FOR:

MY DEAREST FATHER AND MOTHER FOR THEIR

STRUGGLE AND SACRIFICES IN EDUCATING ME

AN INVESTIGATION OF MINERAL SUPPLEMENTATION OF DIET ON THE GROWTH AND METABOLISM OF CARP

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A Thesis Submitted to The University of Aston in Birmingham

For The Degree of Doctor of Philosophy

SEPTEMBER

1980

CONTENTS

Summary

The importance of trace elements in animal metabolism is becoming more and more evident. Trace elements as nutrients play a valuable role in the body, participating in the formation of such highly active biological materials as enzymes, hormones and vitamins. Many investigations have been published on the effect of trace elements on various animals. Recently, there have also been experiments on fish, showing that trace elements influence the growth rate and survival of fish. The difficulty in applying trace elements lies in the dosage; only optimal concentrations have beneficial effects.

The present investigation attempted to determine the optimal concentration of cobalt and iodide for development and growth of carp, Cyprinus carpio.

The results of the present study demonstrate that cobalt is an essential trace element in the diet for carp. The fish fed a diet containing no cobalt showed an extremely low growth rate and high mortality. A large number of the fish on cobalt-deficient diet were found to suffer from malformation in the tail even when the diet was supplemented with vitamin B_{12}. The dietary cobalt also influenced the haematological characteristics and strengthened resistance to disease in carp. The proximate composition of the fish body and the contents of the cobalt in various organs varied according to the dietary cobalt levels in the diet. Judging from the growth rate of the fish, an adequate amount of cobalt, as cobalt chloride, in the diet of carp was estimated to be 3 mg per kg dry diet.

For iodide, the results showed that a diet which was insufficient to maintain adequate thyroid iodide storage in carp did not interfere with the animals' normal growth. However, the results obtained showed that the optimal iodide requirement for carp was 1.2 mg per kg dry diet.

Key Words

Carp (Cyprinus carpio) ; Cobalt ; Iodide ; Growth ; Survival ; Vitamin B_{12} ; Metabolism .

ACKNOWLEDGEMENTS

I wish to express my deepest gratitude to my supervisor, Dr. C. C. Thornburn for his kind guidance throughout the course of this study. In fact, his encouragement at times was the only light which ultimately culminated into successful completion of this study.

I would also like to thank Professor A. J. Matty for his encouragement and understanding; Dr. A. Tacon for his co-operation and help during the course of this study, and Dr. N. R. Bromage for his help in acquiring the fish.

I am grateful to the Iraqi Ministry of Higher Education and Research for funding me during the course of this study.

I greatly appreciate the co-operation and help extended to me by the administrative and technical staff of the department. My thanks also go to Lynda Page for typing this thesis.

Finally, I am thankful to all my colleagues and friends for cheerful companionship.

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CHAPTER ONE

GENERAL INTRODUCTION

1.1. INTRODUCTION

There are many published papers dealing with the mineral requirements of mammals (Mills, C. F., 1956, Ellis, W. C., Pfander, W. H., Muhrer, M. E., and Picketl, E. E., 1958, Ullrey, D. E., Miller, E. R., Thompson, O. A., Ackerman, I. M., Schmidt, D. A., Hoefer, J. A., and Luecke, R. W., 1960, Bremner, I., and Dalgaron, A. C., 1973) and birds (Frost, D. V., Overby, L. R., and Spruth, H. C., 1955, Hopkins, L. L., and Mohr, H. E., 1973, Peterson, R. D., and Jensen, L. S., 1975), but few referring to the needs of fish (Shekhanova, I. A., 1961, Andrews, J. W., Murai, T., and Campbell, C., 1973, Arai, S., Nose, T., and Hashimoto, Y., 1974, Ogino, C., and Kamizono, M., 1975, Page, J. W., 1978). This is not because of lack of desire on the part of investigators, but rather because of the difficulty in experimentally controlling the uptake and excretion of inorganic elements by these animals. Inorganic elements, as nutrients, are difficult to study; many trace minerals are required in such small amounts that it is too difficult to design test rations free from the elements under study (Phillips, A. M., and Podoliak, H. A., 1957). The exchange of ions from the environment across the gills and skin of fish complicates the determination of quantitative dietary requirements. Thus the topic of mineral requirements in fish appears to be very complicated, since metabolism and nutrition of minerals are closely related not only to those present in the diet but also to those dissolved in the water. All these reasons made the standard techniques, developed for other species of animals, unsatisfactory for the accurate determination of mineral requirements in fish until 1950, when the Fish and Wild Life

Service at the Cortland Laboratory in U.S.A. used radio active isotope techniques in mineral studies (Phillips, A. M., 1959).

Most studies of the influence of inorganic elements on fish have until recently been concerned with either toxicity or osmoregulation, and the nutritional aspects have been rather neglected (Zaugg, W. S., and McLain, L. R., 1970).

Nutritional diseases of fish are those which can be attributed to deficiency, excess, or improper balance of components present in the diet or available in the water. Symptoms of such diseases do not appear until one or more components of the diet drop below the minimum requirement. The absence of minerals from the diet may cause many formerly incurable diseases; among these are: (1) dietary anemia; (2) subnormal bone development; (3) improper blood coagulation; (4) numerous problems in growth. According to Phillips, A. M., 1959 "Mineral studies upon fish will surely be productive and such information should result in more vigorous and healthy animals." Phillips further noted that soft waters which are poor in minerals necessitate either fertilization of the water with the needed minerals or suitable mineral supplementation of fish diet. Research dealing with fish growth through the addition of growth stimulants to supplement feeds has greatly increased during recent years. Thus the importance of the trace elements in animal metabolism is becoming more and more evident. Despite the minuteness of the amounts of trace elements in the organism, they play a valuable role in the formation of such important biological compounds as enzymes, hormones, and vitamins. Trace elements are greatly important in reproduction, hemopoiesis, and a number of other vital physiological functions.

In view of this the present study is undertaken to investigate the role and the influence of dietary trace elements supplements on the growth and metabolism in fish, and in particular to determine the optimum concentration of cobalt, as cobalt chloride, and iodine, as potassium iodide, for their development and growth.

1.2. NUTRITIONAL ASPECTS OF MINERALS IN FISH NUTRITION.

1.2.1. CLASSIFICATION OF MINERALS.

All forms of living matter require many inorganic elements for their normal life processes. Virtually all the elements of the periodic table have been found in living cells, though not all are necessarily essential to life. Many mineral elements occur in the living tissues in such small amounts that the early workers were unable to find their precise concentration with the analytical methods which were available at their time; therefore these minerals were described as occurring "in trace" and the term "trace elements" arose to describe them. At the present time about 26 elements are known to be essential for animal life (Underwood, E. J., 1977); these consist of 11 major elements, namely carbon, hydrogen, oxygen, nitrogen, sulfur, calcium, phosphorus, potassium, sodium, chlorine, and magnesium; the 15 elements generally accepted as trace elements are iron, zinc, copper, manganese, nickel, cobalt, molybdenum, selenium, chromium, iodine, fluorine, tin, silicon, vanadium, and arsenic. In addition, boron is essential for higher plants but has not yet been shown to be necessary for animals (Warrington, K., 1926, Rajaratnam, J. A., Lowry, J. B., Avadhani, P. N., and Corley, R. H. V., 1971). The nutrients which are commonly referred to as mineral elements, or as inorganic nutrients, and which have definite demonstrable metabolic function, include calcium, phosphorus, magnesium, sodium, potassium, sulfur, chlorine, iron, copper, cobalt, iodine, manganese, and zinc. Fluorine, molybdenum, and selenium are essential, at least for experimental animals (Heinz, H. G., 1970). Aluminum, arsenic, nickel, and silicon might be mentioned as

elements which occur very consistently in the body, though for them there exists no proof of body need.

The mineral, or inorganic, nutrients are interrelated and balanced against each other in the body; they cannot be considered as single elements with circumscribed function, just as proteins, carbohydrates, fats, and vitamins do not play independent and self-sufficient roles in the body. The different minerals needed by the body must all be derived from the diet; the three whose supply is most likely to be critical are calcium, iron, and iodine. Minerals may be divided into two groups:

1. Major elements:

Which are present in the body at fairly high levels and are needed in large continuous supply.

2. Trace elements:

Which are present in the body at very low levels, and are needed in small amounts.

Both trace and major elements are essential for life and the amount of a mineral that is needed by the body is not a criterion of necessity or importance, as life cannot exist without either the major or trace elements. Thus cobalt or iodine, trace minerals required in small amount, are as necessary for life as calcium or phosphorus, major elements required in large amounts. Major and trace elements occur in most diets in sufficient quantities to satisfy the metabolic requirement of the body, and mineral deficiencies are rare and generally easily alleviated when discovered. There are areas where the soil is deficient in one or more minerals, and animals that are reared on food grown in such areas must receive supplemental minerals in their diet. Constant use of an area for agricultural crops

will eventually reduce the supply of minerals, making it necessary to replenish the soil through artificial fertilization or to supplement the diet with deficient minerals. Minerals may be classified according to use in the animal as:

(1) Essential minerals:

Those minerals whose function in the body is known. They may be grouped as follows:

A. Structural minerals:

These are the minerals that form the hard and supporting structures of the animal body (the bone and teeth). The principal components of these structures are calcium and phosphorus. These minerals must be fed at a ratio of two parts of calcium to one of phosphorus, since improper balance causes incomplete utilization. Small amounts of fluorine and magnesium are also essential for the formation of bones and teeth.

B. Respiratory minerals:

The supply of the red blood cells in the blood stream depends on iron, copper, and cobalt. All are trace elements, and a deficiency of any one will cause anaemia. Iron, in the presence of copper, combines with protein to form hemoglobin, the red blood cell material that carries oxygen throughout the body. An anaemic animal lacks hemoglobin, so that the oxygen-carrying capacity of its blood is reduced. In a severe case of anaemia, the animal eventually dies from suffocation because of insufficient oxygen for normal tissue respiration.

C. Body regulators:

Minerals play an important role in regulating body processes. Minerals regulate the osmotic pressure of the body cells so that the cells may remain turgid and the body form may

be maintained. The regulators of osmotic pressure include sodium, chlorine, and potassium; about 90% of each of these elements in the body is found in the body fluids (Krogh, A., 1939, Phillips, A. M., 1957, Smith, R. L., and Paulson, A. C., 1977).

D. Reproductive minerals:

Minerals are directly concerned with the successful development of the young in the mother's body. Calcium and phosphorus are needed for the development of the bony part of the foetus, and other minerals are needed to establish, maintain, and regulate the life processes of the developing young. The adult requires larger amounts of minerals than normal in the diet during reproduction, due to the formation of milk, eggs, etc. (Phillips, A. M., 1957, Nose, T., 1972, 1976, Ogino, C., and Takeda, H., 1976).

E. Minerals for special body porcesses:

Chlorine, a constituent of gastric juices, is needed for digestion. Calcium is required for blood coagulation, copper is a constituent of important enzymes. Magnesium and phosphorus activate some of the digestive enzymes; also magnesium has a specific laxative effect on the digestive tract. Zinc is needed for normal development of the hair and proper regulation of tissue respiration. Iodine is a component of thyroxine, a product of the thyroid gland that regulates the rate of metabolism in the animal body. An iodine deficiency causes goitre , poor growth, and excessive fat. The phospholipids, which are needed for normal growth, contain phosphorus.

(2) Minerals which may be essential

In addition to the minerals known to be essential, there are others which appear necessary, but whose exact role is unknown yet. It is believed that bromine is necessary for normal development

of the young (Bosshardt, D. K., and Barnes, R. H., 1956). The amount of arsenic in the blood stream is correlated with pregnancy and menstruation in human beings (Frost, D. V., Overby, L. R., and Spruth, H. C., 1955, Frost, D. V., 1967, Nielsen, F. H., Givand, S. H., and Myron, D. R., 1975). There is some evidence that boron may be needed for normal health and growth (Orent-Keiles, E., 1941, Follis, R. H., 1945). Although large amounts of silicon are present in the animal body, a silicon-deficient diet has failed to produce pathological effects (Carlisle, E. M., 1970, Schwarz, K., and Milne, D. B., 1972).

1.2.2. THE NATURE OF TRACE ELEMENTS

It is believed, on present evidence, that evolution may have selected certain elements for the essential functioning of living organisms and has rejected or ignored others; the basis for this selection or rejection is far from clear.

An element is considered by Mertz, W., (1970) to be essential if its deficiency consistently results in impairment of a function from optimal to suboptimal. Cotzias, G. C., (1967) states the position more completely; he maintains that a trace element can be considered essential if it meets the following criteria:

1. It is present in all healthy tissues of all living things.

2. Its concentration from one animal to the next is fairly constant.

3. Its withdrawal from the body induces reproducibly the same physiological and structural abnormalities regardless of the species studied.

4. Its addition either reverses or prevents these abnormalities.

5. The abnormalities induced by deficiency are always accompanied by pertinent, specific biochemical changes; these biochemical changes can be prevented or cured when the deficiency is prevented or cured.

Some 20-30 trace elements which do not meet the above criteria occur more or less constantly in variable concentration in living tissues. They include aluminium, antimony, cadmium, mercury, germanium, rubidium, solver, gold, bismuth, titanium, zirconium, and others. They are believed to be acquired by the body of the animal as environmental contaminants and to reflect the contact of the organism with its environment. Liebscher, K., and Smith, H., (1968) said that the shape of the distribution curve for a trace element in tissues could be used as a method of determining whether the element is essential or not; they have supported this proposal with evidence obtained from a study of the levels of several essential and nonessential trace elements in the tissues from healthy adults who died as a result of violence and who had no known industrial exposure to the element in question. For the essential elements an internal control mechanism was postulated, leading to normal or symmetrical distribution. For the nonessential trace elements, external control of tissue concentration arising from contamination would occur, leading to a distribution pattern similar to the environmental level.

All the trace elements are toxic if ingested or inhaled at sufficiently high levels and for a long enough period. This was recognized many years ago by Bertrand, G., (1912). Venchikov, A. I., (1960) has expanded this concept and presented the dose response

in the form of a curve with two maxima. The first part of the curve, showing an increasing effect with increasing concentrations until a plateau is reached, expresses the 'biological' action of the element, and the plateau expresses optimal supplementation and normal function. The width of the plateau is determined by the homeostatic capacity of the animal. With further increasing dose the element enters a phase of irritation and stimulation of some function, expressing its 'pharmacological' action. In this phase the element acts as a drug independent of a deficiency. At still higher doses this is followed by the appearance of signs of toxicity, expressing the 'toxicological' action of the element. More recently, Venchikov, A. I., (1974), on the basis of numerous experiments with several animal species and tissues, has redefined the concept to establish three zones of action of trace elements, named (a) biological action zone, (b) inactive zone, and (c) pharmacotoxicological action zone.

1.2.3. THE DISCOVERY OF TRACE ELEMENTS.

The interest in trace elements in animal physiology began over a century ago with the discovery of a number of special compounds which contained various metals not previously suspected to be of biological significance in the living organisms. These included turacin, a red porphyrin pigment occurring in the feathers of certain birds and containing no less than 7% copper (Church, A. W., 1869); hemocyanin, a copper-containing compound found in the blood of snails (Harless, E., 1847); sycotypin, a zinc-containing compound which is found in blood pigment in Mollusca (Mendel, L. B., and Bradley, H. C., 1905); and a vanadium-containing respiratory compound present in the blood of sea squirts (Henze, M., 1911).

Such discoveries did a little to stimulate studies of the significance of these elements in the body. Bernard, C., (1857), and MacMunn, C. A., (1885), with their work on cell respiration and iron oxidative processes, pointed the way to later studies of metal-enzyme catalysis and of metallo-enzymes which help greatly to understand the function of trace elements within the tissues. Early discoveries about the function of the trace elements in the body were made by Raulin, J., (1869), on the essentiality of zinc in nutrition, by Chatin, A., (1850-1954), on iodine and its relationship to goiter, and by Fowler, W. M., (1936), on the necessity of iron in the blood.

During the first quarter of the present century, more studies were done on iron and iodine relating to human health and nutrition, such as the work of Kendall, E. C., (1919), who isolated thyroxine from the thyroid gland, and the successful use of supplemental iodine to control goiter in man and animals.

As analytical techniques improved during this part of the century, it was possible to estimate by emission spectrography about 20 elements in low concentrations in the tissues of the animal under study (Wright, N. C., and Papish, J., 1929, Stitch, S. R., 1957). These studies were responsible for:

1. Defining the wide limits of concentration of many of the trace elements found in the food and tissues.
2. Illuminating the significance of such factors as age, location, disease, and industrial contamination in influencing those concentrations.
3. Stimulating studies of the possible physiological significance of several elements previously unsuspected of biological action potentiality.

4. Discriminating between those elements most likely to have such potential and those which were more likely to be environmental contaminants.

During the second quarter of this century there were notable advances in the knowledge of the nutritional importance of trace elements; these advances came from basic studies with laboratory species designed to investigate the total nutrient needs of these animals. Hart, E. B., Steenbock, H., Waddell, J., and Elvehjem, C. A., (1928) showed that supplementary copper, as well as iron, is necessary for growth and hemoglobin formation. The same group after a few years (1931) showed that manganese and then zinc (1934) were dietary essentials for mice and rats. The third quarter of this century has seen other advances in the physiology of trace elements, besides the great significance of metabolic interaction. These advances have mostly been highly dependent on concurrent developments in analytical techniques, such as atomic absorption, neutron activation and microelectron probe analysis; with such procedures the concentration and distribution of most elements in the tissues, cells, and even organelles of the cells can be determined accurately. During the last decade there has been a rapid increase in the number of trace elements shown to be essential and a great surge of interest and activity in their significance or potential significance in animal health and nutrition. Thus six trace elements, namely, tin, silicon, fluorine, nickel, vanadium, and arsenic, have been added to the list of dietary essential trace elements.

1.2.4. MODE OF ACTION OF TRACE ELEMENTS.

Trace elements occur and function in the living tissues in low concentrations. These normal tissue concentrations vary greatly in magnitude and are characteristic for each element. They are usually expressed as parts per million (ppm) or billion (ppb). The characteristic concentrations and functional forms of the trace elements must be maintained within narrow limits if the functional and structural integrity of the tissues is to be safeguarded and the growth, health, and fertility of the animals are to remain unimpaired. Continued ingestion of diets, or continued exposure to total environments that are deficient, imbalanced, or excessively high in a particular trace element, induces changes in the functioning forms, activities, or concentrations of that element in the body tissues and fluids so that they fall below or rise above the permissible limits. In these circumstances biochemical defects develop, physiological functions are affected, and structural disorders may arise in ways which differ with different elements, with the degree and duration of the dietary deficiency or toxicity, and with age, sex, and species of animal involved. To prevent ultimately deleterious changes the animal must be supplied with a diet that is palatable and nontoxic, as well as containing the required elements in adequate amounts, in proper proportions, and in an available form.

Trace elements act primarily as catalysts in enzyme systems, where they serve many functions. In the metalloenzymes the metal is firmly associated with the protein and there is a certain number of atoms per each molecule of protein, which cannot be replaced by any other metal. Evidence is accumulating that protein-metal interactions not only enhance the catalytic activity

of enzymes but also may increase the stability of the protein moiety to metabolic turnover (Harris, E. D., 1976). However, many clinical and pathological disorders in the animal as a consequence of trace element deficiency or excesses cannot yet be explained in biochemical or enzymatic terms. This suggests either that there are many metabolically significant trace element-dependent enzymes which have still to be discovered, or that these elements participate in the activity and structure of other vital compounds in the tissues (Underwood, E. J., 1977).

1.2.5. TRACE ELEMENT NEEDS AND TOLERANCES

The minimum requirements of animals for trace elements are commonly expressed in proportion or concentrations of total dry matter consumed daily. Their requirements, or tolerances, are arrived at by relating the growth, health, fertility, or any other relevant criteria in the animal to varying dietary mineral concentrations. The latter are found by application of analytical techniques that measure the total amounts of the elements in the diet or their component foods (Underwood, E. J., 1977).

According to Underwood, (1977) appetite, toxic material, utilization, level of the other elements or nutrients, age, species, and sex may influence the availability or even utilization of the minerals. Thus, Mills, C. F., (1966) in his study on copper toxicity in pigs found that it is very important to balance the dietary trace elements in order to find correctly the toxic level.

1.2.5.1 Trace elements and their physiological role in animals

Thirty years ago, Green, D. E., (1941) said that enzymic catalysis is the only rational explanation of the fact that low traces of some substances can produce profound biological effects.

Since that time the trace elements have been shown to participate in a wide range of enzymic processes involving many aspects of the intermediary metabolism and physiological function of the organism. The trace elements act as catalysts or cofactors in enzyme systems with roles ranging from relatively weak, non-specific effects (metal-ion activated enzymes) to highly specific associations (metallo-enzymes) in which the metal is firmly attached to the protein in a fixed number of atoms per molecule. Examples of metallo-enzymes are given in Table 1.1 to illustrate the diversity of functions in which trace elements are involved. The trace elements as a group are not functionally related in the animal. Even such physico-chemically similar elements as Ni and Co, Zn and Cd, or Se and Te are quite dissimilar physiologically. The manifestations of dietary deficiency would therefore be expected to differ greatly from element to element as an expression of breakdown or impairment of the different physiological functions in which the elements are involved. With severe deficiencies of some trace elements, characteristic clinical and pathological changes in the animal do arise. However, the situation is much more complex than this and further investigation would yield valuable information in this area. The physiological role of a trace element most noticeably affected by a deficiency of that element, as evidenced by the pathological changes that become apparent, varies markedly with the intensity and duration of the dietary deficiency, age, species, and sex of the animal. According to Mills, C. F. and Murray, G. (1960), specific lesions in the wool may be the only clinical evidence of Cu deficiency in sheep, while with rats at particular levels of Cu intake, depigmentation

TABLE 1.1

Some metallo-enzymes and metallo-proteins, and their physiological functions

Element	Enzyme	Function
Iron	Cytochromes	Electron transfer
	Succinate dehydrogenase	Aerobic oxidation of carbohydrates
	Catalase	Protection against H_2O_2
Copper	Cytochrome oxidase	Principal terminal oxidase
	Lysyl oxidase	Lysine oxidation
	Tyrosinase	Skin pigmentation
	Ceruloplasmin	Iron utilization
	Cytocuprein	Superoxide (O_2^-) dismutation
Zinc	Carbonic anhydrase	CO_2 formation; regulation of acidity
	Carboxypeptidase	Protein metabolism
	Alcohol dehydrogenase	Alcohol metabolism
	Cytocuprein	Superoxide (O_2^-) dismutation
Manganese	Arginase	Urea formation
	Pyruvate carboxylase	Pyruvate metabolism
Molybdenum	Xanthine oxidase	Purine metabolism
	Sulphite oxidase	Sulphite oxidation
Selenium	Glutathione peroxidase	Protection against haemoglobin oxidation

of hair is similarly the only sign.

The physiological roles of most trace elements are mediated through their presence in a wide range of active organic compounds of different function. The animal body has normally the ability to synthesize these complex substances from simple inorganic or ionic forms of the element in adequate amounts and concentrations for the functional requirements of the tissues. This ability can only be discharged if the diet contains adequate quantities of the element in available forms and in proper balance with other elements and nutrients. With I and Co, the only known function of I is as a constituent of tetraiodothyronine (Thyroxine) and triiodothyronine (the thyroid hormones) and the only function of Co is as a constituent of the active cobalamins (vitamin B_{12} group). The physiological roles of I in the animal are therefore apparently confined to those of the thyroid gland hormone and the physiological roles of Co to those of vitamin B_{12}.

1.2.5.2. Trace element interactions in animals

The nutritional significance of a trace element is determined not only by its specific physiological and biochemical role in animal metabolism, but also by its capacity to interact with other elements in ways which can be beneficial or harmful in terms of animal health (Suttle, N. F., 1975). Till now there has been little understanding about the involvement of these trace elements with each other and with other nutrients, particularly under conditions which are relevant to practical nutrition.

According to Suttle, N. F. (1975), four areas for research can be identified in any trace element interaction; they are concerned with:

1. Identifying the dietary components involved.
2. Determining factorially the dose:response relationship for each component.
3. Locating the site(s) of the interaction.
4. Determining the mechanism(s) of interaction.

There are some trace elements, namely, Cu/Mo/S and Cu/Mo whose interactions have been studied in depth, but many areas have been totally neglected.

The interactions between trace elements can be grouped into six categories according to the type of mechanism involved:

1. Formation of insoluble complexes between dissimilar ions.
2. Competition for metabolic pathways between similar ions.
3. Induction of metal-binding proteins.
4. Changes in the metal component of metallo-enzymes.
5. Facilitation of trace element transport.
6. Enhancement of trace element excretion.

1.3. TRACE ELEMENT METABOLISM IN ANIMALS:

Fish have the ability to absorb inorganic elements from the surrounding water as well as from their diet. This ability to exchange inorganic ions across not only the gill membranes but also the skin makes it difficult to elucidate the nutritional function of dietary minerals. However, dietary requirements of most inorganic elements have not yet been determined. Assessment of the mineral requirement has been difficult because of the difficulty in controlling feed intake, absorption of the element from the environment, a complex interrelationship between some dietary minerals and the limited understanding of the metabolic functions of these inorganic elements in fish.

IRON

Iron is an essential element involved in the respiratory processes, including oxidation-reduction activity and electron transport. Iron exists in the animal body mainly in complex forms bound to protein such as heme compounds (hemoglobin or myoglobin), as heme enzymes (mitochondrial and microsomal cytochromes, catalase and peroxidase), or as non-heme compounds (flavin-Fe-enzymes, transferrin and ferritin).

Iron deficiency causes characteristic hypochromic microcytic anemia in brook trout (*Salmo trutta*) (Kawatsu, H., 1972), yellow tail (*Seriola quinqueradiata*) (Ikeda, Y., Ozaki, H., and Uematsu, K., 1973), red sea bream (*Chrysophrys major*) (Yone, Y., 1974), and eels (*Anguilla anguilla*) (Arai, S. *et al.*, 1974, T. Nose, 1976). The quantitative iron requirement of most finfish has not been established. Arai, S., (1974, 1971); Nose, T., (1976) found that the iron requirement of eels was approximately 17 mg percent of

the diet. In non-ruminant experimental animals iron absorption and its availability is depressed by high dietary levels of phosphates and calcium in the diet.

It is well established that ferrous iron is more readily absorbed than the ferric form. Much of this work has been reviewed by Wiseman, G., (1964).

COBALT

The only established biological function of cobalt relates to its role as a component of the vitamin B_{12} molecule.

Evidence indicates that addition of cobalt chloride and/or cobalt nitrate to the feed or cobalt chloride to the water of fish ponds enhances growth and hemoglobin formation in carp (Frolova, P., 1961; Krymova, R. V., and Farberov, V. G., 1964; Vinogradov, V. K., and Erokhina, L. V., 1962, 1963; Kovalskii, V. V., 1964; Tomntik, E. N., and Batyr, A. K., 1965; Boev, P., 1961).

Cobalt in trace quantities is widely distributed in common feed ingredients.

In plant material the existence of stable cobalt-protein complexes has been found. In feed stuffs derived from animal tissues, cobalt is found in the form of cyanocobalamin (Vitamin B_{12}) and related compounds, meat and fish meals being particularly rich sources.

ZINC

Zinc as an essential trace element in living organisms is involved in nucleic acid synthesis and is required for the activity of many important enzymes such as the dehydrogenases, alkaline phosphatase, carboxypeptidase and carbonic anhydrase. Many other enzymes activated by zinc are widely distributed in nature.

Although there are considerable data on the toxicity of zinc to fish under laboratory conditions, information on zinc requirements is limited.

Ketola (1978) demonstrated that zinc prevents cataract in rainbow trout. They found that trout fed white fish waste meal (containing 60 mg Zn/kg of feed) showed 75% incidence of bilateral cataract. Addition of 150 mg Zn per kg of feed in the form of $ZnSO_4.7H_2O$ completely overcame the problem and markedly improved growth.

Although zinc in trace quantities is found in common feed stuffs, some differences exist in its availability in feed ingredients of plant and animal origin. Plant protein contains phytates and soluble phytates added to animal protein decrease zinc availability in oil seed protein (O'Dell et al., 1972). The effect of phytate on the availability of zinc is accentuated by high levels of dietary calcium (Oberleas et al., 1962, 1966 and Likuski and Forbes, 1965).

IODINE

Iodine was the first element found to prevent a specific mineral deficiency, goiter in salmonids, more than sixty years ago. Marine and Lenhart (1910, 1911) and Marine (1914) correctly diagnosed as simple thyroid hyperplasia what was thought to be thyroid carcinoma in brook trout (Gaylord and March, 1914). It was shown conclusively by the above workers that this disease could be controlled by the administration of minute quantities of dietary iodine.

Woodall and LaRoche (1964) reported that the iodine requirement of chinook salmon (Oncorhynchus tshawytscha) fingerlings and parr were 0.6 µ g/g and 1.1 µg/g of dry feed respectively.

The higher iodine requirement of the advanced parr was mainly due to the increased thyroid activity during smoltification. These workers found no evidence of goiter in fish fed iodine-deficient diets, however, histological examination showed thyroid hyperplasia. Ikeda et al., 1972 demonstrated that addition of potassium iodide (0.023 to 2.33 mg KI/l of water) in the aquaria of goldfish (Carassius auratus) improved the body weight and body scale growth. However, addition of 11.1 mg KI/l of water caused a decrease in growth rate and scale radius.

The concentration of iodine in common feed ingredients is highly variable. Protein concentrates of animal origin other than fish meal contain nutritionally insignificant quantities of iodine. Plant protein concentrates usually contain 100 to 300 µg I/kg and common cereal grains 40 to 100 mg I/kg. Plant materials also contain goitrogenic substances such as thioglucosides, thiocyanates and perchlorates. The mechanism of action of the goitrogenic substances in fish has not been studied. Generally commercial fish feeds containing fish meal do not require iodine supplementation.

OTHER TRACE ELEMENTS

Copper, sulfur, fluorine, manganese and molybdenum deficiencies cause abnormalities in mammals and birds. Little, if any, published information on the needs of finfish for these minerals exists. Page et al. (1978) reported that inorganic sulfate failed to promote growth and spared the requirements of sulfur amino acids in rainbow trout. George (1970) indicates that boron or molybdenum supplementation of carp diet improved growth and survival.

Diet containing low levels of both vitamin E and selenium

has been shown to produce nutritional disorders such as muscular dystrophy and exudative diathesis in several species of domestic animals and poultry (Underwood, 1970). These disorders can be prevented either by adding small amounts of selenium or vitamin E to the diet. Poston et al. (1976) reported that both vitamin E and selenium were necessary to prevent muscular dystrophy in Atlantic salmon. They also found anemia, pale gills, anisocytosis, poikilocytosis, elevated plasma protein, exudative diathesis and dermal pigmentation in vitamin E and selenium deficient fish.

1.4. THE DETECTION AND CORRECTION OF DEFICIENCIES AND TOXICITIES IN ANIMALS

Detection of trace element deficiencies in animals

Trace element deficiencies and toxicities are difficult to diagnose because their effects on the animal are often indistinguishable from those arising from a dietary energy deficiency, and because they are seldom accompanied by specific clinical signs. Chemical determination of the trace element level in animal diets and their components provides a helpful indication of the intake in relation to minimum needs. The determination of the element concentration in tissues helps to give a clear picture of the amount of these trace elements needed by the animals. Pathological and clinical studies will be useful to distinguish between deficiencies of these trace elements.

Control or preventation of trace element deficiencies and toxicities in animals

There are various direct and indirect methods for the prevention and control of trace element deficiencies and toxicities in animals, and the method to be chosen varies with different elements and different animal species and their normal feeding practices. Thus with I deficiency in man direct supplementation through iodination of the domestic salt supplies has proved to be the most convenient and effective procedure. With iron deficiency in man the direct method is also accepted, either through iron fortification of a staple food such as flour and bread or through the prescription of iron tablets given during periods of special iron need.

The indirect methods of controlling trace element intakes by animals are normally raising or lowering the concentration in the plant materials as grown and consumed. Trace element fertilization of the soil is widely practised as a means of raising herbage concentration of Co and Cu to satisfactory levels (Nicholas, D. J. D., and Adrian, R. E., 1975).

Trace element toxicities in animals are usually more difficult to control than deficiencies, especially in farm animals which depend on pastures in their feeding, although various procedures have been successfully used to control trace element toxicities in animals. Thus in endemic fluorosis areas the only practical form of protection is periodic removal of the animals from dependence on the fluoridated water. The control of molybdenosis in animals can be achieved by regular oral dose of copper sulfate or periodic injections of this salt. Preventation and control of trace element deficiencies or excesses can be achieved sometimes by an intelligent use of foods, involving regulation of intakes of items known to be high or low in the element in question.

CHAPTER TWO

COBALT SALT

2.1. INTRODUCTION

The cobalt concentrations reported for normal human tissues are similar to those of other species (Yamagata, N., Murata, S., and Torii, T., 1962; Tipton, I. H., and Cook, M. J., 1963), with the exception of those of Butt, E. M., Nusbaum, R. E., Gilmour, T. C., and DiDio, S. L., (1960) and Leddicotte, G. W., (1958) which are appreciably higher; such differences probably reflect analytical variations, and regional differences in cobalt intakes (Table 2.1).

Little is yet known of the forms in which cobalt exists in the tissues, other than as vitamin B_{12}. The existence of other bound forms of this metal has been demonstrated in the tissues of sheep (Monroe, R. A., Sauberlich, H. E., Comar, C. L., and Hood, S. L., 1952), the blood plasma of the dog, and the intestinal wall of the chick (Lee, C. C., and Wolterink, L. F., 1955).

2.1.1. THE BIOLOGICAL ROLE OF COBALT

Cobalt was first shown to be an essential element for ruminants as the result of studies aimed at finding a cure for Coast disease (otherwise known as wasting sickness or bush sickness) which affects sheep in certain areas of Australia. This condition was endemic amongst sheep which grazed on apparently good pastures and, apart from moving them to alternative grazing, no cure was known. A primary symptom of this condition was anaemia and so iron therapy was applied but this met with only limited success.

Treatment of the pasture with cobalt salts or direct administration of cobalt to mature animals alleviated all the symptoms and the blood was rapidly restored to a normal condition. Strangely, however, when suckling lambs were dosed in a similar fashion, a different response was noted: erythropoiesis (the

TABLE 2.1

Cobalt concentration in tissues[@]

Species	Liver	Spleen	Kidney	Heart	Pancreas
Normal human[b]	0.18	0.09	0.23	0.10	0.06
Healthy sheep[c]	0.15	0.09	0.25	0.06	0.11
Co-deficient sheep[c]	0.02	0.03	0.05	0.01	0.02

[@]Concentration expressed in ppm cobalt on dry basis.

[b]From Tipton and Cook (1963).

[c]From Askew and Watson (1943).

process of formation of the erythrocytes) was stimulated, and when the animals were given prolonged treatment they developed polycythaemia (an increase in the number of erythrocytes). No explanation for these observations was satisfactorily advanced till over twenty years later when the anti-pernicious anaemia factor of raw liver was isolated in 1948. The active constituent was isolated and was then shown to be a cobalt complex. It was given the name vitamin B_{12}; the structure is shown in (Fig. 2.1).

The structure of the coenzyme has now been elucidated and vitamin B_{12} consists of a highly substituted porphyrin-like corrin ring in which a cobalt $^{+3}$ ion is bound to the four nitrogen atoms. The metal ion is additionally bound to the nitrogen of a nucleotide base which is attached to the sugar moiety by an unusual β-glycosidic linkage. In addition to the normal 5,6-dimethylbenzimidazole shown in Fig. 2.1, other bases such as adenine, 2-methyladenine, and guanine have been found in naturally occurring vitamin B_{12} analogues.

This part of the structure, which is reminiscent of many haem-iron compounds, is known as cobalamin, so that vitamin B_{12} itself, which contains a cyanide group in the sixth coordination position, is often known as cyanocobalamin. This compound is now synthesized commercially using various species of Propioni bacteria, replacing earlier methods using Streptomyces. The cyanide ion has no functional significance but is merely an artefact of the isolation procedure and can be displaced by many other ligands.

Subsequent studies showed that injections of vitamin B_{12} were extremely effective in treating Coast disease. The effect

on the erythrocyte count could be detected in as little as six hours. Following this it was quickly shown that the inorganic cobalt supplement was converted to vitamin B_{12} by the bacterial microflora of the sheep's rumen and it was only when constituted in this form that the cobalt had the required activity. This explains why inorganic cobalt produces mainly toxic effects in both suckling lambs and monogastric animals, neither of which have developed suitable bacterial colonies in their digestive systems.

Inorganic cobalt is also beneficial in the treatment of some refractory anaemias, but unlike vitamin B_{12} therapy the administration of cobalt salts always produces polycythaemia. It has been suggested that the cobalt may act by causing hypoxia(anoxia) in the bone marrow and this may well be the case, as the oxygen-binding capacity of simple cobalt (II) complexes is well known (Phipps, D. A., 1976). At any rate, cobalt therapy produces exactly the same effect as high-altitude hypoxia, namely to stimulate the formation of erythrocytes. Aquo-cobalamin, and its analogues with other nucleotides, has a water molecule in the sixth coordination position. These compounds are the active coenzymes for at least three biosynthetic reactions: the synthesis of methionine and acetate and methane formation. In each case the aquo-corrinoid compound appears to undergo transient methylation at the cobalt atom and so far the vitamin B_{12} coenzymes are the only naturally occurring compounds known to contain a metal-carbon bond.

The second class of vitamin B coenzymes also contain a metal-carbon bond. In these the cyanide ion is replaced by the

5'-deoxyadenosyl group which is bound to the metal by the carbon atom of the 5'-position (Fig.2.1). It now seems probable that the adenosyl group is introduced by reduction of the cobalamin to cobalt (I), using ferredoxin as the reducing agent followed by reaction with ATP in the presence of a suitable enzyme.

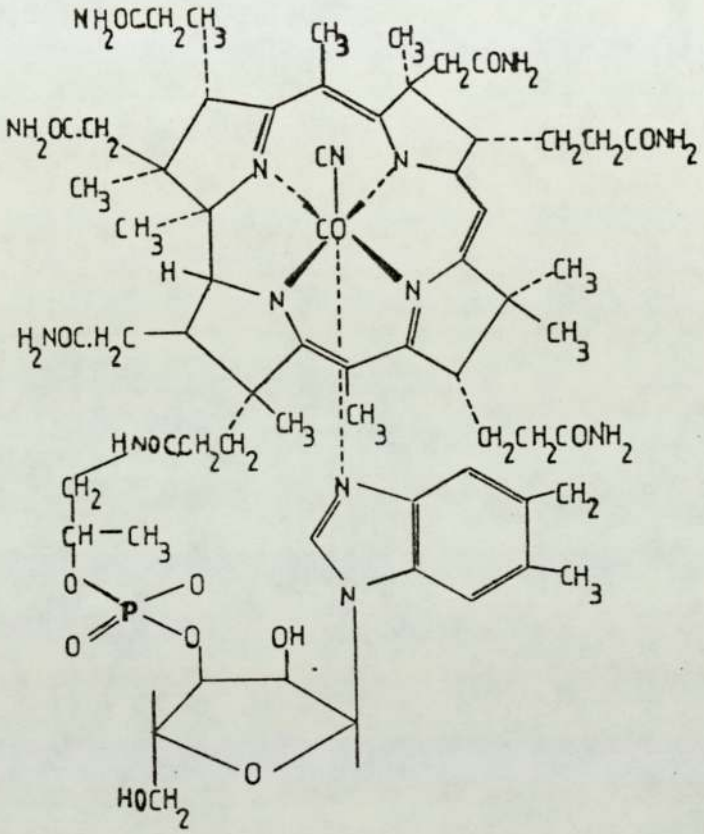
2.1.2. COBALT METABOLISM

In several early studies with farm and laboratory animals, cobalt in the diet or administered orally as soluble salts was reported to be poorly absorbed (Comar, C. L., Davis, G. K., and Taylor, R. F., 1947). More recent investigations indicate that cobalt is well absorbed by small laboratory animals and man. Thus, Toskes, P. P., Smith, G. W., and Conrad, M. E., (1973) found that normal mice on a normal diet absorbed 26.2% of an oral dose of cobalt.

Cobalt absorption increased significantly in iron deficient rats(Pollack, S., George, J. N., Reba, R. C., Kaufman, R. M., and Crosby, W. H., 1965) and man (Valberg, L. S., Ludwig, L., and Glatunbosun, D., 1969). These findings have led to the postulate that cobalt shares a common intestinal mucosal transport with iron, in which acceleration of transport of both elements is governed by the same mechanism (Thomson, A. B. R., Valberg, L. S., and Sinclair, D. G., 1971; Valberg, L. S., 1971).

The actual absorption of the cobalamin is a complex process involving a specific protein carrier. This protein (the intrinsic factor) must combine with vitamin B_{12} (the extrinsic factor) before the vitamin can be absorbed, and this complex seems to act as a carrier, as it is found in an intact form in the tissues. The turnover of the cobalt complex is slow and appears to depend on

Figure 2.1 Vitamin B_{12} (cyanocobalamin)



the exact form of the cobalamin.

The major route of excretion of cobalt in animals and man is the urine (Kent, N. L., and McCance, R. A., 1941; Schroeder, H. A., Nason, A. P., and Tipton, I. H., 1967; Valberg, L. S., et al., 1969), and a direct relationship has been observed between the proportion of an oral dose of cobalt that is absorbed from the intestine and the proportion that is excreted in the urine (Valberg, L. S., 1971). Small amounts of cobalt are also lost by way of the faeces (Valberg, L. S., 1971), sweat (Consolazio, C. F., Nelson, R. A., Matoush, L. O., Hughes, R. C., and Urone, P., 1964), and hair (Schroeder, H. A., et al., 1967).

2.1.3. COBALT TOXICITY

According to Becker, W. S., and Smith, S. E. (1951), cobalt has a low order of toxicity in all species studied, including man. Daily doses of 3 mg/kg body weight, which is approximately 150 ppm cobalt in the dry diet or some 1000 times normal levels, can be tolerated by sheep for many weeks without visible toxic effect. With doses of 10 mg Co/kg body weight, or more, appetite and body weight are severely depressed, the animals become anemic, and death occurs at the higher level.

Rats and various species other than the adult ruminant fed high levels of cobalt salts develop a polycythemia accompanied by hyperplasia of the bone marrow, reticulocytosis, and increased blood volume (Grant, W. C., et al., 1952; Waltner, K., 1929). Inorganic cobalt is mildly toxic, it depresses thyroid activity and a high cobalt diet may cause goiter (Anonymous, 1968). A more serious effect of cobalt toxicity is cardiomyopathy, progressive heart failure caused by excess glycogen (Alexander, C. S., 1969;

Grinvalsky, H. T., and Fitch, D. M., 1969). The cobalt, as Co^{+2}, binds lipoic acid thus preventing glycogen catabolism.

2.2. EFFECT OF COBALT ON MAMMALS

The total cobalt content of the body of adult man has been reported to average 1.1 mg, with about 43% of this total stored in the muscles, 14% in the bones, and the remainder distributed among other tissues (Yamagata, N. et al., 1962). Excessive accumulation does not occur in any particular organ or tissue, but the liver, kidneys, and bones usually carry the highest concentrations of this element (Lee, H. J., 1963; Tipton, I. H., and Cook, M. J., 1963) (Table 2). Bell (1937) investigated the formation of erythrocytes in sheep given cobalt-deficient food and proved that the reduction of the erythrocyte count of sheep blood was influenced by both the season and cobalt deficiency. Kovalskii and Chebaevskaya (1949) found that feeding lambs 4 mg cobalt chloride weekly for 30 days increased their haemoglobin content by 30% above its initial level. In the same period, haemoglobin increased by 13% in the controls. Vsyakikh (1949) found that feeding milk-producing sheep 14 mg cobalt chloride weekly brought about a 15% increase in haemoglobin, while in the control group the haemoglobin content decreased by 13.5%. Bogdanov (1958) found that giving calves cobalt raised their haemoglobin concentration 5% and their erythrocyte count by 3 million, but had no effect on leukocyte count. Bogdanov added also that in calves which received cobalt the protein concentration of the blood was 5% higher than in the control. Robertson (1971) found that ruminants require relatively large quantities of vitamin B_{12} and that this vitamin is manufactured by some of the ruminal flora, cobalt being essential to the process of rumination. Filippov (1973) reported a marked improvement in daily weight

gains and feed efficiency and reduced mortality in young pigs given a supplement of 1 mg/day of cobalt chloride.

2.3. EFFECT OF COBALT ON FISH

Effect of cobalt on the embryonic development in fish

Turdakov and Ozarovskaya (1959) showed that cobalt chloride solution at a concentration of $1 \times 10^{-5} - 1 \times 10^{-6}$ has a favourable effect on the embryogenesis of Schizotherax issykkuli, (Berg) and Rangridibunda, (Pall) and an unfavourable effect on that of Leuciscus bergi, (Kaschkarov). Davis (1962) showed that cobalt retarded the rate of division in trout eggs, and said that this probably happened by inhibition of cell respiration. Shabalina (1963) placed trout eggs in three different concentrations of cobalt chloride, (5, 0.5, 0.05 mg/l itre), and showed that the highest mortality occurred on the 14th day of development at all concentrations of cobalt.

Effect of cobalt on the survival rate and growth of larvae

In many species it has been shown that the early stage of life is characterized by a low rate of survival; it is known that the rate of survival increases with age up to maturity. It has been observed that survival during the early period of life in carp can be significantly enhanced by treatment with minute quantities of vitamin B complex and vitamin B_{12}. Das (1960) suggested that there are certain factors in the B complex which may play an important role in the balance of metabolic processes during the first period of carp life but with increasing age, they become less effective. Das thought that as the ruminant digestive tract is known to contain micro-organisms which synthesise vitamin B_{12}, extract of the ruminant's several stomachs might produce the desired effect. He used a ruminant stomach extract,

which consisted of the juice expressed from the entire contents of four stomachs of a freshly slaughtered goat; Das found that treatment with ruminant stomach extract with cobalt nitrate yielded beneficial results (60% survival rate) when it was given to carp. He suggested that the cobalt nitrate permitted synthesis of vitamin B_{12} by the fry, either from their diet or from the ruminant stomach extract. Shabalina (1963) showed that cobalt has no clear effect on growth of trout larvae (Salmo iridens gibbon), adding that the resorption of the yolk sac at different concentrations of cobalt and in the control proceeded differently, being slowest at a concentration of 5 mg/litre and considerably faster at lower concentrations and in the control. Das (1967) concluded that cobalt is helpful in controlling mortality. Ghosh (1967) concluded that young fry of M. parsia definitely respond to cobalt chloride treatment by improved growth (the net percent increase in growth over that of the control was 228.85% in weight) and survival (the survival percentage was 95%) and that the optimum cobalt requirement for this size group (average length 19 mm) is within the range of 0.6 - 1.0 ppm.

The effect of dietary cobalt on the growth and survival rate of fingerlings

"Research in improvement of fish growth through the addition of growth stimulants to supplementary feeds has greatly increased in recent years" (Ghosh, 1968). Thus Frolova, 1959, 1960, 1961; Sukhoverkhov and Drymova, 1961; Sukhoverkhov, Krymova, and Farberov, 1961; Karpanin and Ushakov, 1961; Vinogradov and Erokhina, 1962; Rimsh, 1963, all found that cobalt influences the growth rate of fish, increases the percentage of haemoglobin,

the erythrocyte count, and the protein concentration in their blood. Trofimova (1962) shows that vitamin B_{12} strengthens resistance to disease in trout fingerlings and increases their weight above that of the controls. Sukhoverkhov (1967) found that the survival rate of carp fingerlings could be enhanced by 30% and the required amount of feed reduced by 20% as a result of using cobalt salts. In the same year, Anon (1967) showed that freshwater fish are being grown to maturity in half the normal time by means of cobalt treatment. The favourable effect of cobalt on the rate of weight gain of salmon fingerlings was demonstrated by Malikova and Kotova (1963); its favourable effect on carp was indicated by Vinogradov and Erokhina (1962) and Shchuvatova (1963). Bow (1971) believed that cobalt stimulates metabolism and growth of fry as well as adults; he added that cobalt chloride or cobalt nitrate, when added to fish food at the rate of 0.08 mg Co/kg fish, increased the growth of the fish by 10 - 32 percent.

CHAPTER THREE

IODINE SALT

3.1. IODINE METABOLISM

3.1.1. INTRODUCTION

Iodine is an essential nutrient for animals; its one recognized function in the body is its role in the formation of thyroid hormone, of which it is an essential component. Thyroid hormones regulate the basal metabolic rate. One of the factors which affect the output of thyroid hormone by the thyroid gland is availability of iodine. In the absence of sufficient iodine, the gland increases its secretory activity in an attempt to compensate for the deficiency; as a result, the gland enlarges and becomes turgid with an iodine-poor secretion. This condition is known as a simple, or endemic, goiter.

Synthesis of the thyroid hormone may be effectively prevented, even in the presence of sufficient iodine in the diet and the circulation, by certain drugs (thiourea, thiouracil), which block the oxidation of iodide to iodine in the thyroid gland.

3.1.2. BLOOD IODIDE

Iodine exists in blood in both inorganic and organic form. The normal range of plasma inorganic iodide (PII) is stated by Wayne, E. J., Koutras, D. A., and Alexander, W. D., (1964) in humans to be 0.08 - 0.60 microgram/100 ml, with values below 0.08 suggesting iodine deficiency and values above 1 microgram/100 ml pointing to exogenous I administration. Leloup and Fontaine (1968) quote values for lower vertebrates of 0.40 - 0.60 µg/100 ml. The organic iodine of the blood, which does not occur in the erythrocytes, is present mainly as thyroxine bound to the plasma proteins. Only a very small proportion, normally about 0.05%,

is free in human serum (Sterling, K., and Bremner, M. A., 1966).

The distribution of iodide in the blood is expressed by the ratio I^{131} per gram of red blood cells (RBC)/I^{131} per gram of plasma. This value varies with the species (Fontaine, M., and J. Leloup, 1957) (Table 3.1). Rall, J. E., M. H. Power, and A. Albert (1950); Courrier, R., F. Morel, and A. Colonge (1954); Owen, C. A., and M. H. Power (1953); and Lachiver, F., and F. Poiviliers (1959) found that the values observed in mammals and birds (0.40 to 0.60) are near that of teleosts (0.45). A very low value (0.098) observed in the salmon during its upstream migration, indicates that the blood iodide is found exclusively in the plasma; this behaviour of iodide is comparable to that generally observed for thyroxine (T_4), which enters RBC very little or not at all.

This leads to the hypothesis that the factor limiting the penetration of iodides into salmon RBC was, as for T_4, a binding of I^- ion with one or several plasma proteins (Leloup, J., 1958). It seems that the plasma iodide of marine teleosts is greater, in general, than that of fresh-water teleosts because of the elevated iodine content of the environment.

3.1.3. INTRATHYROIDAL METABOLISM OF IODINE

Mammals

In 1959, Pitt-Rivers, R., and J. R. Tata showed that the intrathyroidal metabolism of iodine in mammals may be outlined as follows:

1. The thyroid gland concentrates the circulating iodides.

2. Iodides are oxidized and incorporated into tyrosine residues linked in the molecule of thyroglobulin to give MIT and DIT.

Table 3.1

Erythrocyte/Plasma I^- Ratio (H/P) and Dialysis Ratio (R) in Some Fish

Species	H/P ratio	Dialysis ratio (R)
Selachians		
Dogfish (*Scyllium stellare* Flem.)	0.43	1.04
Dogfish (*Scyllium canicula* L.)	0.45	1.00
Teleosts		
Eel (*Anguilla anguilla* L.)	0.47	1.01
Conger (*Conger conger* L.)	0.60	1.04
Carp (*Cyprinus carpio* L.)	0.40	1.03
Sea perch (*Labrax lupus*)	0.49	1.30
Mullet (*Mugil sp.*)	0.23	6.8
Shad (*Alosa alosa* L.) in upstream migration.	0.23	10.3
Trout (*Salmo fario* L.)	0.19	11.3
Rainbow trout (*Salmo gairdnerii* Rich) (immature)	0.23	16.5
Sea trout (*Salmo trutta* L.) in upstream migration.	0.12	25.0
Salmon (*Salmo salar* L.) in upstream migration.	0.098	32.4
Lungfish (*Protopterus annectens*)	0.47	——

Dialysis Ratio : Tissue/Plasma Ratio (R)

3. The coupling of two molecules of DIT or one molecule of MIT and one molecule of DIT leads to the formation of Thyroxine (T_4) and Triiodothyronine (T_3).

