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Effects of varying dietary protein level on the blood parameters of *Cyprinus carpio*

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Abstract. Effects of varying dietary protein levels, 14, 28, 38, 48 and 58%, on the blood parameters of juvenile *Cyprinus carpio* were studied. It was found that the various blood cells and related parameters respond differently to the variation in the dietary protein. Fishes fed with low (14%) and high (58%) level protein showed lesser values of red blood cells, haemoglobin content, haematocrit, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, total leucocyte count (TLC), differential leucocyte count (DLC) and coagulation time when compared with optimum protein level (38%). On the other hand, immature red blood cells, erythrocyte sedimentation rate (ESR) and mean corpuscular volume values of fishes fed with low (14%) and high (58%) level proteins were higher than that of the fish fed with optimum (38%) protein level.

Keywords. Dietary protein levels; *Cyprinus carpio*; haematology.

1. Introduction

Remarkable progress has been made over the past two decades in the study of dietary nutrient requirements of fishes (Halver 1972; Cowey and Sargent 1972, 1979; National Research Council 1981, 1983; Millikin 1982). The growth and health condition of fish is affected by nutritional factors like feeding frequency (Sampath and Pandian 1984), food quantity (Pandian and Raghuraman 1972) and food quality (Vivekanandan 1977). However, studies on the effect of dietary protein level on blood parameters are meagre. Piscine haematology is increasingly gaining significance due to its importance in the study of fish health under different conditions of life and environment, as elaborated by Hickey (1976) and Joshi *et al* (1980). Hence the present investigation is an attempt to relate the protein requirement of fish to the health condition with special reference to the blood parameters.

2. Materials and methods

Samples of *Cyprinus carpio* were collected from Manimuthar Dam and acclimatized to laboratory conditions for a period of 10 days. Healthy fishes ($10 \pm 1{\cdot}23$ g) were chosen from the stock and divided into 5 groups. They were fed with 5 levels of protein (14, 28, 38, 48 and 58%) in the diet. The experimental diets with different levels of protein (14, 28, 38, 48 and 58%) were prepared by square method of food compounding (Hardy 1980). The important ingredients used for the feed preparation were groundnut oil cake, rice bran, dried fish, dried silkworm pupae and tapioca flour. Since 38% is the optimum protein requirement for *Cyprinus carpio*, the group fed with 38% protein was treated as control group. Experiments were conducted in glass aquaria with 50 l capacity. Five fishes were reared in each aquarium. Three replicates were maintained for each group.

The test fishes were fed with weighed quantity of experimental feeds (3% of body weight) daily at 8 AM. After 2 h feeding period, unfed and faecal matters were removed, dried and weighed. The experiment was conducted for 190 days.

At the end of the experimental period the test fishes were sacrificed and blood was collected by severing the caudal peduncle by means of a standard micropipette (Johnsson-sjobeck and Larson 1978). Haematological tests were conducted by routine clinical methods (Wintrobe 1978). The red cell indices like mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV) were calculated according to Johansson-Sjobeck and Larson (1978).

3. Results

The changes observed in blood parameters of *C. carpio* are given in table 1. The test fishes fed with normal level of protein (38%) showed the maximum total red blood cell (RBC) count and the number declined in both low and high levels of protein. The difference in RBC count between the fish fed with 38% protein and the lowest (14%) and the highest protein levels (58%) was statistically significant (38% vs 14%; $t = 3{\cdot}550$ $P < 0{\cdot}02$; 38% vs 58%; $t = 3{\cdot}070$ $P < 0{\cdot}02$). Similar trend was observed for TLC, thrombocyte, basophil, haemoglobin (Hb), haematocrit (Ht), MCH, MCHC values and coagulation time. On the other hand, immature RBC, ESR, MCV, lymphocyte and neutrophil values showed the opposite trend. Minimum number of immature RBC ($2{\cdot}69 \pm 0{\cdot}64$/1000 cells) was noted in control fish whereas the maximum number ($6{\cdot}67 \pm 0{\cdot}71$/1000 cells) was observed in fishes fed with low (14%) level protein. Similarly the maximum ESR ($2{\cdot}30 \pm 0{\cdot}63\ mm^{-1}$) and MCV ($142{\cdot}53 \pm 1{\cdot}19$ fl) values were observed in fishes fed with low (14%) protein; these values in the control fish averaged to $1{\cdot}29 \pm 0{\cdot}59\ mm^{-1}$ and $113{\cdot}04 \pm 1{\cdot}28$ fl, respectively.

