoto, Y., Sato, M. and Ikeda, S., *Bull. Jpn. Soc. Sci. Fish.*, 1978, **44**, 775.

6. Hilton, J. W., Chow, C. Y. and Slinger, S. J., *J. Fish. Res. Board Can.*, 1978, **35**, 431.
7. Lim, C. and Lovell, R. T. *J. Nutr.*, 1978, **108**, 437.
8. Andrew, J. W. and Murai, T., *J. Nutr.*, 1975, **105**, 557.
9. Agrawal, N. K., *J. Nutr.*, 1980, **110**, 2172.
10. Nakamura, M., *Nogei Kagaku Shi*, 1950, **24**, 1.
11. Van Steirteghem, A. C., Robertson, E. A. and Young, D. S., *Clin. Chem.*, 1978, **24**, 54.
12. Elliott, H. C., *Proc. Soc. Exp. Biol. Med.*, 1982, **169**, 363.

NEWS

THE SOUTH INDIAN SCIENCE ASSOCIATION, BANGALORE AND ITS SERVICE TO THE CAUSE OF SCIENCE FOR OVER THREE DECADES

The South Indian Science Association was formed in 1919 by Sri S. S. Iyer, Sri K. N. Kini and Sri P. Ramaswamy Iyer with Sri P. Ramaswamy Iyer as its first Secretary. During those days there were no scientific societies and this Association was the only forum for scientific lectures. The Managing Committees spared no effort to fulfil the aspirations of scientists. Many eminent scientists both from India and abroad (Sir C. V. Raman, Sir K. S. Krishnan, Prof. M. N. Saha, Dewan Bahadur N. N. Iyengar, Prof. Engelhardt, Dr R. W. Williams to mention a few) have addressed the members of the Association. It is a matter of great pride that Sir C. V. Raman announced for the first time the Nobel Prize winning discovery 'New Radiation' (later described as Raman Effect) on the 16th March 1928 under the auspices of this Association. The Association celebrated the Silver Jubilee of the Raman Effect on the 16th March 1953 in a befitting manner on which occasion Sir C. V. Raman addressed the members of the Association.

As time advanced specialization started, new societies devoted to every branch of science came up with the result there was no need for a general society like the South Indian Science Association. The Association had served its purpose for over three decades.

The General Body of the Association which met under the chairmanship of Prof. M. R. A. Rao on the 24 the Feb 1987 at the Chemistry Department, Central College, Bangalore decided to terminate the Association and donate the balance amount belonging to the Association to the Chemical Society, Central College, Bangalore. On this occasion the Association would like to place on record its gratitude to various scientists responsible for the activities of the Association, to the late Prof. B. Sanjiva Rao who was President of the Association for over 20 years and to the Department of Chemistry, Central College, Bangalore at which most of the lectures were held.

R. S. SUBRAHMANYA
Hon. Secretary