Once ingested, iodine is absorbed from the small intestine. It is found in the blood as inorganic iodide and as protein-bound iodine. It is taken up by the thyroid gland as the iodide ion and is selectively concentrated there, oxidized to elemental iodine, and incorporated into the amino acid tyrosine in the thyroglobulin, which, after conversion to mono- and di-iodotyrosine, triiodothyronine, and thyroxine (Fig. 3.1), becomes part of the thyroglobulin complex - the biologically active hormone.

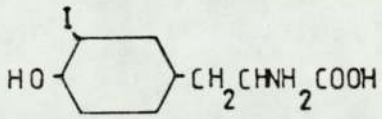
4. The thyroglobulin is hydrolyzed by a protease that liberates iodinated aminoacids. T_4 and T_3 pass into the blood, MIT and DIT are deiodinated by a deiodinase, and the liberated iodide is probably reutilized for hormonal synthesis.

The outline for mammals can be applied to the lower vertebrates in the following respects:

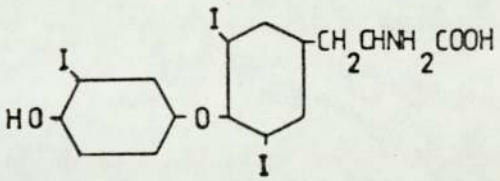
1. The thyroid of all lower vertebrates concentrates iodides and its concentration ability is superior to that of other tissues having the same affinity (notochord in cyclostomes, the gills, stomach, and bile in selachians, the skin in Amphibia, and the ovary in cyclostomes, teleosts, and Amphibia).

2. By chromatographic study of saline extracts of the endostyle of the ammocoete and the thyroid of adult lampreyes, different teleosts, and toads, Leloup, J. (1955); and Donoso, A. O. and J. C. Trivelloni (1958) observed that 24 hours after injection of I^{131} the greater part of the radioactivity is localized at the origin, suggesting a thyroglobulin-like substance. In hydrolyzates

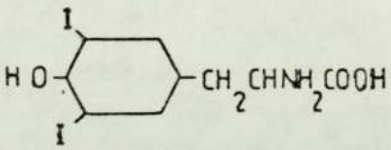
Figure 3.1 Intrathyroidial metabolism of iodine



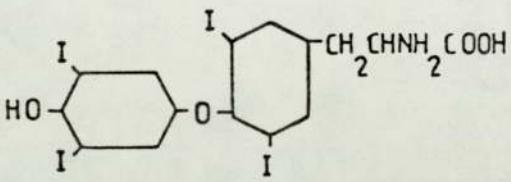
3-mono-iodotyrosine (MIT)



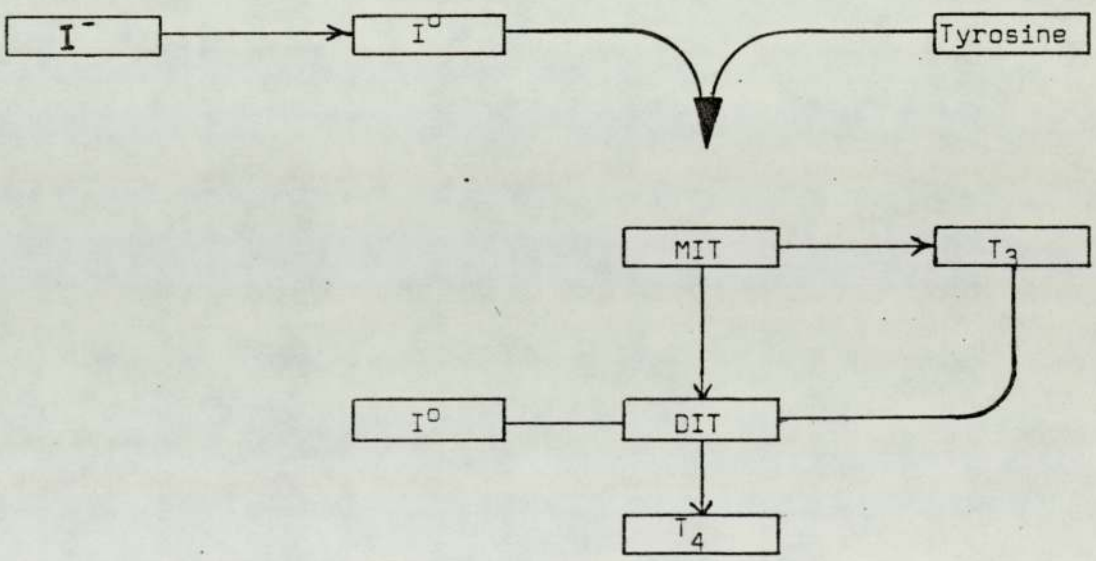
3,4,3-Tri-iodothyronine (T_3)



3,5-di-iodotyrosine (DIT)



3,5,3,5-tetra-iodothyronine (T_4) Thyroxine



of the thyroid of cyclostomes, selachains, teleosts, lungfish, Amphibia, and reptiles a short time after injection of I^{131} the presence of MIT and DIT was shown; after a longer period of time T_3 and T_4 were demonstrated (Berg, O., A. Gorbman, and H. Kobayashi, 1959).

3. The existence of a protease in the endostyle of the ammocoete and in the thyroid of the adult lamprey and of the dogfish (Clements, M. and A. Gorbman, 1955; and Clements, M., 1957). Furthermore, the existence of free T_4 has been shown in the nonhydrolyzed extract of the endostyle of the ammocoete and the thyroid of the adult lamprey (Leloup, J., 1955).

4. The existence of T_4 and T_3 in notable quantities in the plasma of ammocoete, lamprey, trout, and lungfish (Leloup, J., 1955, 1958).

Teleosts

The order of teleosts is probably the most studied from the point of view of thyroid function in lower vertebrates, especially by Gorbman in New York and Fontaine in Paris. The maximal uptake of iodine is generally less in the marine teleosts than the fresh-water teleosts (Hickman, C. P., 1958). However, some fresh-water species seem to have a very slow intrathyroidal metabolism, such as sunfish (Berg, O. et al., 1959) and goldfish (Berg, O. and A. Gorbman, 1954). Moreover, most of the I^{131} present in the thyroid of the carp Cyprinus carpio, is in the inorganic form and a significant fraction is probably in the nonthyroidal tissue associated with the glandular tissue (Olivereau, M., 1954). In certain species, the proportion of T_4 and T_3 can be small and difficult to show. This fact can be due

to the slow iodine metabolism (as in C. auratus and Lepomis gibbosus) and also to the rapid excretion of hormones into the blood (as in Periophalmus) (Berg, O. et al., 1959; and Leloup, J., 1956). Therefore the presence of small quantities of T_4 and T_3 in the thyroid is not necessarily an indication of a slow hormonal biosynthesis.

3.1.4. EXTRATHYROIDAL DISTRIBUTION OF IODINE

In mammals, a number of tissues in addition to the thyroid have the property of concentrating iodide, namely the gastric mucosa, the salivary gland, the lactating mammary gland and the placenta (Pitt-Rivers et al., 1959). In lower vertebrates the same property is found in various tissues. Since iodine concentration in the ovary had been found also in birds (Roche, J. and G. Desruisseaux, 1951; and Roche, J., O. Michel, R. Michel and M. Marois, 1951), and the ovary of mammals is rich in iodine (Roche, J. et al., 1950) it thus seems to be a general phenomenon in the vertebrates.

The maturing ovary of the rainbow trout can accumulate up to 70% of the I^{131} injected (Robertson, O. H. et al., 1953) (Table 3.2). The lesser accumulation in the ovary of the salmon and the low concentration ability in regard to the plasma are probably due to high blood iodine and the greater binding of the iodides to plasma protein. Determination of I^{127} shows the concentration of iodide in the ovaries of salmon, trout, and mature sea-run trout of California (Robertson, O. H., 1953).

3.1.5. EXCRETION OF IODIDES

Mammals

In mammals, the major route of excretion of iodide is through the kidney (Pitt-Rivers, R. et al., 1959). In fish the kidney

Table 3.2

I^{131} and I^{127} Content of Ovary of Some Teleosts

Species	Body weight (gm)	Ovary weight (gm)	Hours after injection	Injected I^{131} recovered in Ovary %	I^{131} per gm Ovary / I^{131}/gm Plasma	I^{127} per gm Ovary / I^{127}/gm Plasma
Rainbow trout (Salmo gairdnerii)						
Maturing	150	12.6	72	70	17.4	14.2
Ripe and spent	-	-	-	-	-	7.8
Sea-run steelhead	-	-	-	-	-	9.4
Salmon (Salmo salar L.) Maturing	5.000	27	24	7	1.33	-
	4.900	37	24	6.7	0.72	3.4
	4.100	22.1	72	7.75	2.00	5.5
	7.100	-	72	-	2.90	-

Data from Robertson and Chaney (1953)

often plays a small role in excretion while the gills may be important.

Teleosts

A. Marine Teleosts

In the mullet (Mugil auratus) 25 and 45 percent of the dose is excreted in 24 and 72 hours respectively. On the other hand, in the eel (Conger conger), excretion seems much lower. In the two species, treatment with antithyroid compounds strongly accelerates the elimination of iodide (Leloup, J., 1952), in accordance with observation in mammals (Brown, J., 1956).

B. Fresh-water Teleosts

Excretion in this group is generally rapid (Table 3.3). However, for a related temperature, it seems to be slow in the eel, which is perhaps a characteristic of the apod teleosts (Order Apodes). The addition of iodine to the environment accelerates the excretion (platyfish, rainbow trout). The temperature seems to be an important factor, for at 24°C the eel excretes much more than at 6°C. Hypophysectomy decreases the excretion in the eel, which agrees with the report of Albert, A.,A. A. Tenney and N. Lorenz (1952), who observed a decrease of the renal clearance in the hypophysectomized rat. The administration of TSH to the hypophysectomized eel at 24°C increases excretion, but does not have a marked effect on the normal animal (Leloup, J., 1968). Excretion is increased in the young salmon during the transformation from parr to smolt. Chavin, W. (1956) reported that the excretion of iodides is much slower in the goldfish when I^{131} is administered in the form of tagged albumin. This result is in favour of the previously mentioned hypothesis that the binding of iodine in certain teleosts considerably reduces loss.

Table 3.3

I^{131} Excretion in Fresh-Water Teleosts

Species	Tempt. °C	Treatment	Excretion of injected I (%) 24 hours	72 hours
Goldfish (Chavin, 1956)				
Carassius auratus	22-24	Radioiodinated human serum albumin	13	-
C. auratus	22-24	0	65	-
Platyfish (Berg *et al.* 1953)				
Xiphophorus maculatus	?	0	40	60
X. maculatus	?	Iodide-enriched water (60 ppm) KI	78	100
Swordtail (Berg *et al.* 1954				
Xiphophorus montezumae	?	0	50	60
Eel (Leloup, 1958, 1959)				
Anguilla anguilla	24	0	18	34
A. anguilla	24	Hypophysectomized	4.3	9.3
A. anguilla	24	Hypophysectomized and TSH	8.6	16.6
A. anguilla	6	0	3.7	11.0
A. anguilla	6	Hypophysectomized	4.8	8.2
Salmon (Leloup *et al.* 1968)				
Parr	14	0	26	39
Smolt	14	0	43	-
Trout (Leloup *et al.* 1968)				
Salmo fario	14	0	24	28
S. gairdnerii	20	0	33.5	-
S. gairdnerii	20	Iodide-enriched water (60 µg I^- /1000 cc	68.4	-

3.1.6. IODINE DEFICIENCY AND TOXICITY

Iodine deficiency

It is known that the functional significance of iodine is always accounted for by its presence as thyroid hormones, thus the manifestations of iodine deficiency are those of a deficient supply of those hormones within the organism. Many factors can inhibit the capacity of the thyroid gland to accumulate iodine and convert it into active compounds (the thyroid hormones); these factors may act independently of iodine supply, or may become apparent in circumstances of borderline I deficiency.

Iodine deficiency in fish

This deficiency was, according to Gaylord and Marsh (1914), reported for brook trout as early as 1891 by Scott who identified throat lesions as a form of cancer. Marine and Lenhart (1910, 1911, 1914) said that this disease was a form of goiter, but Gaylord and Marsh believed it to be a neoplasic disease, as had Scott. After much controversy as to its etiology and many experiments later, the dignosis of goiter was confirmed. It was shown conclusively that the disease could be controlled by the administration of minute quantities of dietary iodine. Davis (1953) explains the confusion that had attended the fish goiter controversy by pointing out the diffuse nature of the teleost thyroid tissue, which is scattered along the course of the ventral aorta and its main branches to the gills, especially the second and third branchial arches. Hyperplasia of the thyroid or goiter presented a picture remarkably similar to that of an invasive neoplasm of the branchial arch region. MacIntyre (1960) includes a synopsis of thyroid tumours in captive and free-living teleosts.

Iodine Toxicity

Iodine toxicity has been studied in man (Vought, R. L., 1971), cattle (Newton, G. L. and Clawson, T. A., 1974), pigs (Arrington, L. R. et al., 1965), rats, rabbits, hamsters (Arrington, 1965), and poultry (Marcilese, N. A., 1968).

According to Wolf, J. (1969), there are four degrees of iodide excess in animals as follows:

(a) Relatively low levels which lead to temporary increases in the absolute I uptake by the thyroid and the formation of organic I, until such time as the thyroid is required to reduce iodide clearances.

(b) A larger amount which can inhibit I release from the thyrotoxic animal thyroid or from thyroids in which I release has been accelerated by TSH.

(c) A slightly greater intake which leads to inhibition of organic I formation and which probably causes iodide goiter.

(d) Very high levels of iodide which saturate the active transport mechanism for this ion. The acute pharmacological effects of iodide can usually be demonstrated before saturation becomes significant.

3.2 THYROID HORMONES

3.2.1 INTRODUCTION

There is a considerable controversy in the literature as to whether or not the fish thyroid is essential for growth. Growth and morphogenetic processes are slowed down when thiourea or other antithyroid drugs are administered to fish (Goldsmith, 1949; Hoar and Bell, 1950; Gaiser, 1952; Hopper, 1952; Vivien and Gaiser, 1952; Scott, 1953; Smith, Sladek and Kellner, 1953; Dales and Hoar, 1954; Honma and Murakawa, 1955, 1957; LaRoche, et al., 1966; Sage, 1967). Chemical thyroidectomy with radioiodine also arrested the growth in different fish species (LaRoche and Leblond, 1954; Gross, Fromm and Roelofs, 1963; LaRoche et al., 1966; Norris, 1969). The antithyroid compounds and chemical thyroidectomy have direct toxic effects on the fish and their individual effects have not been worked out adequately in interpreting their action in fish growth. Sometimes the negative effects of either antithyroid compounds or radiothyroidectomy on growth of fish can be abolished by treatment with thyroxine (Gaiser, 1952; LaRoche et al., 1966; Sage, 1967; Norris, 1969).

Fontain and Baraduc (1955) and Norris (1969) have shown that thyroxine (T_4) promotes growth in Salmo gairdneri. The same results have been reported for Lepomis cyanellus (Gross et al., 1963) and Poecilia reticulata (Sage, 1967). Growth increments have also been achieved with thyroid powder, minced beef thyroid, or iodinated caesin given to S. gairdneri (Fontain and Baraduc, 1955; and Barrington, Barron, and Piggins, 1961), Salmo salar (Piggins, 1962), P. reticulata (Hopper, 1952; and Lam, 1973) and Xiphophorus helleri (Lam, 1973). Recently

Higgs, Donaldson, Dye and McBride (1976) have shown that thyroxine at a dose of 1 µg/g /week and 10 µg/g /week induced significant length and weight gains in under yearlings of Coho Salmon. They also showed that there was no significant difference in the two doses tested and that T_4 in higher dose produced growth anomaly in the skull and fin structure as well as high mortality, and they recommended that 1 µg/g /week of T_4 can be used for promoting growth of coho salmon at $10^{o}C$.

The effect of thyroxine on nitrogen metabolism in fish has not received much attention. Hoar (1958) noted that treatment with T_4 increased the ammonia excretion to 100% in goldfish. Thornburn and Matty (1963) reported that ammonia production of goldfish immersed in T_4 solution increased markedly at $21^{o}C$. T_4 had no effect on nitrogen excretion of trout at $14^{o}C$. Apart from ammonia excretion, T_4 also produced an increase in the free amino acids in the liver and muscle while no change in keto acids was found. Further, thyroxine produced 18% greater uptake of 1-C^{14}-DL-leucine *in vitro* than the controls. Narayansingh and Eales (1975) have reported that T_4 and T_3 can stimulate protein synthesis in brook and rainbow trout plasma and tissues.

3.2.2. CIRCULATION OF THYROID HORMONE

Nature

The thyroid of lower vertebrates has been the object of numerous studies but the study of the circulating hormone has received less attention. T_4 has been characterized in the plasma of the ammocoete (Leloup, 1955), the adult of *L. planeri*, rainbow trout, *S. gairdnerii*, and the lungfish (Leloup, 1958).

T_3 found in the same species exists in less quantities than T_4.

Plasma Content of Hormonal Iodine-I^{127}

The plasma content of hormonal iodine is very variable in lower vertebrates (Table 3.4). In the marine lamprey and in the lungfish it is of the same order as in the mammals and birds. In fresh-water and marine teleosts elevated values are observed in some species and low values in others. High values are usually found in migratory species (mackerel, mullet); the typical migrators (salmon, sea trout, shad) show the greatest content in hormonal iodine, as they have also the greatest content of iodide (Fontaine and Leloup, 1952). This peculiarity is related to the intense muscular activity manifested by these fish in upstream swimming. It has been shown that the upstream battle increases the need for thyroid hormone in the rainbow trout (Fontaine and Leloup, 1959).

Binding of Thyroid Hormones to Plasma Proteins

The existence of a thyroxine-binding protein (TBP) has been well demonstrated in several species of mammals. Apter et al. (quoted by Robbins and Rall, 1957) studied the serum of two reptiles; these two species have two proteins binding T_4, of which one is albumin. Tata (Leloup and Fontaine, 1968) demonstrated the existence of TBP in two species of teleosts, plaice and brown trout.

Distribution of Thyroid Hormones T_3 and T_4 between RBC and Plasma

This distribution according to Leloup et al., (1968) has been studied in the carp, eel, salmon, and lungfish (Leloup, 1958), and it seems that it varies with the species (Table 3.5). T_4 does not penetrate into the RBC of the eel or the lungfish,

Table 3.4

Plasma Iodine in Lower Vertebrates

Order or Species	µg I^{127}/100 g Plasma			
	Total		PBI	
	Min.	Max.	Min.	Max.
Cyclostomes				
Lamprey (Fontaine and Leloup, 1950)				
Petromyzon Marinus	5.4	14.8	2.4	10.1
Selachians				
Torpedo (Leloup, 1949)				
Torpedo marmorata	2.7	24.0	2.4	10.8
Dogfish (Leloup, Fontaine, 1968)				
Scyllium canicula	8.2	51.0	2.4	12.5
Teleosts				
Fresh-water (Robertson and Clements, 1953; Fontaine and Leloup, 1952; Fontaine, 1956)	2.7	46.0	1.0	15.0
Marine (Leloup, 1949)	15.0	91.5	6.0	35.0
Fish on Upstream Migration				
Salmon (Robertson et al., 1953; Fontaine, 1956; Fontaine and Leloup, 1950)	36.0	257.0	10.0	80.0
Sea trout (Robertson et al., 1953 Fontaine and Leloup, 1952)	260.0	570.0	45.0	109.0
Shad (Fontaine and Leloup, 1950; 1952; Fontaine, 1956)	284.0	2300.0	17.2	70.0
Lungfish (Leloup, 1958)				
Protopterus annectens	4.2	6.4	1.0	3.2

Table 3.5

H/P ratio of I^{131}-labelled iodide, triiodothyronine and thyroxine in some teleosts

Species	H/P ratio		
	Iodide	Triiodothyronine	Thyroxine
Carp	0.43	0.41	0.14
Eel	0.53	0.19	0.07
Salmon (adult)	0.06	0.17	0.15

but will do so into those of the salmon (H/P=0.15) and carp (H/P=0.14). The case of the salmon is particularly remarkable since the H/P is higher for T_4 than for iodide. With T_3 the H/P is always higher than for T_4, but the difference is small in the case of the salmon. However, in the carp T_3 penetrates into RBC as do iodides.

3.2.3. DISTRIBUTION AND METABOLISM OF THYROID HORMONES

The distribution and metabolism of T_3 and T_4 labelled with I^{131} have been studied in the salmon, and of T_4 in the rainbow trout and the normal hypophysectomized eel. The variation in radioactivity in different tissues as compared to plasma, 24 and 72 hours after the injection of labelled T_4 into the migrating adult salmon, is presented in (Table 3.6).

In the young salmon, T_3 is metabolised more rapidly than T_4 and its concentration in the bile is greatly elevated. A more rapid disappearance of T_3 has been found equally in man and different mammals (Pitt-Rivers et al., 1959). The temperature distinctly influences the metabolism of T_4 in the eel. At 24°C, T_4 disappears more quickly from the blood and is deiodinated more rapidly than at 6°C (Olivereau, 1955). Hypophysectomy does not seem to affect the speed of utilization of T_4, contrary to the results obtained with the rat (Van-Arsdel and Willaims, 1956).

3.2.4. PITUITARY CONTROL OF THYROID FUNCTION

Hypophysectomy

Hypophysectomy produces in the goldfish embryo (Vivien and Rechenmann, 1954), the eel (Fontaine, Leloup and Olivereau, 1953), the goldfish (Chavin, 1956), and the toad (Donoso and

Table 3.6

Concentration[@] of radioactivity in various tissues of salmon after injection of I^{131}-labelled thyroxine

Tissue	24 hours		72 hours	
	Tissue/Plasma ratio (R)	I^{131} organic %	Tissue/Plasma ratio (R)	I^{131} organic %
Ovary	0.24	67.5	4.71	14.2
Liver	1.66	98.0	1.95	95.0
Bile	150.4	-	606.6	-
Intestine	1.28	91.0	5.86	85.0
Kidney	0.54	85.0	1.88	81.0
Muscle	-	-	0.31	71.0
Thyroid	6.86	94.5	146.4	98.0
Pituitary	0.55	-	0.82	-
Hypothalamus	0.12	93.0	0.17	80.0
Plasma	1.00	82.0	1.00	41.0

[@] Expressed as the ratio of the radioactivity per gram of organ weight to the trichloracetic-insoluble radioactivity per gram of Plasma.

Trivelloni, 1958) a marked decrease in the thyroid uptake of I^{131} in comparison to that in normal animals, 5 - 8 days after operation. In the eel the reduction of I^{131} uptake is accentuated with time, although the histological aspect of the gland is not characteristically modified. The organic binding of iodine is considerably retarded because the percentage of inorganic I^{131} in the gland is always considerable. The synthesis of T_4 and its liberation into the plasma are equally slow. Hypophysectomy in the eel therefore seems to depress the different phases of intrathyroidal I^{131} metabolism in the same manner as in the rat (Taurog, Tong and Chaikoff, 1858). The only difference seems to be a slower evolution of these processes in the eel, probably due to the influence of temperature.

Influence of TSH

Numerous authors have shown an increase in the uptake of I^{131} in the thyroid of various marine and fresh-water teleosts after administration of TSH: conger at 15 - 16°C (Leloup, 1952), C. auratus at 23 - 24°C (Chavin, 1956; Berg and Gorbman, 1954), rainbow trout at 20 - 21°C (Fontaine, Baraduc and Fontaine, 1955; Fontaine and Fontaine, 1956; Olivereau, 1955), and the normal and hypophysectomized eel at 25°C (Leloup and Fontaine, 1956). The increase of the uptake of I^{131} is proportionately greater in the hypophysectomized animal (Fig. 3.2), as where the pituitary-thyroid axis is put at rest by the addition of iodinated casein to the diet or by fasting (Fontaine et al., 1955; Fontaine et al., 1956). In the normal eel, at 25°C, TSH accelerates the organic binding of iodine and the synthesis of T_4 soon after the injection of I^{131}.

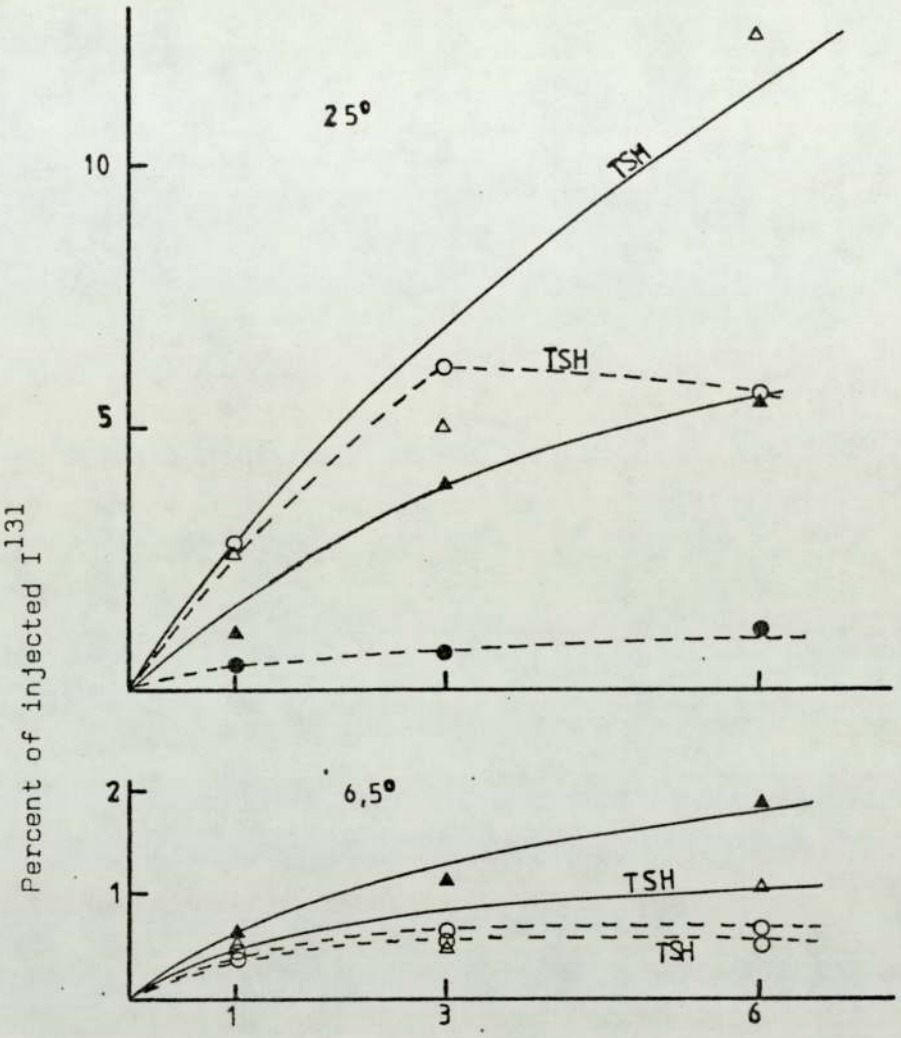


Figure 3.2 Influence of thyrotropic hormones on the uptake of I by the thyroid of normal (lines) and hypophysectomized eel (dotted lines) at 6.5°C and 25°C.

CHAPTER FOUR

GENERAL MATERIALS AND METHODS

4.1 THE EXPERIMENTAL ANIMALS

The fish used in the present study were Carp, Cyprinus carpio. Carp live in fresh water; generally they are thermophilic, gregarious fish dwelling mostly at the bottom of the ponds. They thrive best in well warmed waters at lower elevations. They can adapt to coarser conditions (reduction in ambient oxygen, exposure to disease, and variation in water temperature), which, however, impair their growth. With a rise of water temperature (between 17 - 22^{o}C) their food intake increases; at a temperature of 2 - 3^{o}C the carp take no food at all, gathering at the bottom in selected places.

The carp is not demanding for oxygen requirement; in winter 3 ml per litre of water is sufficient, while in the summer the requirement rises to a maximum of 7 - 8 ml per litre of water. From the economic point of view the carp is one of the best and most important fish of fish ponds. It has rapid growth, tasty flesh, good productive ability, breeding ability (easy to breed with other fish from the same family and can be bred in captivity), resistance to diseases and modest requirements for food and oxygen: all these characteristics make it very popular and have led to the carp becoming the staple fish of warm water fisheries.

4.2 THE EXPERIMENTAL FACILITIES

Holding fish in a restricted volume of water causes deleterious changes in water quality that must be rectified. These changes include increases in the concentration of dissolved organic material, solid faecal matter and dissolved carbon dioxide, as well as a decrease in the dissolved oxygen content of the water. The first limiting change is the depletion of oxygen due to the

metabolic requirements of the fish. This situation is easily corrected by mechanical agitation and aeration of water. Huisman (1974) found that for mirror carp, Cyprinus carpio, no depression of the growth or toxic effects occurred provided that dissolved oxygen concentration in excess of 3 mg per litre was maintained. The second limiting change is the production of ammonia by the fish, which is their primary method of nitrogen elimination. The safe level for carp is reported by Huisman (1969) to be 2 mg per litre at undefined temperature, dissolved oxygen, or pH. Smith (1972) reports a value of 1 mg per litre as a safe limit for rainbow trout, Salmo gairdneri, with oxygen concentration in excess of 7 mg per litre; as the dissolved oxygen of the water falls, so the threshold of ammonia toxicity falls (Larmoyeaux and Piper, 1973).

On the basis of the above considerations it was considered that if the total ammonia remained below 0.5 mg per litre it would have no toxic or growth inhibitory effect on mirror carp between temperatures of 20 - 30^{o}C at an approximate pH of 7 and a level of dissolved oxygen in excess of 5 mg per litre.

System 1

The arrangement of this system is shown in Figure 4.1. This system was used to accommodate the fish after their arrival from quarantine in order to acclimatize them with the changed environment.

The 1200 litre stocking tank was placed on the floor in a tropical room (25 - 27^{o}C); water passed from the abstraction in the centre of the tank to the surface of the biological filter. This filter consisted of a 300 litre tank containing broken gravel (1.5 cm) supported on a corrugated perforated plate which was, in

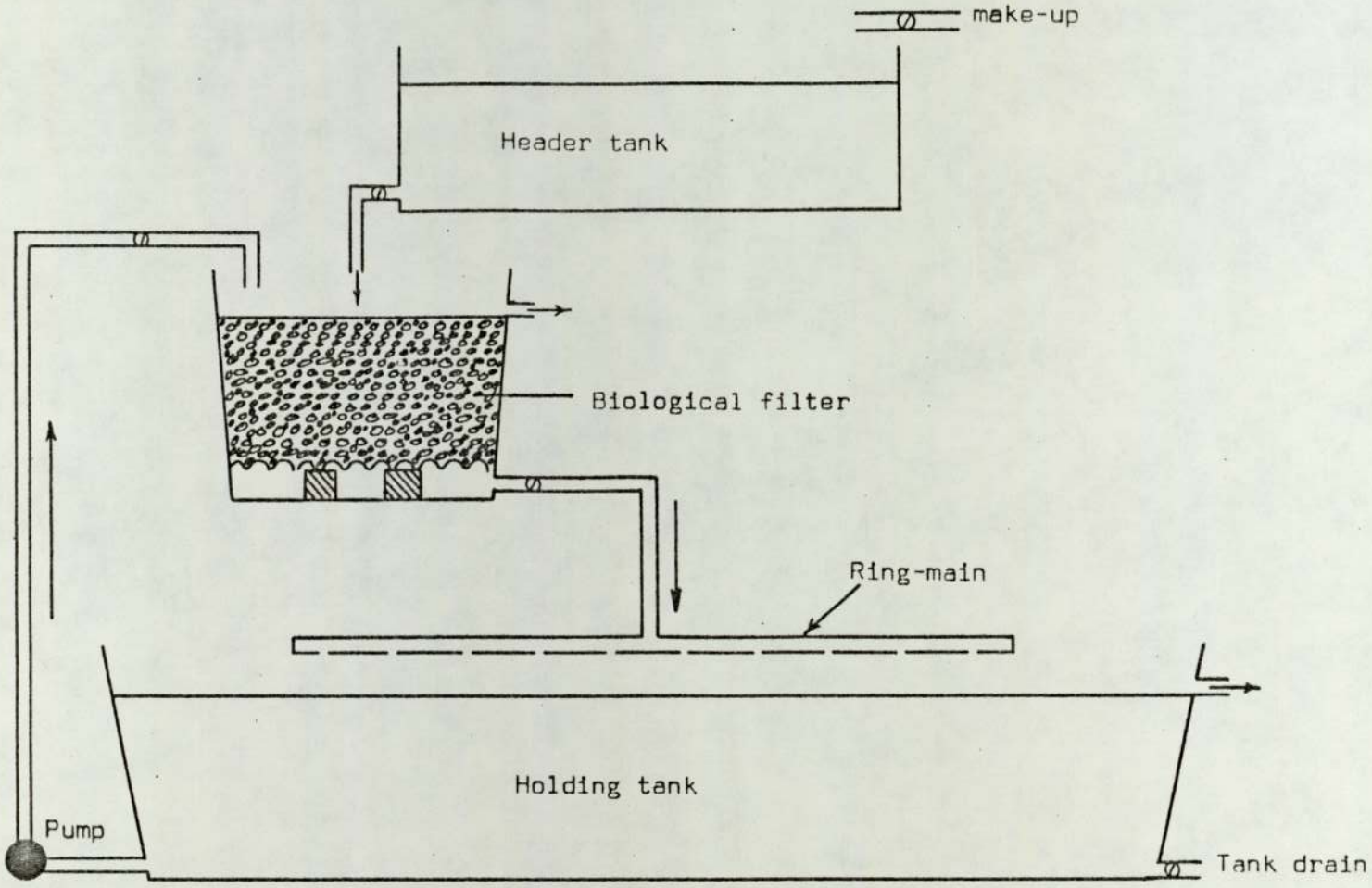


Figure 4.1. A diagrammatic representation of the recycling units (System 1).

turn, supported on house-bricks. Water was drawn down through the filter gravel into the cavity below the filter plate and then to the stocking tank through small holes in order to increase aeration.

Make-up water was continuously added to the header tank both to compensate for losses through splahing and evaporation and to keep ammonia levels within the acceptable limits. Two thirds of the gravel in the filter was dug over once a month and displaced detritus siphoned off.

During the stocking the fish were fed on a pelleted commercial trout diet; the proximate analysis of the food as supplied by the manufacturers was as follows: Protein, 50%; Fat, 6.5%; Fibre, 3.0%; Vitamin A, 1500 IU/kg; Vitamin D, 1500 IU/kg; Vitamin E, 90 IU/kg.

Several water quality parameters were measured and the values recorded (average of reading, twice a week) are shown in Table 4.1.

Table 4.1

Water Quality Criteria in System 1

Temperature	25 ± 1.0°C
Dissolved Oxygen	More than 7 mg per litre
Total Ammonia	Less than 0.2 mg per litre
pH	6.8 - 7.2

System 2

The arrangement of this system is shown in Figure 4.2. This system was constructed in a small tropical room and thus ambient room and water temperature fluctuation were greatly reduced.

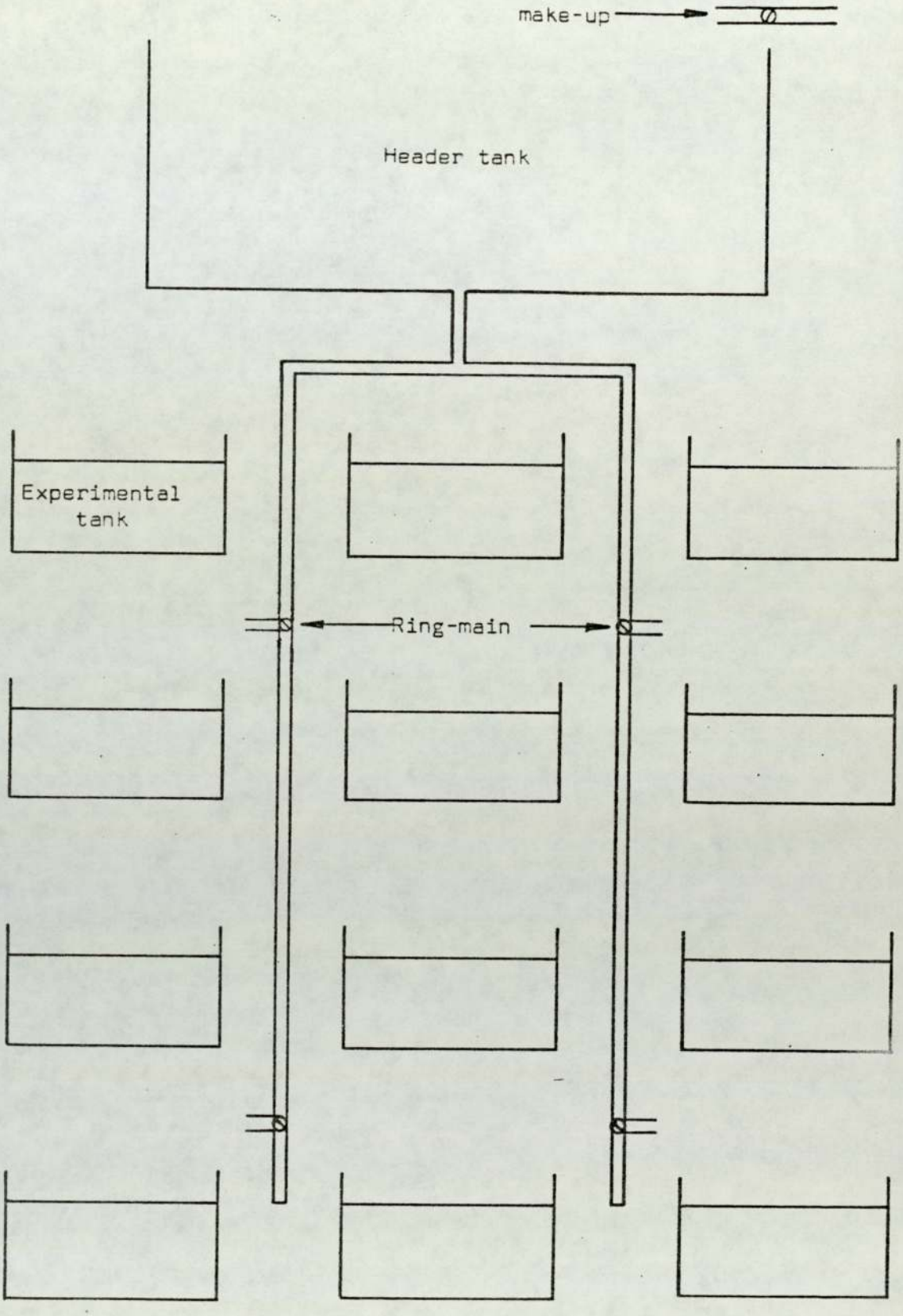


Figure 4.2. A diagrammatic representation of System 2

It was decided to construct using the available materials, Angle-iron and glass Aquaria, 30 x 30 x 60 cm, capacity 25 litres. The tanks were placed on shelves in the tropical room; each tank was supplied with a small plastic filter (Figure 4.3), in order to increase the aeration. The filters were cleaned weekly. Tap water was placed in the header tank for 24 hours before use to equilibrate to the room temperature.

Water quality parameters were measured and the values recorded (average of reading, twice a week) are shown in Table 4.2.

Table 4.2

Water Quality Criteria in System 2

Temperature	24 ± 1.0°C
Dissolved Oxygen	More than 8.0 mg per litre
Total Ammonia	Less than 0.3 mg per litre
pH	7.1 - 7.5

System 3

The arrangement of this system is shown in Figure 4.4. This system was used in experiment 3 (Chapter 7); the principles employed in this system are related to the nature of this experiment which required changing the temperature of the water every four weeks. Thus a glass tank 30 x 60 x 60 cm was used, and four small glass tanks 15 x 20 x 30 cm were placed inside the first tank and filled with water to the same level. Temperature control was achieved by a heater-cooler thermocirculator (Churchill Instruments, Middlesex) which abstracted water, heated or cooled it to a thermostatically regulated temperature, and then returned it to the surface of the tank. The principles employed in the small

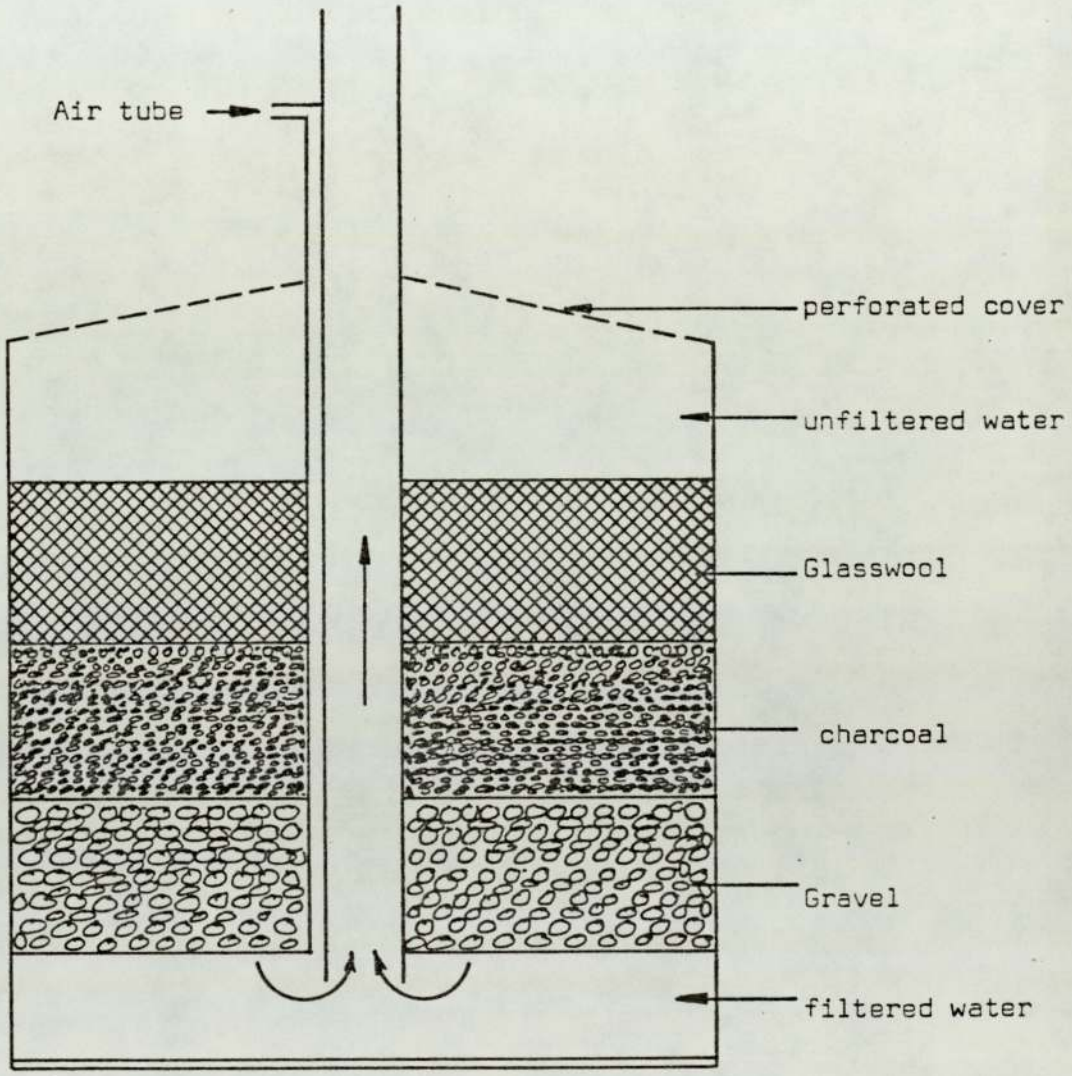


Figure 4.3. A transverse section of the filter

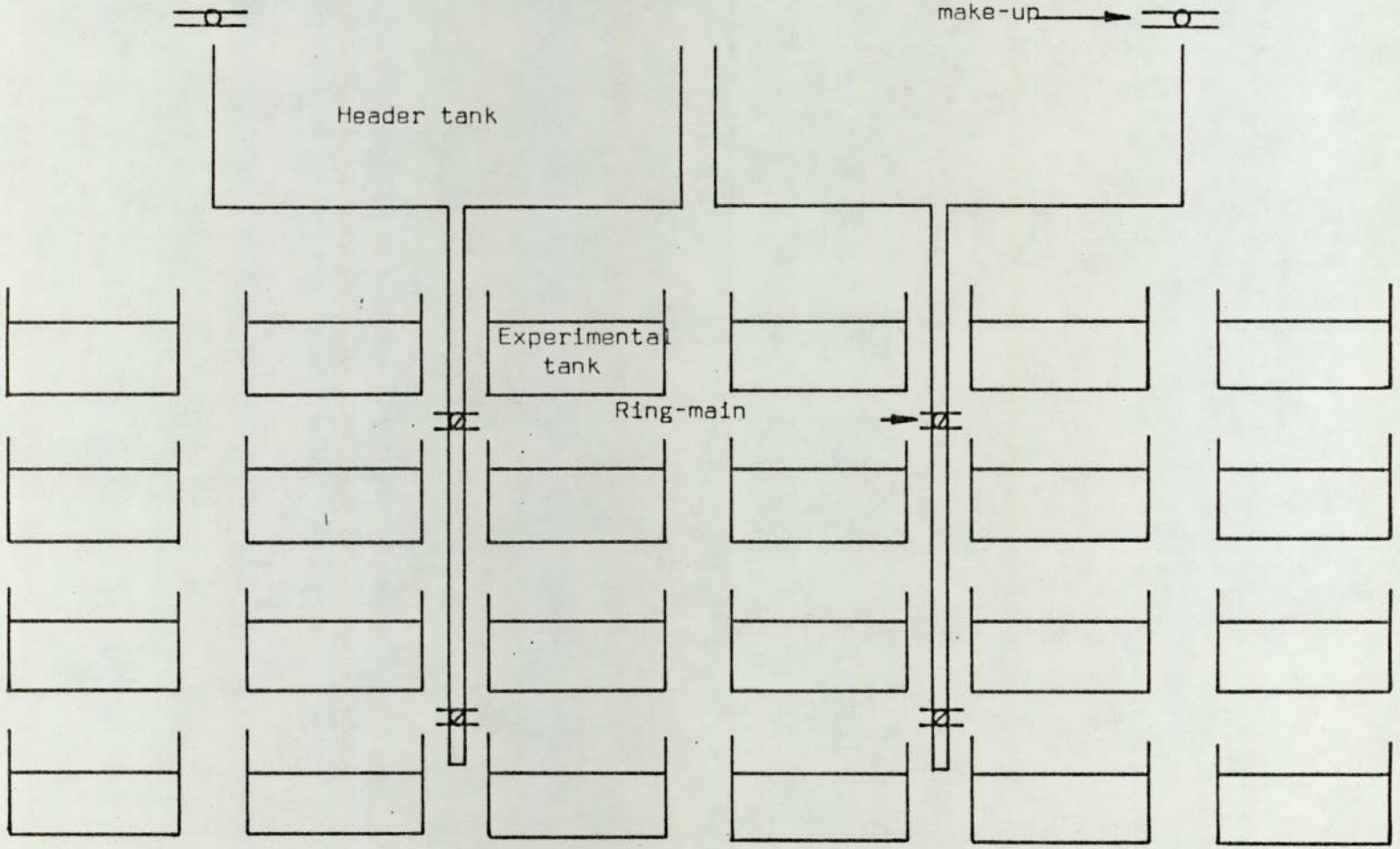


Figure 4.4. A diagrammatic representation of System 3.

glass tanks are the same as those described for system 2. Water quality was measured (average of reading, twice a week) and the values obtained are presented in Table 4.3 .

Table 4.3

Water Quality Criteria in System 3

Temperature	As pre-set ± 0.5°C
Dissolved Oxygen	More than 8.0 mg per litre
Total Ammonia	Less than 0.1 mg per litre
pH	6.9 - 7.2

System 4

The arrangement of this system is shown in Figure 4.5. The principles employed in this system are the same as those described for system 2. This system was used in experiment 4 (Chapter 8) during which water quality was measured twice a week; the values obtained are presented in Table 4.4.

Table 4.4

Water Quality Criteria in System 4.4

Temperature	23 ± 1.0°C
Dissolved Oxygen	More than 7.0 mg per litre
Total Ammonia	Less than 0.2 mg per litre
pH	6.9 - 7.1

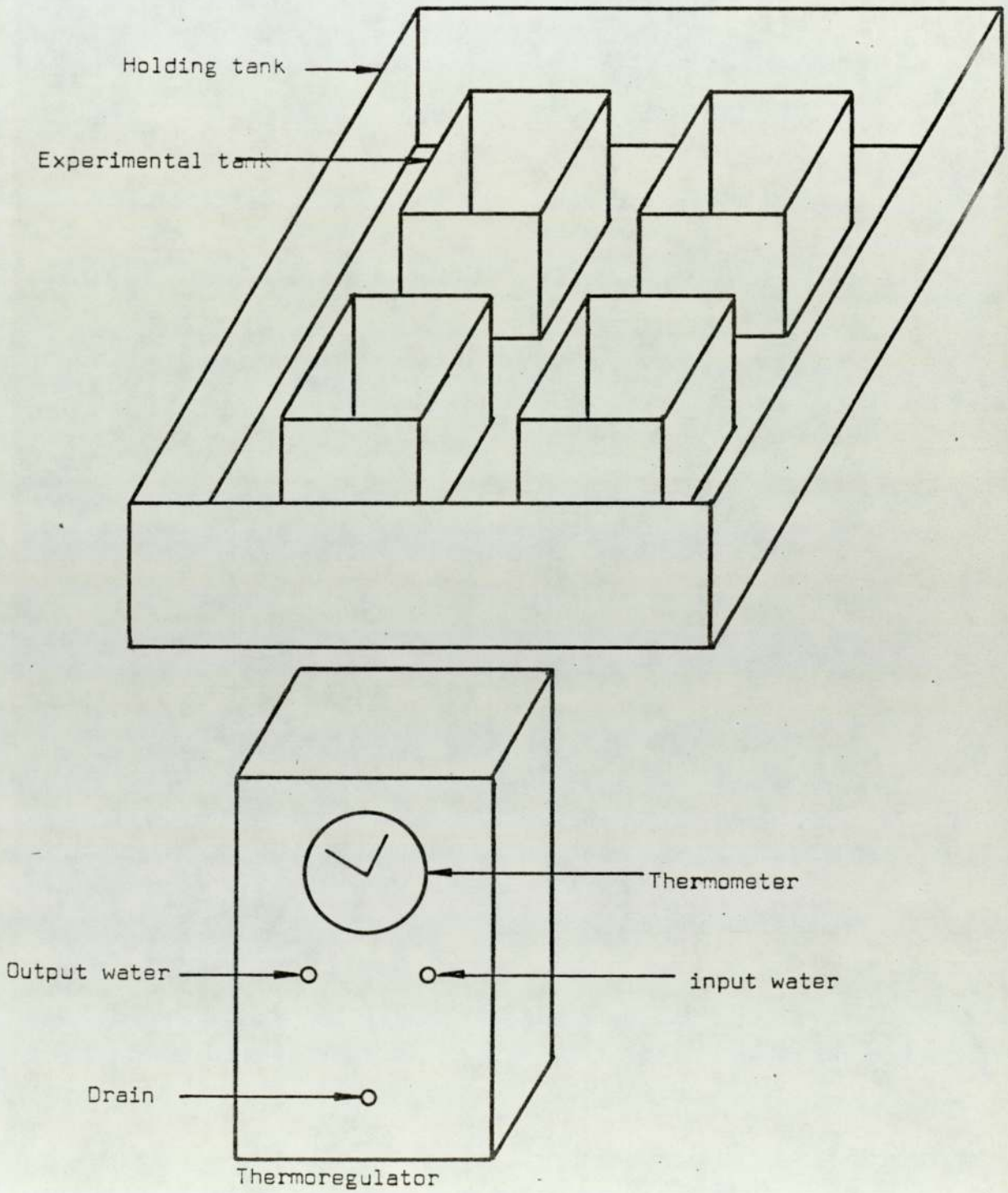


Figure 4.5. A diagrammatic representation of System 4.