Table 1. Effects of varying dietary protein level on the blood parameters of *C. carpio*.

Blood parameters	Protein levels				
	14%	28%	38%	48%	58%
Total RBC ($\times 10^6/mm^{-3}$)	$2{\cdot}33 \pm 0{\cdot}31$	$3{\cdot}43 \pm 0{\cdot}42$	$3{\cdot}58 \pm 0{\cdot}39$	$3{\cdot}47 \pm 0{\cdot}33$	$2{\cdot}45 \pm 0{\cdot}35$
Immature RBC (per 1000 cells)	$6{\cdot}67 \pm 0{\cdot}71$	$2{\cdot}89 \pm 0{\cdot}51$	$2{\cdot}69 \pm 0{\cdot}64$	$2{\cdot}99 \pm 0{\cdot}69$	$5{\cdot}94 \pm 0{\cdot}73$
Hb content (%)	$5{\cdot}21 \pm 1{\cdot}06$	$10{\cdot}16 \pm 1{\cdot}22$	$10{\cdot}73 \pm 1{\cdot}31$	$10{\cdot}08 \pm 1{\cdot}22$	$6{\cdot}53 \pm 0{\cdot}93$
ESR (mm^{-1})	$2{\cdot}30 \pm 0{\cdot}63$	$1{\cdot}36 \pm 0{\cdot}36$	$1{\cdot}29 \pm 0{\cdot}59$	$1{\cdot}38 \pm 0{\cdot}38$	$1{\cdot}41 \pm 0{\cdot}61$
Haematocrit PCV (%)	$33{\cdot}21 \pm 1{\cdot}23$	$40{\cdot}32 \pm 1{\cdot}42$	$40{\cdot}47 \pm 1{\cdot}31$	$40{\cdot}21 \pm 1{\cdot}23$	$34{\cdot}11 \pm 1{\cdot}49$
MCV Ht/RBC × 10 (fl)	$142{\cdot}53 \pm 1{\cdot}19$	$117{\cdot}55 \pm 1{\cdot}13$	$113{\cdot}04 \pm 1{\cdot}28$	$115{\cdot}87 \pm 1{\cdot}25$	$139{\cdot}22 \pm 1{\cdot}18$
MCH Hb/RBC × 10 (pg)	$22{\cdot}36 \pm 1{\cdot}15$	$29{\cdot}62 \pm 1{\cdot}34$	$29{\cdot}97 \pm 1{\cdot}12$	$29{\cdot}04 \pm 1{\cdot}34$	$26{\cdot}65 \pm 1{\cdot}15$
MCHC Hb/Ht × 100 (g/lt)	$15{\cdot}68 \pm 1{\cdot}04$	$25{\cdot}19 \pm 1{\cdot}18$	$26{\cdot}51 \pm 1{\cdot}42$	$25{\cdot}06 \pm 1{\cdot}32$	$19{\cdot}14 \pm 1{\cdot}19$
Total white blood cells ($\times 10^3/mm^{-3}$)	$55{\cdot}68 \pm 1{\cdot}03$	$61{\cdot}21 \pm 1{\cdot}34$	$61{\cdot}36 \pm 1{\cdot}03$	$60{\cdot}45 \pm 1{\cdot}08$	$56{\cdot}11 \pm 0{\cdot}98$
Thrombocytes (%)	$53{\cdot}66 \pm 1{\cdot}43$	$54{\cdot}67 \pm 1{\cdot}42$	$54{\cdot}73 \pm 1{\cdot}08$	$54{\cdot}18 \pm 1{\cdot}14$	$52{\cdot}13 \pm 1{\cdot}06$
Lymphocytes (%)	$42{\cdot}72 \pm 1{\cdot}09$	$39{\cdot}97 \pm 1{\cdot}13$	$39{\cdot}62 \pm 1{\cdot}07$	$39{\cdot}98 \pm 1{\cdot}15$	$41{\cdot}99 \pm 1{\cdot}23$
Basophil (%)	$37{\cdot}21 \pm 1{\cdot}44$	$39{\cdot}66 \pm 1{\cdot}61$	$41{\cdot}98 \pm 1{\cdot}14$	$37{\cdot}39 \pm 1{\cdot}23$	$37{\cdot}31 \pm 1{\cdot}52$
Neutrophil (%)	$24{\cdot}91 \pm 0{\cdot}92$	$23{\cdot}68 \pm 1{\cdot}32$	$23{\cdot}48 \pm 1{\cdot}62$	$23{\cdot}52 \pm 1{\cdot}04$	$24{\cdot}09 \pm 1{\cdot}43$
Coagulation time (s)	$22{\cdot}07 \pm 0{\cdot}92$	$23{\cdot}48 \pm 1{\cdot}12$	$23{\cdot}69 \pm 0{\cdot}98$	$23{\cdot}18 \pm 1{\cdot}32$	$22{\cdot}99 \pm 1{\cdot}43$