4.3 DIET FORMULATION

For all the experiments a purified, nutritionally complete, ration was prepared in the laboratory. The method of formulation adopted was based on Halver's (1972) recommendations and the findings of Ogino et al. (1975, 1976) with a slight modification, by which it was hoped to minimise as much as possible the leaching of nutrients from the diet through the water and to establish a purified diet containing no traces of the elements under study.

Addition of the salt-mixture at dietary levels of 4% was found by Ogino and Kamizona (1975) to be optimal for growth and prevention of deformity in carp when casein was used as the sole dietary protein source.

Table 5.1 shows the individual ingredients used to formulate this ration and their amount present in the basal ration. This basal ration was regarded by Ogino et al. (1975) as a test diet for mineral studies in carp.

Dietary cobalt was adjusted to 3, 6 and 30 mg cobalt chloride ($CoCl_2.6H_2O$) per kg dry diet by alpha-cellulose. Dietary iodide was adjusted to 0.1, 0.3, 1.2, 3.0, 6.0, 24.0 and 48.0 mg potassium iodide (KI) per kg dry diet by alpha-cellulose. The amount of moisture in each dietary ingredient was determined analytically and the quantity in the diet subtracted from the total so that formulation was on a dry weight basis for all ingredients.

To all diets was added 4% of cod liver oil and 5% Vitamin mixture (without Vitamin B_{12}). The latter was supplied as a commercial premixed fish diet additive by Coopers Nutrition Products. 1% binder (Carboxy Methyl Cellulose), was added. The remainder of the diet was made up by a mixture of glucose, dextrin, starch and alpha-cellulose to achieve the desired energy level.

4.4 DIET PREPARATION

Diet preparation is one of the most important operations of a fish nutrition research programme. The best ingredients obtainable are useless without the proper preparation for feeding. "Diet preparation and presentation can become as important as the diet constituents in that results may become distorted due to the loss of the water soluble components" (Halver, 1970).

The amount of diet required for each experiment was estimated from the starting weight of the fish and the expected maximum growth rate assuming a food conversion ratio (g dry food fed per g live weight gain) of 1. 10 percent was added to this figure to allow for losses during pelleting, drying and sieving of the dry diet to the required size, as well as the taking of samples for analysis. All dietary ingredients were sieved to a particle size of less than 1 mm^3 prior to weighing and pelleting to ensure that a homogeneous mixture was obtained. The dry ingredients were then weighed out, according to the formulation, placed in the bowl of a Kenwood food mixer and thoroughly blended for 3 - 5 minutes. To this mixture was added the weighed quantity of Cod liver oil and blending continued for a further 3 minutes. Gelatin dissolved in warm water (35^{o}C) was then added to the diet, with mixing, until a stiff dough was obtained. The stiff dough was extruded through the mincer attachment of the food mixer, using a 6 mm die, into long spaghetti-like strands, which were dried on porous trays by having warm air (about 40^{o}C) blown over them from an electric fan convector heater.

When almost dry to the touch the strands were broken into pieces approximately 3 - 5 mm in length by rubbing them between

the hands and then further dried (Figure 4.6). The dry diet was sieved to remove fines, a sample was taken for proximate analysis, sec. (4.9) and the remainder stored in sealed polythene bags at $4^{o}C$ until required for feeding.

4.5 QUARANTINE PROCEDURES

Upon arrival each batch of fish was placed in a prepared, sterilised tank situated in a building separate from the main fish culture room (the tropical room). Access to this area was via a caustic foot bath and all the equipment used was sterilised and maintained only for use in the quarantine area.

For the first ten days after arrival the fish were fed a specailly prepared antibiotic diet, to eliminate bacterial pathogens, and for the remainder of the time in quarantine they were fed on a commercial fish feed.

On the 5th and 6th days after arrival the fish were bathed in 150 ppm formalin for 1 hour. After a further 5 days the fish were bathed on three successive days in 2 ppm malachite green for 1 hour. These treatments with formalin and malachite green were to eliminate external parasites and were accompanied by vigorous aeration.

Throughout the quarantine period fish were carefully observed for any abnormalities and samples of them were examined microscopically for pathogens. Fish were held in quarantine for a total of 21 days before being transferred to the system 1 (4.2).

4.6 ANAESTHESIA

Although there are no absolute guidelines established for anaesthetising fish, there are a number of factors governing the

Figure 4.6. The fish pellets

choice of anaesthetic agent and its application. A major consideration is the relative tolerance or intolerance of the species of fish to the anaesthetic agent. Lake trout, Salvelinus namaycush, and a few strains of rainbow trout, have a very low tolerance to tricaine methanesulfonate (MS-222) (Marking, 1967). In addition, most species acquire some degree of increased tolerance to repeated anaesthesia. An increasing variety of chemicals, including most of the mammalian anaesthetics, have been used to anaesthetise many species of fish (Klontz, 1964; Bell, 1964, 1967; Smith and Bell, 1967; McIarland and Klontz, 1968). The majority of these drugs can be utilised safely to induce and to maintain anaesthesia at a desired depth in nearly any species of fish.

In our experiments two kinds of anaesthetic were used:

1. Tricaine methanesulfonate (MS-222, Tricaine). This agent is the most frequently used fish anaesthetic. The anaesthetic dose is 50 - 100 mg per litre of water. Induction requires 1 - 3 minutes, recovery requires 3 - 15 minutes.

2. Phenoxyethanol. This is an oily liquid having a faint aromatic odour and a slight water solubility (2.5 ml per 100 ml of water). The recommended anaesthetic dose for fish is 0.1 - 0.5 ml per litre of water. Induction of anaesthesia requires 10 - 30 minutes; maintenance is not difficult; recovery is uneventful and requires 5 - 15 minutes.

4.7 TAGGING AND WEIGHING PROCEDURES

In experiment 1 (Chapter 5) and 2 (Chapter 6) the fish were weighed a tank at a time. The fish were anaesthetised (4.6) a whole tank at a time; they were allowed to drain for 10 - 15 seconds in a net, then the excess of water was removed with an adsorbent

paper towel, before being transferred to a tared bucket on a top-pan balance (accurate to ± 0.01 g) weighing up to 500 g . After weighing, the total length of each fish was measured to the nearest 0.1 cm. The fish were returned to their tanks, and they recovered completely from the anaesthetic within 3 - 10 minutes.

In experiment 3 (Chapter 7) and 4 (Chapter 8) the fish were individually tagged. The tags were made out of 5 mm wide "Dymo" embossing tape trimmed to an oval shape with a 1.5 mm diameter hole punched in the middle top end. Various combinations of tape colours and symbols (letters and numbers) enabled identification of each individual fish. The tags were attached by a loop of 0.5 mm diameter silver wire inserted just in front of, and below, the dorsal fin (Figure 4.7). Fish were anaesthetised, and after removal from the anaesthetic a 1 mm diameter hypodermic needle was inserted through the fish at the desired tag attachment position. One end of a 5 cm length of silver wire was inserted into the needle and drawn through the fish by withdrawal of the needle. The tag was then threaded on to the silver wire and the wire bent over the back of the fish, allowing a generous loop for growth. The free ends were then securely twisted together and the excess trimmed off. Individual weighing of fish permitted the distribution of growth rate in different populations to be statistically compared. In addition tagging enabled instant identification of any fish which succeeded in escaping from the experimental tanks, and allowing, in the case of mortalities, adjustment of the daily feeding rate and calculation of growth parameters.

Individual weighing of fish was achieved by removing the fish from their tank and anaesthetising them, a few at a time, in

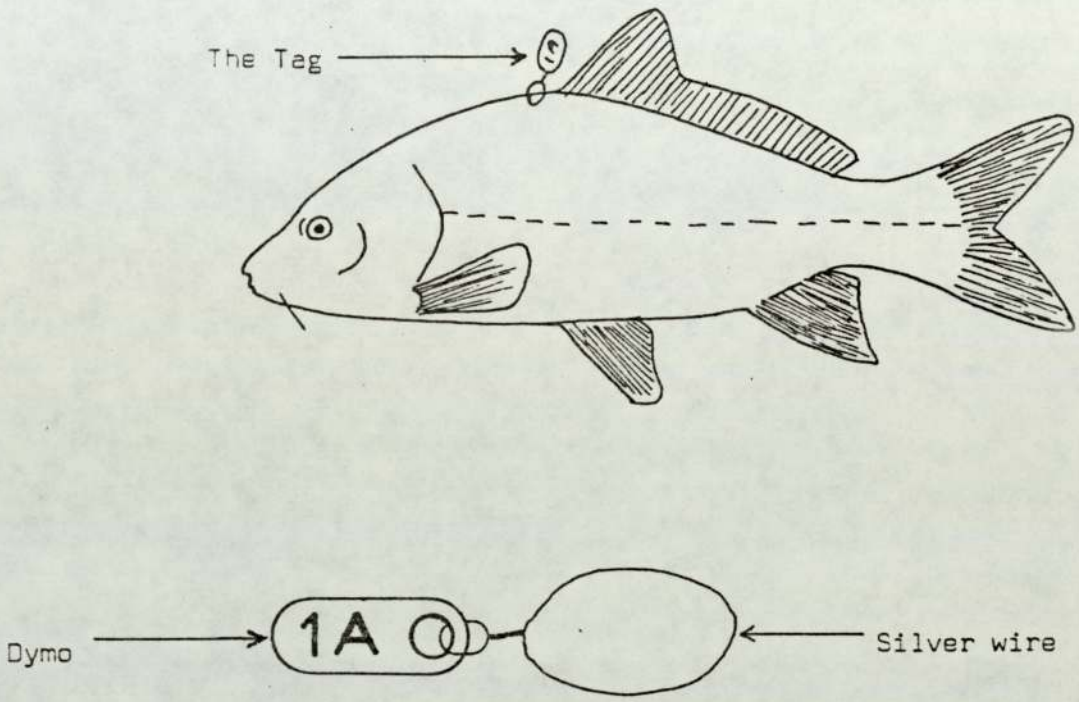


Figure 4.7. The fish tag

MS-222 or phenoxyethanol. They were then removed from the anaesthetic, lightly blotted on a paper towel, and weighed to an accuracy of ±0.01 g using a top pan balance; the total length of each fish was measured to the nearest 0.1 cm, then the fish were returned to their tank where complete recovery occurred within 3 - 10 minutes.

In all experiments the fish were starved for 12 hours prior to weighing.

4.8 TEMPERATURE AND LIGHT

The temperature of the fish room (Tropical Room) was kept constant at 28 ± 1°C, when the water temperature in the experimental tanks was 24 ± 1°C. The photoperiod was set at 12 hours day and 12 hours night (8 am - 8 pm - 8 am). For day light, fluorescent lights were used. A roof fan was used throughout the experiments in order to ensure a better distribution of heat inside the tropical room.

4.9 METHODS OF PROXIMATE ANALYSIS

Proximate analysis of diets, dietary ingredients and fish and haematological characteristics of fish blood, were carried out by the following procedures:

Moisture

Moisture contents were determined according to the recommended AOAC methods (1975). A sample was placed in a preweighed aluminium foil pan and oven dried at 105°C for 48 hours to constant weight; the difference between the weight of the pan with the sample before and after drying was taken as the water content of the sample.

Crude Protein

The protein contents were determined by a microkjeldahl method for determining nitrogen (AOAC methods, 1970), and applying the

empirical factor of 6.25 to the results to convert total nitrogen to total crude protein.

Crude Lipid

Crude lipid content was determined by extracting dried samples for 4 hours, using a soxhlet apparatus (A. Gallenkamp and Co. Ltd.), with 40 - 60°C boiling range petroleum ether and measuring, by weight difference, the amount of the ether soluble material extracted.

Ash

A sample of known weight was placed in a preweighed crucible. The crucible was then placed in a muffle furnace (FR 610 A, A. Gallenkamp and Co. Ltd.), at a temperature of 500 - 550°C, for 12 hours. By reweighing the crucible, the ash content of the original sample was calculated.

Nitrogen Free Extractives (NFE) and Fibre

NFE and fibre were determined by difference:

NFE = 100 - (% Moisture + % Crude Protein + % Crude lipid + % Ash) + fibre

Cobalt Content in the Fish

Cobalt content of the body and some of the organs was determined by using the oxygen flask technique followed by atomic absorption spectrophotometry.

An accurately weighed sample (5 - 10 mg) contained in a wrapped filter paper is fixed into a hinge of platinum gauze and then ignited in a 500 ml flask (Figure 4.8) which has previously been flushed with oxygen, and which contains 15.5 ml of deionised water, 1.0 ml of hydrogen peroxide (50%), and 1.0 ml of Conc. hydrochloric acid. After ignition, the flask is shaken until the

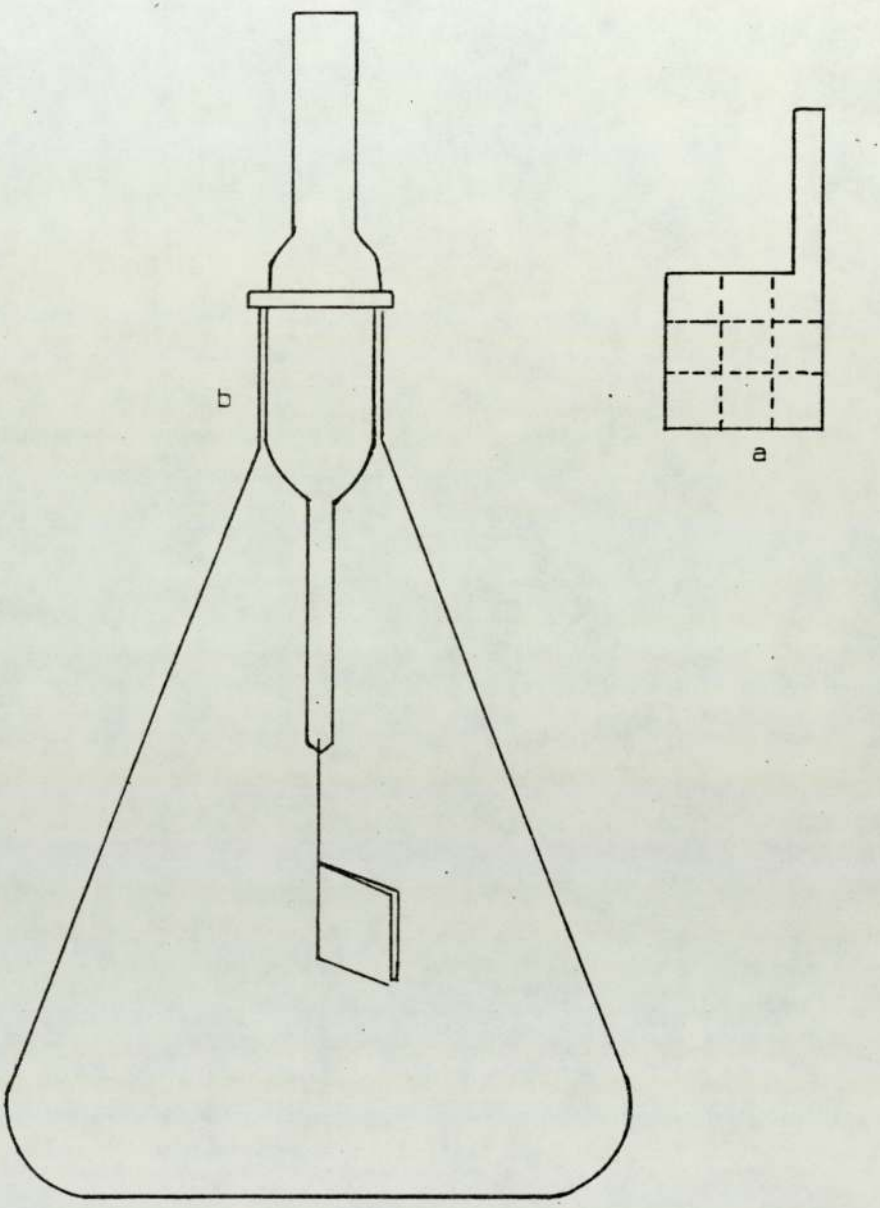


Figure 4.8. a. Ashless filter paper used for wrapping samples with fold lines indicated.

b. Conical flask with platinum sample holder.

decomposition products are absorbed in the aqueous peroxide/ hydrochloric acid solution, then this aqueous solution is transferred to a volumetric flask and the volume made up to 50 ml with deionized water.

The absorption of the solution or a suitable further dilution is determined by the atomic absorption spectrophotometer at 345 nm (Figure 4.9).

Iodine Content in the Fish

Numerous methods for the determination of aqueous iodide and iodine solutions have been summarised in recent reviews (Fishman and Robinson, 1969; Fishman and Erdmann, 1971; and Fishman et al., 1973). A few methods have shown a high sensitivity for iodide in the presence of large excesses of chloride. Schnepfe, 1972, has determined iodide as well as iodate in sea water in the submicrogram range, but this procedure is quite lengthy (Lambert, Hatch and Mosier, 1975). Lambert et al. (1975), describe a very simple procedure for the determination of iodine at part per billion level. A Beckman Model DB Spectrophotometer was used for all the measurements.

Standard iodide solutions were prepared from sodium iodide dried overnight at 110°C. Solutions were freshly prepared before each experiment using the dried salt as a primary standard.

A weighed sample (10 - 20 mg) of the tissue is homogenised in 10 ml dionised ice-cold water and the homogenate is centrifuged at 5000 rpm for 5 minutes. To 5 ml of the supernatant, 5 ml stock solutions of hydrogen peroxide, sodium thiocyante, and perchloric acid were added and made up to 25 ml in a volumetric flask. Absorbance is determined at 302 nm. Maximum absorbance is reached in about 2 minutes.

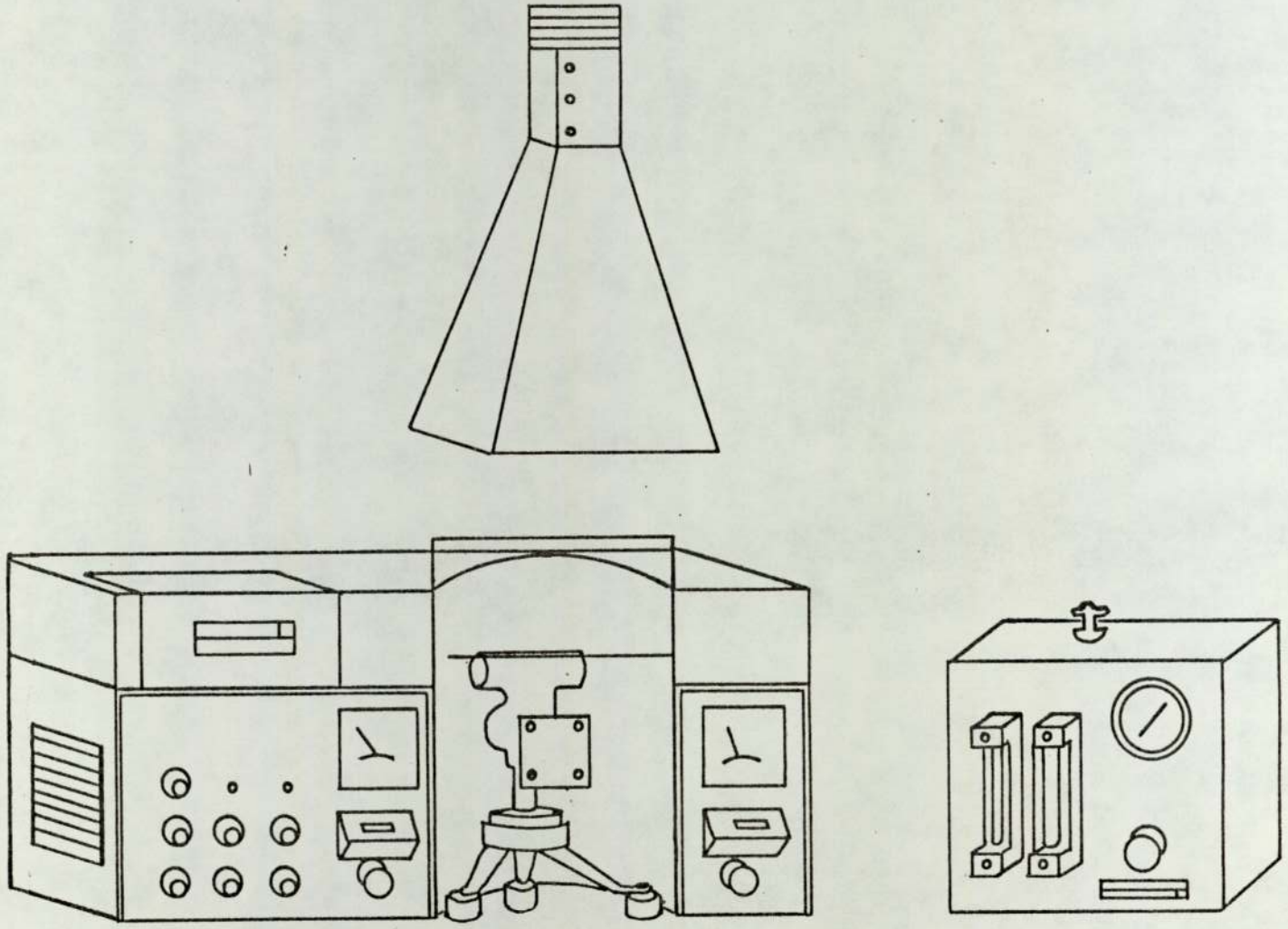


Figure 4.9. The double beam Perkin-Elmer model 303.

Haematological indices

Great difficulty was experienced in drawing and preserving satisfactory blood samples from the fish for later analysis. Each of the haematological values is the mean of samples taken from 3 fish. The blood was drawn by heart puncture or by cutting off the fish head in some cases. The anticoagulants used were heparin and sodium citrate.

Erythrocyte counts were carried out on fresh blood by means of a haemocytometer (Hendricks, 1952; Korzhnev, 1962). The number of leucocytes per mm were counted by the indirect method from stained smear slides using Leishmann-Giemsa stain (Chlebeck and Phillips, 1969; McKnight, 1966).

4.10 ANALYSIS OF EXPERIMENTAL DATA

The index of fatness or leaness, the condition factor of the fish, was computed from the formula:

$$C = \frac{W}{L^3}$$

where:

C = Condition factor (metric)

W = Weight in grams

L = Length in centimetres

For daily increments in weight and length the relationship is:

$$W_t = W_o (1 + \alpha/100)^t$$

where:

W_t = Weight or length at time (t)

W_o = Weight or length at the start of the experiment (o)

t = Time in days between t and o

α = Specific growth rate

The weight and length gain over control were calculated by the formulae:

$$W\ (\%) = \frac{W - w}{w} \times 100 \text{ (percent increase in weight or length)}$$

$W_n - W_o$ = percent gain over control for group n .....

where:

W = Weight or length (Mean of each group)

w = Initial weight or length (Mean of each group)

W_o = Percent increase in weight or length for control

W_n = Percent increase in weight or length for group n

The Craniosomatic index (CSI), Hepatosomatic index (HSI), Renosomatic index (RSI), Viscerosomatic index (VSI), and Fat in the body index (FBI) were calculated by the formula:

(Weight of the corresponding organ / Weight of the body) x 100

The food utilization efficiency (FUE), with which fish were able to utilise their food was determined by calculation of the weight gain (g wet weight) and the weight of the total food given:

$$FUE = \frac{\text{Weight gain, g wet weight}}{\text{Food given, g}}$$

4.11 STATISTICAL METHODS

Statistical comparisons between means were made by the single factor analysis of variance (ANOVA) according to Sokal and Rohl (1969).

For all the biochemical analysis samples were taken from at least two different fish and in addition all analyses were performed on duplicate samples.

CHAPTER FIVE

THE EFFECT OF COBALT IN THE DIET WITHOUT VITAMIN B_{12}, ON GROWTH PERFORMANCE, FOOD UTILIZATION, BODY COMPOSITION AND HAEMATOLOGICAL CHARACTERISTICS OF CARP, *Cyprinus carpio*

5.1 INTRODUCTION

Nutrition plays an important role under conditions of heavy stocking, when the natural food supply declines. The artificial feed should be physiologically balanced and rich in protein, carbohydrate, fat, etc. and should also contain minerals. Sen (1972) and Sen and Chatterjee (1976) observed that survival and growth of major Indian carp fry can be enhanced significantly by addition of cobalt chloride and to a lesser extent by starch and manganese to the feed. Das (1959, 1960, 1967) and Das and Krishnamurthy (1959) have observed that minute quantities of cobalt nitrate and yeast significantly enhance the survival of post-embryonic Indian carp fry. IN USSR, Korneeva (1963) and Leonov (1963) have used amino acids, vitamins, cobalt chloride and cobalt nitrate for enhancement and stimulation of growth in carps.

The present 32-week growth study was undertaken to determine the optimum dietary level of cobalt, as cobalt chloride, in the diet without vitamin B_{12}, for growth, metabolism and survival of carp, Cyprinus carpio.

5.2 MATERIAL AND METHODS

5.2.1 THE EXPERIMENTAL SYSTEM AND ANIMALS

The experimental facility used in the work reported here was system 2, described in detail in Chapter 4 (4.2). 250 fingerling carp (7.00 $\pm$ 0.08 cm) were obtained from Cotswold Carp Farm, Bourton-on-the Water, Gloucestershire. The fish were subjected to quarantine and prophylaxis, as described in Chapter 4 (4.5), and then transferred to a tropical room and kept in a recycling system, System 1, as described in Chapter 4 (4.2). A

sufficient number of them (20 fish) of an appropriate size (7.00 cm) were selected and transferred to 12 of the 25 litre glass tanks of system 2 at the prevailing ambient temperature of 24°C. During this period the fish were fed actively on a commercial trout diet; 12 fish were removed for proximate carcass analysis, Chapter 4 (4.9). The density of stocking was found to be 2.4 g per litre. The experiment was carried on for a period of 6 months; throughout this time pH and dissolved oxygen were checked 3 times a week using a pH meter and the Winkler method; they were found to range from pH 7.1 - 7.5 and 8 - 10 ppm respectively. Room and water temperature were also recorded daily and found to range from 27 - 29°C and 23 - 25°C respectively. Some losses occurred during temperature acclimatisation. Photoperiod was controlled at 12 hours day and 12 hours night (8 am - 8 pm - 8 am) throughout the experiment.

5.2.2 THE EXPERIMENTAL DIET

Formulation of the diet was carried out by the general procedure described in Chapter 4 (4.4). The ingredients used are presented in Table 5.1. It was necessary to make sure that all the ingredients used for the formulation of the diet were free from cobalt salts and Vitamin B_{12}. Cobalt, as cobalt chloride, was used and added to the diet at 3 levels, 3 ppm, 6 ppm, and 30 ppm, by spraying a solution over 500 grams of the food pellets using a chromatographic sprayer. For the controls, an identical procedure was followed using distilled water only. After the spray, the food was thoroughly dried and stored at -4°C.

The proximate analysis was performed on this diet and the results are presented in Table 5.2.

Table 5.1

Composition of the Test Diet

Main Mixture		Vitamin Supplement		Mineral Mixture	
Ingredients	Parts	Vitamin	mg*	Mineral	mg**
Casein	400	Riboflavin	200	$CaCO_3$	250
α-starch	180	Thiamin-HCl	50	KCl	4,670
Dextrin	190	Pyridoxin-HCl	50	KH_2PO_4	4,000
Cod Liver Oil	40	Folic Acid	15	Na_2HPO_4	3,090
Vitamin Mixture	50	Ascorbic Acid	100	$MgSO_4$	2,475
Mineral Mixture	40	Pantothenic Acid	500	$FeSO_4.7H_2O$	250
Methionine	10	Inositol	2,000	$ZnSO_4.7H_2O$	220
Tryptophan	5	Nicotinic Acid	750	$MnSO_4.7H_2O$	92
α-cellulose	15	Biotin	5	$CuSO_4$	20
Carboxymethyl cellulose	10	Choline Chloride	4,000	KI	1
		Vitamin A	40	Na_2SeO_3	0.2
Gelatin	60	Vitamin E	5,000 IU	$(NH_4)_6Mo_7O_{24}.4H_2O$	0.4
Total	1,000	Vitamin D_3	40	$CoCl_2.6H_2O$***	-
		Vitamin K	150		
		Glucose (as a carrier)	4,200		

*mg/kg dry diet

** mg/kg of diet
*** dietary cobalt was adjusted to: 3, 6, 30 ppm by $CoCl_2.6H_2O$ and α-cellulose

Table 5.2

Composition of the Basal Ration for Carp

Diet Composition in percent %	Concentration of Cobalt Chloride in the diet, mg/kg			
	Control (0)	3	6	30
Water	10.50	10.60	10.40	10.50
Crude Protein	42.10	42.20	42.15	42.10
Total Fat	4.15	4.20	4.16	4.18
Ash	5.25	5.30	5.30	5.35
Nitrogen Free Extract (NFE) and fibre	38.00	37.70	37.99	37.87

5.2.3 FEEDING RATE

The fish were fed three times per day, at 10 am, 2 pm and 6 pm, except for Sundays, when they were fed twice a day at 10 am and 6 pm. Each feeding continued for 15 minutes, including a 5-minute recess in the middle. The fish were fed at a rate that allowed them to consume the food as it fell through the water. All groups were fed 6% of their body weight per day. The quantity of food delivered per day was adjusted after each weekly weighing and fed for the subsequent 7 days. When the fish were unwilling to consume the whole day's ration, they were fed only the amount they would consume within the 45 minutes total feeding period, and the food not consumed was weighed and deducted from the daily ration.

5.2.4 WEIGHING AND SAMPLING

Details of the weighing procedure was presented in Chapter 4 (4.7). Fish were batch weighed ($\pm$ 0.01 g), under anaesthesia, after 12 hours starvation every 7 days for 24 weeks, and at the end of this period samples were removed from each group for proximate carcass analysis and determination of cranio-somatic (CSI), Hepato-somatic (HSI), Reno-somatic (RSI), and Viscero-somatic (VSI) indices. In addition, haematological characteristics were determined.

At the termination of the experiment (32 weeks) 3 fish were taken from each group and the same analyses were done on them; in addition, body composition and cobalt concentration in fish body and organs were determined.

5.2.5 STATISTICAL METHODS AND ANALYSIS OF GROWTH DATA

These were performed as detailed in Chapter 4 (4.10 and 4.11).

5.3 RESULTS

5.3.1 ACCELERATION OF GROWTH

Weight and Length

The results of the feeding trails with carp are shown in Tables 5.3 and 5.4 and Figures 5.1 and 5.2. For the first and the second week the fish in all treatment groups responded to the cobalt treatment by increasing their weight and length above that of the controls, up to the fourth week. At this time the fingerlings in all sections contracted Ichthyophthirius multifiliis, and at the end of the fifth week their weight had become lower. Those which were receiving cobalt in their diet were more resistant to the disease and therefore their weight and survival were less severely affected than those in the control group. The disease was treated by administering quinine sulphate, 1 mg per liter of water. Throughout the experiment, fish which had been given cobalt in their diet were heavier than the controls, the group which had been given 3 mg cobalt chloride per kg dry diet being the heaviest. By the end of 12 weeks (intermediate stage of growth) the difference between the control and the treated groups were 125.5%, 100.9% and 107.7% respectively (Figure 5.3) in weight and 23.8%, 19.2% and 12.6% respectively (Figure 5.4) in length. At the end of 24 weeks (when cobalt was withdrawn from the diet) the difference between the control and treated groups were 513.5% and 429.6% respectively in weight and 37.4% and 24.2% respectively in length. At this time all the fish in the group which received 30 mg cobalt chloride per kg dry diet had died, at this dose the inhibitory effect of cobalt being in evidence.

In order to check whether the increase in weight in the treated groups was due to the treatment with cobalt, all the groups

Table 5.3

Changes in the Body Weight of Carp Fed diet Supplemented with Cobalt Chloride. Values given are the mean weight of 20 fish ± S.E.

Duration in weeks	Concentration of Cobalt Chloride in the Diet, mg per kg dry diet						
	Control (0)	3		6		30	
	Weight (g)	Weight (g)	% Gain*	Weight (g)	% Gain	Weight (g)	% Gain
0	3.00 ± 1.08	3.16 ± 0.05	-	3.10 ± 0.15	-	3.00 ± 0.16	-
1	3.40 ± 0.92	3.84 ± 0.27	8.2	3.70 ± 0.27	6.0	3.60 ± 0.10	6.7
2	3.41 ± 0.93	4.20 ± 0.41	19.3	4.00 ± 0.13	6.0	4.00 ± 0.09	19.7
3	3.80 ± 0.90	5.63 ± 0.80	51.5	4.50 ± 0.07	15.4	3.90 ± 0.10	3.3
4	2.70 ± 0.24	5.00 ± 0.43	48.2	4.70 ± 0.06	18.5	3.80 ± 0.09	16.7
5	2.65 ± 0.46	4.60 ± 0.37	33.9	3.90 ± 0.03	41.6	3.40 ± 0.06	1.7
6	2.61 ± 0.44	5.00 ± 0.51	45.2	4.88 ± 0.07	14.1	4.10 ± 0.11	23.7
7	2.72 ± 0.28	6.41 ± 0.55	93.5	5.90 ± 0.07	44.4	4.80 ± 0.15	50.7
8	3.01 ± 0.55	6.54 ± 0.31	106.6	6.00 ± 0.06	48.7	5.10 ± 0.20	69.7
9	3.02 ± 0.21	6.80 ± 0.40	114.5	6.18 ± 0.05	93.2	6.00 ± 0.20	99.3
10	2.87 ± 0.35	7.00 ± 0.18	117.2	6.20 ± 0.05	98.7	6.10 ± 0.19	99.0
11	3.08 ± 0.36	7.07 ± 0.19	121.1	6.31 ± 0.11	95.7	6.20 ± 0.21	104.0
12	3.07 ± 0.46	7.20 ± 0.26	125.5	6.50 ± 0.09	100.9	6.30 ± 0.23	107.7
13	3.10 ± 0.46	7.36 ± 0.19	129.6	6.80 ± 0.05	107.3	6.43 ± 0.22	111.7
14	3.20 ± 0.28	7.50 ± 0.24	136.7	7.20 ± 0.55	116.0	7.60 ± 0.18	126.7
15	3.50 ± 0.52	7.70 ± 0.40	127.0	7.30 ± 0.12	125.6	7.20 ± 0.20	123.3

Table 5.3 (cont'd.)

Duration in weeks	Concentration of Cobalt Chloride in the Diet, mg per kg dry diet						
	Control (0)	3		6		30	
	Weight (g)	Weight (g)	% Gain*	Weight (g)	% Gain	Weight (g)	% Gain
16	3.98 ± 0.56	9.50 ± 0.21	168.0	10.00 ± 0.11	118.8	8.00 ± 0.33	164.0
17	4.86 ± 0.14	12.00 ± 0.33	217.7	13.00 ± 0.10	189.9	12.00 ± 0.40	238.0
18	5.15 ± 0.12	17.00 ± 0.46	366.3	15.60 ± 0.11	257.4	13.20 ± 0.55	268.3
19	6.97 ± 0.16	20.00 ± 0.80	400.6	18.00 ± 0.09	348.3	16.50 ± 0.50	317.7
20	8.47 ± 0.13	23.00 ± 0.53	445.5	20.00 ± 0.07	362.8	20.00 ± 0.60	383.7
21	9.79 ± 0.23	25.50 ± 0.85	480.6	23.20 ± 0.05	422.0	21.50 ± 0.95	390.3
22	11.44 ± 0.16	28.00 ± 1.34	504.7	25.00 ± 0.09	425.1	-	-
23	13.97 ± 0.19	30.10 ± 1.17	486.9	27.10 ± 0.09	408.5	-	-
24**	14.50 ± 0.22	31.50 ± 1.15	513.5	28.30 ± 0.11	429.6	-	-
32	20.50 ± 0.17	43.50 ± 1.36	693.3	35.55 ± 0.27	463.4	-	-

*percent weight gain over control, they were calculated by the formula, $W_2 - W_1/W_1 \times 100$; where W_1, the initial weight; W_2 the weight at time T.

**Cobalt Chloride withdrawn after 24 weeks of feeding on the experimental diet.

Table 5.4

Changes in the Body Length of Carp Fed Diet Supplemented with Cobalt Chloride. Values given are the Mean Length of 20 fish ± S.E. of the mean

Duration in weeks	Concentration of Cobalt Chloride in the Diet, mg per kg dry diet						
	Control (0)	3		6		30	
	Length (cm)	Length (cm)	% Gain*	Length (cm)	% Gain*	Length (cm)	% Gain*
0	6.93 ± 0.68	7.05 ± 0.14	-	7.10 ± 0.15	-	6.95 ± 0.55	-
1	7.22 ± 0.68	7.53 ± 0.37	2.6	7.40 ± 0.27	0.1	7.36 ± 0.50	1.7
2	7.23 ± 0.69	7.75 ± 0.23	5.6	7.65 ± 0.18	3.4	7.60 ± 0.35	5.0
3	7.50 ± 0.64	8.63 ± 0.39	14.2	8.25 ± 0.13	8.0	7.70 ± 0.56	2.6
4	7.51 ± 0.28	8.60 ± 0.26	13.6	8.30 ± 0.11	8.5	7.75 ± 0.60	3.2
5	7.50 ± 0.30	8.55 ± 0.24	13.1	8.30 ± 0.12	8.7	7.70 ± 0.67	2.6
6	7.50 ± 0.37	8.53 ± 0.28	12.8	8.35 ± 0.17	9.4	7.75 ± 0.66	3.3
7	7.55 ± 0.33	8.93 ± 0.31	17.7	8.60 ± 0.27	12.2	7.90 ± 0.70	4.7
8	7.52 ± 0.43	8.99 ± 0.18	19.0	8.70 ± 0.27	14.0	7.99 ± 0.65	6.2
9	7.51 ± 0.44	9.10 ± 0.18	20.7	8.80 ± 0.26	15.6	8.10 ± 0.70	8.2
10	7.55 ± 0.41	9.15 ± 0.11	20.8	8.81 ± 0.25	15.1	8.15 ± 0.75	8.3
11	7.60 ± 0.29	9.25 ± 0.15	21.5	8.80 ± 0.26	14.3	8.20 ± 0.80	8.5
12	7.59 ± 0.37	9.31 ± 0.10	22.5	8.90 ± 0.21	15.8	8.30 ± 0.90	9.8
13	7.60 ± 0.37	9.41 ± 0.19	23.8	9.15 ± 0.29	19.2	8.50 ± 0.85	12.6
14	7.80 ± 0.34	9.50 ± 0.21	22.2	9.20 ± 0.25	19.9	8.70 ± 0.95	12.6
15	7.80 ± 0.23	9.65 ± 0.12	24.3	9.20 ± 0.23	17.0	9.00 ± 1.00	12.9

Table 5.4

Changes in the Body Length of Carp Fed Diet Supplemented with Cobalt Chloride. Values given are the Mean Length of 20 fish ± S.E. of the mean

Duration in weeks	Concentration of Cobalt Chloride in the Diet, mg per kg dry diet						
	Control (0)	3		6		30	
	Length (cm)	Length (cm)	% Gain*	Length (cm)	% Gain*	Length (cm)	% Gain*
16	8.00 ± 0.36	10.00 ± 0.22	29.3	9.50 ± 0.32	21.3	9.20 ± 1.10	16.9
17	8.10 ± 0.36	10.20 ± 0.29	29.2	9.70 ± 0.31	21.2	9.40 ± 1.11	18.4
18	8.30 ± 0.34	10.30 ± 0.25	29.2	9.90 ± 0.30	22.6	9.50 ± 1.20	16.9
19	8.30 ± 0.34	10.50 ± 0.22	29.2	10.10 ± 0.33	22.5	9.50 ± 1.20	16.9
20	8.50 ± 0.43	10.70 ± 0.25	29.1	10.30 ± 0.40	22.4	10.00 ± 1.30	21.2
21	8.90 ± 0.47	11.00 ± 0.31	27.6	10.70 ± 0.55	22.3	10.20 ± 1.25	18.3
22	9.00 ± 0.44	11.50 ± 0.35	33.3	10.90 ± 0.51	23.7	-	-
23	9.50 ± 0.46	12.00 ± 0.42	33.1	11.00 ± 0.45	17.8	-	-
24*	9.50 ± 0.47	12.30 ± 0.45	37.4	11.45 ± 0.35	24.2	-	-
32	12.00 ± 0.77	17.00 ± 0.22	38.1	15.00 ± 0.44	38.8	-	-

*percent length gain over control, they were calculated by the formula, $W_2 - W_1 / W_1 \times 100$; where W_1 is the initial length and W_2 the length at time T.

**Cobalt chloride withdrawn after 24 weeks of feeding on the experimental diet.

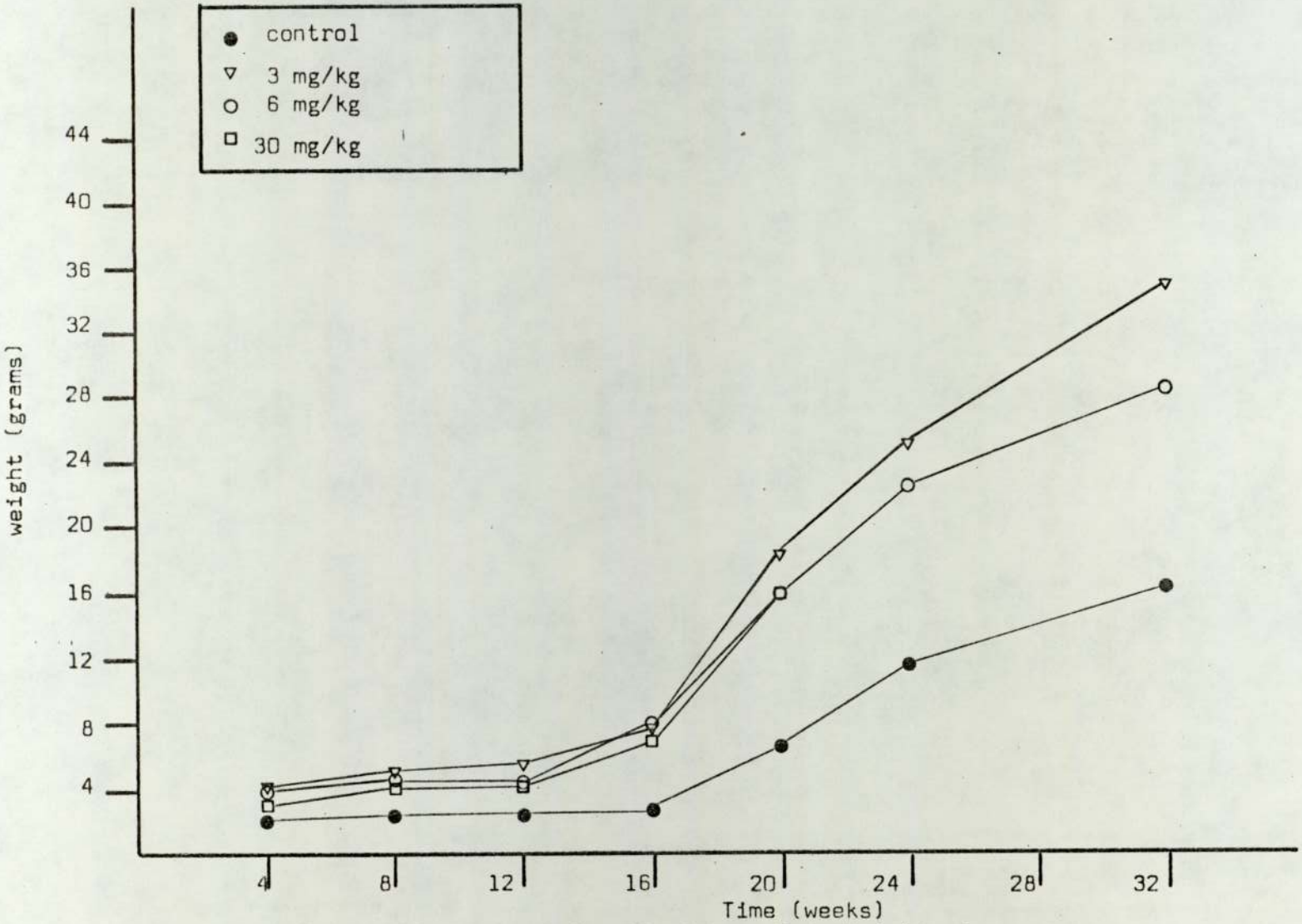


Figure 5.1. Effect of cobalt chloride on Body Weight of carp

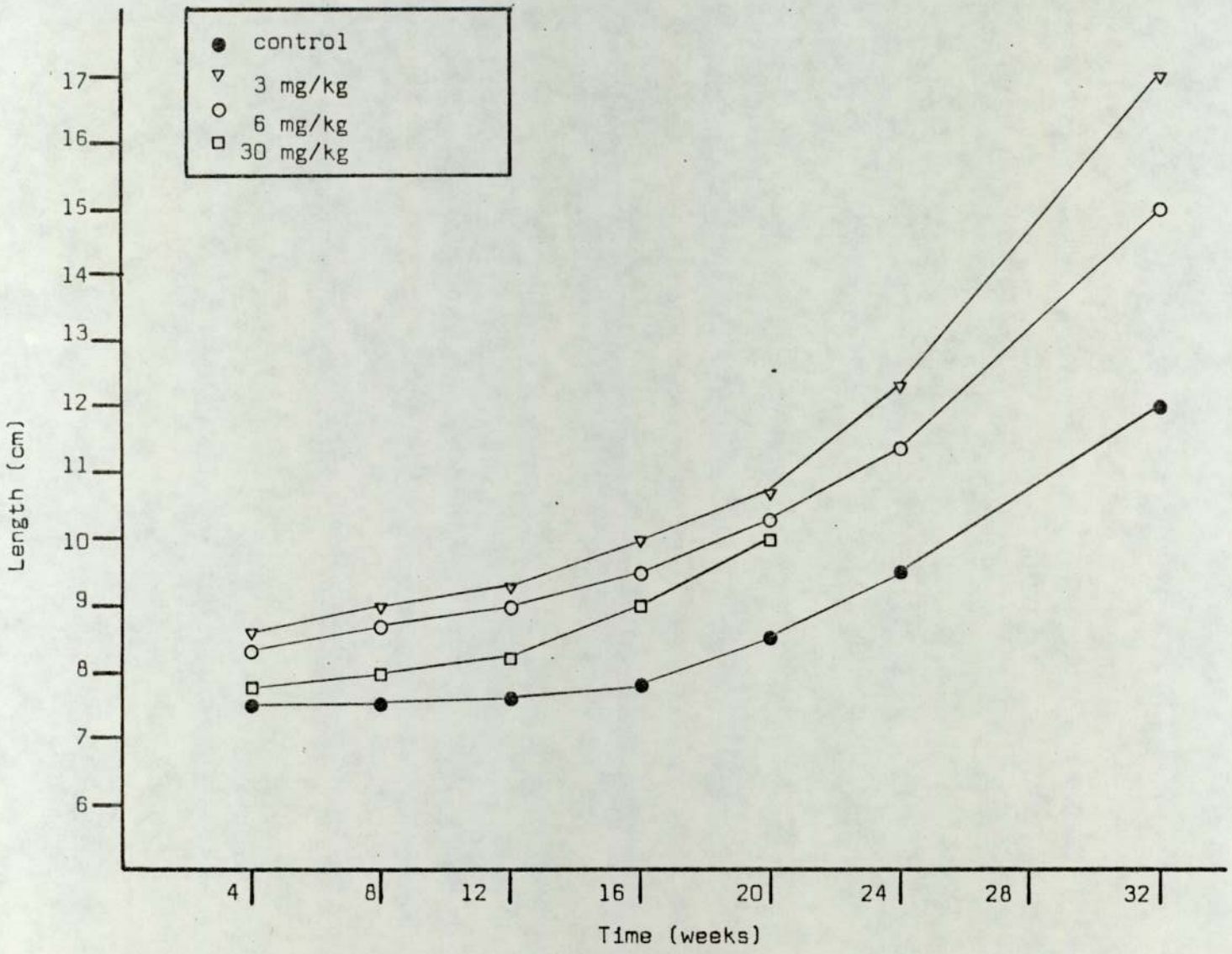


Figure 5.2. Effect of cobalt chloride on Body Length of carp.

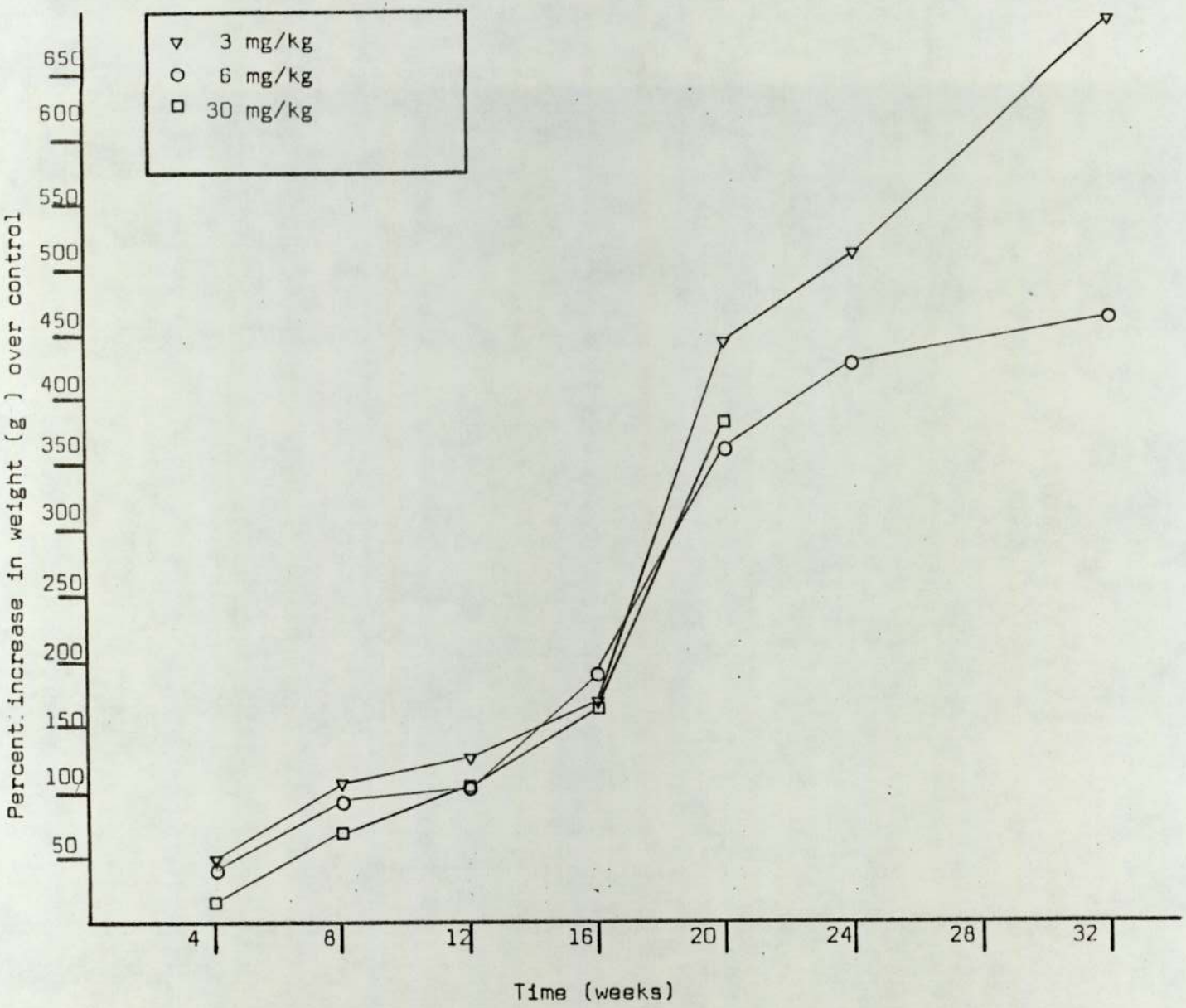


Figure 5.3. Effect of cobalt chloride on the percentage increase over controls in weight of carp

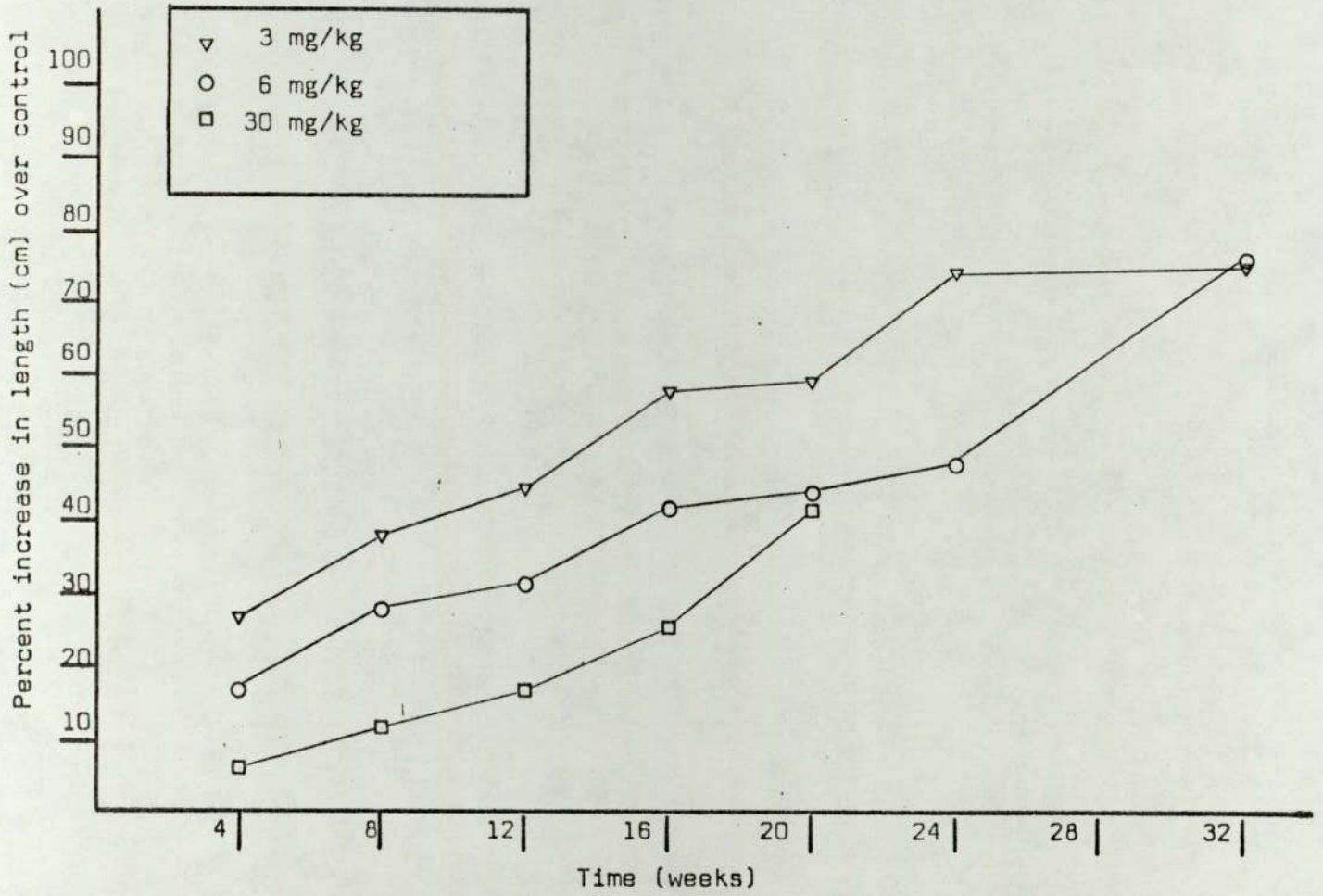


Figure 5.4. Effect of cobalt chloride on the percentage increase over control in length of carp.

were fed normal trout diet size 2, for two months. The results obtained show that there was a considerable increase in the weight of the control group; this indicated a positive effect of cobalt on the growth of carp.

Growth Rate

The mean daily growth rate for all the treated groups was greater than the control until the experiment was terminated. The greatest increase was in the group receiving 3 mg cobalt chloride per kg dry diet where it was 6.18 g per 100 g initial weight at 24 - 32 weeks compared with 2.83 g per 100 g initial weight in the control (Table 5.5).

The treated fish exhibited an increase in length in the first 4 weeks compared with the controls, but the rate of increase lessened in the treated groups from the eighth week till the 24th week of feeding (Table 5.6).

Condition Factor

At the start of the experiment the mean value of the "condition factor" which relates the weight to the length was 0.090 among all groups. For the first 4 weeks all the treated groups showed a decrease in condition factor (Table 5.7); by the end of the 12th week the condition factorsof the treated groups were higher than the starting point and higher than the control, where there was a decrease in the condition factor. At the end of the experiment the condition factor was higher in the treated groups, the highest increase being observed in the group that had received 6 mg cobalt chloride per kg dry diet (Figure 5.5).

Food Utilisation Efficiency (FUE)

Table 5.8 shows that there are considerable increases in the

Table 5.5

Effect of oral Administration of Cobalt Chloride on Daily increase of Body Weight of Carp

Period in weeks	Daily increase of mean Body Weight, g per 100 g initial weight			
	Concentration of Cobalt Chloride in the Diet, mg Per kg Dry Diet			
	Control (0)	3	6	30
0 - 4	1.03	3.01	1.74	1.15
4 - 8	-0.16	1.90	1.67	1.11
8 - 12	0.03	1.59	1.33	1.37
12 - 16	0.16	1.38	1.30	1.35
16 - 20	1.02	4.10	3.70	3.46
20 - 24	2.34	5.46	4.96	-
24 - 32	2.83	6.18	5.08	-

Table 5.6

Effect of oral Administration of Cobalt Chloride on Daily increase of Body Length of Carp

Period in weeks	Daily increase of Mean Body Length, cm per 100 cm initial length			
	Concentration of Cobalt Chloride in the Diet, mg per kg Dry Diet			
	Control (0)	3	6	30
0 - 4	0.32	0.86	0.62	0.41
4 - 8	0.17	0.49	0.39	0.25
8 - 12	0.12	0.40	0.31	0.23
12 - 16	0.12	0.35	0.28	0.24
16 - 20	0.15	0.38	0.33	0.28
20 - 24	0.24	0.45	0.35	-
24 - 32	0.36	0.69	0.54	-

Table 5.7

Effect of oral Administration of Cobalt Chloride on the condition factor of carp. Values given are the mean ± S.E.

Duration in weeks	The condition factor			
	Concentration of Cobalt Chloride in the Diet, mg/kg Dry diet			
	Control (0)	3	6	30
0	0.090 ± 0.002	0.090 ± 0.003	0.090 ± 0.002	0.090 ± 0.002
4	0.090 ± 0.001	0.088 ± 0.002	0.080 ± 0.002	0.085 ± 0.002
8	0.063 ± 0.003	0.090 ± 0.003	0.093 ± 0.002	0.097 ± 0.003
12	0.070 ± 0.002	0.089 ± 0.003	0.093 ± 0.003	0.112 ± 0.001
16	0.073 ± 0.001	0.086 ± 0.001	0.094 ± 0.002	0.109 ± 0.001
20	0.122 ± 0.003	0.173 ± 0.002	0.175 ± 0.003	0.156 ± 0.002
24	0.163 ± 0.003	0.174 ± 0.002	0.204 ± 0.002	-

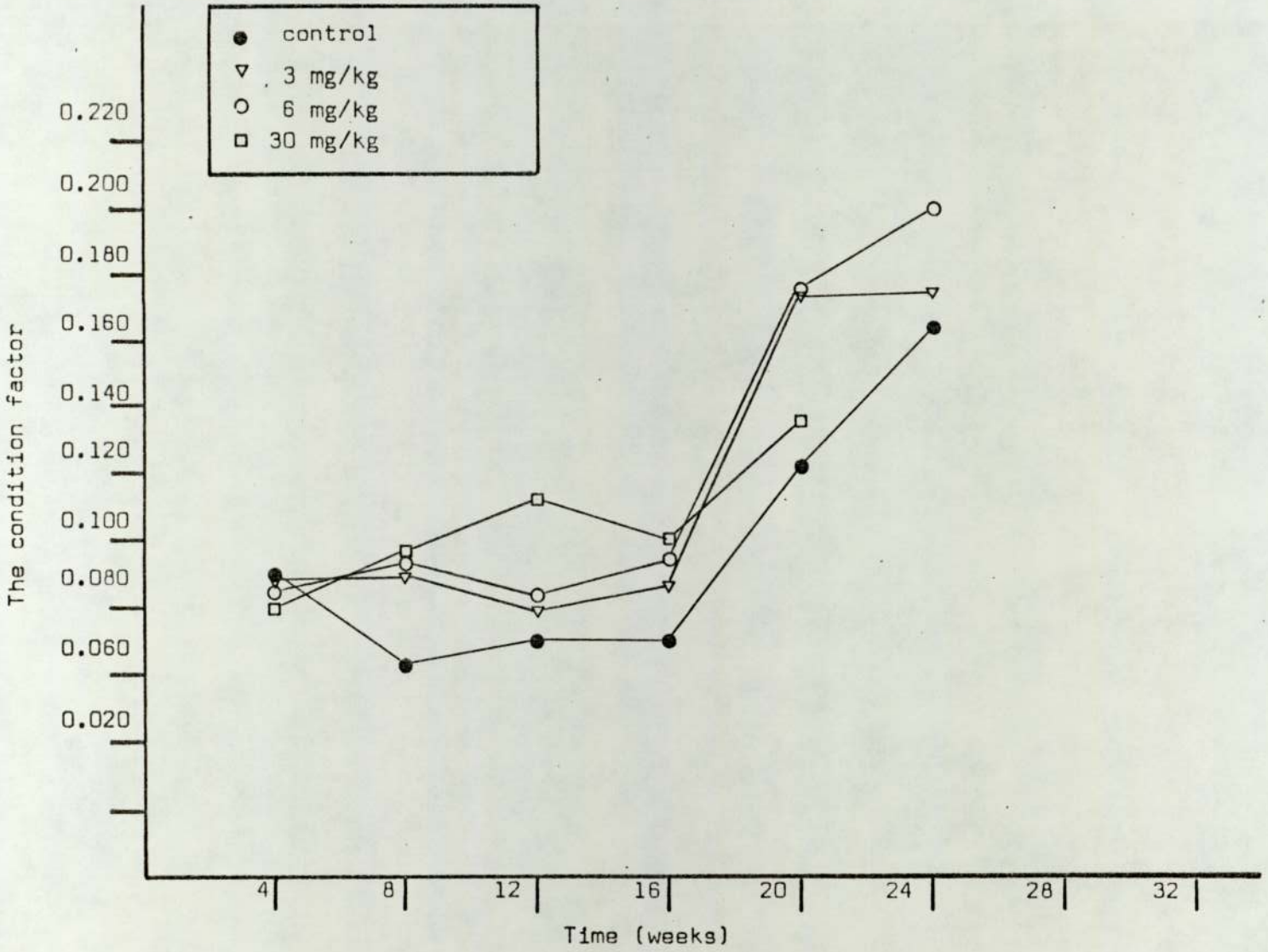


Figure 5.5. Effect of cobalt chloride on the condition factor of carp.

Table 5.8

Effect of oral Administration of Cobalt Chloride on food utilization efficiency in Carp

Period in weeks	Food utilization efficiency, weight gain/food given			
	Concentration of Cobalt Chloride in the Diet, mg/kg Dry Diet			
	Control (0)	3	6	30
0 - 4	0.73	2.14	1.24	0.82
4 - 8	-0.28	1.39	1.30	1.10
8 - 12	0.10	1.51	1.39	1.41
12 - 16	0.39	1.61	1.58	1.60
16 - 20	1.68	2.31	2.27	2.24
20 - 24	2.15	2.45	2.43	-
24 - 32	2.34	2.54	2.50	-

FUE values of carp fed cobalt, as cobalt chloride, for the first 4 weeks. This increase declined in the second 4 weeks in the controls and 3 mg cobalt chloride per kg dry diet group till the end of 20 weeks, when there was an increase in the FUE values in both groups. However, the FUE values in the group that received 3 mg cobalt chloride per kg dry diet were higher than in other treated groups and controls during the whole period of the experiment.

5.3.2 SURVIVAL RATE

Results of the survival rate of carp fingerling fed different levels of cobalt chloride without vitamin B_{12} are presented in Table 5.9. There was a considerable decrease in survival in the control and the group receiving 30 mg cobalt chloride per kg dry diet throughout the experiment. The survival rate decline in the control group was accompanied by weight decreases, whereas there was no such decrease in the group receiving 30 mg cobalt chloride per kg dry diet, but the survival in this group was more affected. The only thing one can assume is that the decline in survival rate in this group was due to the toxicity of cobalt in the diet, and that the high dose of cobalt (30 mg per kg) did not depress the growth of the fish. On the other hand it was obvious from Table 5.9 that cobalt, as cobalt chloride, in the diet has a great effect on the resistance of carp to the *Ichthyophthirius multifiliis* that was contracted in week 5 of the experiment.

5.3.3 CRANIO-SOMATIC (CSI), HEPATO-SOMATIC (HSI), RENO-SOMATIC (RSI) AND VISCERO-SOMATIC (VSI) INDICES

The ratios of different body organs to body weight are given in Table 5.10. The Cranio-somatic index (CSI) decreased in the

Table 5.9

Changes in survival rate of carp fed diet supplemented with cobalt chloride

Duration in weeks	Survival of Carp							
	Concentration of cobalt chloride in the diet, mg per kg dry diet							
	Control (0)		3		6		30	
	No. of fish	%	No. of fish	%	No. of fish	%	No. of fish	%
0	20	100	20	100	20	100	20	100
4	19	95	20	100	20	100	20	100
8	16	80	20	100	20	100	18	90
12	15	75	19	95	18	90	15	75
16	13	65	19	95	17	85	8	40
20	10	50	19	95	17	85	2	10
24	10	50	17	85	16	80	-	0
32	5	25	17	85	16	80	-	0

Table 5.10

Cranio-somatic index (CSI), Hepato-somatic index (HSI), Viscero-somatic index (VSI) of Carp Fed Diet Supplemented with Cobalt Chloride

	AT THE START OF THE EXPERIMENT			
	Concentration of Cobalt in the Diet mg/kg			
	Control	3	6	30
Number of samples	3	3	3	3
Weight of fish (g), Mean ± S.E.	3.00 1.08	3.16 0.05	3.10 0.15	3.00 0.16
Brain weight (g); CSI	0.02;0.66	0.04;1.26	0.03;0.96	0.01;0.33
Liver weight (g); HSI	0.04;1.33	0.06;1.89	0.05;1.61	0.03;1.00
Kidney weight (g); RSI	0.01;0.33	0.02;0.63	0.01;0.32	0.02;0.66
Viscera weight (g); VSI	0.13;4.33	0.15;4.74	0.13;4.19	0.15;5.00

	AFTER 24 WEEKS OF FEEDING			
	Concentration of Cobalt in the Diet mg/kg			
	Control	3	6	30
Number of samples	2	2	2	2
Weight of fish (g), Mean ± S.E.	14.50 0.22	31.50 1.15	28.30 0.11	21.50 0.95
Brain weight (g); CSI	0.10;0.69	0.09;0.28	0.08;0.28	0.15;0.69*
Liver weight (g); HSI	0.12;0.83	0.49;1.55**	0.39;1.37*	0.50;2.32**
Kidney weight (g); RSI	0.07;0.48	0.05;0.15	0.04;0.14	0.12;0.55**
Viscera weight (g); VSI	0.65;4.48	1.40;4.44**	1.10;3.88**	1.10;5.11**

	AFTER 32 WEEKS OF FEEDING			
	Concentration of Cobalt in the Diet mg/kg			
	Control	3	6	30
Number of samples	3	3	3	-
Weight of fish (g), Mean ± S.E.	20.50 0.17	43.50 0.36	35.55 0.27	
Brain weight (g); CSI	0.16;0.78	0.15;0.34**	0.12;0.33**	
Liver weight (g); HSI	0.20;0.98	0.80;1.83**	0.65;1.82**	
Kidney weight (g); RSI	0.12;0.59	0.11;0.25	0.08;0.22	
Viscera weight (g); VSI	1.15;5.61	2.12;4.87*	2.10;5.90	

* = $P < 0.05$ ** = $P < 0.01$

groups receiving 3 and 6 mg cobalt chloride per kg dry diet, and there was an increase in CSI in the other two groups during the first 24 weeks of feeding. At the end of 32 weeks there was a slight increase in CSI index in the treated groups compared with controls (Figure 5.6).

The Hepato-somatic index (HSI) decreased in the control and the 3 and 6 mg cobalt chloride per kg dry diet groups, while there was an increase in the 30 mg group, during the first 24 weeks of feeding. At the end of the experiment there was an increase in HSI in all groups including controls, and the differences between the treated groups was very small (Figure 5.7).

The Reno-somatic index (RSI) decreased in all groups including the controls during the whole period of the experiment. The lowest decrease was observed in the 3 and 6 mg cobalt chloride/kg groups (Figure 5.8).

The Viscero-somatic index (VSI), which includes the weight of the digestive tract, swim bladder, and the heart, decreased in the groups receiving 3 and 6 mg cobalt chloride per kg dry diet in the first 24 weeks, while it increased in the control and the 30 mg (cobalt chloride) groups. At the end of the experiment there was an increase in all the groups including the controls (Figure 5.9).

5.3.4 HAEMATOLOGICAL CHARACTERISTICS

There is a great deal of speculation whether or not cobalt salts have any effect on the haematological indices in fish. The results obtained over a period of 24 weeks of feeding carp a cobalt-enriched diet show that the treatment has affected the hematological characteristics (Table 5.11). There was an increase in haemoglobin percentage in all the treated groups (Figure 5.10), and in the erythrocyte counts (Figure 5.11) over controls; while there was a decrease in the leucocyte count (Figure 5.12). The

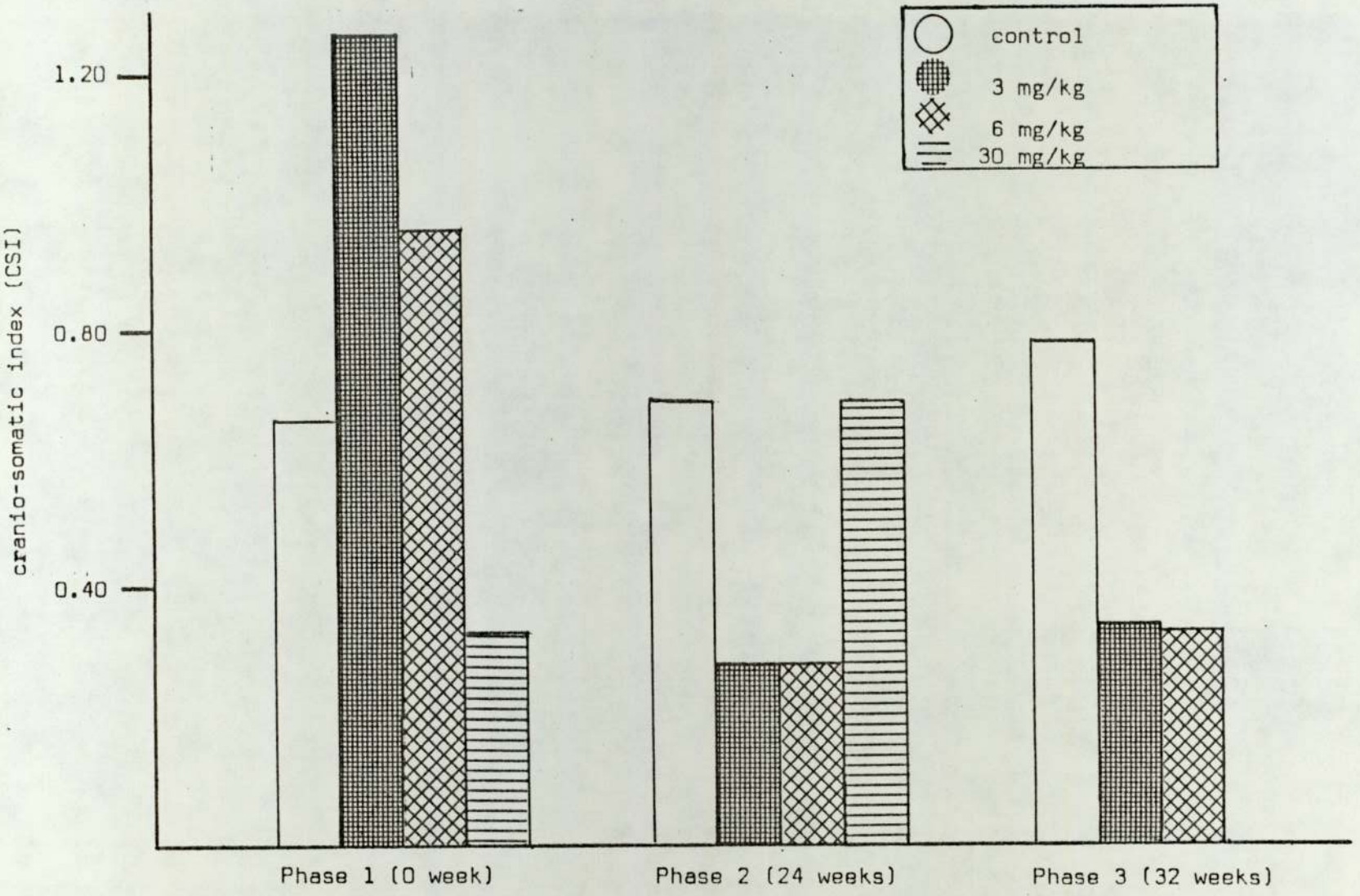


Figure 5.6. Effect of cobalt chloride on the CSI of carp

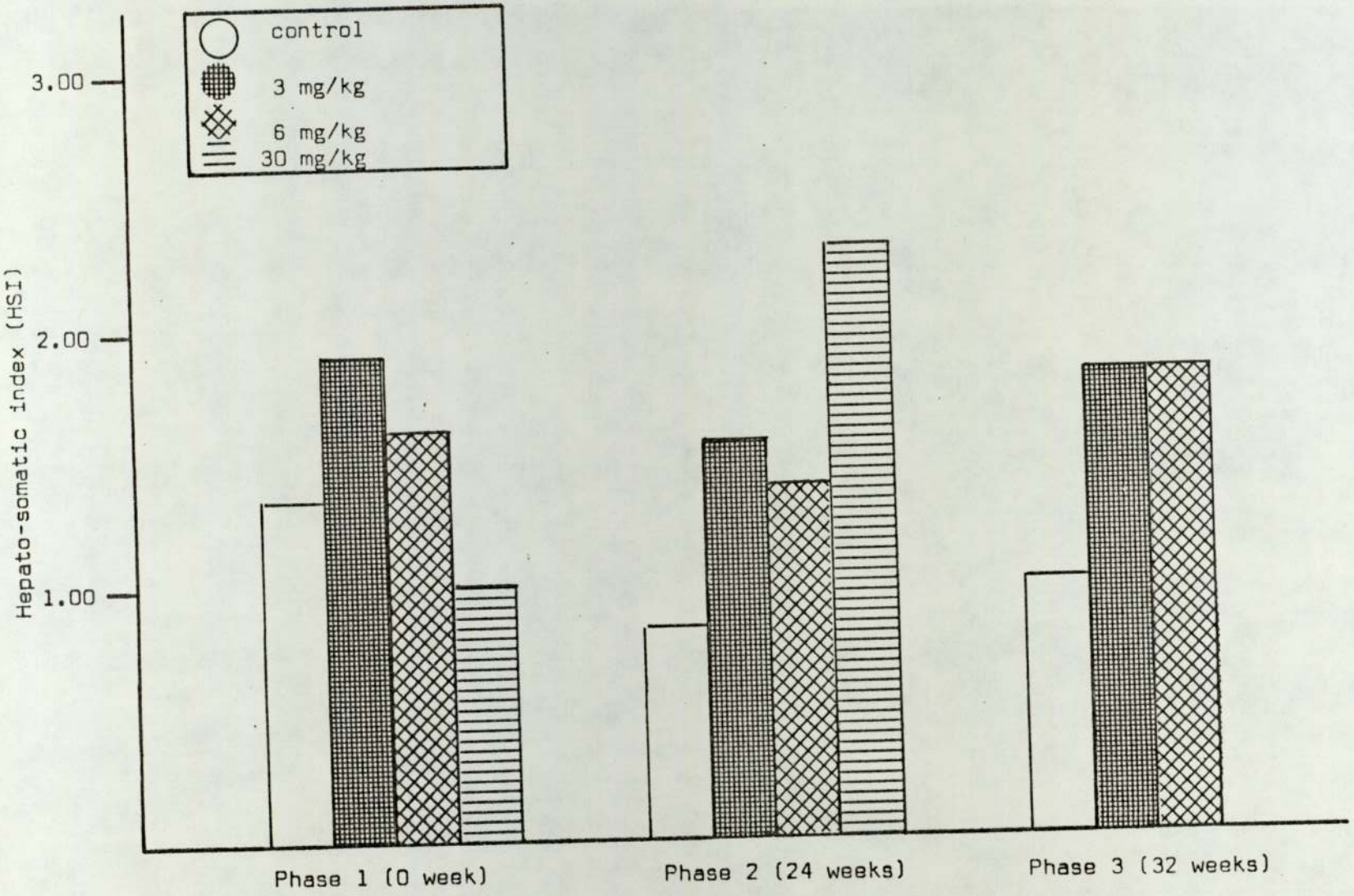


Figure 5.7. Effect of cobalt chloride on the HSI of carp.

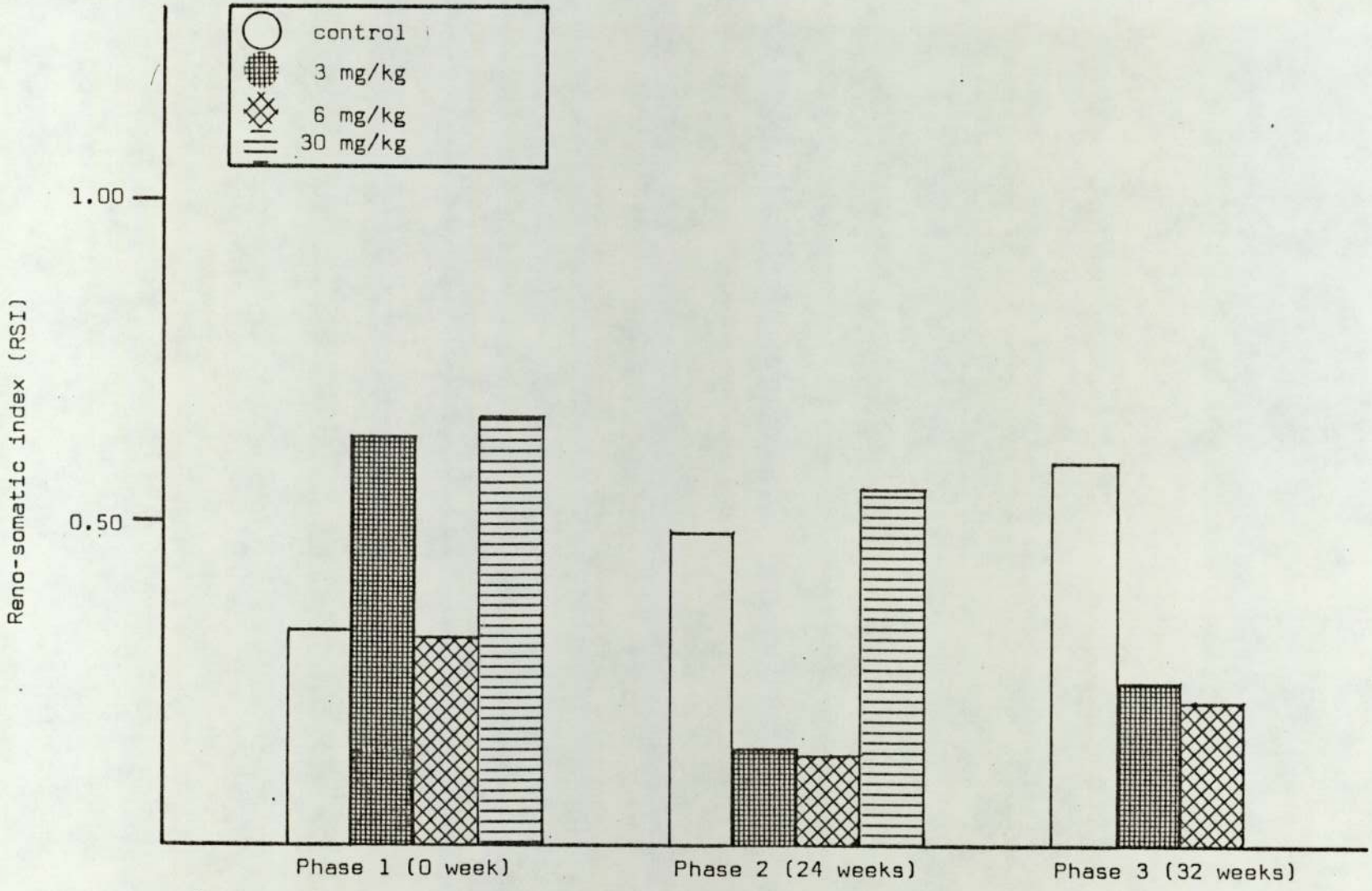


Figure 5.8. Effect of cobalt chloride on the RSI of carp.

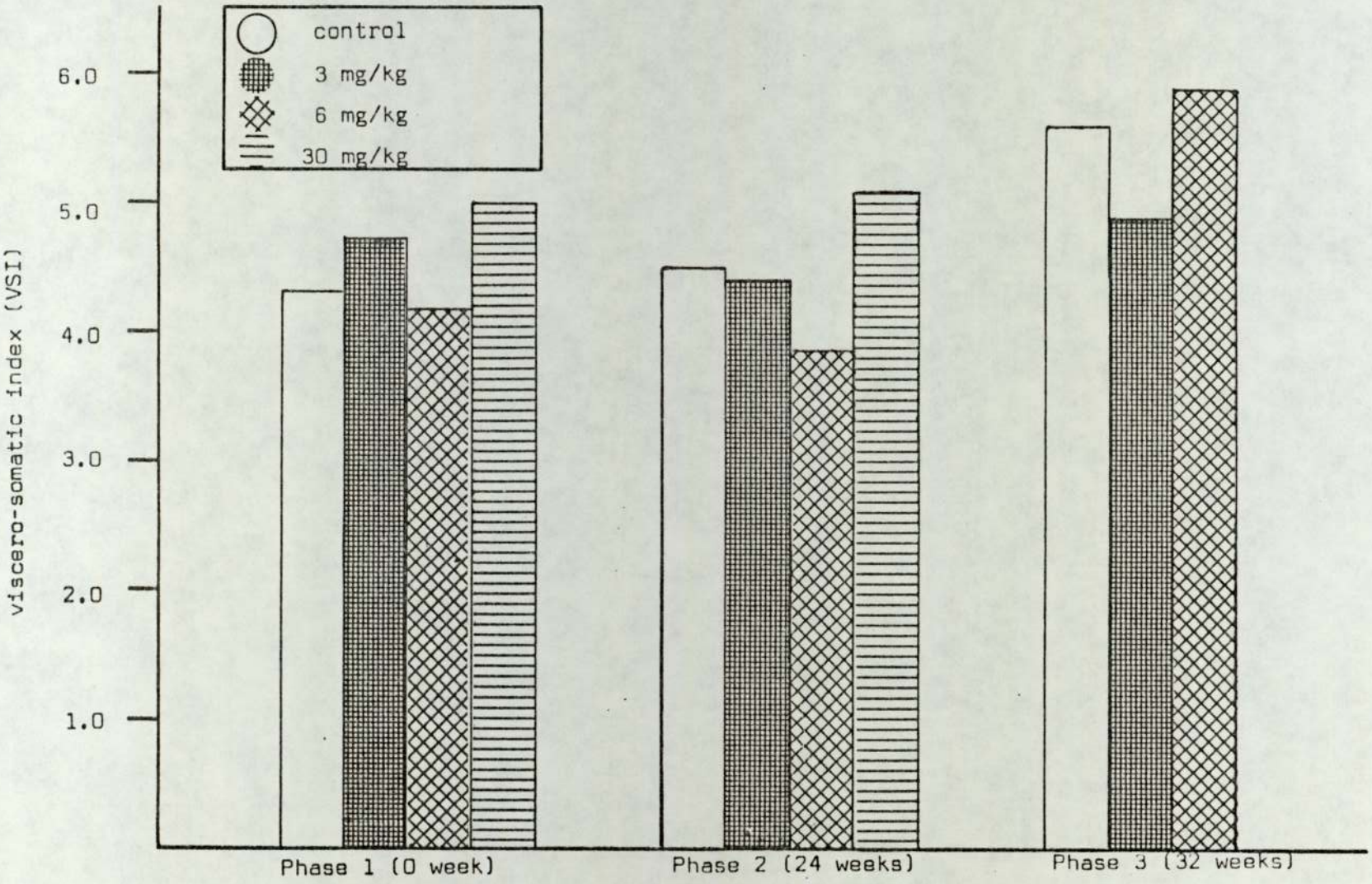


Figure 5.9. Effect of cobalt chloride on the VSI of carp.

Table 5.11

Hematological indices of carp grown on cobalt-enriched diet. Values given are the mean ± S.E.

Hematological	Concentration of Cobalt Chloride in the Diet, mg/kg			
	Control (0)	3	6	30*
Hemoglobin Concentration (%)	30.8 ± 1.47	34.8 ± 2.15**	35.4 ± 2.11**	34.2 ± 1.34**
Erythrocyte (Thousands/cm^3)	838.2 ± 78.47	1536.7 ± 68.25**	1800.0 ± 125.29**	1783 ± 165.02**
Leucocyte (Thousands/cm^3)	26.3 ± 0.97	21.0 ± 1.17**	20.0 ± 0.81**	19.3 ± 0.81***

*Samples taken after 21 weeks of feeding.

** = $P < 0.05$

*** = $P < 0.01$

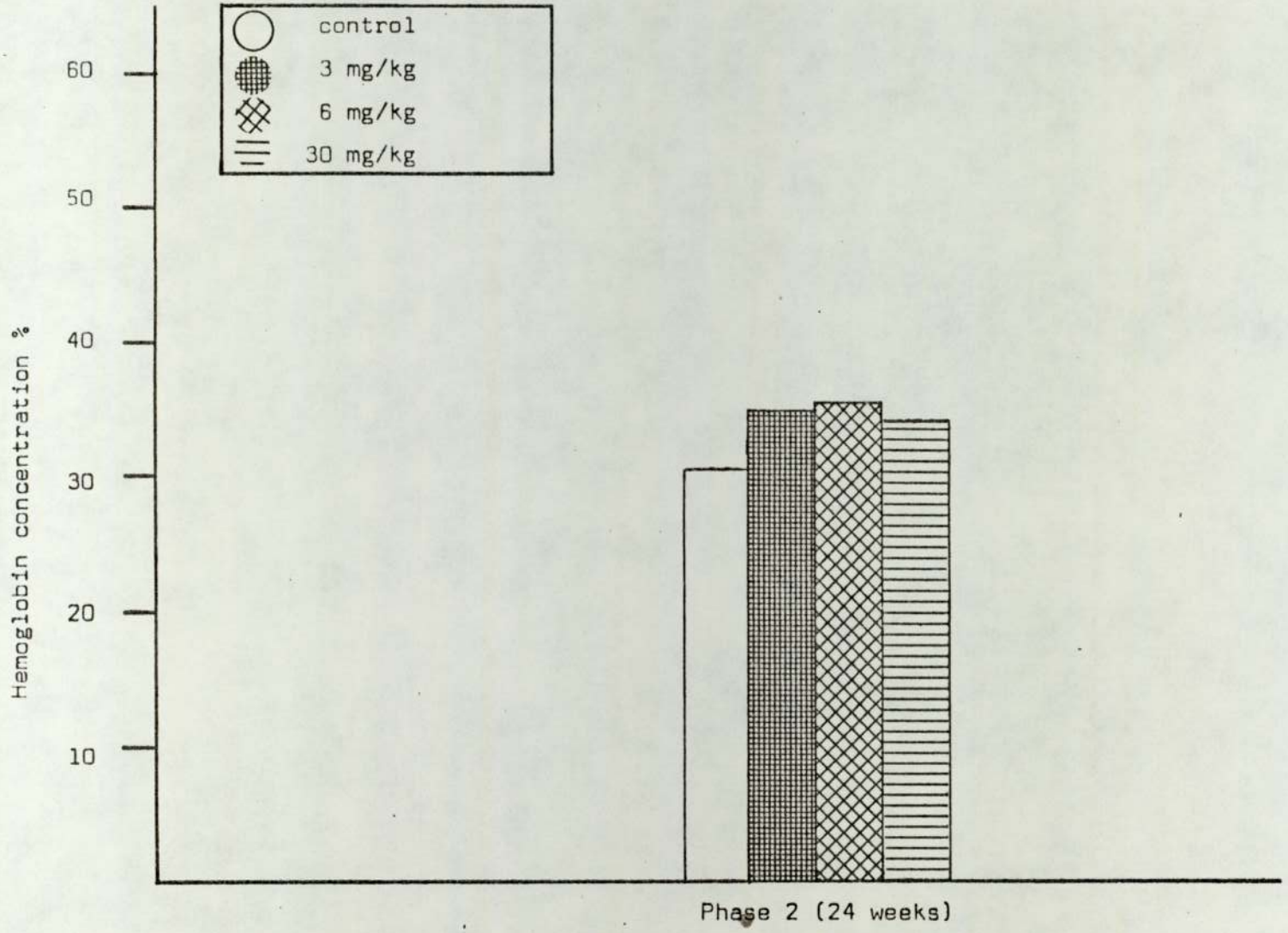


Figure 5.10. Effect of cobalt chloride on the hemoglobin percentage in carp.

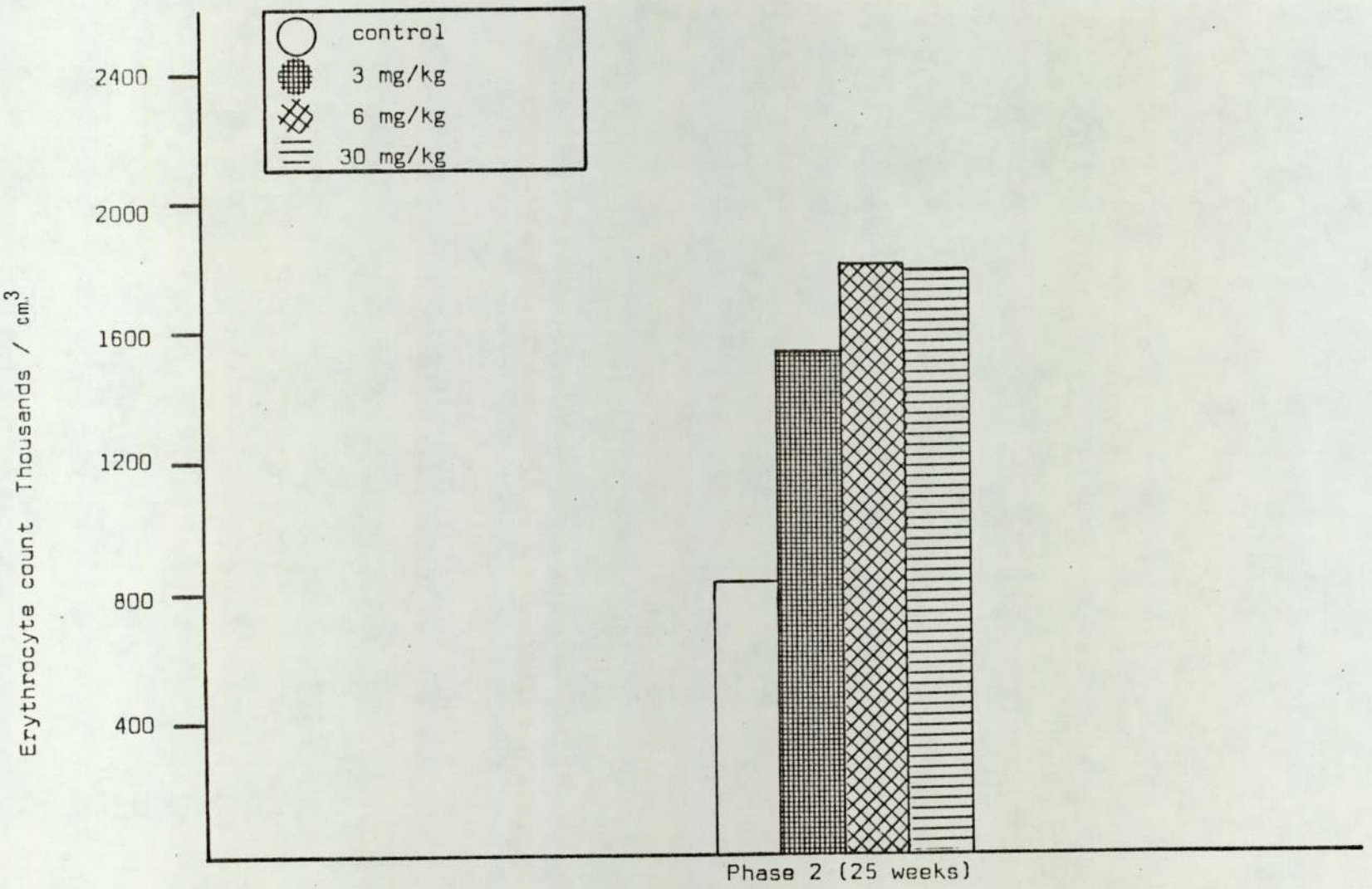


Figure 5.11. Effect of cobalt chloride on the Erythrocyte count in carp.

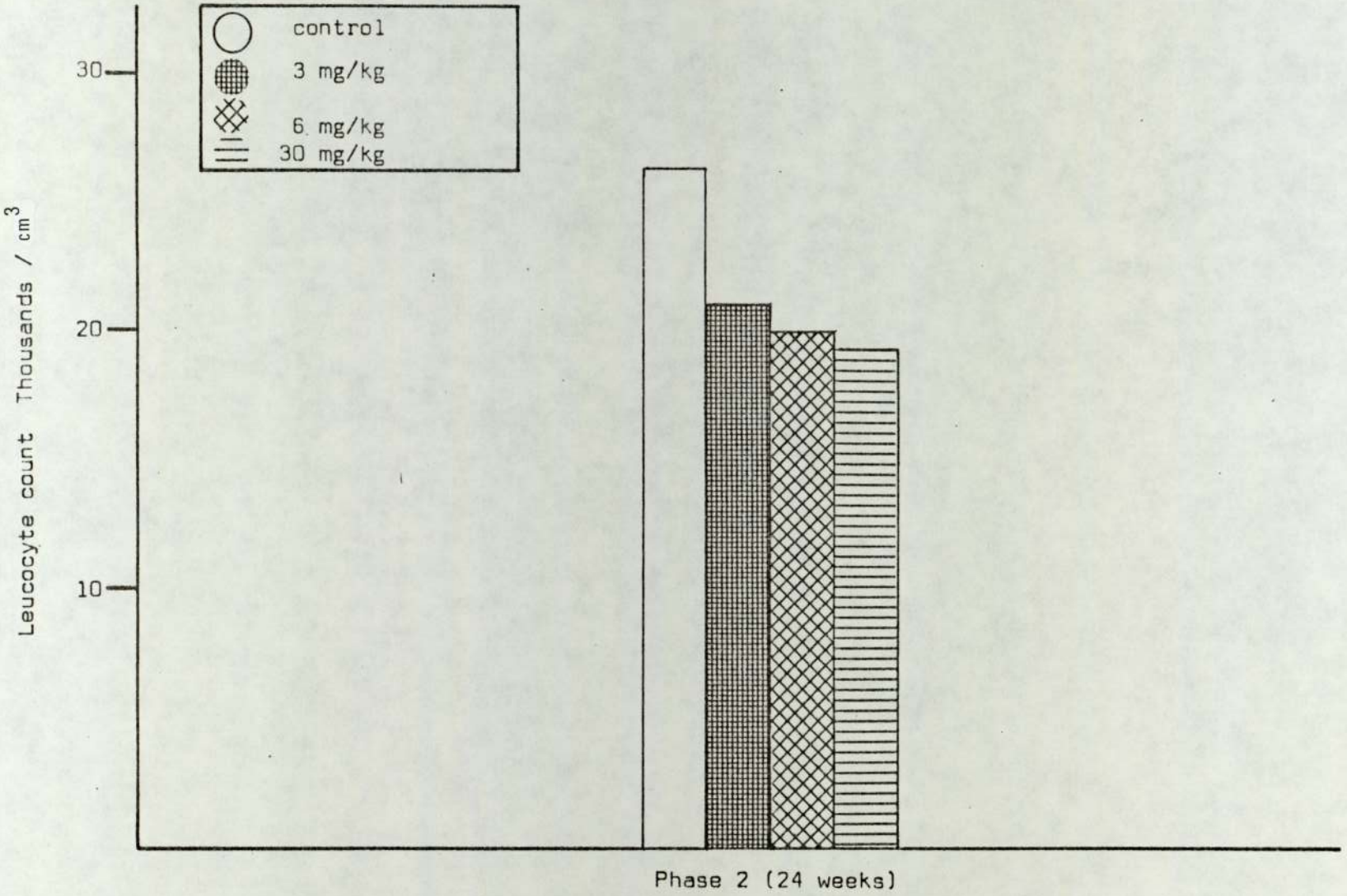


Figure 5.12. Effect of cobalt chloride on the Leucocyte count in carp.

highest haemoglobin percentage and erythrocyte values were obtained in the 6 mg cobalt chloride group, and the lowest value of leucocytes was noted in the 30 mg group, after 24 weeks of feeding. These results agree with the fact that cobalt promotes haemopoiesis in the animal.

5.3.5 BODY COMPOSITION

Body analyses are shown in Table 5.12; they indicate an increase in total protein in all treated groups. Protein was higher in the 3 mg cobalt chloride per kg dry diet group than in other treated groups. There was an increase in ash content of the body in all treated groups, this increase being in proportion to the amount of cobalt presented in the diet. There was little difference in water content of the body between the treated groups and the controls. Fat in the body was almost the same in treated groups, where it was lower in the control group.

5.3.6 COBALT CONTENT IN CARP AND ITS ORGANS AND TISSUES

Cobalt Content in Carp

The concentration of cobalt stored in the fish body is shown in TAble 5.13. This amount was in a way a reflection of the amount of cobalt presented in the diet; when the amount of cobalt was high in the diet, the amount of cobalt stored in the body was high and vice-versa (Figure 5.13).

Cobalt Content in Carp Organs and Tissues

The distribution of cobalt within the fish was uneven (Table 5.14); it was high in kidney (Figure 5.14) and low in muscle (Figure 5.15) compared to the amount stored in the liver (Figure 5.16). There was a direct relationship between the

Table 5.12

Body Composition of Carp fed diet Supplemented with Cobalt Chloride. Values given are the mean of 3 fish ± S.E.

Body Composition of Carp	Concentration of Cobalt Chloride in the Diet, mg per kg Dry Diet			
	Control (0)	3	6	30
Water	75.10 ± 1.10	75.80 ± 0.76	74.10 ± 0.90	74.80 ± 0.68
Crude Protein	9.20 ± 1.05	14.20 ± 0.97	13.60 ± 0.95	13.20 ± 0.90
Total fat	4.30 ± 0.88	6.50 ± 1.00	7.20 ± 0.91	6.40 ± 0.90
Ash	2.20 ± 0.10	2.80 ± 0.10	3.30 ± 0.20	3.60 ± 0.15
NFE + Fibre	9.20	0.70	1.80	2.00

Table 5.13

Cobalt Concentration in Carp fed Cobalt Chloride supplemented diet. Values given are the mean ± S.E.

Concentration of cobalt in carp mg /kg dry matter	Concentration of cobalt chloride in the diet, mg/kg dry diet			
	Control (0)	3	6	30
	0.48 ± 0.076	2.48 ± 0.333**	2.91 ± 0.202*	3.05 ± 0.123**

* = $P < 0.05$

** = $P < 0.01$

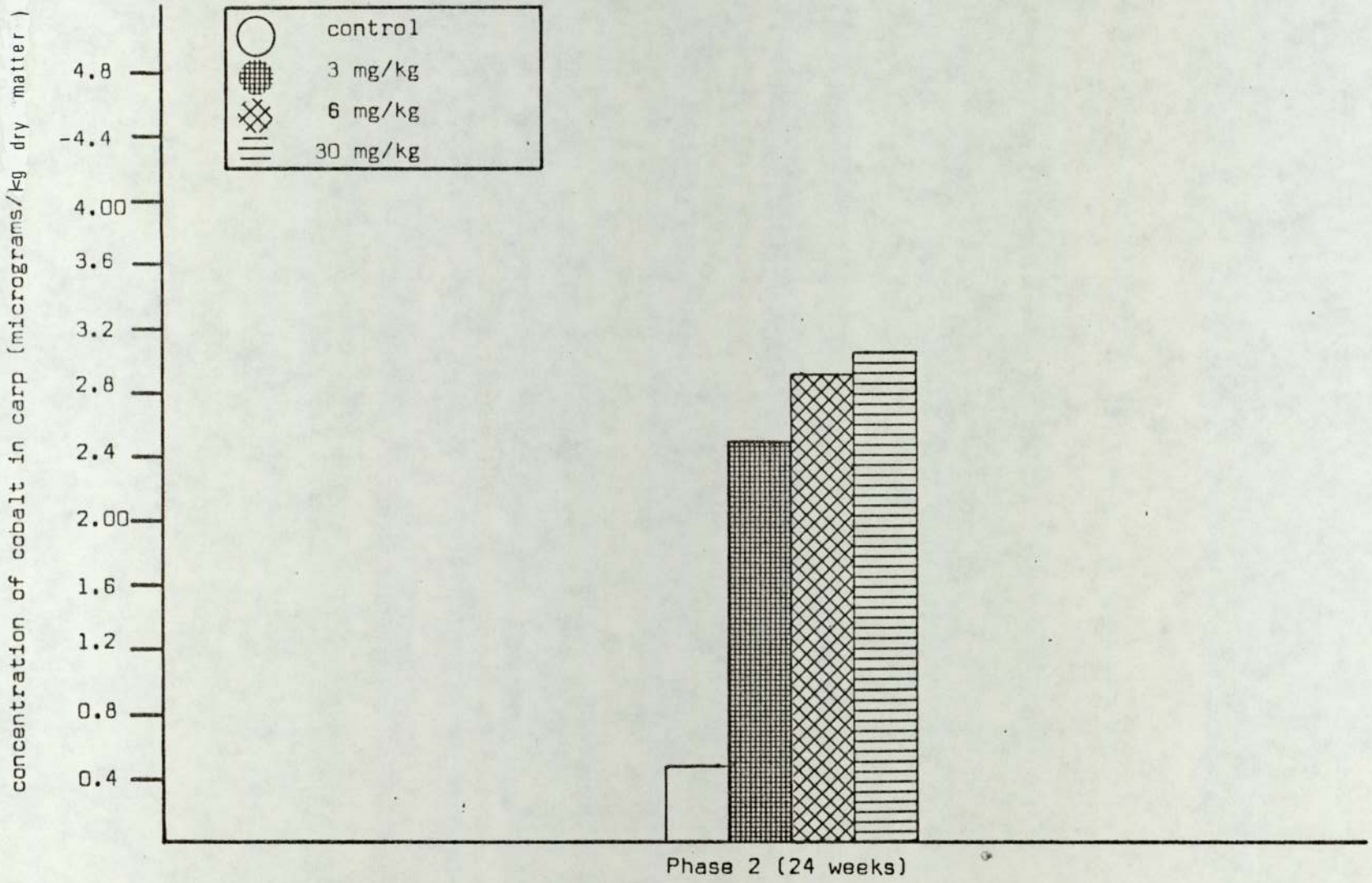


Figure 5.13. Concentration of cobalt in carp fed diet supplemented with cobalt chloride.