Each value is the average ($\bar{X} \pm SD$) of 3 observations.

4. Discussion

The present observations clearly show that protein levels in the diet affect the blood cells of *C. carpio*. A significant reduction of red cells was noted in test fishes fed with low (14%) and high (58%) levels of protein. This reduction may be caused either by the inhibition of erythropoiesis or by the destruction of red cells. An increase in the number of immature RBC indicates that the erythropoiesis is not affected by the protein variations. But the higher MCV value, on the other hand suggests that the anaemic condition may be due to the destruction of red cells. An increased MCV value in the present study may be considered as an index of RBC destruction leading to anaemia (Johnsson-Sjobeck and Larson 1979). The abnormalities observed in the mature and immature RBC in the present investigation are similar to the observations of Smith (1968) who has reported many abnormal RBCs with distorted nuclei in the population of matured and old cells in salmonids, carp and cat fishes exposed to the conditions of protein deficiency. This observation is also in accordance with the monochromic normocytic anaemia reported by Smith *et al* (1974) in rainbow trout *Salmo gairdnerii* exposed to pyridoxine deficient food.

A significant reduction in white cells was observed in test fishes fed with low protein. Similar decrease in white cells leading to leukopenia has been reported by Hawkins and Evans (1952) in rat and dog fed with vitamin deficient food (see also Agarwal and Mahajan 1982).

Even though the total leukocyte count showed a remarkable reduction in number in low protein fed fishes, the leukocyte types like lymphocytes and neutrophils showed a marked increase. The increase in lymphocytes may be due to the stimulated lymphopoiesis or due to an increased release of lymphocytes from lymphomycloid tissues (Ellis 1976). In the present investigation the increase in lymphocyte value may be the result of direct stimulation of lymphopoiesis caused by protein variations in the experimental diets.

In the case of mammals, the neutrophils are said to be responsible for phagocytosis and disposal of foreign bodies or damaged tissues. McLeay and Brown (1974) have attributed a similar role for the neutrophils in fishes. According to them the elevated neutrophil count in fish is an index of tissue damage or the entry of foreign bodies into the blood stream. In the present investigation, the elevated neutrophil count may be the result of tissue damage caused by physiological stress due to protein deficiency.

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