Table 5.14

Concentration of cobalt in carp tissues and organs. Values given are the mean ± S.E. of the mean

Concentration of cobalt in organs nanograms /g wet weight	Concentration of Cobalt Chloride in the Diet, mg/kg dry diet			
	Control (0)	3	6	30
Muscle	0.98 ± 0.10	7.60 ± 2.07	8.36 ± 1.72**	8.10 ± 0.95**
Liver	10.26 ± 7.08	50.60 ± 19.60**	55.56 ± 13.25**	73.83 ± 10.04**
Kidney	53.93 ± 17.74	213.86 ± 64.82*	277.00 ± 25.16**	443.65 ± 70.33**

* = $P < 0.05$

** = $P < 0.01$

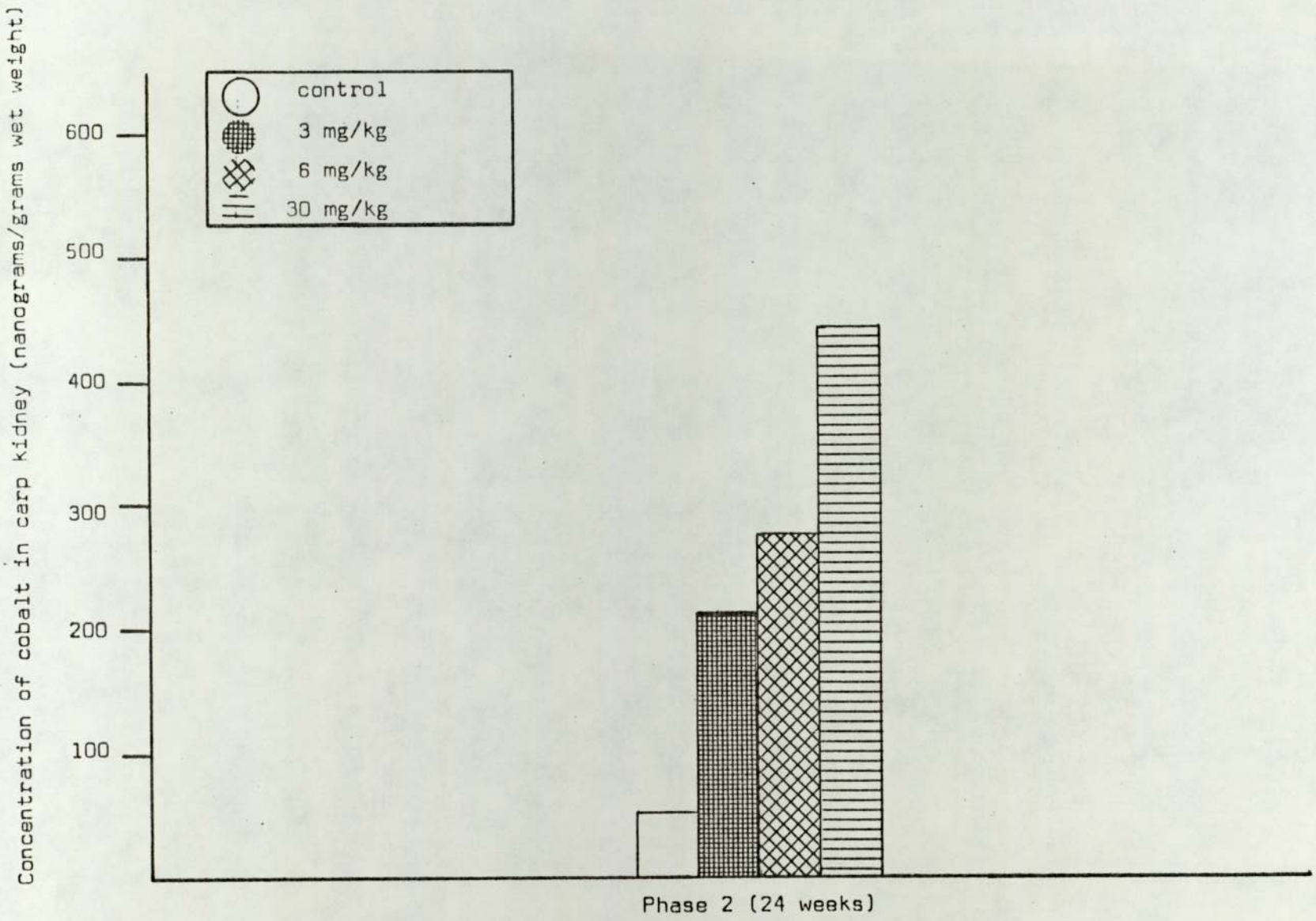


Figure 5.14. Concentration of cobalt in carp kidney

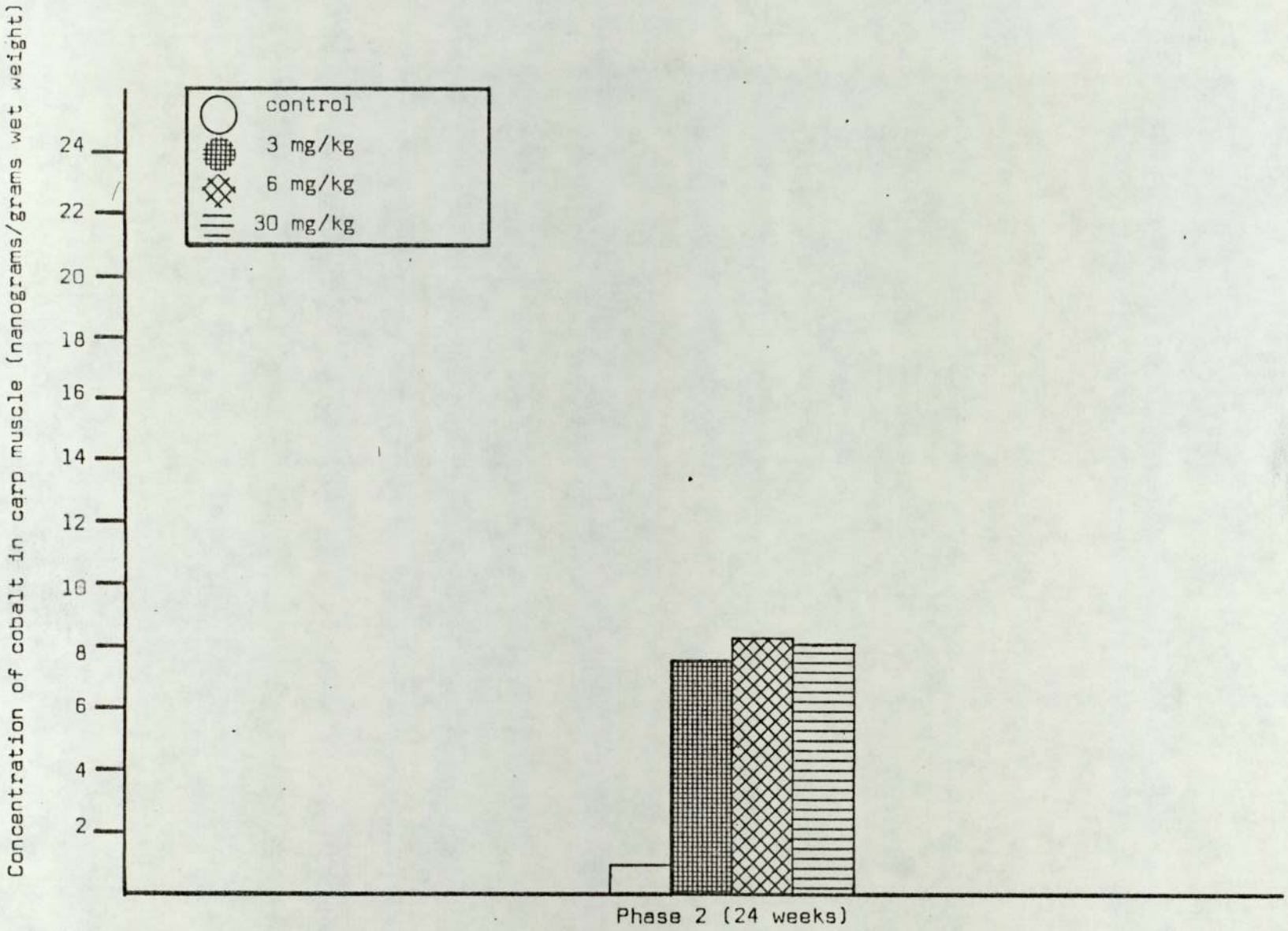


Figure 5.15. Concentration of cobalt in carp muscle

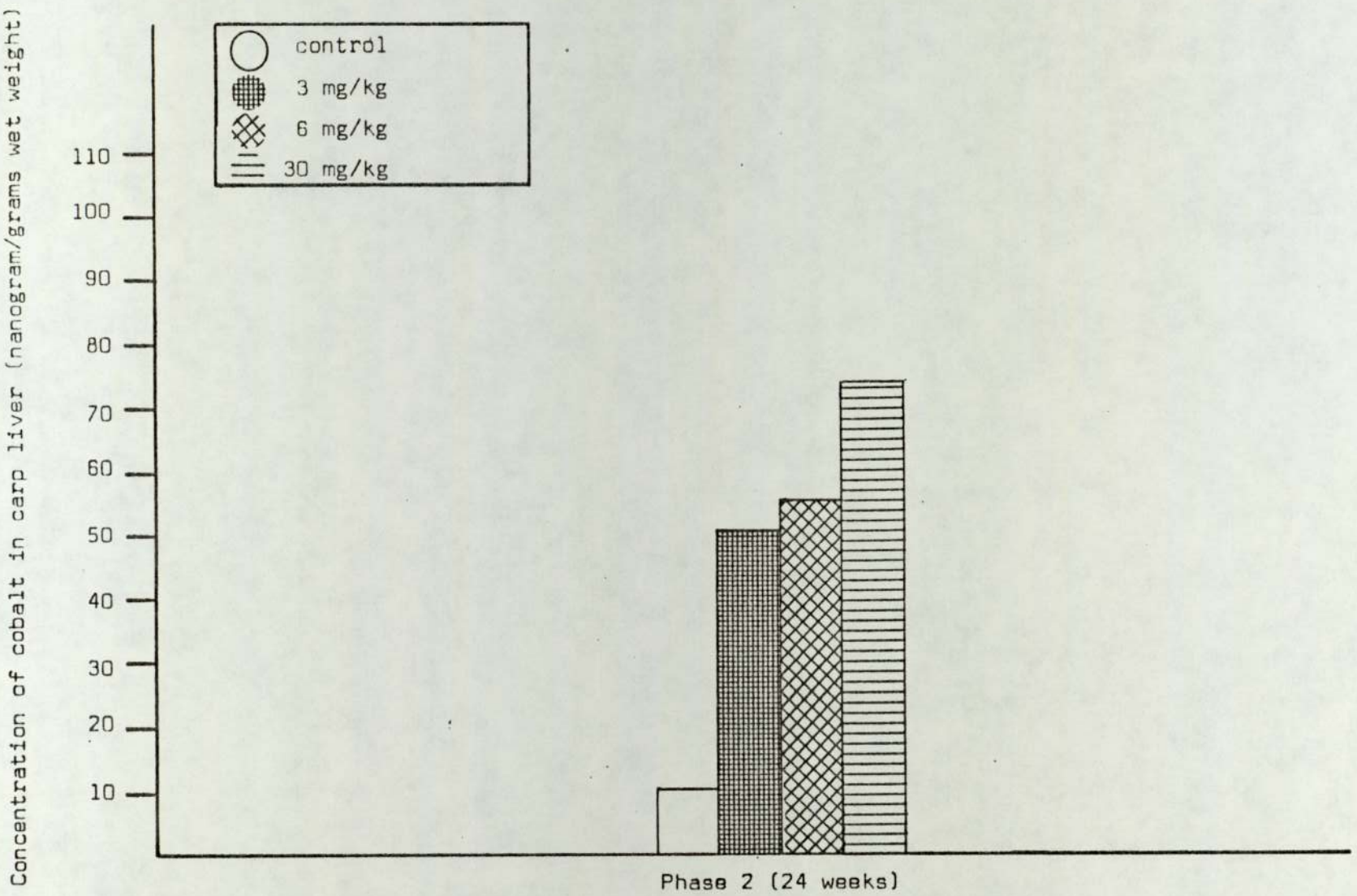


Figure 5.16. Concentration of cobalt in carp liver.

amount of cobalt given to the fish in the diet and the amount contained in their organs and tissues.

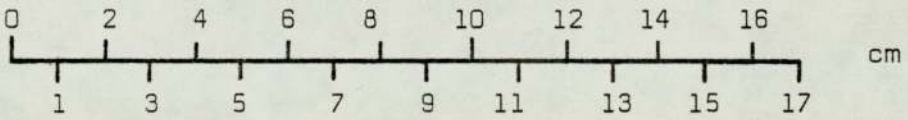
5.3.7 DEFICIENCY SYMPTOMS

Carp which were fed on a basal diet lacking in cobalt lost their appetite and became sluggish after 8 weeks. After 12 weeks, some of the fish exhibited convulsions which finally terminated in death; malformation of tail and head developed in almost 60% of these fish, malformations of the tail appearing as early as the 4th week (Figure 5.17).

5.4 DISCUSSION AND CONCLUSIONS

From the results of the present study it may be inferred that the optimum level of cobalt (as cobalt chloride) in the diet for growth and metabolism of carp is 3 mg cobalt per kg dry diet. This finding is in agreement with that of other authors whose results are summarised in Table 5.15. The effects of cobalt on body composition were not as profound in this study as in those reported by other authors. Bican (1977) found that cobalt in carp reared in Bohemia was from 2.9 - 2.4 mg per kg dry matter which is in fact ten times the amount that was found in this experiment. In the present study, the control fish, which received no supplement of cobalt in their diet, had a final carcass cobalt content of 0.48 microgram per kg dry matter, which suggests that cobalt can be concentrated from the water either directly or via the microflora in the fish gut. At higher levels of cobalt the results agree with those of Shabalina (1964) and Frolova (1961) that at high doses of cobalt (more than 5 mg per kg fish weight) the inhibitory effects of this element were in evidence.

a



b

Figure 5.17

a. Normal fish fed on cobalt enriched diet

b. Malformation in carp grown on cobalt deficient diet

Table 5.15

The Effect of Cobalt on the Rate of Weight Gain

Dose(s) mg per kg	Effect(s)	Author(s)
3 - 6	Optimum for growth	Frolova, 1961
0.5 mg/litre	Faster resorption of the yolk sac	Shabalina, 1964
0.08 mg per kg fish weight	Weight gain, Promoted growth	Farberov, 1965
1 ppm with 5 cc ruminant stomach extract	Weight gain, Survival	Das, 1967
3 - 6 ppm	Promoted the growth	Ghosh, 1968
3 - 30 ppm	Increase in the growth rate, better storing	Grosev and Joschev, 1971

Some general conclusions may be drawn from the results presented here:

1. It is possible to reduce mortality in carp at a dietary cobalt level of 3 mg per kg dry diet, with improved food utilization, haematological characteristics, and resistance to disease; results are better than with doses of 6 mg or 30 mg cobalt per kg dry diet.

2. When the dose of cobalt was increased to 30 mg cobalt per kg dry diet, both the growth and the survival rates were less than in other treated groups and all the fish finally died.

CHAPTER SIX

THE EFFECT OF COBALT IN THE DIET, WITH VITAMIN B12, ON GROWTH PERFORMANCE AND METABOLISM OF CARP, *Cyprinus carpio*.

6.1 INTRODUCTION

The only established biological function of cobalt relates to its role as a component of the vitamin B_{12} molecule. Vitamin B_{12} is very important in the lives of farm animals, but its role in fish is far from clear. Fish generally do not show any deficiency symptoms even after 2 - 3 months on a diet without this vitamin (Halver, 1957).

The present 24 weeks growth study was undertaken to determine the optimum dietary level of cobalt, as cobalt chloride, in the diet with vitamin B_{12} for growth and metabolism of carp and the role of cobalt with vitamin B_{12} in the body.

6.2 MATERIALS AND METHODS

6.2.1 THE EXPERIMENTAL SYSTEM AND ANIMALS

The experimental facilities used in the present study was System 2 as described in Chapter 4 (4.2).

250 fingerling carp, Cyprinus carpio, were obtained from Avion farm, Aries ford. The fish were subjected to quarantine and prophylaxis, as described in Chapter 4 (4.5). Then they were transferred to a tropical room and kept in a recycling system, System 1, as described in Chapter 4 (4.2).

A sufficient number of them (20 fish) of an appropriate size (9.26 ± 0.11 cm) were selected and transferred to 12 of the 25 litre glass tanks, System 2, at the prevailing ambient temperature of 23°C. During this period the fish were fed on a commercial trout diet, and 12 were removed for proximate carcass analysis, Chapter 4 (4.9). The density of stocking was found to be 8.40 ± 1.16 g /litre. The water was aerated and filtered by using a small plastic filter.

The experiment was carried on for a period of 24 weeks; throughout this time pH and dissolved oxygen were checked once a week using a pH meter and the Winkler method. They were found to range from pH 7.1 - 7.5 and 8 - 10 ppm respectively. Room and water temperature were also recorded daily and found to range from 25 - 27°C and 23 - 25°C respectively. No losses occurred during temperature acclimatization. Photo period was controlled at 12 hours day and 12 hours night (8 am - 8 pm - 8 am) throughout the experiment.

6.2.2 THE EXPERIMENTAL DIET

Formulation of the diet was carried out by the general procedure described in Chapter 4 (4.4); the ingredients used are presented in Table 6.1. Vitamin B_{12} was included at 25 microgram per kg diet (Halver, 1970). Cobalt, as cobalt chloride ($CoCl_2.6H_2O$) was added to the diet at 3 levels: 3 ppm, 6 ppm, and 30 ppm. A solution of suitable concentration was sprayed over 500 gm of the food pellets, using a chromotographic sprayer; for the control, an identical procedure was followed using distilled water only. After dyring, food was stored at -4°C. The proximate analysis was performed on this diet and the results are presented in Table 6.2.

6.2.3 FEEDING RATE

The fish were fed three times a day, at 10 am, 2 pm and 6 pm; except for Sunday when they were fed twice a day at 10 am and 6 pm. Each feeding continued for 15 minutes including 5 minutes recess in the middle. The fish were fed at a rate that allowed them to consume the diet as it fell through the water. All the groups were fed 6% of their body weight per day. The quantity of food delivered

Table 6.1

Composition of the Test Diet

Main Mixture		Vitamin Supplement		Mineral Mixture	
Ingredients	Parts	Vitamin	mg*	Mineral	mg*
Casein	400	Riboflavin	200	$CaCO_3$	250
α-starch	180	Thiamin-HCl	50	KCl	4670
Dextrin	190	Pyridoxin-HCl	50	KH_2PO_4	4000
Cod liver oil	40	Folic acid	15	Na_2HPO_4	3090
Vitamin mixture	50	Ascorbic acid	100	$MgSO_4$	2475
Mineral mixture	40	Pantothenic acid	500	$FeSO_4.7H_2O$	250
Methionine	10	Inositol	2000	$ZnSO_4.7H_2O$	220
Tryptophan	5	Nicotinic acid	750	$MnSO_4.H_2O$	92
α-cellulose	15	Biotin	5	$CuSO_4$	20
Carboxymethyl-cellulose	10	Choline chloride	4000	KI	1
		Vitamin A	40	Na_2SeO_3	0.2
Gelatine	60	Vitamin E	40	$(NH_4)_6MO_7O_{24}.4H_2O$	0.4
Total	1000	Vitamin D_3	5000 IU	$CoCl_2.6H_2O$**	-
		Vitamin K	150		
		Vitamin B_{12}	25		
		Glucose (as a carrier	4200		

* mg/kg diet

** Dietary cobalt was adjusted to 3, 6, 30 ppm by cobalt chloride, $CoCl_2.6H_2O$, and α-cellulose.

Table 6.2

Composition of the Basal Ration for Carp

Diet Composition in percent %	Concentration of Cobalt Chloride in the diet, mg/kg			
	Control (0)	3	6	30
Water	11.5	12.0	11.8	11.4
Crude Protein	42.0	41.9	42.2	42.2
Total fat	4.2	4.3	4.2	4.2
Ash	5.2	5.1	5.2	5.3
Nitrogen free + extract (NFE)* fibre	37.1	36.7	36.6	36.9

*Calculated by difference

to the fish per day was adjusted after each weighing and fed for the subsequent days. When the fish were unwilling to consume the whole day's ration, they were fed only the amount they would consume, and uneaten food was weighed and deducted from the daily ration.

6.2.4 WEIGHING AND SAMPLING

Details of the weighing procedure are presented in Chapter 4 (4.7). Fish were batch weighed (0.01 g), under anaesthesia, after 12 hours starvation every seven days for 24 weeks. At the end of the 12th week, an intermediate sample (3 fish) was removed from each group for proximate carcass and haematological characteristic analysis. At the end of the experiment 3 fish were removed from each group, for carcass analysis and determination of haematological characteristics, body composition, and the concentration of cobalt in the body and in individual organs.

6.2.5 STATISTICAL METHODS AND ANALYSIS OF GROWTH DATA

They were performed as detailed in Chapter 4 (4.10 and 4.11).

6.3 RESULTS

6.3.1 ACCELERATION OF GROWTH

Weight and Length

The results of the feeding trials are shown in Table 6.3 and 6.4. The fish in all the treated groups responded to the cobalt treatment with Vitamin B_{12} by increasing their weight and length above that of the controls up to the end of the experiment (Figures 6.1 and 6.2). By the end of the 12th week (intermediate stage) the difference between the control and the treated groups

Table 6.3

Changes in Body Weight of Carp fed diet supplemented with Cobalt Chloride and Vitamin B_{12} over a period of 24 weeks. Values given are the mean weight of 20 fish ± S.E. of the mean

Duration in weeks	Concentration of Cobalt Chloride in the diet, mg per kg dry diet						
	Control (0)	3		6		30	
	Weight (g)	Weight (g)	% Gain*	Weight (g)	% Gain	Weight (g)	% Gain
0	12.50 ± 0.35	9.00 ± 0.20	-	10.50 ± 0.50	-	9.50 ± 0.70	-
1	13.50 ± 0.40	10.00 ± 0.32		14.00 ± 0.70		10.70 ± 0.60	
2	14.80 ± 0.55	12.50 ± 0.40		15.80 ± 0.65		12.30 ± 0.56	
3	15.90 ± 0.57	15.00 ± 0.50		17.00 ± 0.50		14.00 ± 0.66	
4	16.20 ± 0.45	17.10 ± 0.53	60	19.50 ± 0.55	56	15.60 ± 0.73	35
5	17.00 ± 0.62	19.00 ± 0.64		21.05 ± 0.60		17.00 ± 0.80	
6	17.90 ± 0.58	21.20 ± 0.71		23.00 ± 0.70		18.40 ± 0.91	
7	18.10 ± 0.64	22.30 ± 0.80		24.35 ± 0.82		19.20 ± 0.93	
8	18.50 ± 0.65	24.00 ± 0.95	119	25.10 ± 0.90	91	20.00 ± 0.99	63
9	20.40 ± 0.77	25.50 ± 1.23		26.02 ± 0.85		21.15 ± 1.10	
10	22.00 ± 0.82	26.90 ± 1.50		28.00 ± 0.98		22.00 ± 1.20	
11	22.05 ± 0.98	29.00 ± 1.70		29.69 ± 1.05		23.30 ± 1.45	
12	22.70 ± 1.50	31.20 ± 1.90	165	31.10 ± 1.40	115	25.00 ± 1.50	82
13	23.15 ± 1.45	35.00 ± 2.05		33.00 ± 1.35		27.00 ± 1.70	
14	24.00 ± 1.55	37.30 ± 2.15		35.00 ± 1.42		28.05 ± 1.90	
15	25.10 ± 1.53	38.00 ± 2.00		36.70 ± 1.46		30.00 ± 2.10	

Table 6.3 (cont'd.)

Duration in weeks	Concentration of Cobalt Chloride in the diet, mg per kg dry diet						
	Control (0)	3		6		30	
	Weight (g)	Weight (g)	% Gain*	Weight (g)	% Gain	Weight (g)	% Gain
16	25.90 ± 1.50	39.40 ± 2.30	231	38.80 ± 1.50	162	33.00 ± 2.40	140
17	26.25 ± 1.56	40.00 ± 2.40		39.10 ± 1.64		34.50 ± 2.70	
18	27.10 ± 1.70	41.00 ± 2.30		40.80 ± 1.73		35.10 ± 2.80	
19	28.15 ± 1.90	42.10 ± 2.50		41.40 ± 1.90		36.20 ± 2.90	
20	28.90 ± 2.00	44.00 ± 2.60	258	42.00 ± 2.00	169	37.10 ± 3.10	159
21	30.00 ± 2.20	45.00 ± 2.70		43.80 ± 2.10		-	
22	32.00 ± 2.25	47.00 ± 2.65		45.00 ± 2.15		-	
23	33.80 ± 2.40	48.00 ± 2.73		46.80 ± 2.30		-	
24	35.00 ± 2.60	50.00 ± 2.80	276	47.90 ± 2.25	176	-	-

*Percent weight gain over control

Table 6.4

Changes in Body Length of Carp fed diet supplemented with cobalt chloride and vitamin B_{12} over a period of 24 weeks. Values given are the mean length of 20 fish ± S.E. of the mean

Duration in weeks	Concentration of Cobalt Chloride in the Diet, mg/kg dry diet						
	Control (0)	3		6		30	
	Length (cm)	Length (cm)	% Gain*	Length (cm)	% Gain	Length (cm)	% Gain
0	9.30 ± 0.20	9.20 ± 0.12	-	9.40 ± 0.14	-	9.15 ± 0.10	-
1	9.40 ± 0.25	9.20 ± 0.05		9.50 ± 0.10		9.30 ± 0.07	
2	9.60 ± 0.28	9.50 ± 0.08		9.80 ± 0.11		9.50 ± 0.08	
3	9.80 ± 0.11	10.00 ± 0.12		10.00 ± 0.12		9.60 ± 0.13	
4	10.00 ± 0.12	10.30 ± 0.12	3	10.20 ± 0.14	1	9.80 ± 0.13	-0.4
5	10.10 ± 0.14	10.50 ± 0.15		10.40 ± 0.17		10.00 ± 0.14	
6	10.38 ± 0.14	10.90 ± 0.16		10.60 ± 0.17		10.50 ± 0.15	
7	10.93 ± 0.18	11.11 ± 0.19		10.70 ± 0.16		10.70 ± 0.17	
8	11.00 ± 0.20	11.90 ± 0.23	11	11.30 ± 0.20	2	11.20 ± 0.20	4
9	11.20 ± 0.17	12.30 ± 0.26		11.55 ± 0.22		11.30 ± 0.23	
10	11.50 ± 0.20	12.80 ± 0.27		11.90 ± 0.30		11.45 ± 0.25	
11	11.70 ± 0.25	13.10 ± 0.29		12.30 ± 0.31		11.50 ± 0.30	
12	12.00 ± 0.27	13.70 ± 0.30	20	12.75 ± 0.37	7	11.80 ± 0.35	-0.1
13	12.10 ± 0.27	14.20 ± 0.37		13.00 ± 0.39		12.00 ± 0.41	
14	12.25 ± 0.30	14.70 ± 0.35		13.30 ± 0.47		12.25 ± 0.49	
15	12.30 ± 0.32	15.10 ± 0.40		13.40 ± 0.50		12.40 ± 0.50	

Table 6.4 (cont'd.)

Duration in weeks	Concentration of Cobalt Chloride in the Diet, mg/kg dry diet						
	Control (0)	3		6		30	
	Length (cm)	Length (cm)	% Gain*	Length (cm)	% Gain	Length (cm)	% Gain
16	12.70 ± 0.33	15.60 ± 0.42	33	13.90 ± 0.53	11	12.70 ± 0.58	2
17	13.00 ± 0.31	15.80 ± 0.45		14.15 ± 0.59		12.80 ± 0.61	
18	13.30 ± 0.35	15.95 ± 0.50		14.70 ± 0.55		13.10 ± 0.63	
19	13.60 ± 0.40	16.10 ± 0.51		14.95 ± 0.61		13.55 ± 0.67	
20	13.90 ± 0.44	16.30 ± 0.56	28	15.30 ± 0.74	13	14.00 ± 0.70	4
21	14.10 ± 0.60	16.30 ± 0.56		15.40 ± 0.75		-	
22	14.25 ± 0.68	16.40 ± 0.60		15.55 ± 0.80		-	
23	14.40 ± 0.72	16.50 ± 0.63		15.70 ± 0.82		-	
24	14.70 ± 0.80	16.50 ± 0.65	21	15.80 ± 0.83	10	-	-

*Percent length gain over control.

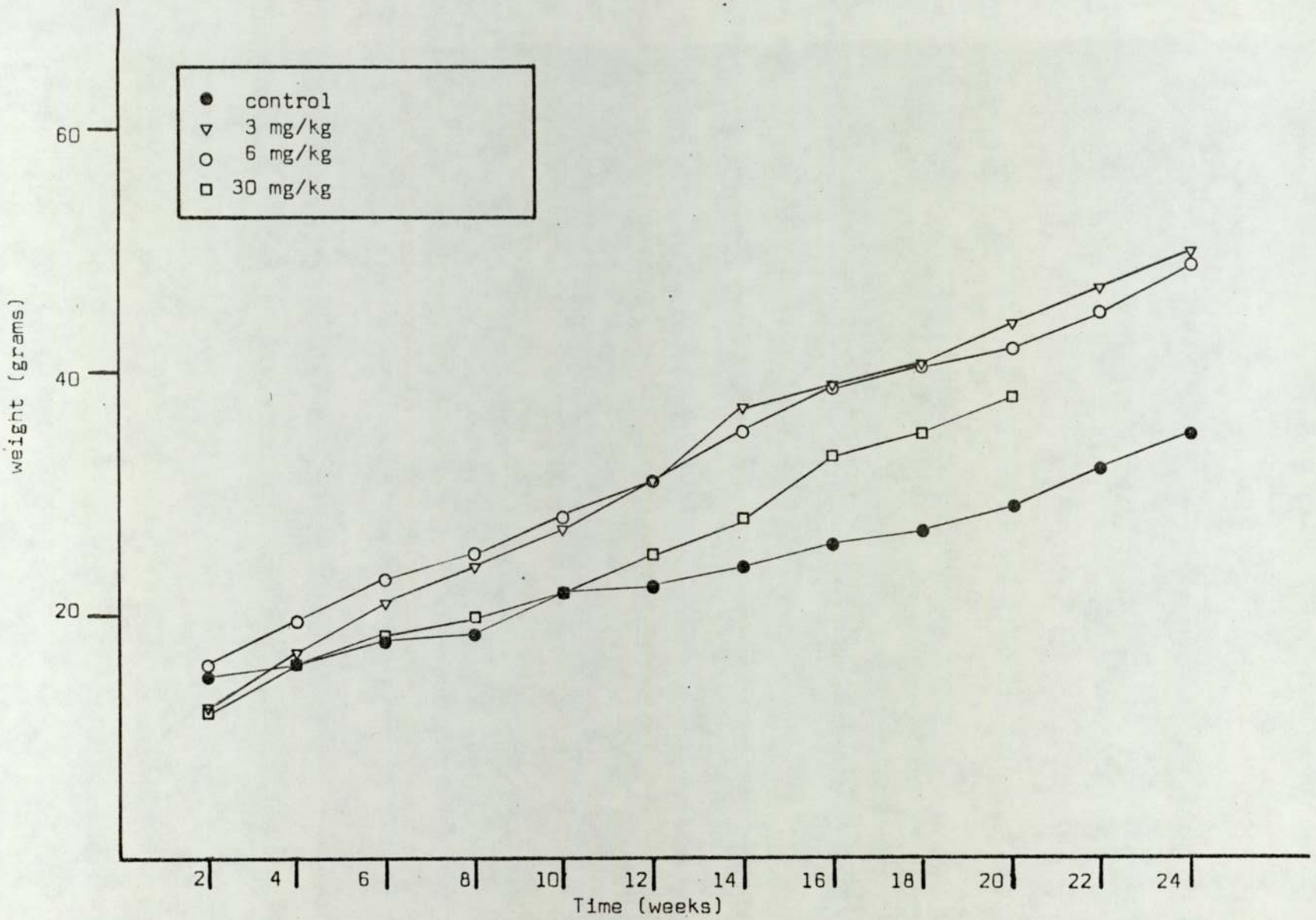


Figure 6.1. Effect of cobalt chloride with vitamin B12 on body weight of carp

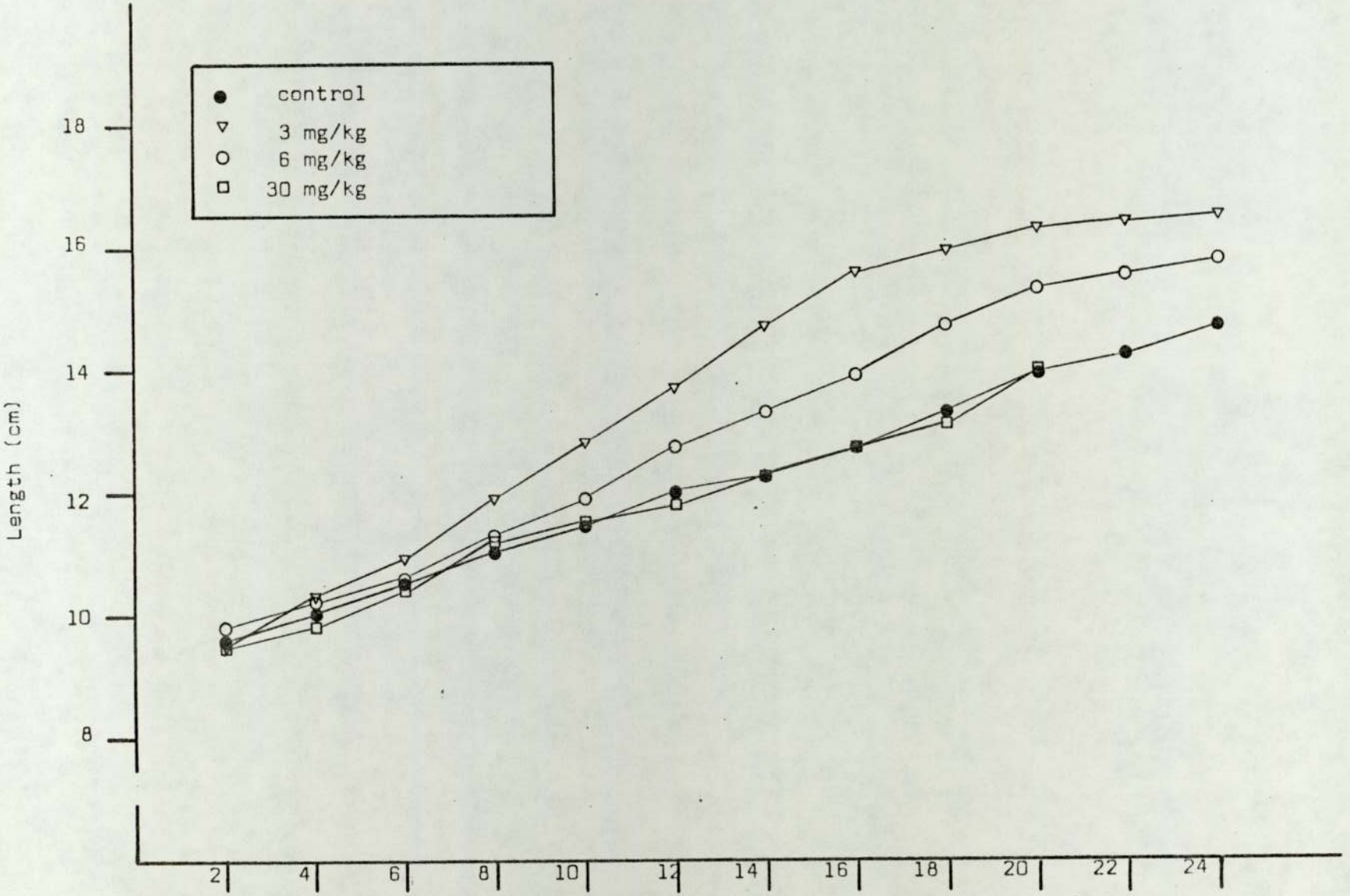


Figure 6.2. Effect of cobalt chloride with vitamin B_{12} on Body Length of carp.

was 165%, 115% and 82% in weight (Figure 6.3) and 20%, 7%, and -0.1% in length (Figure 6.4) respectively. At the end of the experiment the differences between the control and the treated groups were 276%, 176% in weight and 21%, 10% in length. All the fish in the group which was receiving 30 mg cobalt per kg dry diet had died after 20 weeks of starting the feeding on the experimental diet; at this dose the toxic effect of cobalt was in evidence.

Growth Rate

The mean daily growth rate for all the treated groups was greater than that of the control until the termination of the experiment. The greatest daily increases in weight and length were in the group receiving 3 mg cobalt at the end of 12 weeks. At the end of the experiment, this group also showed the greatest increase in weight, and the 6 mg group showed the greatest increase in length (Table 6.5 and 6.6).

Condition Factor

At the start of the experiment the mean value of the condition factor among all the groups was 0.013. For the first 4 weeks all the treated groups showed an increase in the condition factor whereas its value in the controls remained constant (Table 6.7). After the 4th week there was a general decrease in condition factor in all groups until the end of the experiment (Figure 6.5).

Food Utilization Efficiency (FUE)

Table 6.8 shows that there is a considerable increase in the FUE of carp fed cobalt with vitamin B_{12} for the whole period of this experiment. The increase in this value in the control was less than in the treated groups; it was greatest in the last 12

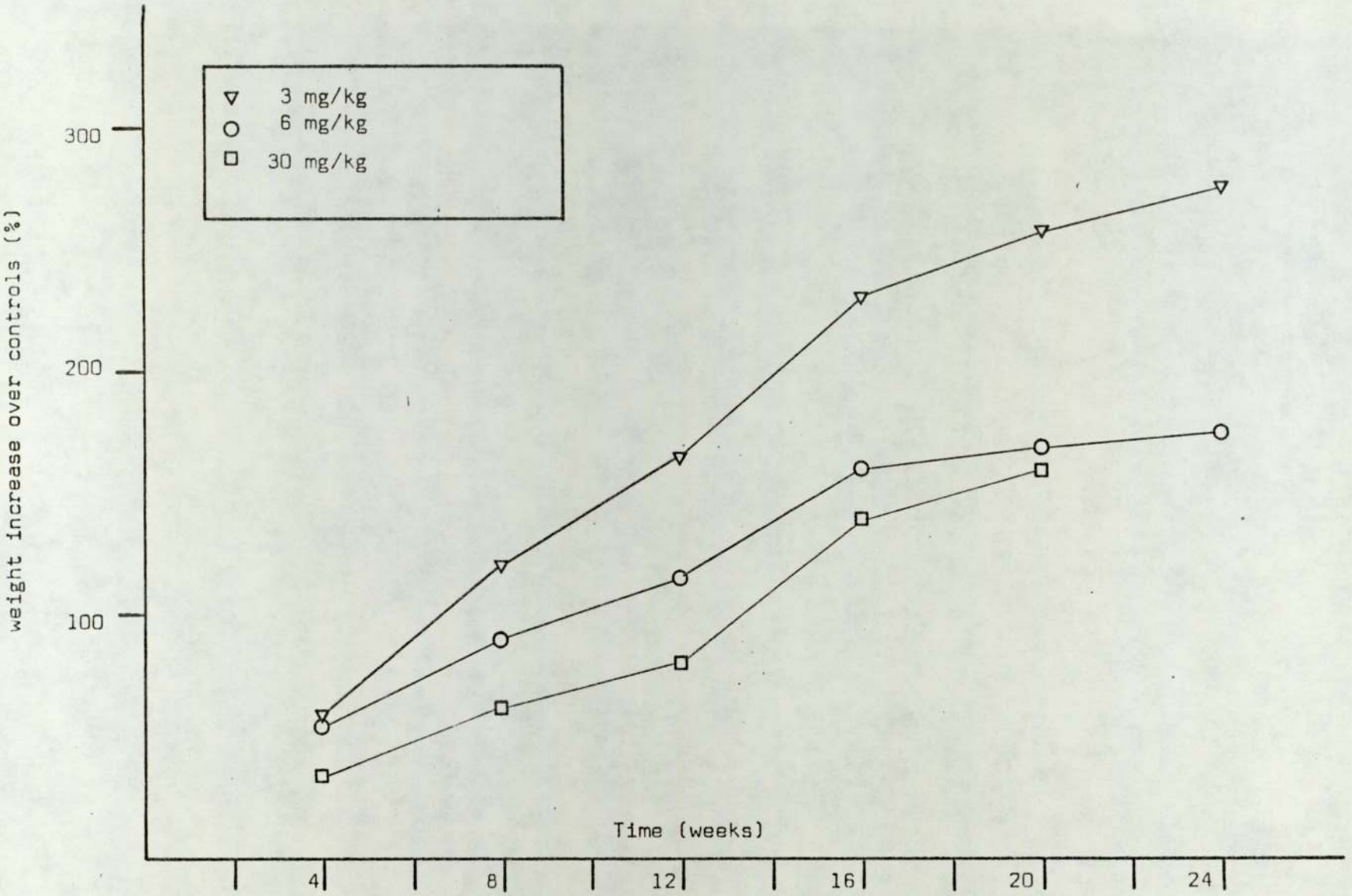


Figure 6.3. Effect of cobalt chloride with vitamin B_{12} on the percentage increase over controls in weight of carp.

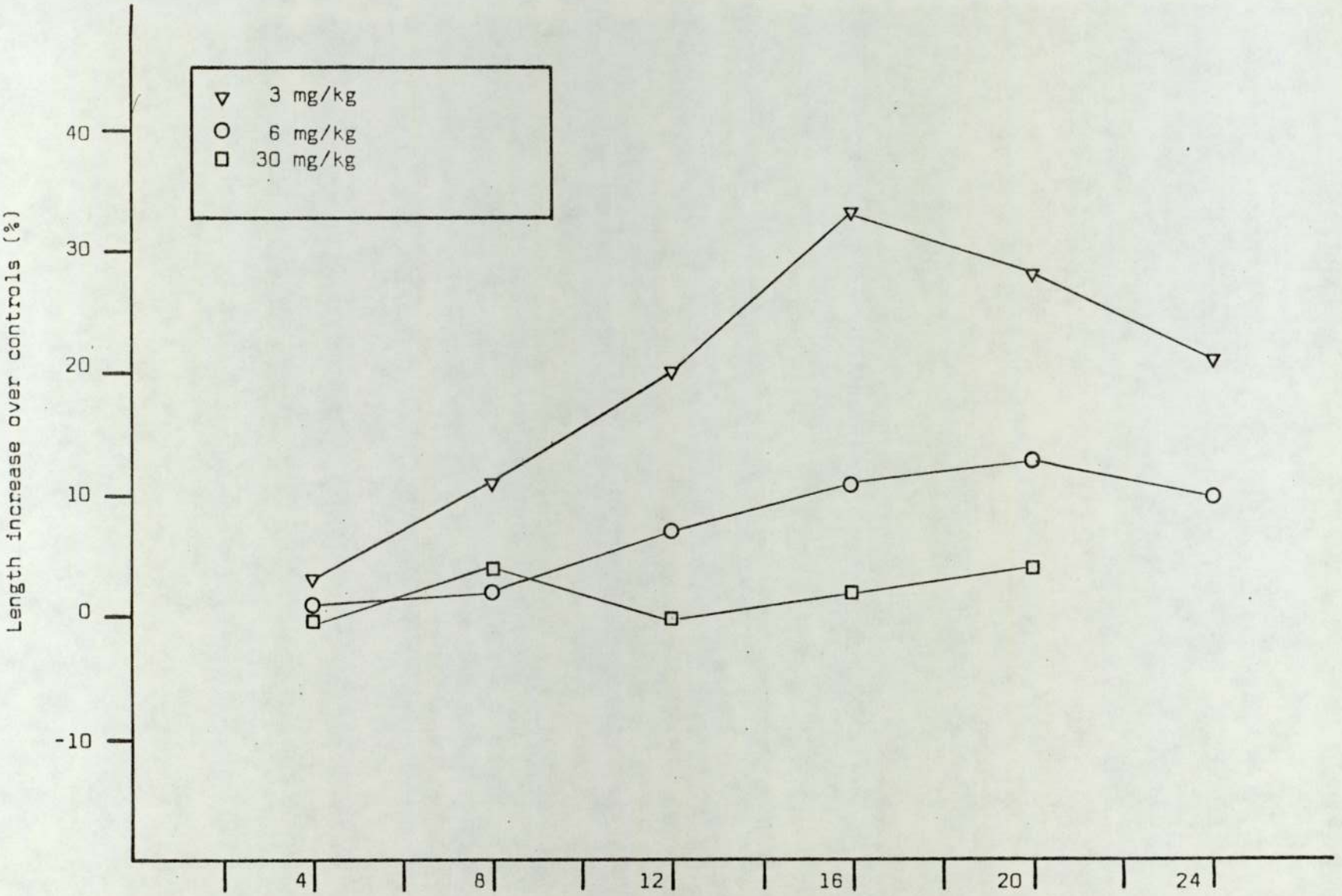


Figure 6.4. Effect of cobalt chloride with vitamin B_{12} on the percentage increase over controls in Length of carp.

Table 6.5

Effect of Administration of Cobalt Chloride with Vitamin B_{12} on Daily increase in Body Weight of Carp over 4-weekly period

Period in weeks	Concentration of Cobalt Chloride in the diet, mg/kg dry diet			
	Daily increase of mean Body Weight, g per 100 g initial weight			
	Control (0)	3	6	30
0 - 4	7.40	22.50	14.00	16.05
4 - 8	4.60	19.17	11.20	11.58
8 - 12	8.40	20.00	12.00	13.16
12 - 16	6.40	22.78	15.40	21.05
16 - 20	6.00	12.77	6.40	10.79
20 - 25	12.20	16.67	11.80	-

Table 6.6

Effect of Administration of Cobalt Chloride with Vitamin B_{12} on Daily increase of Body Length of carp over a 4-weekly period

Period in weeks	Daily increase of mean Body Length, cm per 100 cm initial length			
	Concentration of Cobalt Chloride in the diet, mg/kg dry diet			
	Control (0)	3	6	30
0 - 4	1.88	2.99	2.13	1.78
4 - 8	2.69	4.35	2.93	3.83
8 - 12	2.69	4.89	3.86	1.64
12 - 16	1.88	5.16	3.06	2.46
16 - 20	3.23	1.90	3.72	3.55
20 - 24	2.15	0.55	1.33	-

Table 6.7

Effect of oral Administration of Cobalt Chloride with Vitamin B_{12} on the condition factor of carp over a period of 24 weeks. Values are the means ± S.E.

Duration in weeks	The Condition Factor			
	Concentration of Cobalt Chloride in the diet, mg per kg dry diet			
	Control (0)	3	6	30
0	0.016 ± 0.001	0.012 ± 0.002	0.013 ± 0.001	0.012 ± 0.002
4	0.016 ± 0.002	0.016 ± 0.003	0.018 ± 0.003	0.017 ± 0.004
8	0.014 ± 0.002	0.014 ± 0.001	0.017 ± 0.002	0.014 ± 0.003
12	0.013 ± 0.001	0.012 ± 0.002	0.015 ± 0.003	0.015 ± 0.003
16	0.013 ± 0.003	0.010 ± 0.002	0.014 ± 0.002	0.012 ± 0.002
20	0.011 ± 0.003	0.010 ± 0.001	0.012 ± 0.001	0.014 ± 0.003
24	0.011 ± 0.003	0.011 ± 0.002	0.012 ± 0.001	-

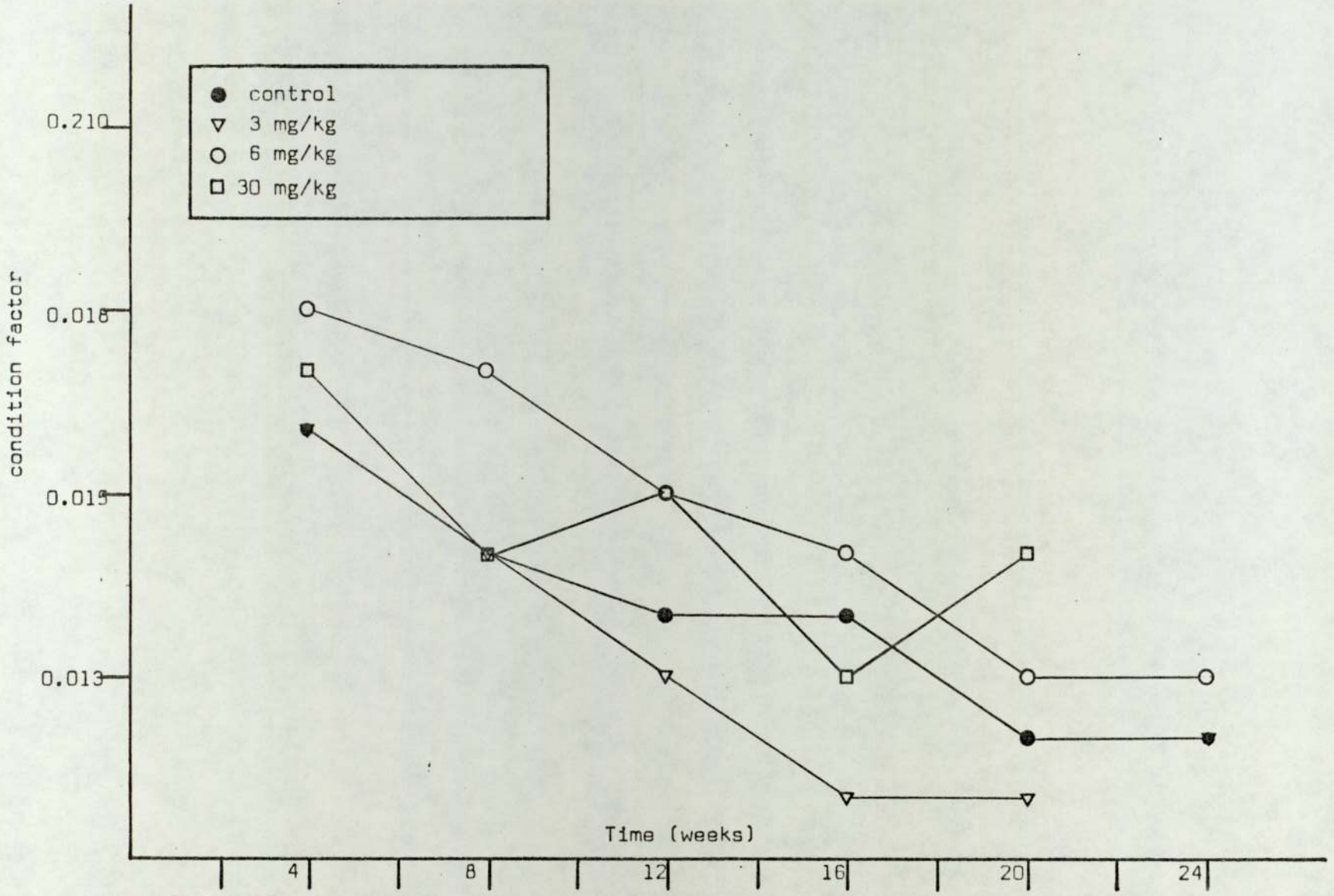


Figure 6.5. Effect of cobalt chloride with vitamin B_{12} on the condition factor of carp.

Table 6.8

Effect of oral administration of Cobalt Chloride and Vitamin B_{12} on food utilization efficiency of Carp over a period of 24 weeks

Period in weeks	Food Utilization Efficiency, weight gain (g)/food given (g)			
	Concentration of Cobalt Chloride in the diet, mg/kg dry diet			
	Control (0)	3	6	30
0 - 4	0.49	0.81	0.70	0.68
4 - 8	0.60	1.00	1.05	1.05
8 - 12	0.68	1.13	1.09	1.03
12 - 16	0.89	2.13	2.05	2.00
16 - 20	0.96	2.17	2.18	2.05
20 - 24	1.30	2.37	2.18	-

weeks of the experiment. The highest values were observed throughout the experiment in the group that received 3 mg cobalt per kg diet.

6.3.2 SURVIVAL RATE

Results for the survival of carp fed cobalt chloride with vitamin B_{12} are presented in Table 6.9. There was a steep decline in the survival percentage throughout the experiment in the group that received 30 mg cobalt chloride per kg diet. All the fish in this group had died by the end of the experiment; death was always accompanied by blood spots on the eyes. It can be assumed (as mentioned earlier) that these deaths were due to the toxic effects of cobalt.

6.3.3 CRANIO-SOMATIC, HEPATO-SOMATIC, RENO-SOMATIC, AND VISCERO-SOMATIC INDICES

The ratios of the different body organs to the weight over a period of 24 weeks of growth are given in Table 6.10. The cranio-somatic (CSI) and the reno-somatic (RSI) indices decreased, whereas the hepato-somatic index increased, in all groups of fish, including the controls, during the experiment (Figures 6.6, 6.7 and 6.8). The viscero-somatic index (VSI) decreased in the groups receiving 3 and 30 mg cobalt per kg diet, but it increased in the other two groups (Figure 6.9).

6.3.4 HAEMATOLOGICAL CHARACTERISTICS

Cobalt as cobalt chloride in a diet supplemented with vitamin B_{12} at a level of 25 micrograms per kg dry diet has a great effect on the haematological characteristics of the blood of carp (Table 6.11). The haemoglobin concentration and the erythrocyte

Table 6.9

Changes in Survival rate of carp fed cobalt chloride and vitamin B_{12} supplemented diet over a period of 24-weeks

Duration in weeks	Survival of Carp							
	Concentration of Cobalt Chloride in the diet, mg/kg dry diet							
	No. of fish	%	No. of fish	%	No. of fish	%	No. of fish	%
0	20	100	20	100	20	100	20	100
4	20	100	20	100	20	100	16	80
8	19	95	18	90	18	90	14	70
12	16	80	18	90	16	80	10	50
16	16	80	18	90	16	80	6	30
20	16	80	18	90	16	80	2	10
24	14	70	18	90	16	80	-	0

Table 6.10

Cranio-somatic (CSI), Hepato-somatic (HSI), Reno-somatic (RSI) and Viscero-somatic indices of carp fed diet supplemented with Cobalt Chloride and Vitamin B_{12} over a period of 24 weeks. Values given are the mean ± S.E. of the mean

	At the start of the Experiment			
	Concentration of cobalt in the diet, mg/kg			
	Control (0)	3	6	30
Number of samples	3	3	3	3
Weight of fish (g), mean ± S.E.	12.33 ± 0.20	8.87 ± 0.32	12.50 ± 0.50	8.67 ± 0.76
Brain weight (g); CSI	0.09 ± 0.01 0.73 ± 0.07	0.06 ± 0.005 0.72 ± 0.09	0.11 ± 0.02 0.88 ± 0.13	0.06 ± 0.02 0.65 ± 0.13
Liver weight (g); HSI	0.10 ± 0.01 0.81 ± 0.07	0.08 ± 0.003 0.94 ± 0.06	0.12 ± 0.03 0.98 ± 0.16	0.11 ± 0.02 1.00 ± 0.10
Kidney weight (g); RSI	0.06 ± 0.005 0.46 ± 0.04	0.05 ± 0.004 0.53 ± 0.08	0.07 ± 0.02 0.53 ± 0.10	0.05 ± 0.03 0.57 ± 0.23
Viscera weight* (g); VSI	0.56 ± 0.05 4.56 ± 0.34	0.44 ± 0.01 5.00 ± 0.06	0.56 ± 0.09 4.49 ± 0.50	0.49 ± 0.12 5.66 ± 0.61

	After 12 weeks of the Experiment			
	Concentration of cobalt in the diet, mg/kg			
	Control (0)	3	6	30
Number of samples	3	3	3	3
Weight of fish (g), mean ± S.E.	22.50 ± 1.10	30.60 ± 1.70	30.00 ± 1.10	24.50 ± 1.55
Brain weight (g); CSI	0.17 ± 0.08 0.75 ± 0.06	0.20 ± 0.05 0.64 ± 0.03[a]	0.16 ± 0.02 0.54 ± 0.03[b]	0.20 ± 0.06 0.80 ± 0.09
Liver weight (g); HSI	0.56 ± 0.06 2.50 ± 0.23	0.53 ± 0.04 1.72 ± 0.12	0.56 ± 0.07 1.85 ± 0.11	0.31 ± 0.09 1.25 ± 0.15
Kidney weight (g); RSI	0.08 ± 0.01 0.36 ± 0.04	0.10 ± 0.05 0.34 ± 0.31[a]	0.08 ± 0.03[b] 0.25 ± 0.09[b]	0.15 ± 0.09 0.61 ± 0.06
Viscera weight* (g); VSI	1.01 ± 0.05 4.50 ± 0.39	1.14 ± 0.33 3.72 ± 0.21[a]	1.33 ± 0.25 4.42 ± 0.04[a]	1.23 ± 0.70 5.00 ± 0.62

	At the termination of the Experiment			
	Concentration of cobalt in the diet, mg/kg			
	Control (0)	3	6	30**
Number of samples	3	3	3	2
Weight of fish (g), mean ± S.E.	35.00 ± 2.00	49.77 ± 1.37	45.40 ± 1.93	37.10 ± 2.99
Brain weight (g); CSI	0.17 ± 0.04 0.48 ± 0.08	0.26 ± 0.05 0.53 ± 0.03	0.18 ± 0.05[b] 0.39 ± 0.09[b]	0.12 ± 0.04 0.39 ± 0.07
Liver weight (g); HSI	0.75 ± 0.10 2.14 ± 0.15	0.74 ± 0.08 1.50 ± 0.21	0.85 ± 0.15 1.87 ± 0.26	0.60 ± 0.10 1.61 ± 0.14
Kidney weight (g); RSI	0.09 ± 0.03 0.26 ± 0.06	0.18 ± 0.04[b] 0.36 ± 0.07[b]	0.14 ± 0.05[b] 0.30 ± 0.10[b]	0.09 ± 0.01 0.28 ± 0.02
Viscera weight* (g); VSI	1.64 ± 0.19 4.69 ± 0.28	1.97 ± 0.02 3.95 ± 0.15[b]	2.39 ± 0.12 5.26 ± 0.09	1.66 ± 0.31 4.46 ± 0.49

Table 6.10 (cont'd.)

* = Gut, swim bladder, and the heart weight (g).

** = Samples were taken after 20 weeks of feeding

a = $P < 0.05$

b = $P < 0.01$

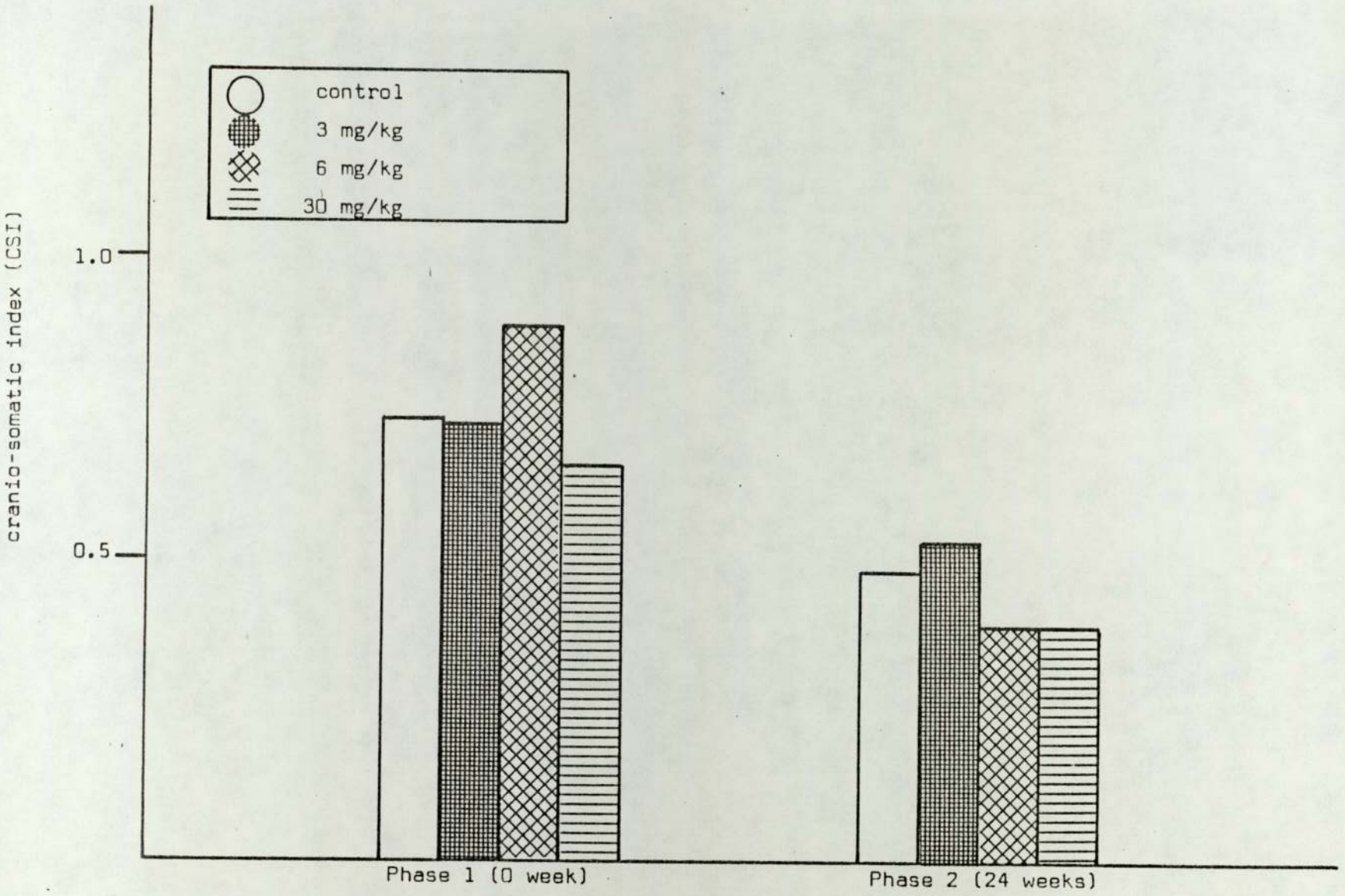


Figure 6.6. Effect of cobalt chloride with vitamin B_{12} on the cranio-somatic index in carp.

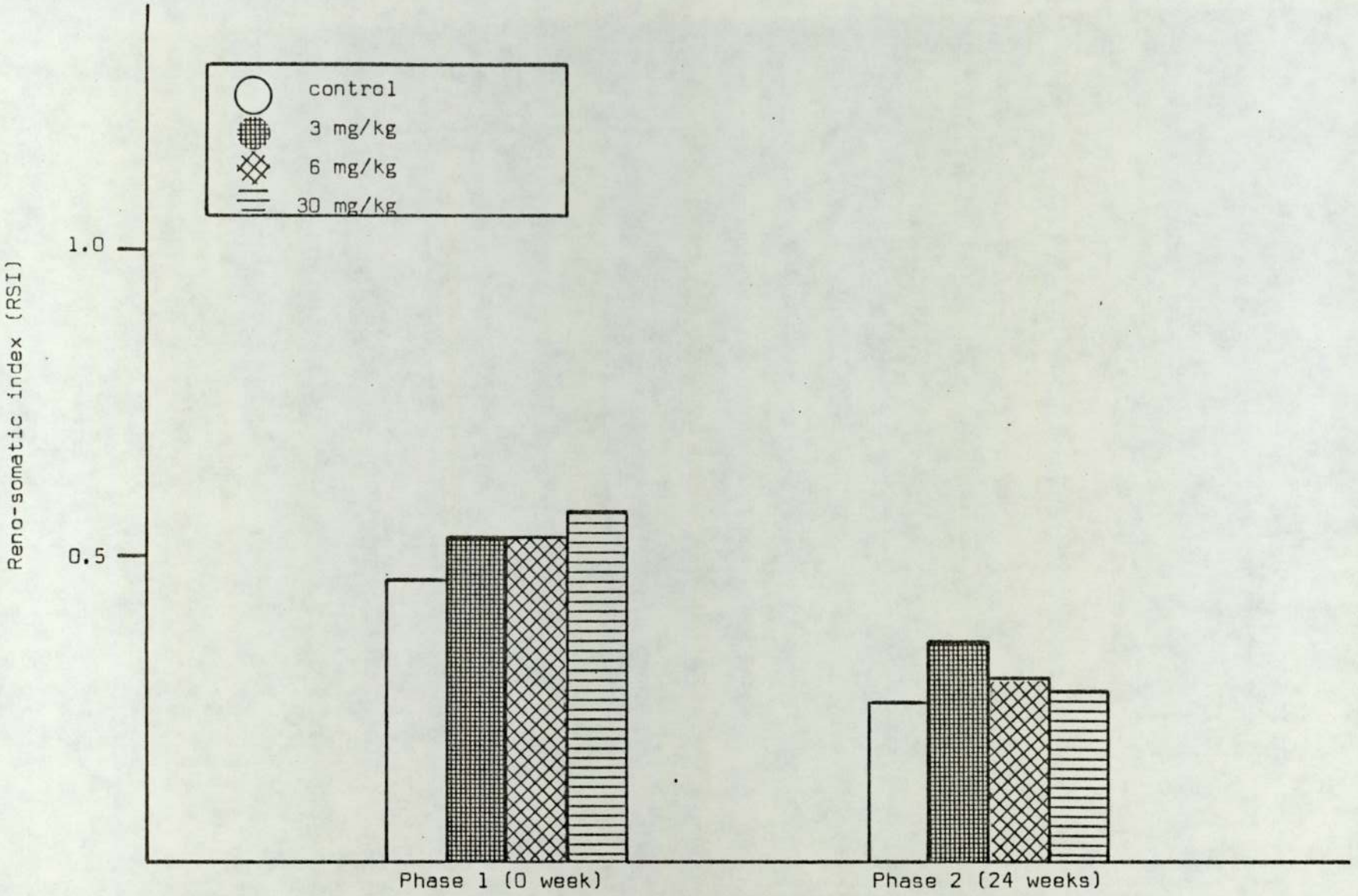


Figure 6.7. Effect of cobalt chloride with vitamin B_{12} on the reno-somatic index in carp.

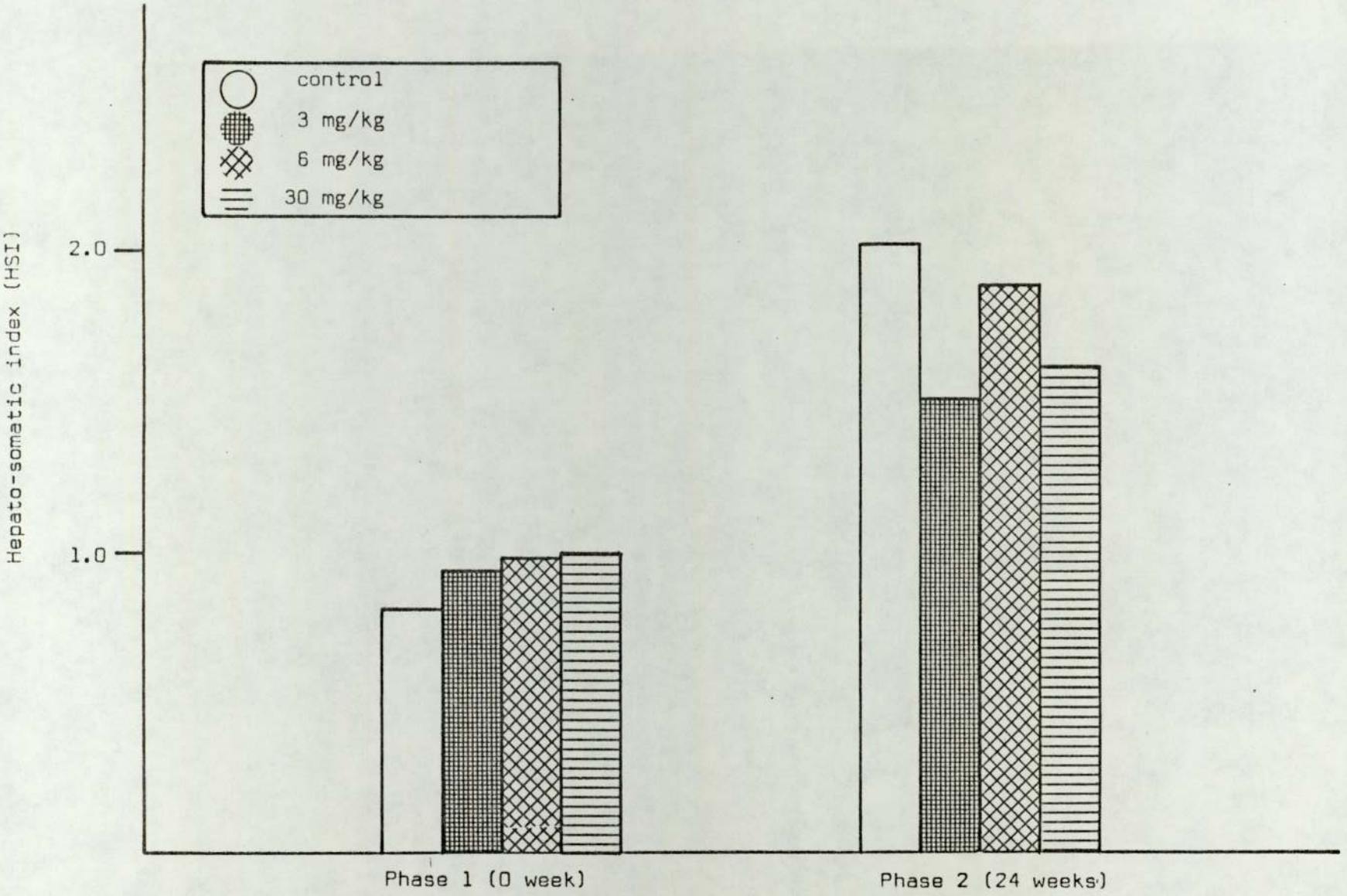


Figure 6.8. Effect of cobalt chloride with vitamin B_{12} on the hepato-somatic index in carp.

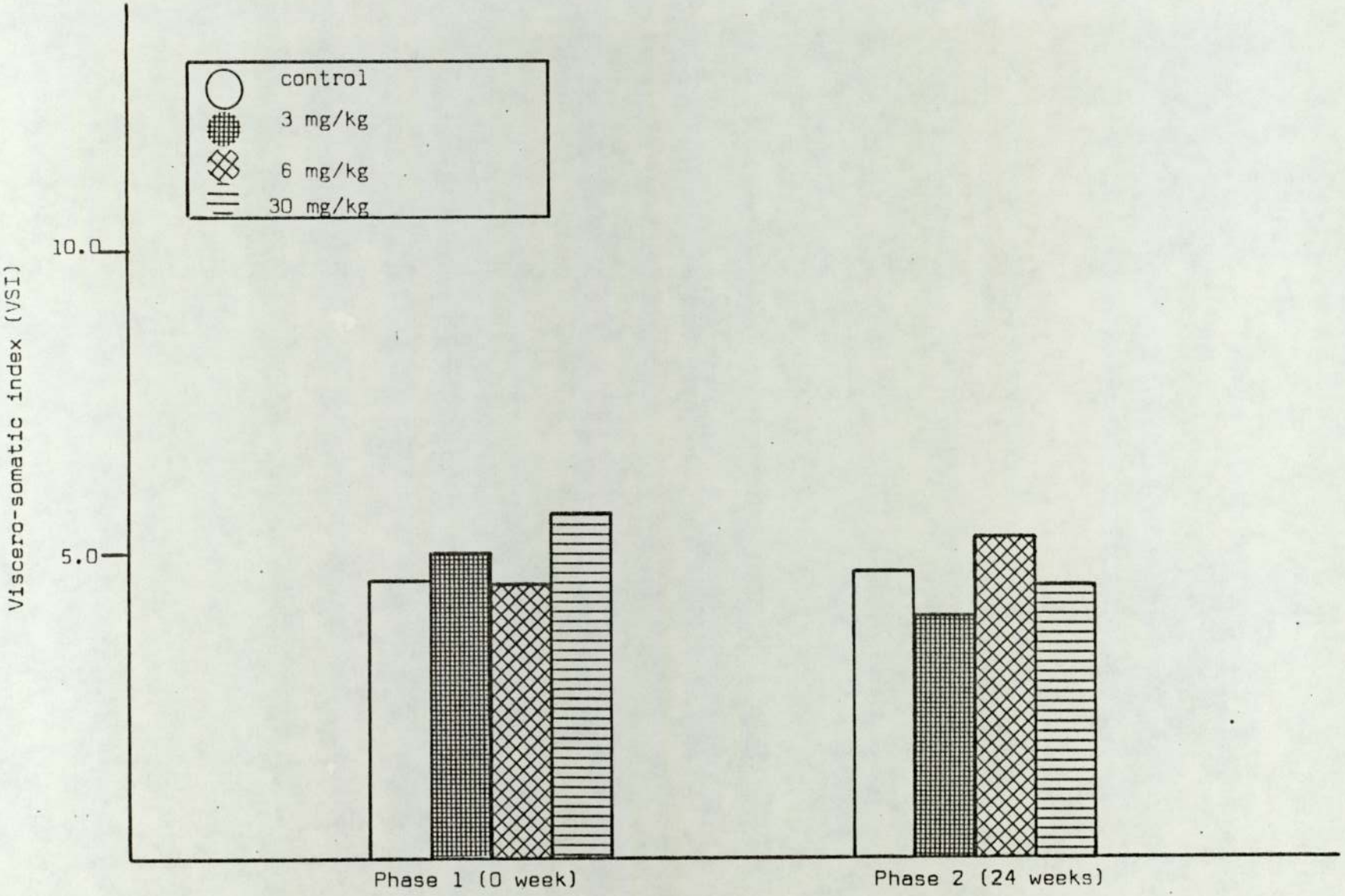


Figure 6.9. Effect of cobalt chloride with vitamin B_{12} on the viscero-somatic index in carp.

Table 6.11

Hematological indices of carp grown on cobalt-enriched diet supplemented with vitamin B_{12} over a period of 24 weeks, values given are the mean ± S.E.

At the end of the 12 weeks of feeding

Hematological indices	Concentration of cobalt in the diet, mg/kg			
	Control (0)	3	6	30
Hemoglobin Concentration (%)	30.10 ± 1.57	32.80 ± 1.50*	32.40 ± 1.78*	32.30 ± 1.90*
Erythrocyte (Thousands/cm^3)	1040.2 ± 75.80	1350.7 ± 85.70**	1371.61 ± 90.15**	1122.55 ± 77.90**
Leucocytes (Thousands/cm^3)	25.3 ± 1.30	20.10 ± 0.95*	20.20 ± 1.15*	17.15 ± 1.20**

* = $P < 0.05$
** = $P < 0.01$

At the termination of the Experiment

Hematological indices	Concentration of cobalt in the diet, mg/kg			
	Control (0)	3	6	30
Hemoglobin Concentration (%)	32.40 ± 2.40	36.80 ± 2.15**	36.25 ± 8.10**	35.30 ± 1.95**
Erythrocytes (Thousands/cm^3)	1220.6 ± 80.40	1811.8 ± 75.25**	1785.8 ± 95.90**	1780.3 ±150.20**
Leucocytes (Thousands/cm^3)	42.50 ± 1.50	22.60 ± 0.85**	20.70 ± 0.90**	17.15 ± 0.75

*Samples taken after 20 weeks of feeding
** = $P < 0.01$

count increased in all groups, including the controls; the increase was greatest in the 3 mg cobalt group (Figures 6.10 and 6.11). The leucocyte count increased considerably in the controls, but only slightly in the cobalt treated groups (Figure 6.12), the increase becoming less as the dose increases. These results suggest that cobalt plus vitamin B_{12} promotes production of erythrocytes but inhibits leucocyte formation.

6.3.5 BODY COMPOSITION

Body analyses, shown in Table 6.12, indicate that there is an increase in the total body protein in all treated groups compared with the controls. Body protein was highest in the group receiving 3 mg cobalt chloride with 25 micrograms of Vitamin B_{12} per kg diet than in the other treated groups.

The total ash content of the treated groups also increased, this increase being in proportion to the amount of cobalt present in the diet. There was little difference in the total water content of the body between the treated groups and the controls. Fat in the body is slightly increased in all the groups.

6.3.6 COBALT CONTENT OF THE BODY AND ITS ORGANS

The body content of cobalt in carp fed diets supplemented with cobalt as cobalt chloride and vitamin B_{12} is shown in Table 6.13; it increased both with time and with the amount of cobalt in the diet. The distribution of cobalt within the fish was uneven (Table 6.14); it increased in the order muscle < liver < kidney, and also with time and with the amount of cobalt in the diet (Figures 6.14, 6.15 and 6.16). There was a direct relationship between the amount of cobalt given to the fish in their diet and the amount stored in their body (Figure 6.13).

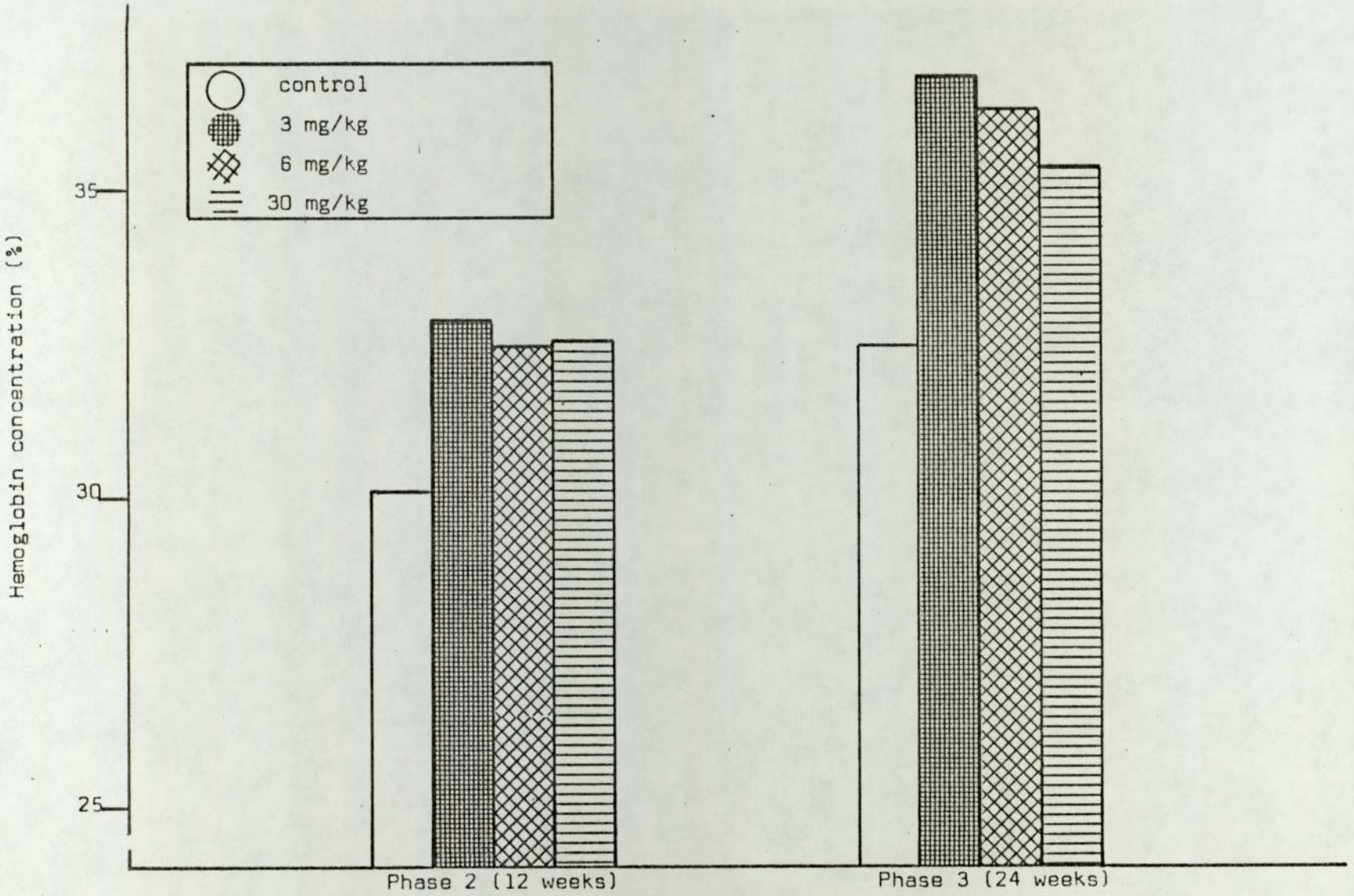


Figure 6.10. Effect of cobalt chloride with vitamin B_{12} on the hemoglobin percentage in carp.

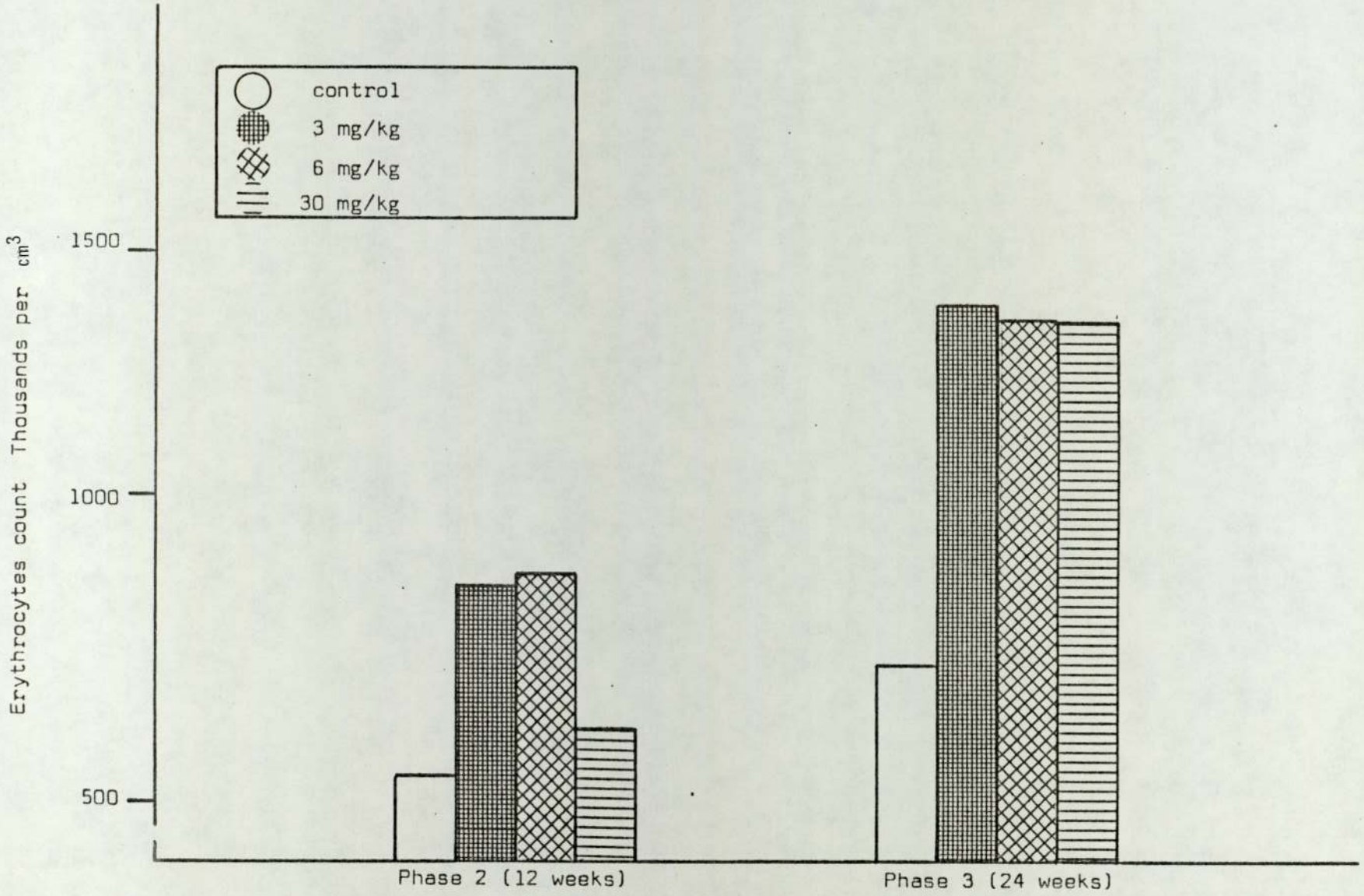


Figure 6.11. Effect of cobalt chloride and vitamin B_{12} on the erythrocytes count in carp.

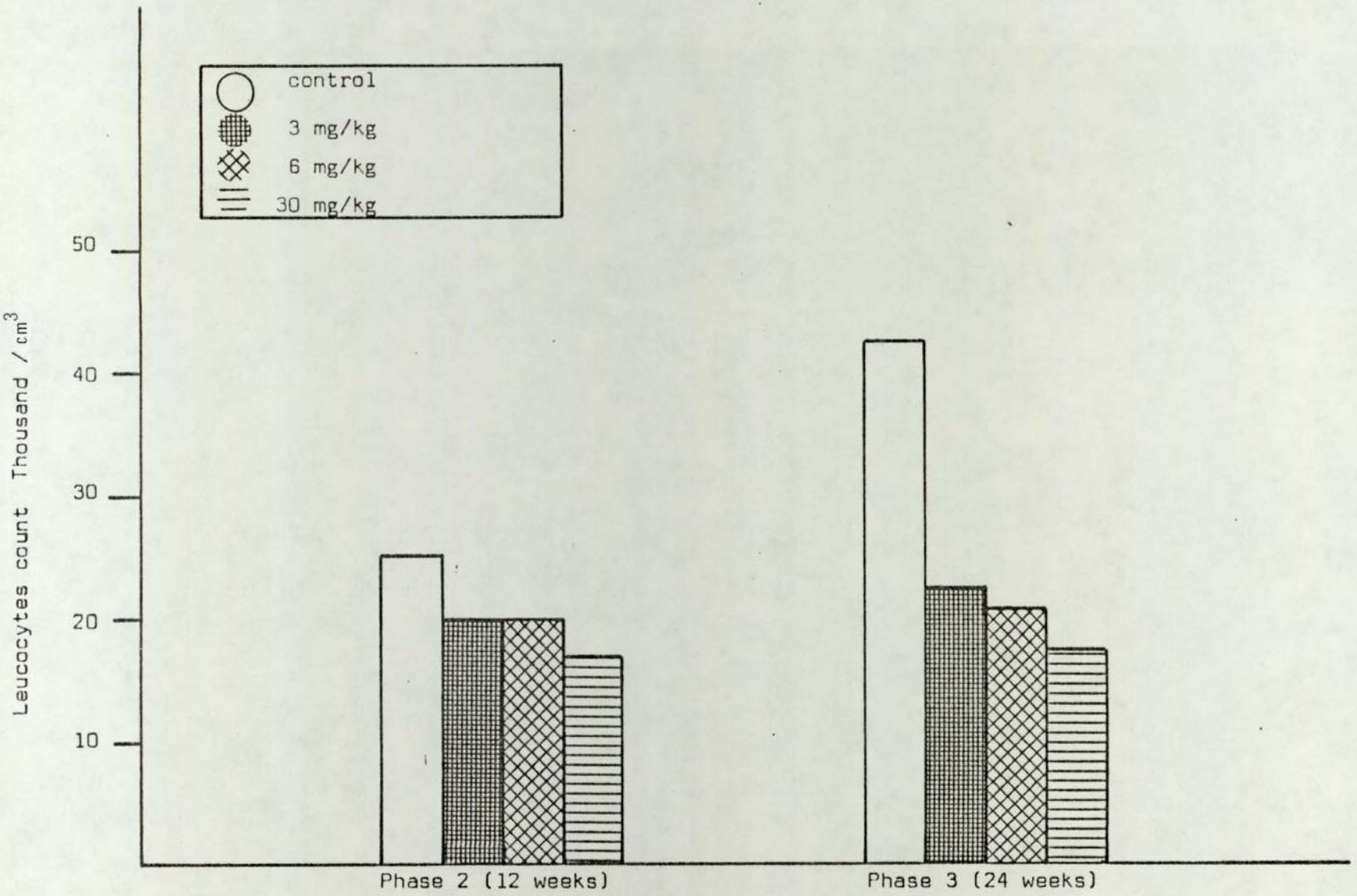


Figure 6.12. Effect of cobalt chloride with vitamin B_{12} on the Leucocytes count in carp.

Table 6.12

Body Composition of carp fed diet supplemented with cobalt chloride and vitamin B_{12} for a period of 24 weeks. Values given are the mean of 3 fish ± S.E. of the mean

At the end of 12 weeks of feeding

Body Composition %	Concentration of cobalt in the diet, mg/kg			
	Control (0)	3	6	30
Water	78.40 ± 2.50	75.80 ± 3.00	75.50 ± 2.70	75.80 ± 2.50
Protein	9.50 ± 1.50	13.20 ± 1.65**	13.20 ± 1.55**	12.70 ± 1.95**
Fat	4.10 ± 0.55	5.30 ± 0.65	5.80 ± 0.70	5.20 ± 0.60
Ash	2.50 ± 0.25	2.70 ± 0.31	2.90 ± 0.25**	2.60 ± 0.33**
NFE* + Fibre	5.5 ± 1.50	3.00 ± 1.70	3.10 ± 1.63	3.20 ± 1.55

*Nitrogen free extract (NFE).

At the Termination of the Experiment

Body Composition %	Concentration of cobalt in the diet, mg/kg			
	Control (0)	3	6	30
Water	75.20 ± 1.50	75.00 ± 2.20	75.50 ± 1.85	76.00 ± 1.55
Protein	9.15 ± 1.33	15.70 ± 2.35**	14.00 ± 2.70**	14.15 ± 2.31**
Fat	4.85 ± 0.98	5.80 ± 1.10	6.30 ± 1.40	6.20 ± 1.75
Ash	2.65 ± 0.35	2.95 ± 0.25	3.00 ± 0.42**	3.50 ± 0.56**
NFE* + Fibre	8.15 ± 0.95	0.55 ± 1.25	1.20 ± 1.30	0.15 ± 1.33

*Nitrogen free extract (NFE).

** = $P < 0.05$

Table 6.13

Cobalt concentration of carp fed diet supplemented with cobalt chloride and vitamin B_{12} for a period of 24 weeks. Values given are the mean of 3 fish ± S.E. of the mean

Concentration of cobalt chloride in the diet, mg/kg	Concentration of cobalt in carp, nanograms/kg dry matter	
	After 12 weeks of the Experiment	After 24 weeks of the Experiment
Control (0)	0.28 ± 0.10	0.35 ± 0.09
3	2.50 ± 0.57	2.70 ± 0.45
6	2.80 ± 0.41	2.95 ± 0.31
30	3.50 ± 0.67	3.85 ± 0.45

Table 6.14

Cobalt Concentration in Carp tissues and organs, fed diet supplemented with Cobalt Chloride and Vitamin B_{12} for a period of 24 weeks, values given are the mean of 3 fish ± S.E. of the mean

After 12 weeks of the Experiment				
Concentration of cobalt in carp tissues and organs, nanogram/kg dry matter	Concentration of cobalt in the diet, mg/kg			
	Control (0)	3	6	30
Muscle	0.50 ± 0.14	5.20 ± 1.22*	7.10 ± 2.15**	8.20 ± 1.20**
Liver	4.50 ± 2.30	35.10 ± 10.20**	40.20 ± 20.30**	55.10 ± 35.40**
Kidney	50.50 ± 15.20	155.70 ± 60.50**	253.20 ± 70.40**	451.10 ± 100.55**

After 24 weeks of the Experiment				
Concentration of cobalt in carp tissues and organs, nanogram/kg dry matter	Concentration of cobalt in the diet, mg/kg			
	Control	3	6	30
Muscle	0.90 ± 0.15	7.50 ± 2.06**	9·60 ± 3.15**	10.15 ± 3.45**
Liver	9.50 ± 2.30	56.70 ± 20.60**	60.56 ± 15.30**	89.33 ± 40.10**
Kidney	60.40 ± 10.20	200.100± 70.70**	300.200± 55.15**	500.65 ± 80.33**

* = P <0.05

** = P <0.01

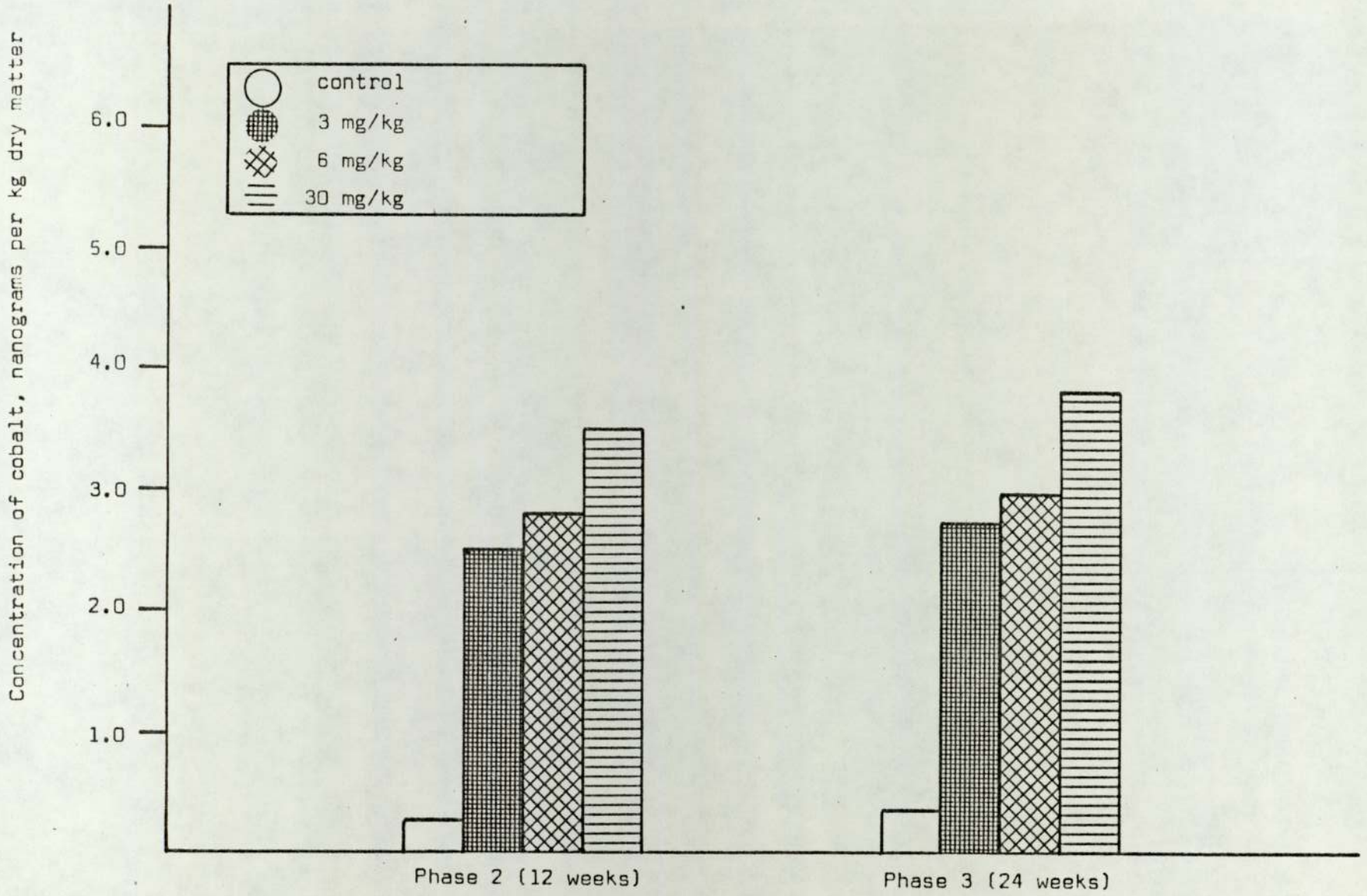


Figure 6.13. Concentration of cobalt in carp fed diet supplemented with cobalt chloride and vitamin B_{12}.

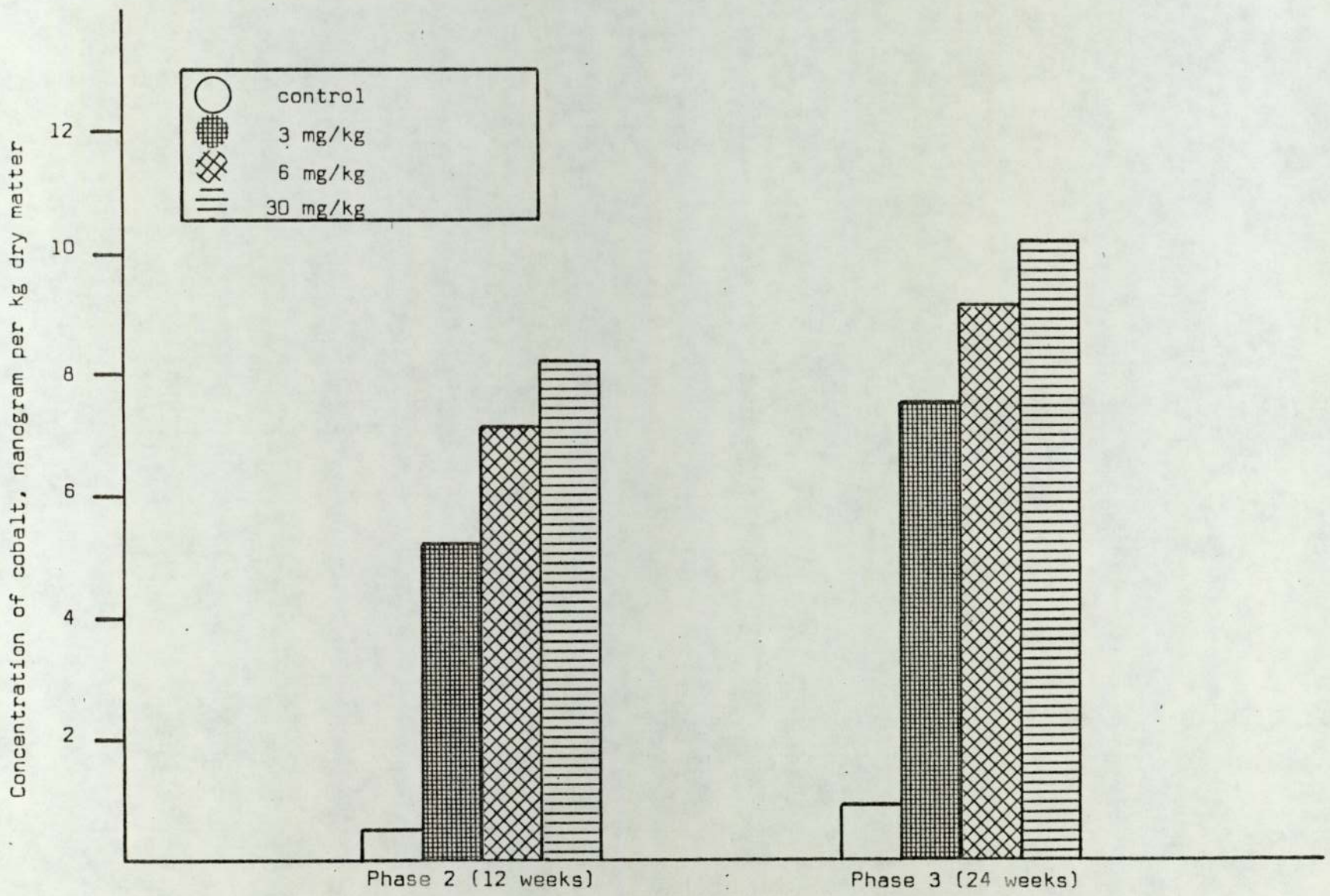


Figure 6.14. Concentration of cobalt in carp muscles.

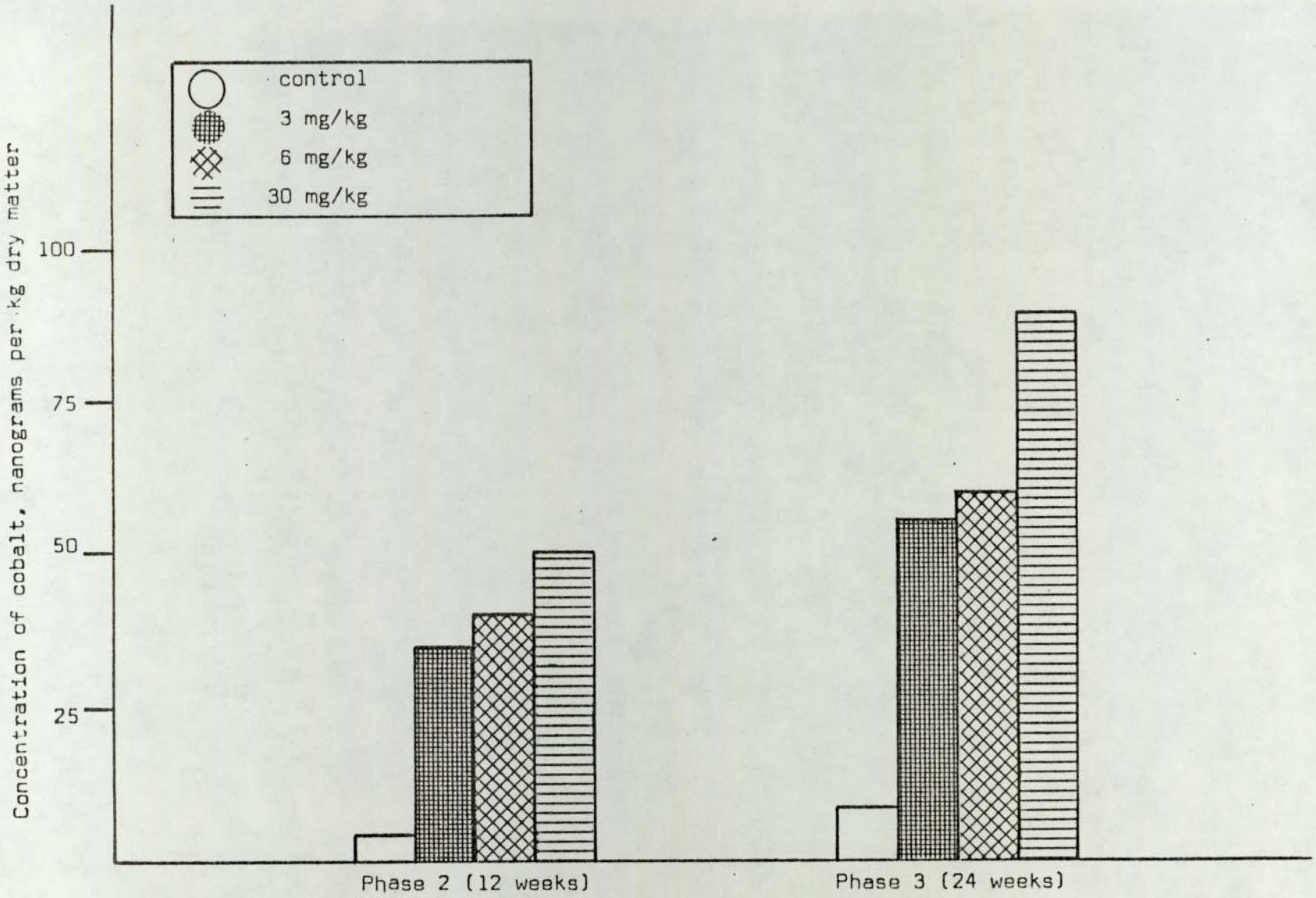


Figure 6.15. Concentration of cobalt in carp liver.

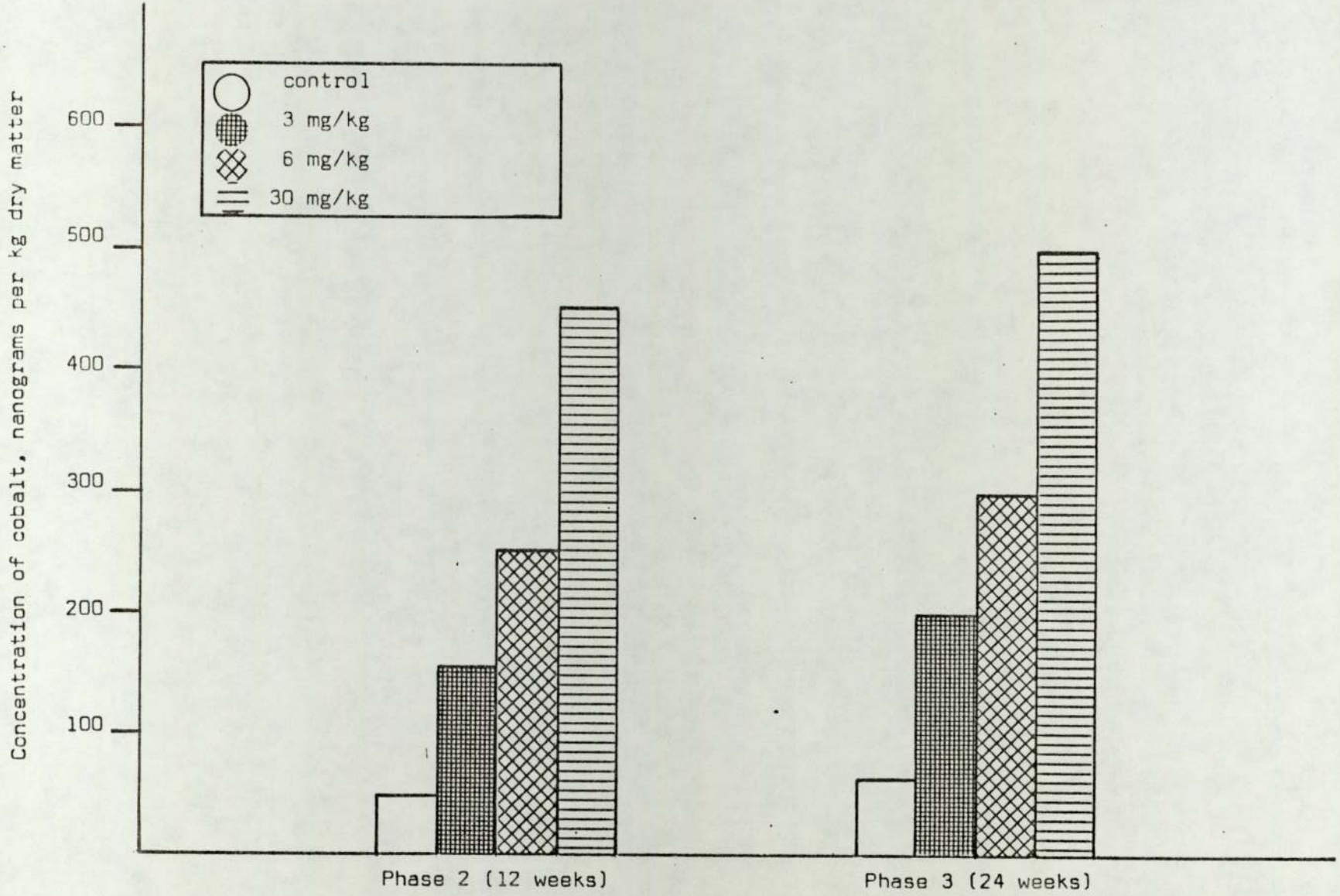


Figure 6.16. Concentration of cobalt in carp kidney

6.3.7 DEFICIENCY SYMPTOMS

Carp which were fed diet supplemented with vitamin B_{12} alone, without cobalt (controls), exhibited malformation in their tail and head which developed in almost 30% of the fish receiving basal diet without cobalt. Malformation occurred in control group 8 weeks after starting the experiment. Their appetite and activity was less than those in the treated groups (Figure 6.17).

6.4 DISCUSSION AND CONCLUSIONS

From the results of the present study one may conclude that the addition of cobalt as cobalt chloride to a diet supplemented with vitamin B_{12} enhanced the growth and the survival rate of carp; survival rate declined below that of controls when the dose of cobalt was 30 mg/kg dry diet. These findings agree with the results obtained by Das and Krishamurthy, 1959; Trofimora, 1962; who showed that vitamin B_{12} strengthens resistance to disease in trout fingerlings and increases their weight above that of controls. Das (1960a) suggested that there are certain factors in the B-Complex which may play an important role in the balance of metabolic processes during the first stage of fish life, but with increasing age, they become less effective. When an extract of ruminant stomach (which is known to contain micro-organisms that synthesise vitamin B_{12}) was given to carp along with cobalt nitrate, it yielded beneficial results; and the survival of the fish was enhanced (Das, 1960b, 1961, 1967). The same results were obtained by Sukhoverkhov et al. (1961) and Tomantik and Batyr (1965).

The results obtained in this study showed that the optimal concentration of cobalt with 25 micrograms of vitamin B_{12} per kg dry diet is 3 mg cobalt chloride per kg dry diet. The presence

Figure 6.17. Malformation in carp reared on vitamin B_{12} enriched diet without cobalt (control)

of vitamin B_{12} alone in the diet does not help to prevent deficiency symptoms (malformations).

With this combination of cobalt and vitamin B_{12}, the haematological characteristics of carp blood are improved, apart from the leucocyte count, agreeing with the results obtained by Rimsh (1963), Karpanin et al. (1961), Frolova (1961) and Shabalina (1963).

There was an increase in total ash content of the body in all treated groups, in direct proportion with the amount of cobalt present in the diet. The distribution of cobalt within the fish was uneven; it accumulated in the kidney more than the other organs examined (liver and muscle), and the amounts of cobalt stored in the organs had a direct relationship to the amount of cobalt present in the diet. These results agree with the findings of Bican (1977) and Harms and Kunze (1977).

CHAPTER SEVEN

EFFECT OF DIET SUPPLEMENTED WITH COBALT SALT ON THE RESISTANCE OF CARP (*Cyprinus carpio*), TO WINTERING (LOW WATER TEMPERATURE) UNDER LABORATORY CONDITIONS

7.1 INTRODUCTION

There is a great deal of speculation about Cobalt salts and their role in controlling fish survival. Das, 1960[a], suggested that among the environmental factors influencing survival and growth, nutrition may be expected to play an important role. He also added that survival during the early period of life in fish can be enhanced by treatment with cobalt nitrate and ruminant stomach extract. Shabalina (1966) indicated that cobalt has a great effect on reducing winter mortality in rainbow trout from 80% to about 20% when added to the water. The same results were found by Karpanin and Ushakov (1961).

The present experiment was carried out to examine the effect of cobalt treatment on survival and growth of carp fingerlings under different water temperatures.

7.2 MATERIALS AND METHODS

7.2.1 EXPERIMENTAL SYSTEM AND ANIMALS

The experimental facilities used in the present investigation were system 3 described in detail in Chapter 4(4.2). 24 fingerling carp, Cyprinus carpio (5.00 ± 0.55) cm, were obtained from Cotswold Carp Farm, Bourton-on-the Water, Gloucestershire. The fish were subjected to quarantine and prophylaxis, as described in Chapter 4 (4.5). Then they were transferred to a tropical room and kept in a recycling system (System 1) as described in Chapter 4 (4.2). Six fish of an appropriate size (5.9 cm) were selected and transferred to 4 of the 4 litre glass tanks of System 3. Tap water was used throughout this experiment; the estimation of dissolved gases and trace elements in water is shown in Table 7.1. The density of stocking was found to be 1.3 g per litre. The

Table 7.1

Estimation of Dissolved Gases and Trace Minerals in Water

Substance	Concentration in Water (ppm)
Oxygen (O_2)	8 - 10
Na^+	6
K^+	1
Ca^{++}	8
Mg^{++}	1
Mn^{++}	<0.1
Fe^{+++}	<0.1
Cl^-	10
NO_3^{-2}	2
SO_4^{-2}	4
PO_4^{-3}	0.5
$(NH_4)^+$	0.05
Cu	<0.05
Co	<0.05
I	<0.05

experiment was carried on for a period of 4 months; throughout this time pH and dissolved oxygen were checked daily using a pH meter and the Winkler method. They were found to range from pH 6.9 - 7.2 and 8 - 10 ppm respectively. Photoperiod was controlled at 12 hours day and 12 hours night throughout the experiment.

7.2.2 THE EXPERIMENTAL DIET

Formulation of the diet was carried out by the general procedure described in Chapter 4 (4.4). The ingredients used for the formulation of the diet are the same as those presented in Table 6.1, Chapter 6 (6.2.2).

7.2.3 FEEDING RATE

The fish were fed three times per day, 10 am, 2 pm, 6 pm, except for Sunday when they were fed twice a day, 10 am, 6 pm. Each feeding continued for 15 minutes; the fish were fed at a rate that allowed them to consume the food as it feel through the water. All groups were fed only the amount they would consume within the 45 minutes total feeding period, and the amount of the remaining food was weighed and deducted from the daily ration.

7.2.4 WEIGHING AND SAMPLING

Details of the weighing procedure are presented in Chapter 4 (4.7). The fish were individually tagged and weighed to the nearest $\pm$ 0.01 g , under anaesthesia, after 12 hours starvation every 15 days for 120 days; at the end of this period three fish were removed from each group for proximate carcass analysis.

7.2.5 STATISTICAL METHODS AND ANALYSIS OF GROWTH DATA

These were performed as detailed in Chapter 4 (4.9 and 4.10).

7.3 RESULTS

7.3.1 ACCELERATION OF GROWTH

Weight and Length

The weight and length data accumulated over a period of 120 days is presented in Tables 7.2 and 7.3. All groups administered cobalt chloride increased both in weight and length faster than controls throughout the experiment even when the temperature was $5^{o}C$ (Figures 7.1 and 7.2).

At the end of the first 30 days, when the water temperature was $30^{o}C$, the fish receiving 3, 6, and 30 mg cobalt chloride per kg dry diet were 167%, 24% and 9% heavier than controls (Table 7.4 and Figure 7.3); they were 27%, 10% and 5% longer than the respective controls. In the next 30 days, when the water temperature was $20^{o}C$, the corresponding figures were 177%, 54% and 13% (heavier) and 26%, 8% and 0.8% (longer). At the end of 90 days, when the water temperature was $10^{o}C$, the fish receiving 3, 6 and 30 mg cobalt chloride were 294%, 125% and 6% heavier and were 30%, 10% and 10% longer than controls. At the termination of the experiment, when the water temperature was $5^{o}C$, all the treated groups were heavier (nearly treble in weight in some cases) and longer than the controls (Table 7.5 and Figure 7.4).

Growth Rate

In phase 1 of the experiment, when the fish were kept at $30^{o}C$ (first 30 days), the daily growth rate was greater over controls in all treated groups, but for the period extending from 31-60 days, phase 2, this increase in weight was more pronounced in the group receiving 6 mg cobalt chloride than the other treated groups; for the period from 61 - 90 days, phase 3, the daily growth

Table 7.2

Changes in Body Weight of Carp fed Cobalt Chloride Supplemented diet under different Water Temperatures. Values given are the mean of five fish ± S.E. of the mean

Duration in Days	Temperature in °C	Concentration of Cobalt Chloride in the Diet, mg per kg dry diet			
		Control (0)	3	6	30
0	30	3.00 ± 0.24	2.50 ± 0.14	3.20 ± 0.05	3.10 ± 0.10
15	30	4.35 ± 0.20	5.00 ± 0.26	5.10 ± 0.15	4.50 ± 0.23
30	30	6.50 ± 0.26	8.70 ± 0.30	7.70 ± 0.20	7.00 ± 0.28
45	20	7.30 ± 0.35	9.70 ± 0.35	9.10 ± 0.35	8.00 ± 0.40
60	20	8.50 ± 0.40	11.50 ± 0.46	10.80 ± 0.48	9.20 ± 0.64
75	10	8.70 ± 0.45	13.00 ± 0.57	12.30 ± 0.67	10.80 ± 0.77
90	10	9.20 ± 0.55	15.00 ± 0.78	13.80 ± 0.70	11.40 ± 0.98
105	5	9.80 ± 0.60	15.60 ± 0.98	14.00 ± 0.87	11.90 ± 1.10
120	5	9.80 ± 0.76	16.50 ± 1.51	15.00 ± 1.26	12.00 ± 1.30

Table 7.3

Changes in Body Length of Carp fed Cobalt Chloride Supplemented Diet under Different Water Temperatures. Values given are the mean of five fish ± S.E. of the mean

Duration in Days	Temperature in °C	Concentration of Cobalt Chloride in the Diet, mg per kg dry diet			
		Control (0)	3	6	30
0	30	6.00 ± 0.07	5.70 ± 0.04	6.00 ± 0.04	6.00 ± 0.05
15	30	6.20 ± 0.12	6.50 ± 0.13	6.30 ± 0.12	6.30 ± 0.13
30	30	7.20 ± 0.15	8.40 ± 0.26	8.00 ± 0.22	7.50 ± 0.26
45	20	7.88 ± 0.25	8.80 ± 0.42	8.30 ± 0.50	8.00 ± 0.46
60	20	8.26 ± 0.47	9.30 ± 0.75	9.00 ± 0.62	8.30 ± 0.53
75	10	8.37 ± 0.66	9.70 ± 0.99	9.20 ± 0.90	8.80 ± 0.78
90	10	8.50 ± 0.55	9.80 ± 0.95	9.40 ± 0.77	9.10 ± 0.85
105	5	8.90 ± 0.73	10.00 ± 1.10	9.60 ± 0.80	9.20 ± 0.87
120	5	8.90 ± 0.71	10.10 ± 1.25	9.70 ± 0.86	9.30 ± 0.92

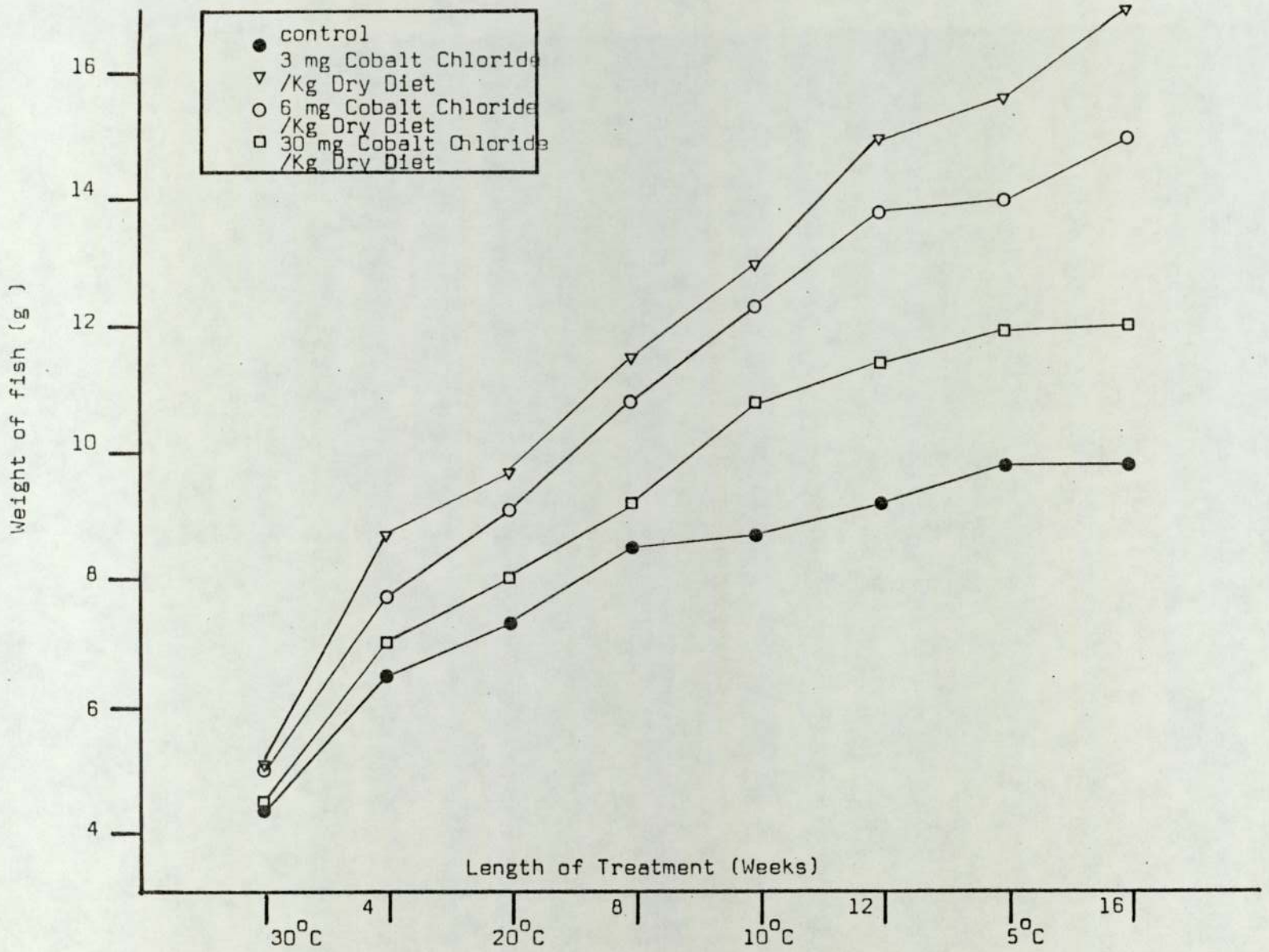


Figure 7.1 Effect of cobalt chloride in Body Weight of carp under different water temperatures.

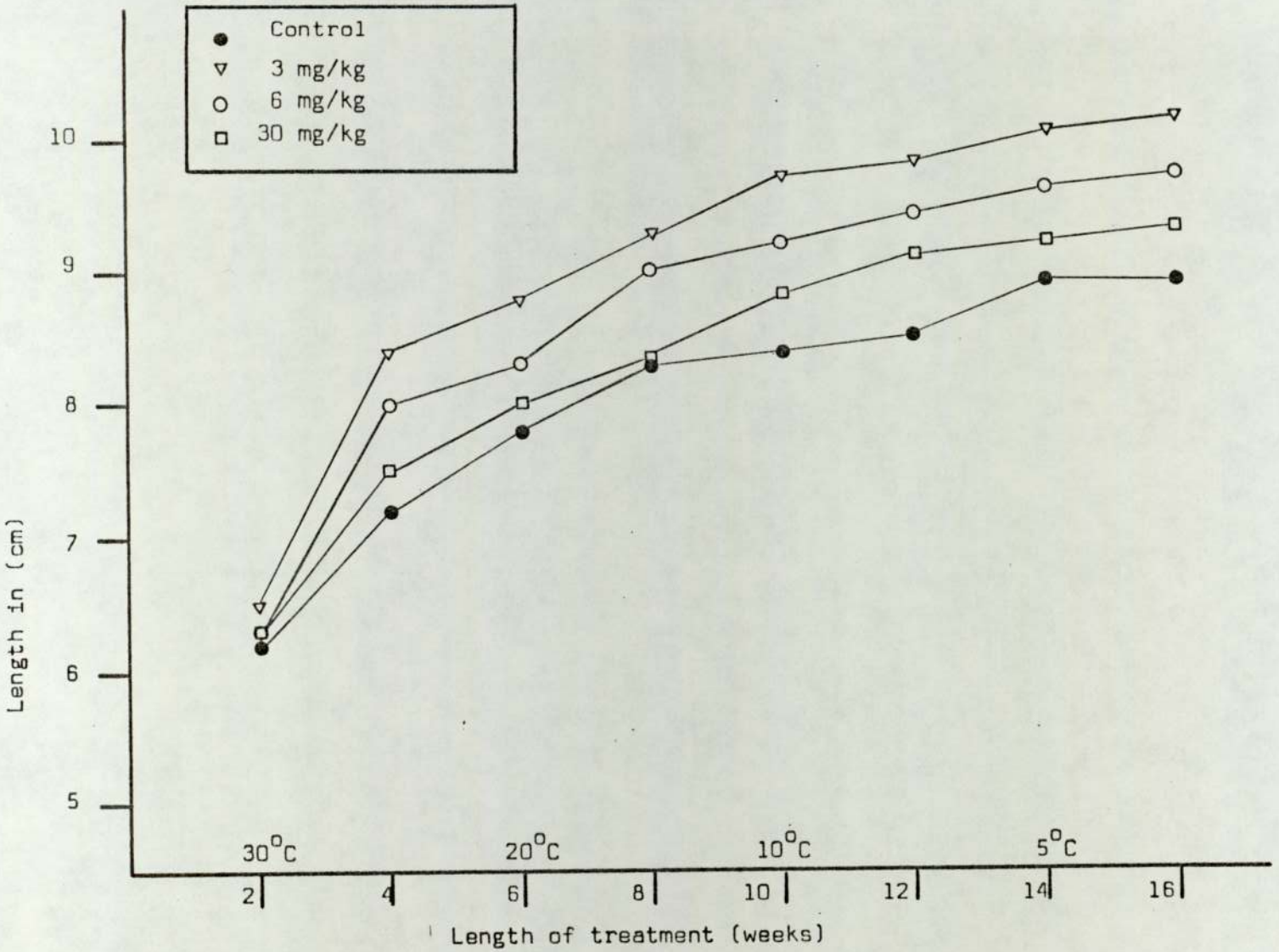


Figure 7.2. Effect of cobalt chloride on Body Length of carp under different water temperatures.

Table 7.4

Percent Weight Gain over Controls of Carp fed Cobalt Chloride Supplemented diet under different Water Temperatures

Duration in Days	Temperature in °C	Percent Weight Gain Over Controls %		
		Concentration of Cobalt Chloride in the Diet, mg per kg dry diet		
		3	6	30
0	30	-	-	-
15	30	55	14	0.16
30	30	167	24	9
45	20	145	41	15
60	20	177	54	13
75	10	230	94	58
90	10	294	125	61
105	5	297	111	58
120	5	333	143	61

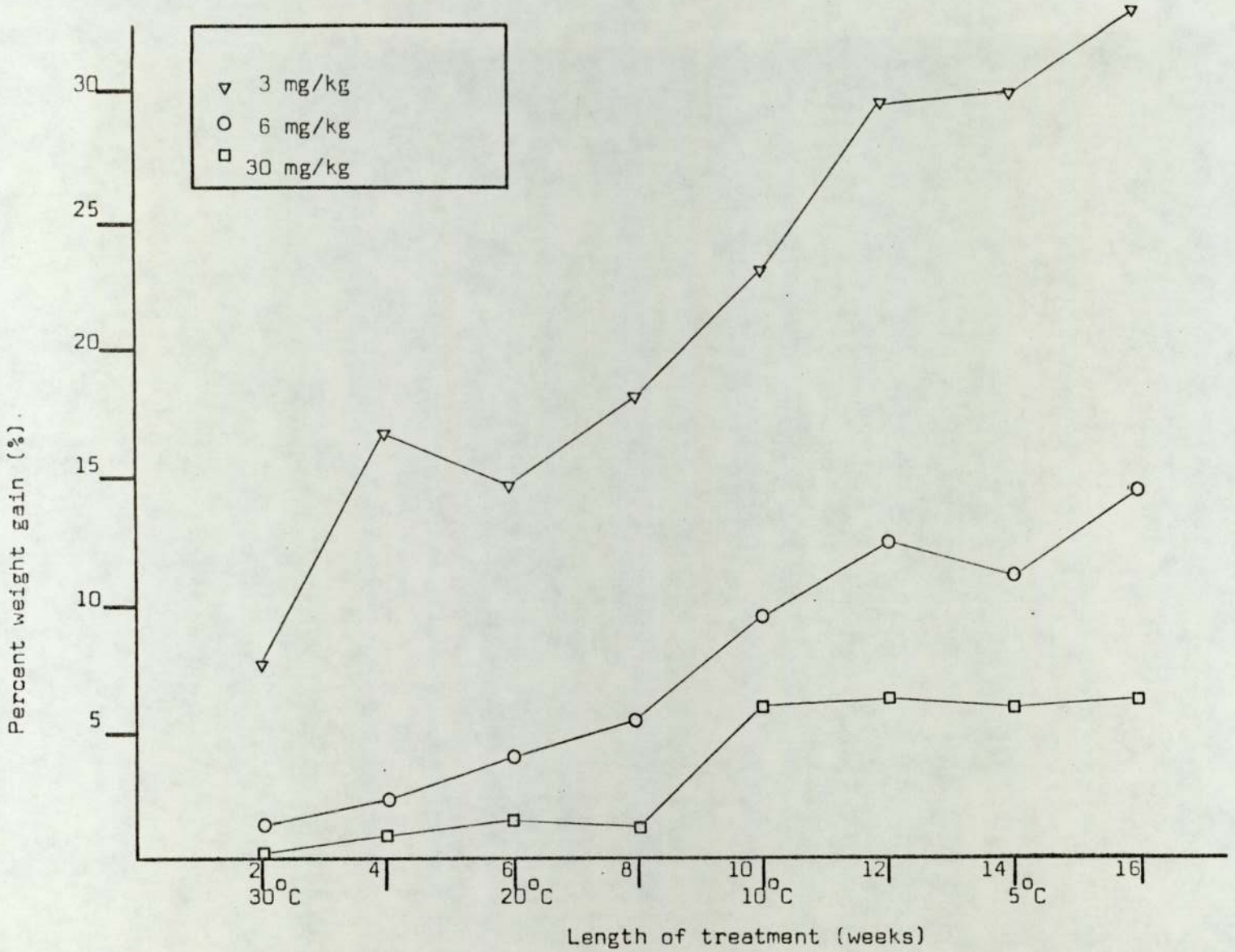


Figure 7.3. Effect of cobalt chloride on the percentage increase over controls in weight of carp.

Table 7.5

Percent Length Gain over Controls of Carp fed Cobalt Chloride Supplemented Diet under Different Water Temperatures

Duration in Days	Temperature in °C	Percent Length Gain Over Controls %		
		Concentration of Cobalt Chloride in the Diet, mg per kg dry diet		
		3	6	30
0	30	-	-	-
15	30	11	2	2
30	30	27	10	5
45	20	23	3	2
60	20	26	8	0.8
75	10	31	9	7
90	10	30	10	10
105	5	27	7	5
120	5	29	8	7

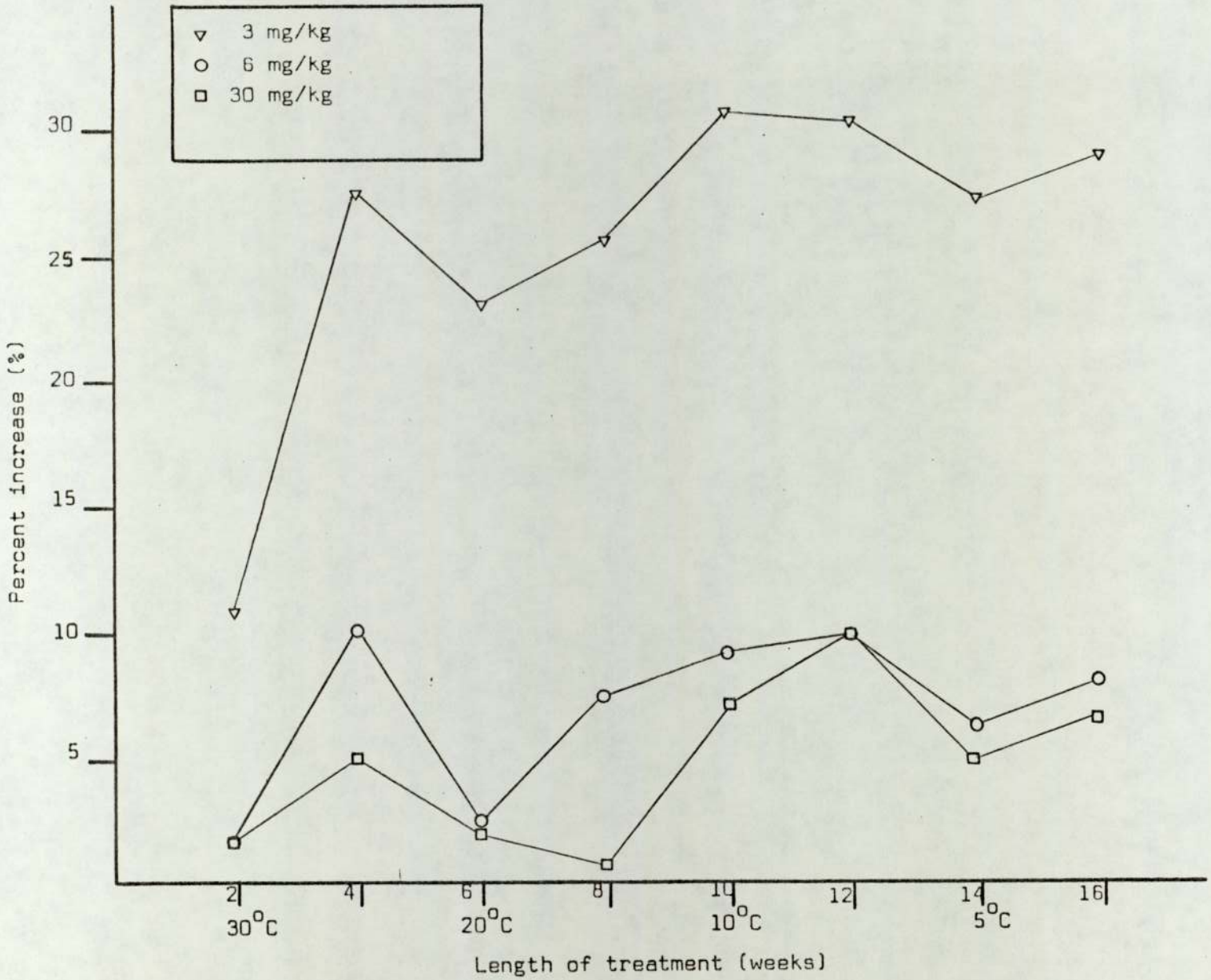


Figure 7.4. Effect of cobalt chloride on the percentage increase over controls in length of carp.

rate was still greater than controls in the treated groups, and was more pronounced in the group receiving 3 mg cobalt chloride. For the period from 91 - 120 days, phase 4, the increase in weight per day between the controls and the group receiving 30 mg cobalt chloride was narrow compared to the difference between the controls and the groups receiving 3 and 6 mg cobalt chloride (Table 7.6).

As far as the increase in length per day is concerned, the treated fish exhibited an increase in length in the first 90 days, phases 1 and 2, but in phases 3 and 4 the increase was much less (Table 7.7).

Condition Factor

At the start of the experiment the mean values of the parameter (a), which related the weight to the length (Condition Factor), extended among all groups from 0.014 to 0.015. For the first phase of the experiment all groups including the controls showed a rise in the condition factor which was more pronounced in the controls. After this initial rise the condition factor declined in controls for the next 30 days. Thereafter, it rose again for the groups receiving 3 and 6 mg cobalt chloride in their diet, but it continued to fall for the controls and the group receiving 30 mg cobalt chloride (Table 7.8 and Figure 7.5).

Food Utilization Efficiency (FUE)

Table 7.9 and Figure 7.6 shows the ratios of total food consumed by the fish to the wet body weight. It is clear that there was a considerable decrease in the ratio as the water temperature declined; at the same time it seems that the treated fish were less affected than the controls. There was a considerable decline in the total food intake per day as the water temperature

Table 7.6

Effect of Oral Administration of Cobalt Chloride on Daily increase of Body Weight of carp kept under different Water Temperatures

Period in Days	Temperature in ^{o}C	Daily Increase of mean Body Weight, g per 100 g initial weight			
		Concentration of Cobalt Chloride in the Diet, mg per kg dry diet			
		Control (0)	3	6	30
0 - 30	30	4.5	9.5	5.4	4.5
31 - 60	20	1.2	1.2	1.5	1.2
61 - 90	10	0.3	1.2	1.1	0.9
91 - 120	5	0.2	0.4	0.3	0.2

Table 7.7

Effect of Oral Administration of Cobalt Chloride on Daily Increase of Body Length of Carp kept under Different Water Temperatures

Period in Days	Temperature in °C	Daily Increase of Mean Body Length, cm per 100 cm Initial Length			
		Concentration of Cobalt Chloride in the Diet, mg per kg dry diet			
		Control (0)	3	6	30
0 - 30	30	0.8	1.8	1.3	1.0
31 - 60	20	0.6.	0.4	0.5	0.4
61 - 90	10	0.1	0.2	0.2	0.4
91 - 120	5	0.2	0.1	0.2	0.1

Table 7.8

Effect of Oral Administration of Cobalt Chloride on the Condition Factor of Carp kept under Different Water Temperature. Values Given are the Mean ± S.E.

Duration in Days	Temperature in °C	The Condition Factor			
		Concentration of Cobalt Chloride in the Diet, mg per kg dry diet			
		Control (0)	3	6	30
0	30	0.014 ± 0.002	0.015 ± 0.002	0.015 ± 0.003	0.014 ± 0.002
30	30	0.017 ± 0.003	0.015 ± 0.003	0.015 ± 0.002	0.016 ± 0.002
60	20	0.015 ± 0.004	0.015 ± 0.002	0.015 ± 0.003	0.016 ± 0.003
90	10	0.015 ± 0.003	0.016 ± 0.003	0.017 ± 0.003	0.015 ± 0.003
120	5	0.014 ± 0.003	0.016 ± 0.004	0.016 ± 0.003	0.015 ± 0.003

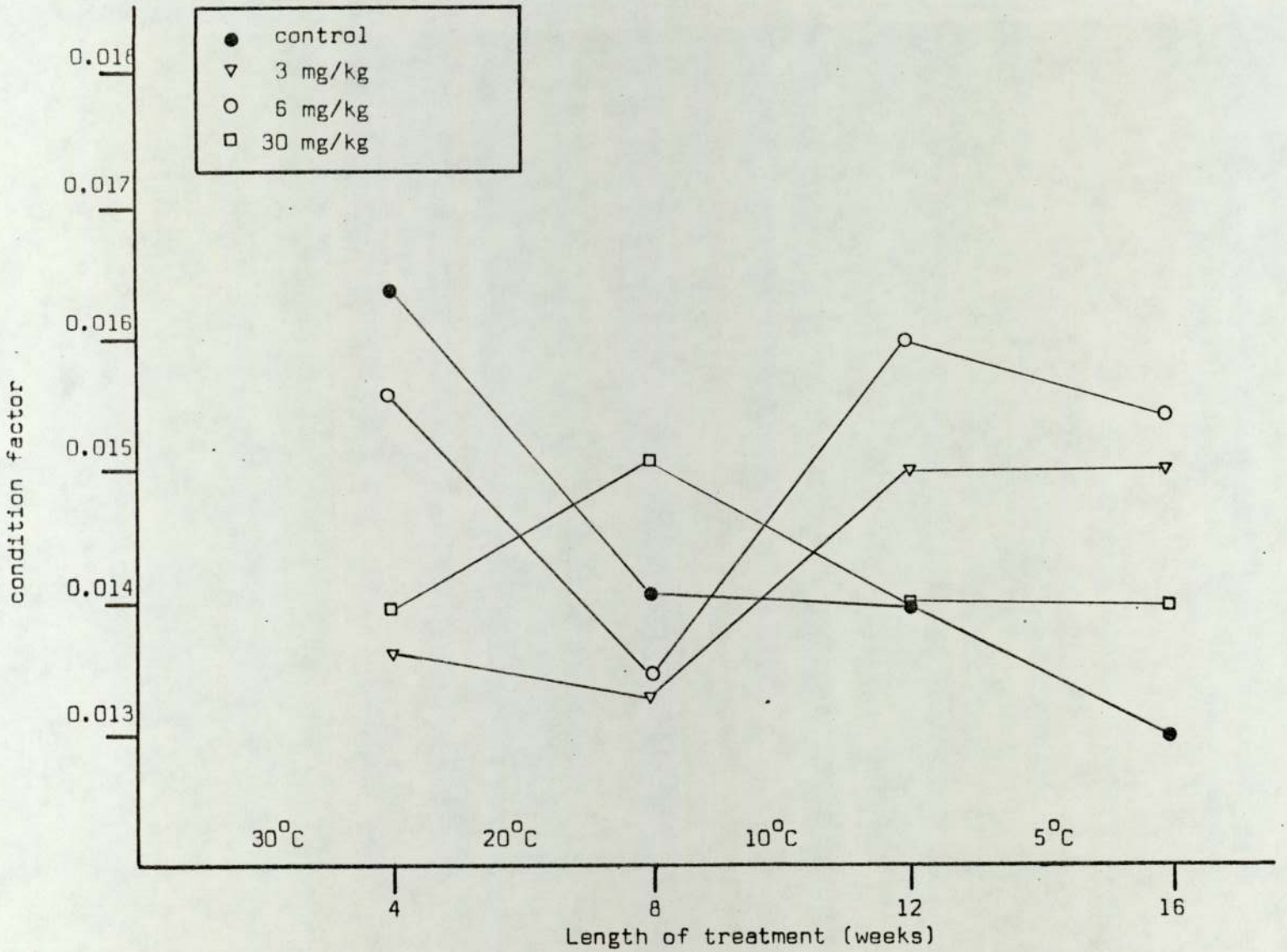


Figure 7.5. Effect of cobalt chloride on the condition factor of carp kept under different water temperatures.

Table 7.9

Effect of Oral Administration of Cobalt Chloride on Food Utilization Efficiency of Carp Kept Under Different Water Temperatures

Period in Days	Temperature in ^{o}C	Food Utilization Efficiency			
		Concentration of Cobalt Chloride in the Diet, mg per kg dry diet			
		Control (0)	3	6	30
0 - 30	30	32	45	35	34
31 - 60	20	13	13	15	13
61 - 90	10	4	13	12	10
91 - 120	5	3	5	4	4

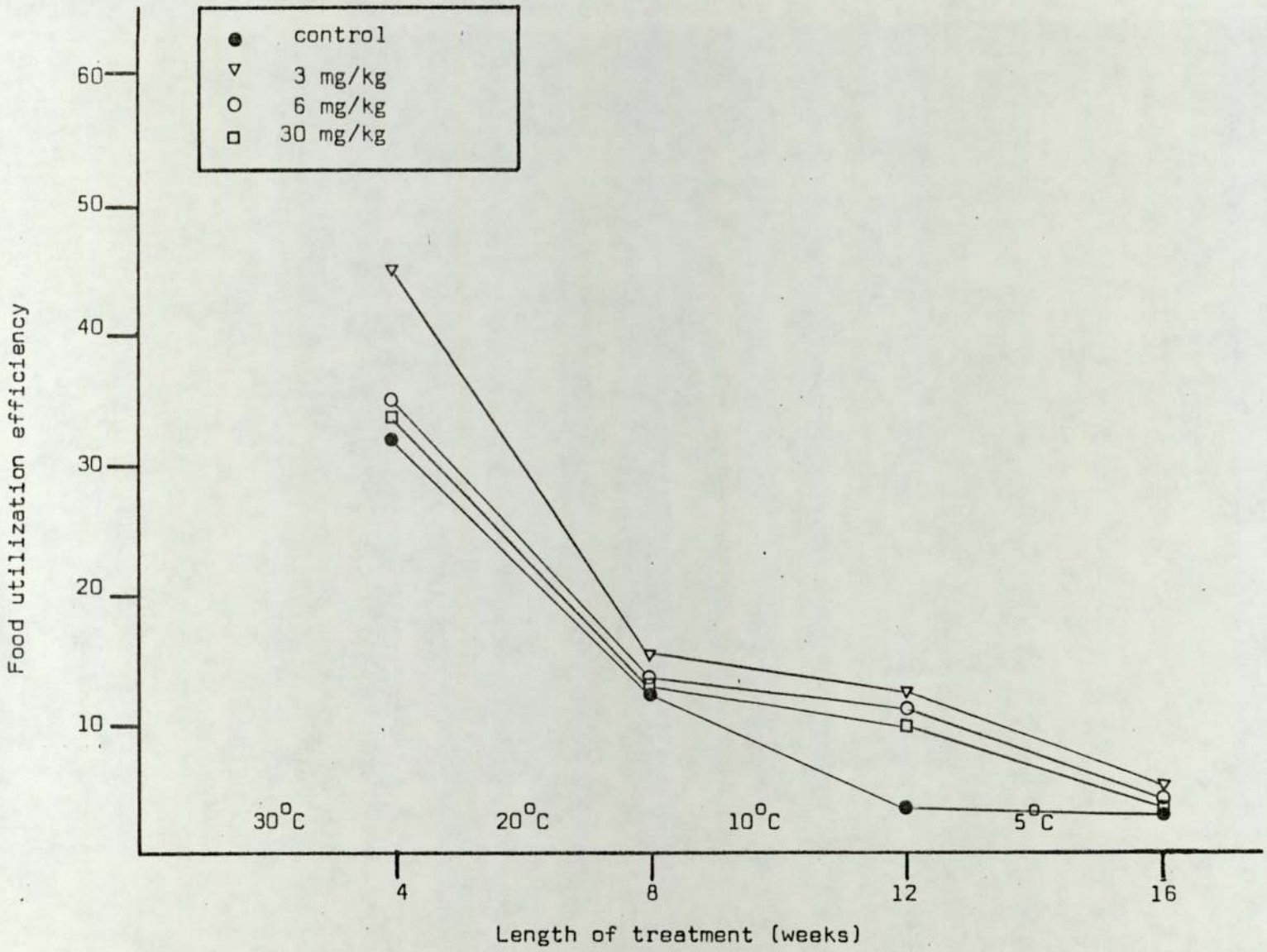


Figure 7.6. Effect of cobalt chloride on the food utilization efficiency of carp kept under different water temperatures.

declined, and it seems also that the treated group was less affected than controls. The best results obtained were in the group receiving 3 mg cobalt chloride per kg dry diet (Figure 7.7 and Table 7.10).

7.3.2 SURVIVAL RATE

As the water temperature was lowered, the survival of the control fish was reduced, and that of the 30 mg cobalt chloride group was reduced even more, whereas there were no deaths whatever in the 3 and 6 mg cobalt chloride treated groups (Table 7.11). It was also noted that the cobalt chloride-treated fish were much more active than the controls.

7.3.3 BODY COMPOSITION

Body analysis is given in Table 7.12; it shows that there is an increase in total body fat in all the treated groups as well as the total ash content of the body.

7.4 DISCUSSION AND CONCLUSIONS

It can be seen that the growth and food utilization efficiency of carp fed a diet supplemented with cobalt chloride and kept under different water temperatures were higher than those of controls. However, when the dose of cobalt chloride was increased to 30 mg cobalt chloride per kg dry diet there were smaller differences between them and the control group than with 3 and 6 mg cobalt chloride groups. At the same time the high dose of cobalt chloride adversely affected the survival of the fish. It can be concluded that carp definitely respond to cobalt treatment when they are kept under temperatures between 30 - 5^{o}C by improving their growth, survival rate, and the food requirements. The optimum for them is 3 mg cobalt chloride per kg dry diet.

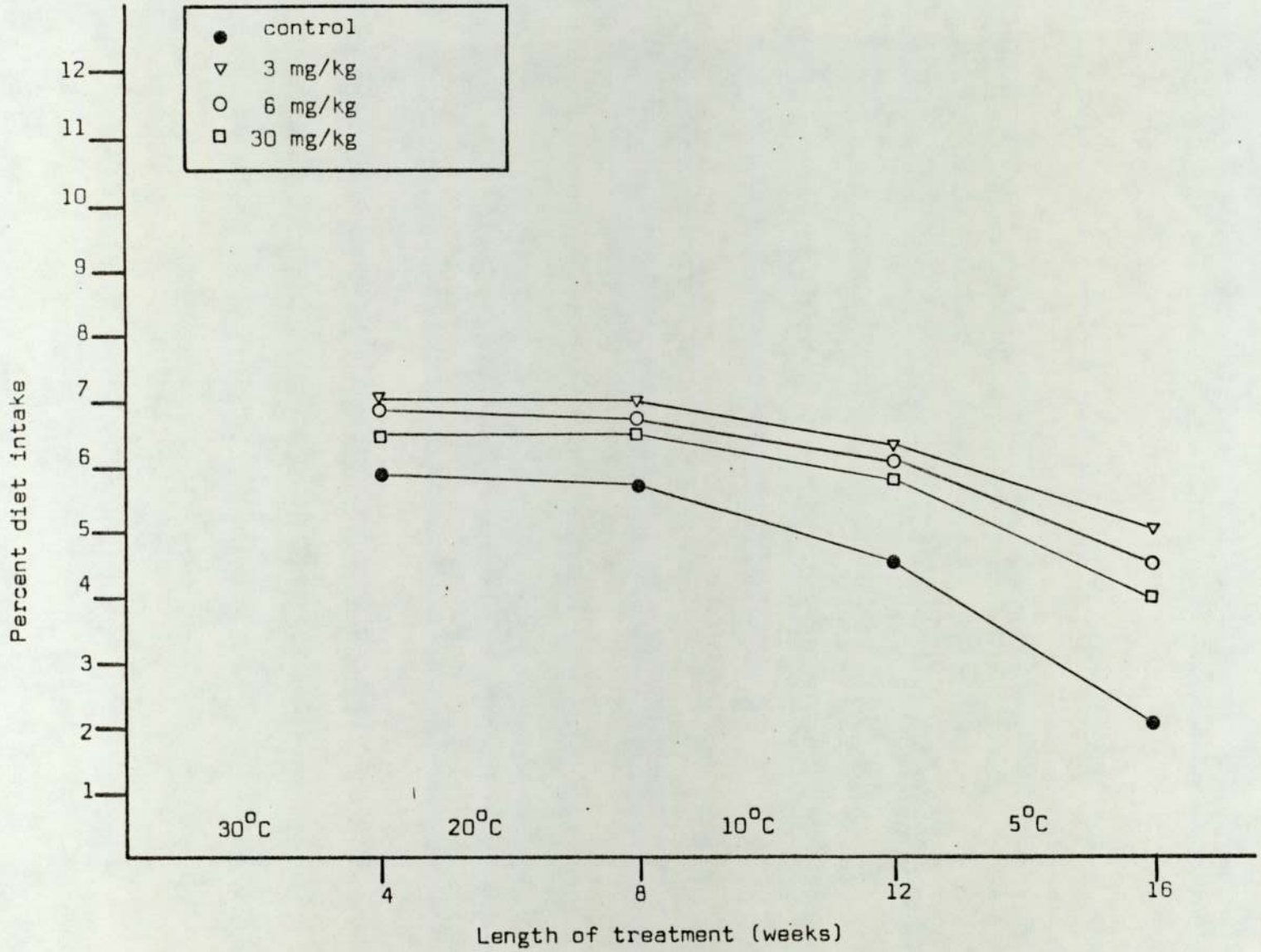


Figure 7.7. Effect of cobalt chloride on total food intake by carp kept under different water temperatures.

Table 7.10

Effect of Oral Administration of Cobalt Chloride on Total Food Intake of Carp kept under different Water Temperatures

Period in Days	Temperature in °C	Food Intake as Percentage of Total Body Weight			
		Concentration of Cobalt Chloride in the Diet, mg per kg dry diet			
		Control (0)	3	6	30
0 - 30	30	5.9	7.1	6.9	6.5
31 - 60	20	5.7	7.0	6.7	6.5
61 - 90	10	4.5	6.3	6.1	5.8
91 - 120	5	2.0	5.0	4.5	4.0

Table 7.11

Effect of Oral Administration of Cobalt Chloride on Survival of Carp kept under different Water Temperatures

Duration in Days	Temperature in °C	Survival of Carp %			
		Concentration of Cobalt Chloride in the Diet, mg per kg dry diet			
		Control (0)	3	6	30
0	30	100	100	100	100
15	30	100	100	100	100
30	30	100	100	100	100
45	20	100	100	100	80
60	20	100	100	100	80
75	10	80	100	100	80
90	10	80	100	100	80
105	5	80	100	100	60
120	5	60	100	100	40

Table 7.12

Effect of Oral Administration of Cobalt Chloride on Body Composition of Carp kept under Different Water Temperatures

Concentration of Cobalt Chloride in the Diet, mg per kg Dry Diet	Body Composition				
	Water	Protein	Fat	Ash	Total
Control (0)	73.5	12.6	5.0	2.4	93.5
3	74.1	13.5	5.7	2.9	96.3
6	74.0	13.2	5.5	3.1	95.8
30	73.7	12.8	5.1	3.3	94.9

Carp receiving Cobalt in their diet produced more fat in their body than those in controls; one may suggest that this fat allows the fish to withstand low water temperatures better than the controls and thus raises their resistance and increases their survival.

CHAPTER EIGHT

EFFECT OF IODIDE ON GROWTH AND METABOLISM OF CARP, *Cyprinus carpio*

8.1 INTRODUCTION

Under certain conditions iodine intake may be inadequate to support normal thyroid function in fresh-water teleosts (Marine and Lenhart, 1911; Hoar and Bell, 1950; Hoar, 1952; Baker-Cohen, 1959; Radulescu et al., 1968; Black and Simpson, 1974; Drongowski et al., 1975; Sonstegard and Leatherland, 1976). It is therefore surprising that despite several studies on dietary iodine requirements (Woodall and LaRoche, 1964; LaRoche et al., 1965) and radioiodide kinetics (Hickman, 1959; Leloup and Fontaine, 1960; Hunn and Reineke, 1964; Leloup, 1970, Gregory and Eales, 1974), no comprehensive study of iodine requirements in warm water fish has been undertaken. Thus the purpose of the present investigation was to determine the dietary iodide requirements of carp, Cyprinus carpio, raised in water low (less than 0.05 micrograms per litre) in iodide.

8.2 MATERIALS AND METHODS

8.2.1 THE EXPERIMENTAL SYSTEM AND ANIMALS

The experimental facility used in the present study was System 4 as described in Chapter 4 (4.2).

480 fingerling carp (5.00 ± 1.00 cm) were obtained from Avion Farm, Aries Ford. The fish were subjected to quarantine and prophylaxis, as described in Chapter 4 (4.5), and then transferred to the tropical room where they were kept in a recycling system, Chapter 4 (4.2). A sufficient number of them (20 fish) of an appropriate size (6.00 ± 0.05 cm) were selected and transferred to 24 of the 25 litre glass tanks (System 4) at the prevailing ambient temperature of 23^{o}C; 12 fish were removed for proximate carcass analysis, Chapter 4 (4.9). The density of

stocking was found to be 4.8 g per litre. The experiment was carried on for a period of 24 weeks. Throughout this time pH and dissolved oxygen were checked once a week using a pH meter and the Winkler method; they were found to range from 6.9 - 7.1 and 7.5 - 9.0 ppm respectively. Room and water temperature were recorded and found to range from 25 - 28°C and 22 - 24°C respectively. No losses occurred during temperature acclimatization; photo-period was controlled at 8 am - 8 pm - 8 am throughout the experiment.

8.2.2 THE EXPERIMENTAL DIET

Formulation of the diet was carried out by the general procedure described in Chapter 4 (4.4); the ingredients used are presented in Table 8.1. Iodine, as potassium iodide (KI), was added to the diet at eight levels: 0.1, 0.3, 0.6, 1.2, 3.0, 6.0, 24.0 and 48.0 mg iodide per kg dry diet; it was sprayed over 1000 g of the food pellets using the same technique as described in Chapter 5 (5.2.2). The proximate analysis was performed on this diet and the results are presented in Table 8.2.

8.2.3 FEEDING RATE

Feeding rate and feeding time were as described in Chapter 5 (5.2.3).

8.2.4 WEIGHING AND SAMPLING

Details of the weighing procedure are presented in Chapter 4 (4.6); fish were weighed individually (4.6). After 12 weeks of feeding, an intermediate sample (3 fish) was removed from each group for proximate chemical analysis; at the end of the experiment (24 weeks) 3 fish were removed from each lot for carcass analysis (body composition, concentration of iodine in the body).

Table 8.1

Composition of the Test Diet

Main Mixture		Vitamin Supplement		Mineral Mixture	
Ingredients	Parts	Vitamin	mg*	Mineral	mg**
Casein	400	Riboflavin	200	$CaCO_3$	250
α-starch	180	Thiamin-HCl	50	KCl	4670
Dextrin	190	Pyridoxin-HCl	50	KH_2PO_4	4000
Cod Liver Oil	40	Folic Acid	15	Na_2HPO_4	3090
Vitamin Mixture	50	Ascorbic Acid	100	$MgSO_4$	2475
Mineral Mixture	40	Pantothenic Acid	500	$FeSO_4.7H_2O$	250
Methionine	10	Inositol	2000	$ZnSO_4.7H_2O$	220
Tryptophan	5	Nicotinic Acid	750	$MnSO_4.H_2O$	92
α-cellulose	15	Biotin	5	$CuSO_4$	20
Carboxymethyl cellulose	10	Choline chloride	4000	KI***	-
		Vitamin A	40	Na_2SeO_3	0.2
gelatin	60	Vitamin E	5000	$(NH_4)_6MO_7O_{24}.4H_2O$	0.4
		Vitamin D_3	40	$CoCl_2.6H_2O$	3
		Vitamin K	150		
		Glucose (as a carrier)	4200		

*mg/kg dry diet
**mg/kg dry diet
***Dietary iodide was adjusted to 0.1, 0.3, 0.6, 1.2, 2.4, 24.0, 48.0 ppm by KI and alpha-cellulose

Table 8.2

Composition of the basal diet for carp

Diet Composition percent %	Concentration of Iodide in the diet, mg/kg dry diet								
	Control (0)	0.1	0.3	0.6	1.2	3.0	6.0	24.0	48.0
Water	12.10	11.80	11.90	11.90	12.0	12.10	12.05	11.95	11.95
Crude Protein	45.00	44.50	44.00	44.90	44.80	45.10	44.90	44.85	44.90
Total Fat	5.10	5.15	5.20	5.10	5.00	5.15	5.15	5.10	5.10
Ash	5.10	5.10	5.10	5.10	5.20	5.25	5.30	5.30	5.35
Nitrogen free extract (NFE) + fibre	32.70	33.45	33.80	33.00	33.00	32.45	32.60	32.80	32.70

8.2.5 STATISTICAL METHODS AND ANALYSIS OF GROWTH DATA

These were performed as detailed in Chapter 4 (4.10 and 4.11).

8.3 RESULTS

8.3.1 ACCELERATION OF GROWTH

Weight and Length

The results of the feeding trial with carp are shown in Tables 8.3 and 8.4. The fish in all treated groups responded to the iodide treatment by increasing their weight and length above that of the controls up to the end of the experiment. The differences between the control and the treated groups in the first eight weeks were relatively small; by the end of the experiment the differences between them were more pronounced (Figures 8.1 (1 - 9) and 8.2 (1 - 9)). The group receiving 1.2 mg iodide per kg dry diet gained somewhat more than other groups (Figures 8.1.5 and 8.2.5), but the statistical evaluation of the growth data by analysis of variance, indicated that the mean gains of the fish in all treated groups did not differ significantly.

Growth Rate

The mean daily growth rate in all treated groups was higher than that of the control up to the end of the experiment. The greatest daily increases in weight and length were observed in the groups receiving 0.1, 0.3, 0.6 and 1.2 mg iodide per kg dry diet (Tables 8.5 and 8.6).

Food Utilization Efficiency (FUE)

Table 8.7 shows that there was no difference in food

Table 8.3

Changes in the Body Weight of Carp fed Potassium Iodide Supplemented Diet for a Period of 24 weeks. Values Given are mean (grams) ± S.E. of 20 Fish. Percent Weight Gain Over Controls is Given in Parentheses under the Actual Values

Duration in weeks	Concentration of Potassium Iodide in the Diet, mg per kg Dry Diet								
	Control	0.1	0.3	0.6	1.2	3.0	6.0	24.0	48.0
0	2.65 ± 0.30	2.60 ± 0.25	2.60 ± 0.22	2.60 ± 0.21	2.65 ± 0.30	2.70 ± 0.27	2.70 ± 0.41	2.75 ± 0.18	2.60 ± 0.15
2	4.15 ± 0.25	4.10 ± 0.30	3.90 ± 0.25	4.25 ± 0.22	4.40 ± 0.25	4.20 ± 0.35	4.30 ± 0.43	4.20 ± 0.20	4.00 ± 0.20
4	6.55 ± 0.20	6.60 ± 0.41 (6.7)	6.65 ± 0.35 (8.6)	6.80 ± 0.25 (14.4)	7.10 ± 0.32 (20.8)	6.90 ± 0.40 (8.4)	6.95 ± 0.50 (10.2)	6.80 ± 0.22 (0.1)	6.60 ± 0.25 (6.7)
6	7.50 ± 0.33	7.75 ± 0.55	7.95 ± 0.40	8.05 ± 0.33	8.30 ± 0.33	8.10 ± 0.50	8.15 ± 0.43	8.00 ± 0.32	7.50 ± 0.30
8	8.10 ± 0.40	8.10 ± 0.53 (5.9)	8.10 ± 0.50 (5.9)	8.65 ± 0.31 (27.0)	8.80 ± 0.25 (26.4)	8.50 ± 0.55 (9.2)	8.60 ± 0.30 (12.9)	8.40 ± 0.42 (-0.21)	8.00 ± 0.30 (2.0)
10	10.50 ± 0.51	10.55 ± 0.60	10.60 ± 0.65	11.00 ± 0.60	11.20 ± 0.35	11.00 ± 0.70	11.10 ± 0.55	10.90 ± 0.60	10.70 ± 0.45
12	14.00 ± 0.53	14.25 ± 0.70 (19.8)	14.30 ± 0.60 (21.7)	14.45 ± 0.55 (27.5)	15.00 ± 0.40 (37.4)	14.50 ± 0.80 (8.7)	14.70 ± 0.75 (16.2)	14.40 ± 0.70 (-4.66)	14.30 ± 0.53 (21.7)
14	18.00 ± 0.75	18.70 ± 0.95	18.70 ± 0.70	19.00 ± 0.75	19.10 ± 0.60	19.00 ± 0.90	18.90 ± 0.90	18.85 ± 0.80	18.75 ± 0.80
16	19.80 ± 0.90	20.35 ± 1.10 (35.5)	20.30 ± 0.85 (33.6)	20.50 ± 0.66 (41.3)	20.60 ± 0.82 (30.2)	20.50 ± 0.90 (12.1)	20.40 ± 1.10 (8.4)	20.40 ± 0.95 (-5.35)	20.40 ± 0.80 (37.4)
18	20.50 ± 1.10	22.50 ± 1.20	22.50 ± 0.95	22.60 ± 0.97	22.70 ± 0.90	22.70 ± 1.05	22.50 ± 1.10	22.40 ± 1.08	22.35 ± 1.37
20	25.00 ± 1.15	27.55 ± 1.28 (116.2)	27.50 ± 1.15 (114.3)	27.65 ± 1.18 (120.1)	27.80 ± 1.12 (105.7)	27.85 ± 1.15 (88.1)	27.70 ± 1.20 (82.5)	27.70 ± 1.10 (63.9)	27.65 ± 1.55 (120.1)
22	28.00 ± 1.30	31.00 ± 1.50	31.00 ± 1.20	31.20 ± 1.20	31.50 ± 1.22	31.40 ± 1.23	31.40 ± 1.35	31.80 ± 1.30	31.30 ± 1.60
24	30.00 ± 1.35	35.00 ± 1.55 (217.1)	35.10 ± 1.40 (217.9)	35.10 ± 1.45 (217.9)	35.50 ± 1.33 (207.5)	35.20 ± 1.34 (171.6)	35.25 ± 1.40 (173.5)	35.20 ± 1.50 (147.9)	35.00 ± 1.70 (214.1)

Table 8.4

Changes in the Body Length of Carp Fed Potassium Iodide Supplemented Diet for a Period of 24 Weeks. Values given are the Mean (grams) ± S.E. of 20 Fish. Percent Length Gain over Controls is given in Parentheses under the Actual Values

Duration in weeks	Concentration of Potassium Iodide in the Diet, mg per kg Dry Diet								
	Control	0.1	0.3	0.6	1.2	3.0	6.0	24.0	48.0
0	6.00 ± 0.05	6.00 ± 0.25	5.90 ± 0.05	6.00 ± 0.06	6.00 ± 0.05	6.00 ± 0.05	6.10 ± 0.04	6.00 ± 0.03	6.00 ± 0.03
2	6.20 ± 0.18	6.15 ± 0.15	6.10 ± 0.19	6.20 ± 0.17	6.20 ± 0.15	6.20 ± 0.15	6.25 ± 0.13	6.20 ± 0.14	6.15 ± 0.12
4	6.80 ± 0.22	6.80 ± 0.20 (0.0)	6.80 ± 0.20 (1.92)	6.85 ± 0.21 (0.84)	6.90 ± 0.19 (1.67)	6.90 ± 0.17 (1.67)	6.95 ± 0.16 (0.6)	6.90 ± 0.15 (1.67)	6.80 ± 0.15 (0.0)
6	7.05 ± 0.25	7.75 ± 0.30	7.70 ± 0.35	7.70 ± 0.40	7.70 ± 0.50	7.60 ± 0.45	7.70 ± 0.50	7.75 ± 0.35	7.75 ± 0.40
8	8.20 ± 0.30	8.25 ± 0.35 (0.83)	8.30 ± 0.30 (4.01)	8.35 ± 0.35 (2.50)	8.30 ± 0.33 (1.66)	8.25 ± 0.40 (0.83)	8.30 ± 0.42 (-0.6)	8.30 ± 0.30 (1.66)	8.30 ± 0.30 (1.66)
10	9.10 ± 0.50	9.30 ± 0.40	9.40 ± 0.43	9.59 ± 0.40	9.50 ± 0.50	9.40 ± 0.52	9.50 ± 0.55	9.50 ± 0.39	9.40 ± 0.50
12	9.50 ± 0.10	9.50 ± 0.15 (0.0)	9.60 ± 0.20 (4.38)	9.80 ± 0.20 (5.00)	9.80 ± 0.15 (5.00)	9.70 ± 0.15 (3.34)	9.70 ± 0.15 (0.69)	9.70 ± 0.30 (3.34)	9.70 ± 0.25 (3.34)
14	10.00 ± 0.20	10.30 ± 0.18	10.30 ± 0.15	10.50 ± 0.16	10.50 ± 0.17	10.50 ± 0.22	10.40 ± 0.23	10.40 ± 0.25	10.40 ± 0.15
16	11.80 ± 0.51	12.00 ± 0.57 (3.33)	12.00 ± 0.62 (6.72)	12.10 ± 0.60 (5.00)	12.20 ± 0.60 (6.66)	12.10 ± 0.58 (5.00)	12.10 ± 0.53 (1.69)	12.15 ± 0.60 (5.83)	12.15 ± 0.55 (5.83)
18	13.00 ± 0.75	13.45 ± 0.60	13.40 ± 0.56	13.60 ± 0.70	13.60 ± 0.70	13.50 ± 0.65	13.50 ± 0.60	13.55 ± 0.70	13.50 ± 0.65
20	14.00 ± 0.80	14.40 ± 0.75 (6.67)	14.30 ± 0.70 (9.04)	14.50 ± 0.80 (8.43)	14.50 ± 0.83 (8.34)	14.40 ± 0.75 (6.67)	14.40 ± 0.77 (2.74)	14.50 ± 0.78 (8.34)	14.50 ± 0.73 (8.34)
22	14.50 ± 0.95	15.10 ± 0.90	15.00 ± 0.95	15.00 ± 0.52	15.20 ± 0.90	15.15 ± 0.89	15.20 ± 0.83	15.10 ± 0.90	15.10 ± 0.90
24	15.00 ± 1.05	15.45 ± 1.10 (7.50)	15.00 ± 1.10 (12.71)	15.70 ± 1.00 (11.67)	15.90 ± 1.10 (15.00)	15.80 ± 1.20 (13.33)	15.90 ± 1.00 (10.66)	15.85 ± 1.05 (14.17)	15.85 ± 1.10 (14.17)

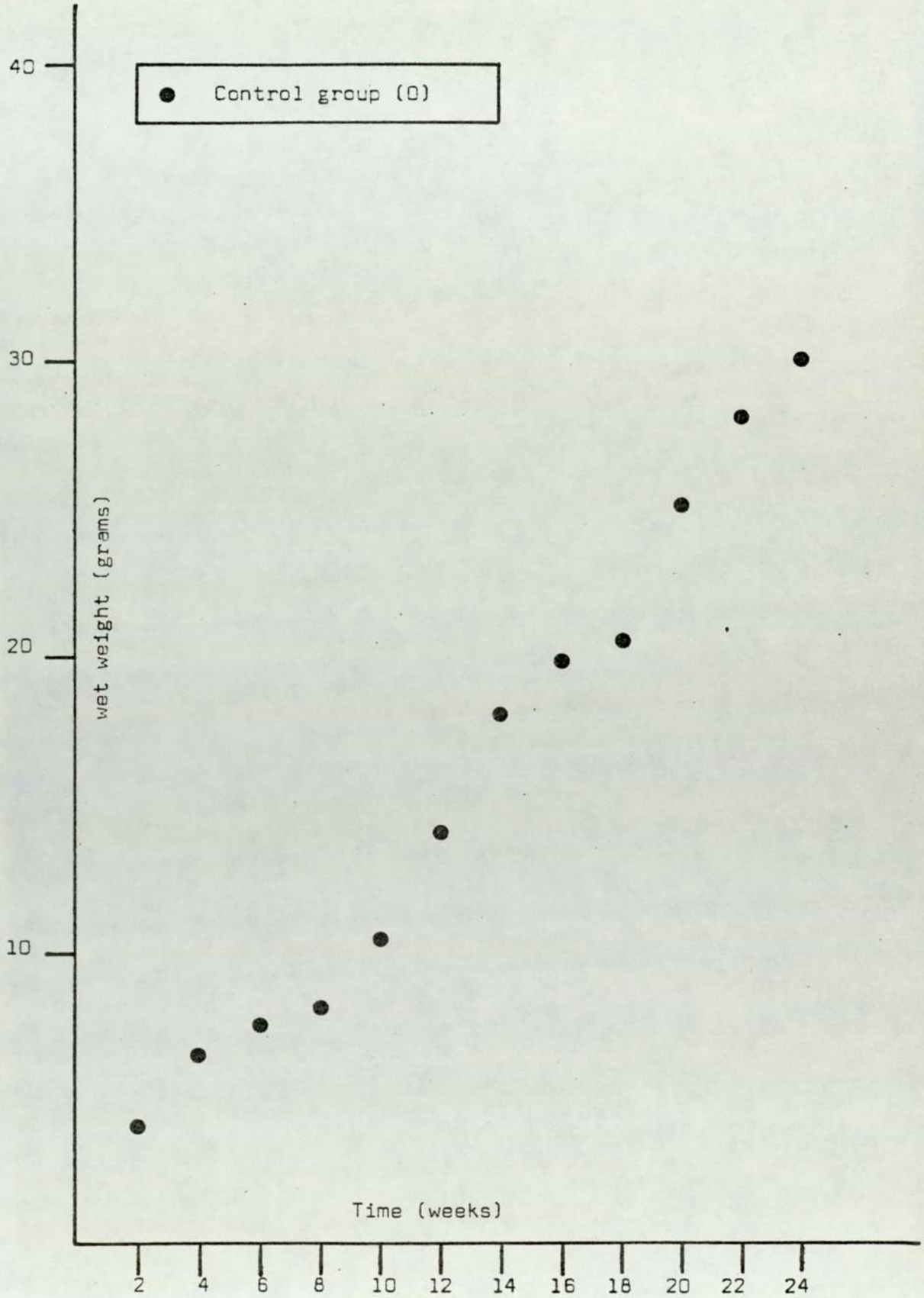


Figure 8.1.1. Effect of Potassium iodide on Body weight of carp.

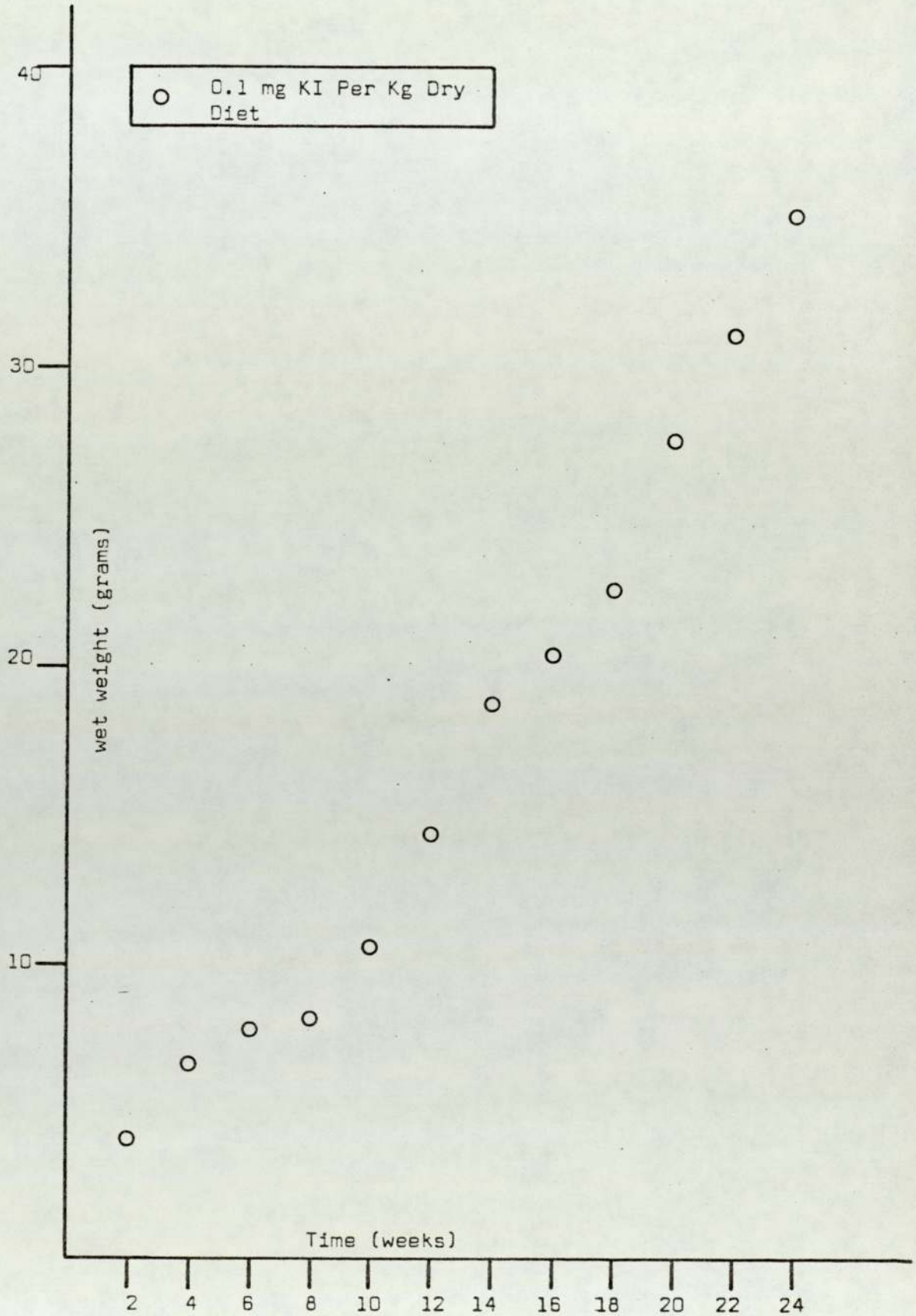


Figure 8.1.2. Effect of Potassium Iodide on Body Weight of Carp.

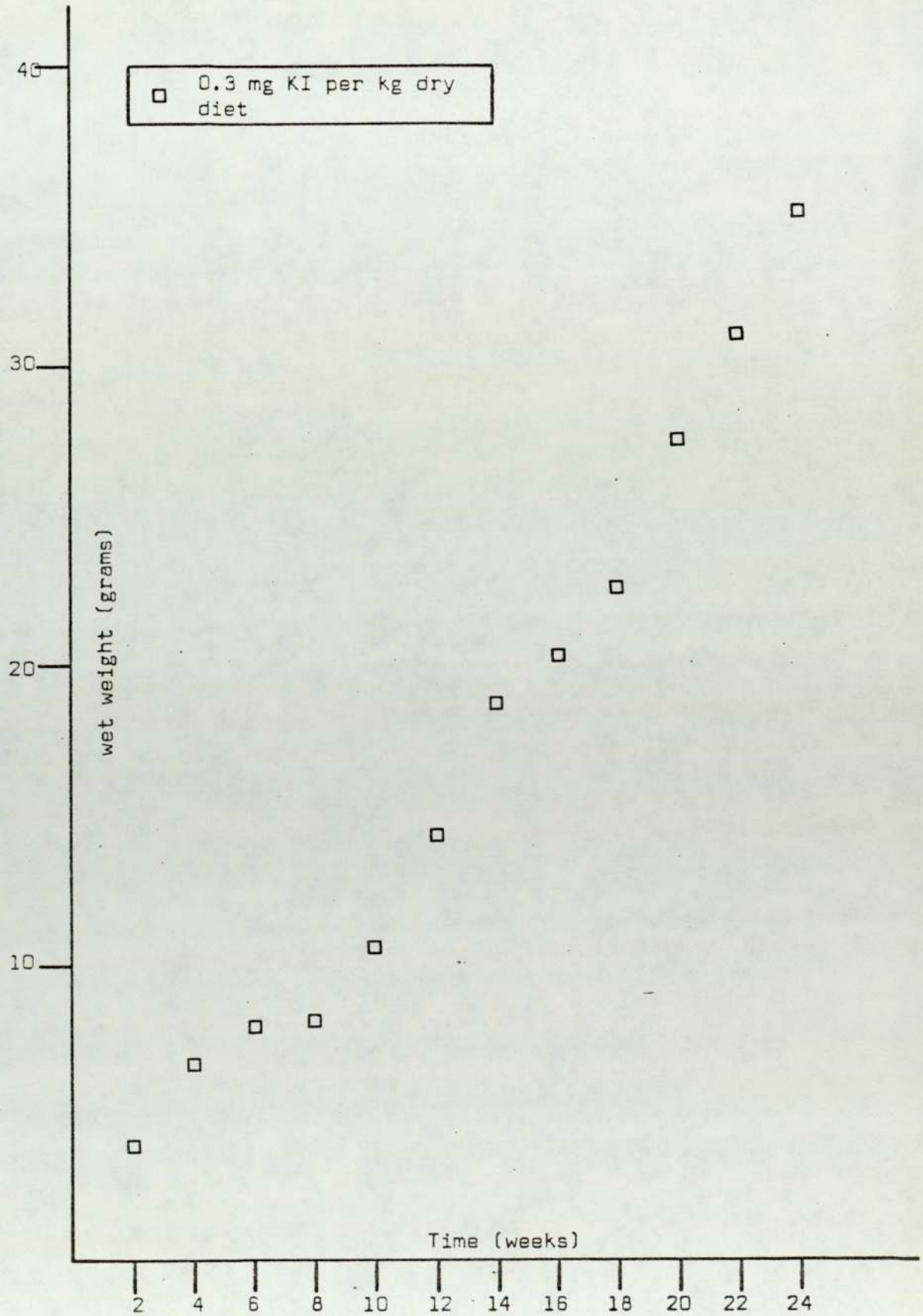


Figure 8.1.3. Effect of Potassium Iodide on Body Weight of carp.

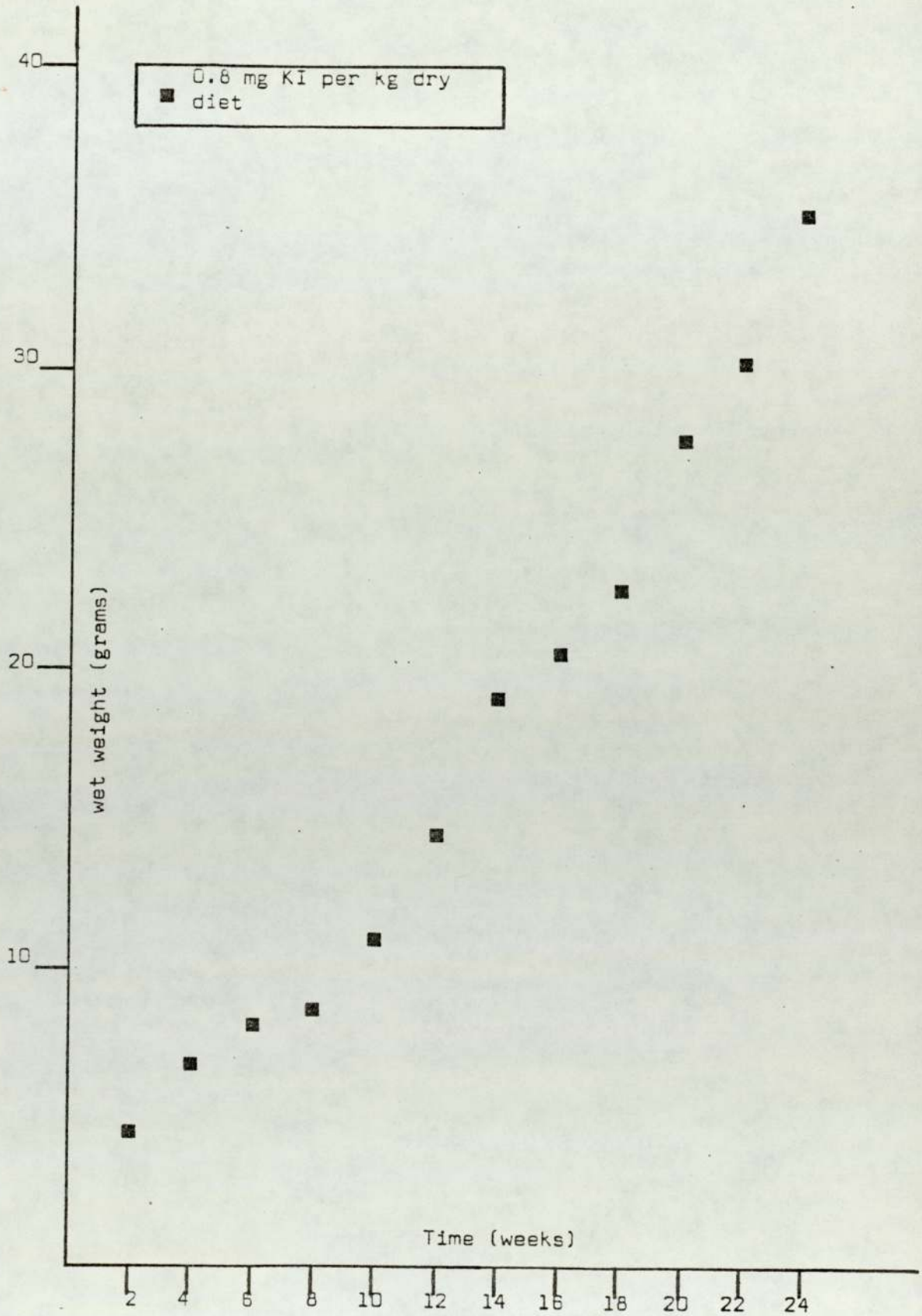


Figure 8.1.4. Effect of Potassium iodide on Body Weight of carp.

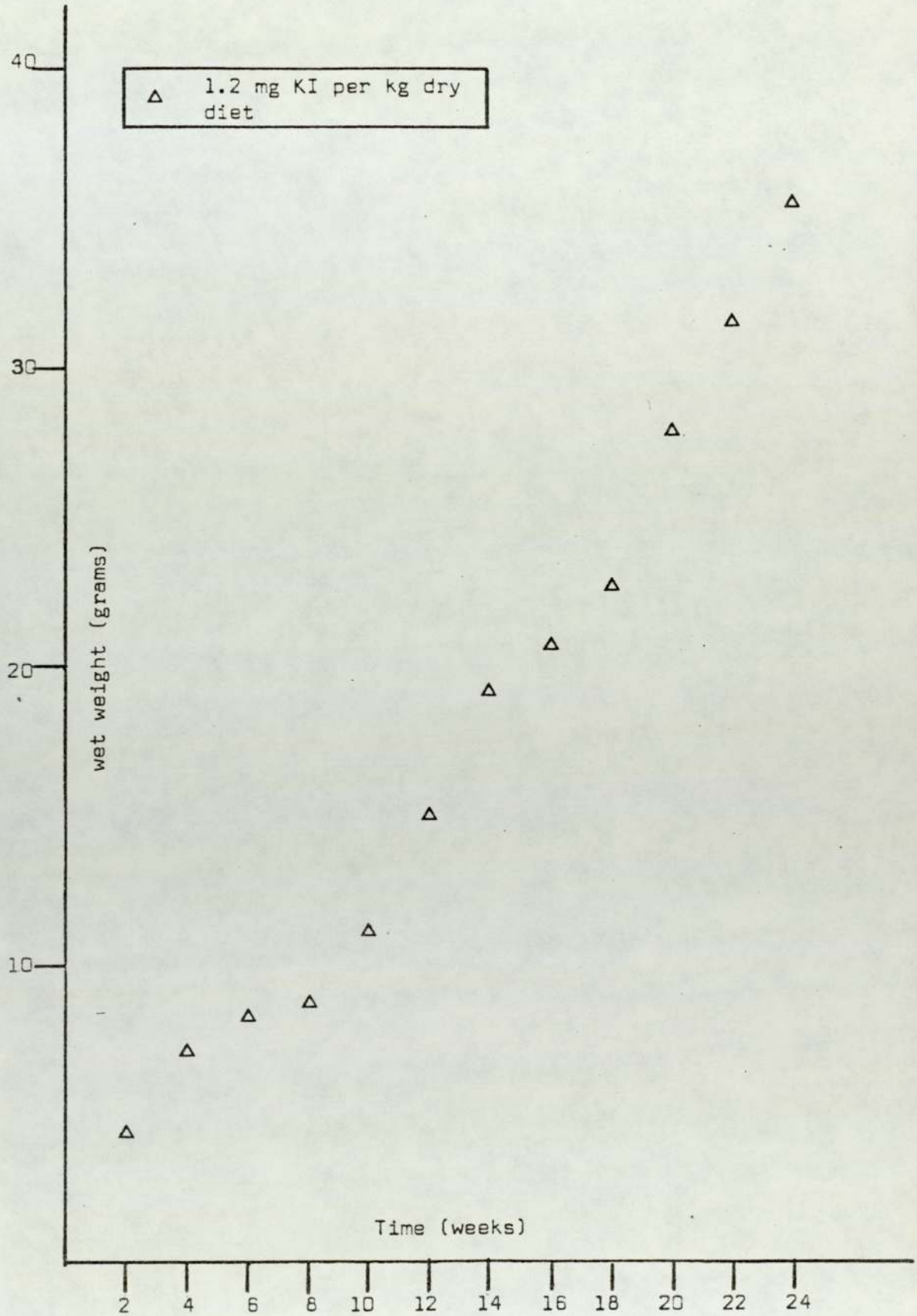


Figure 8.1.5. Effect of Potassium Iodide on Body Weight of carp.

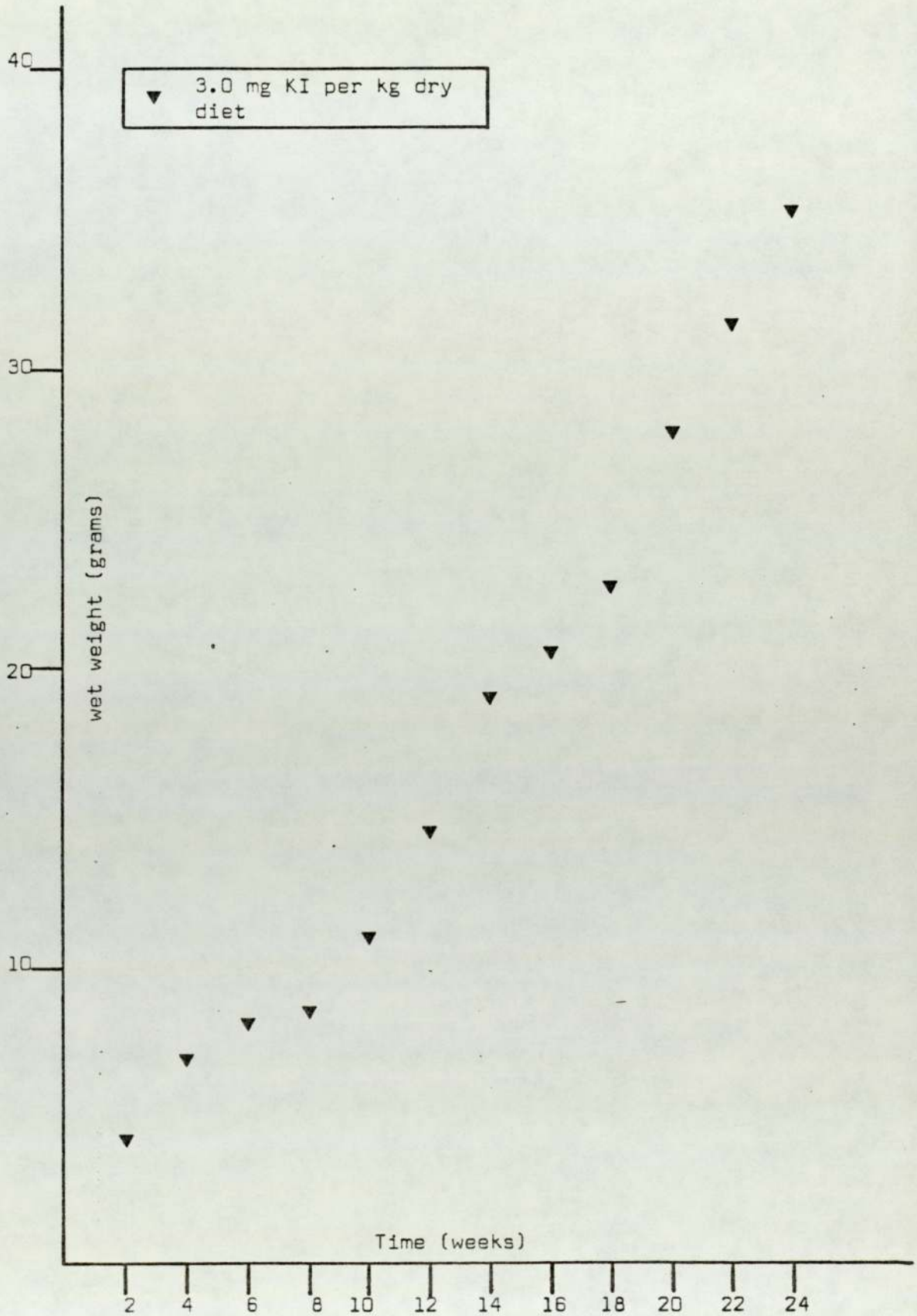


Figure 8.1.6. Effect of Potassium Iodide on Body Weight of carp.

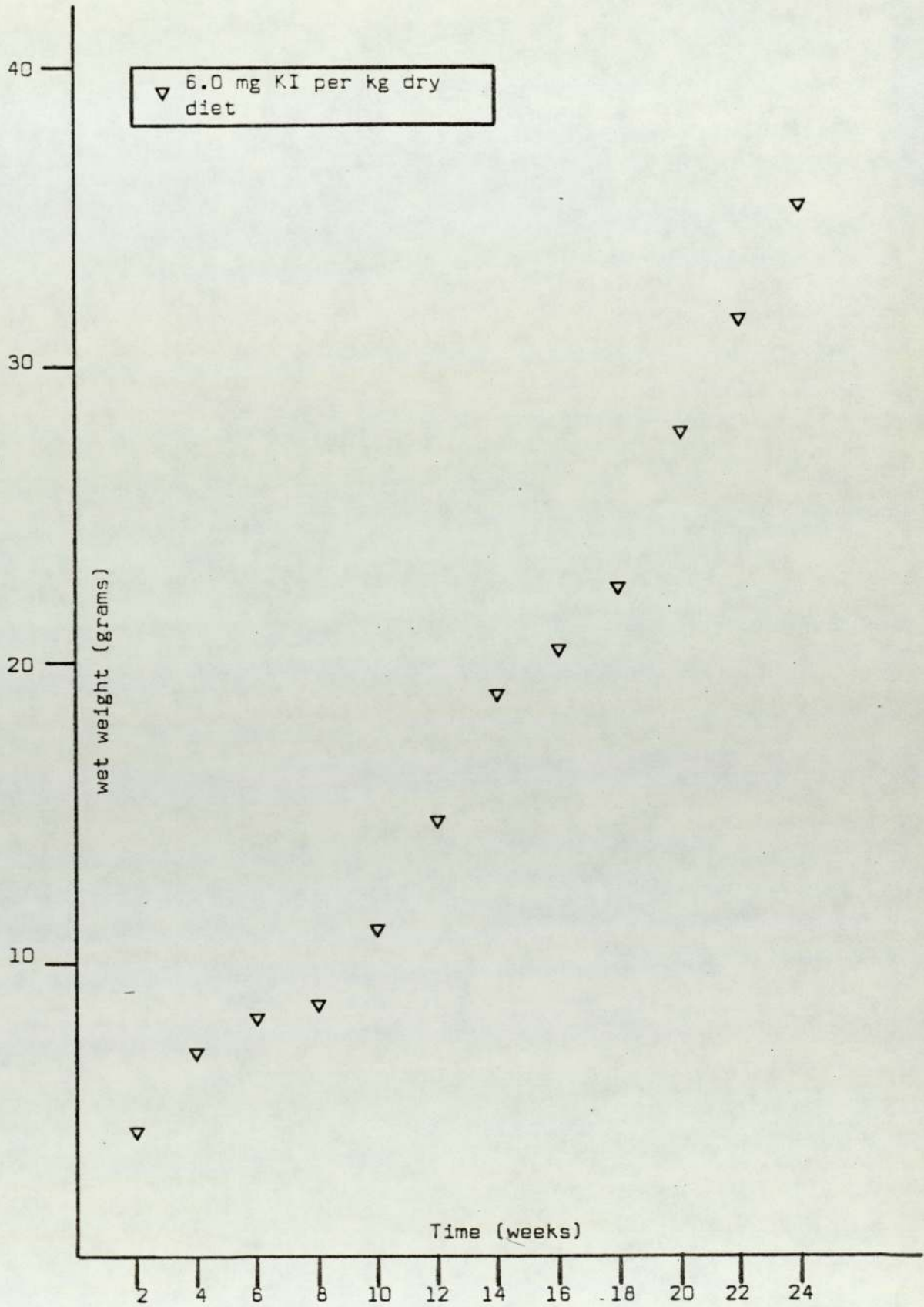


Figure 8.1.7. Effect of Potassium Iodide on Body Weight of carp.

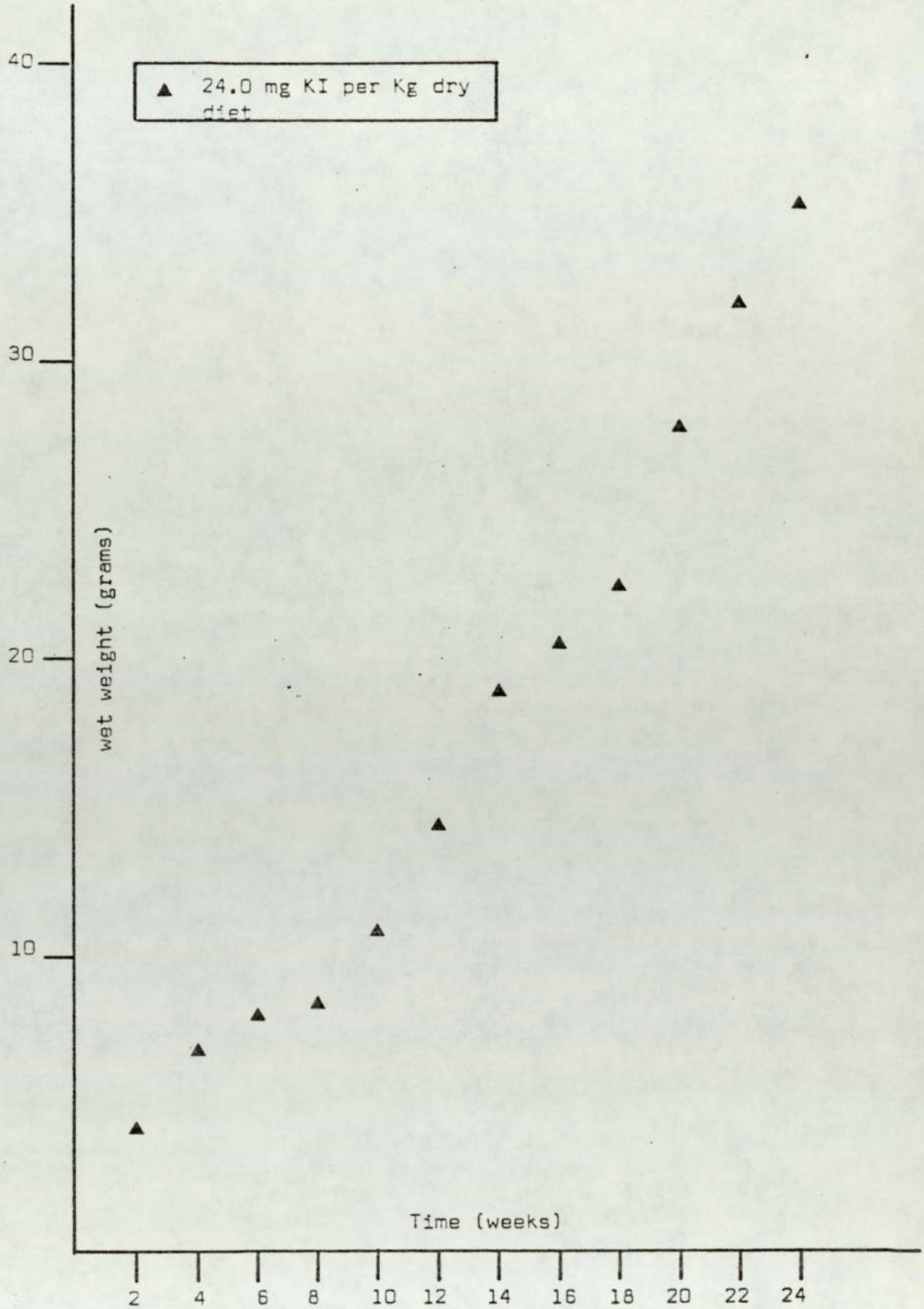


Figure 8.1.8. Effect of Potassium Iodide on Body Weight of carp.

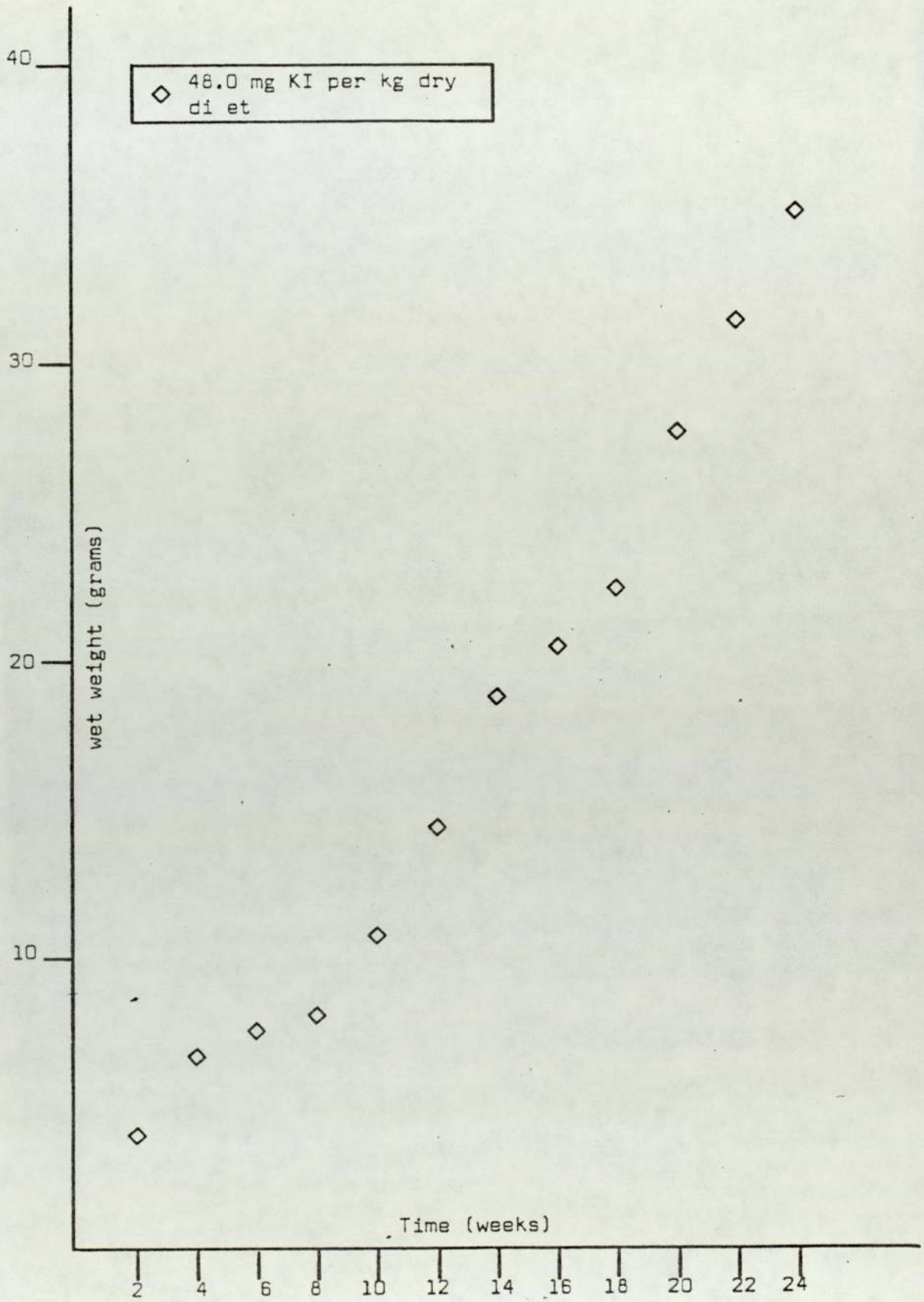


Figure 8.1.9. Effect of Potassium iodide on Body Weight of carp.

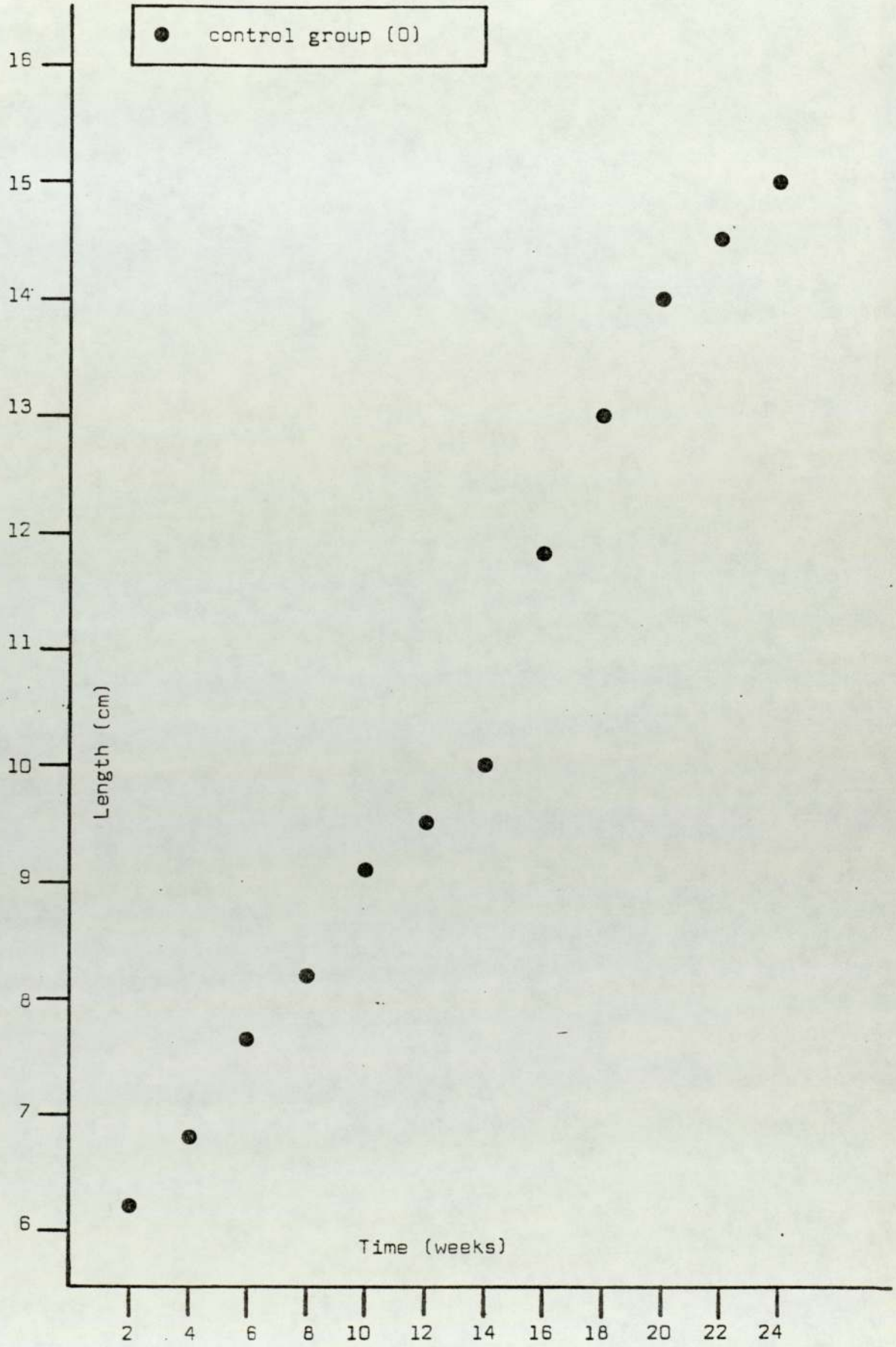


Figure 8.2.1. Effect of Potassium Iodide on Body Length of carp.

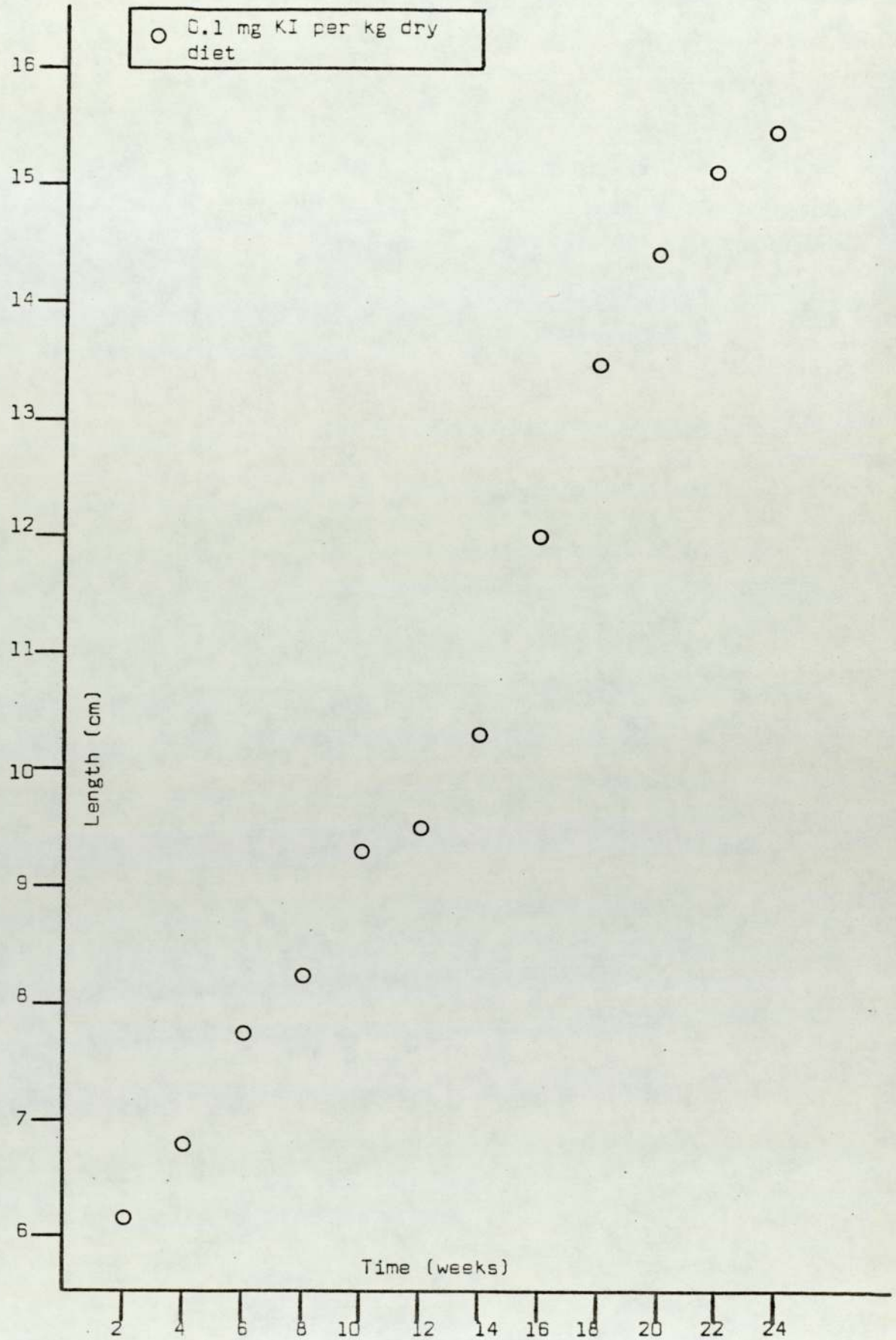


Figure 8.2.2. Effect of Potassium Iodide on Body Length of carp.

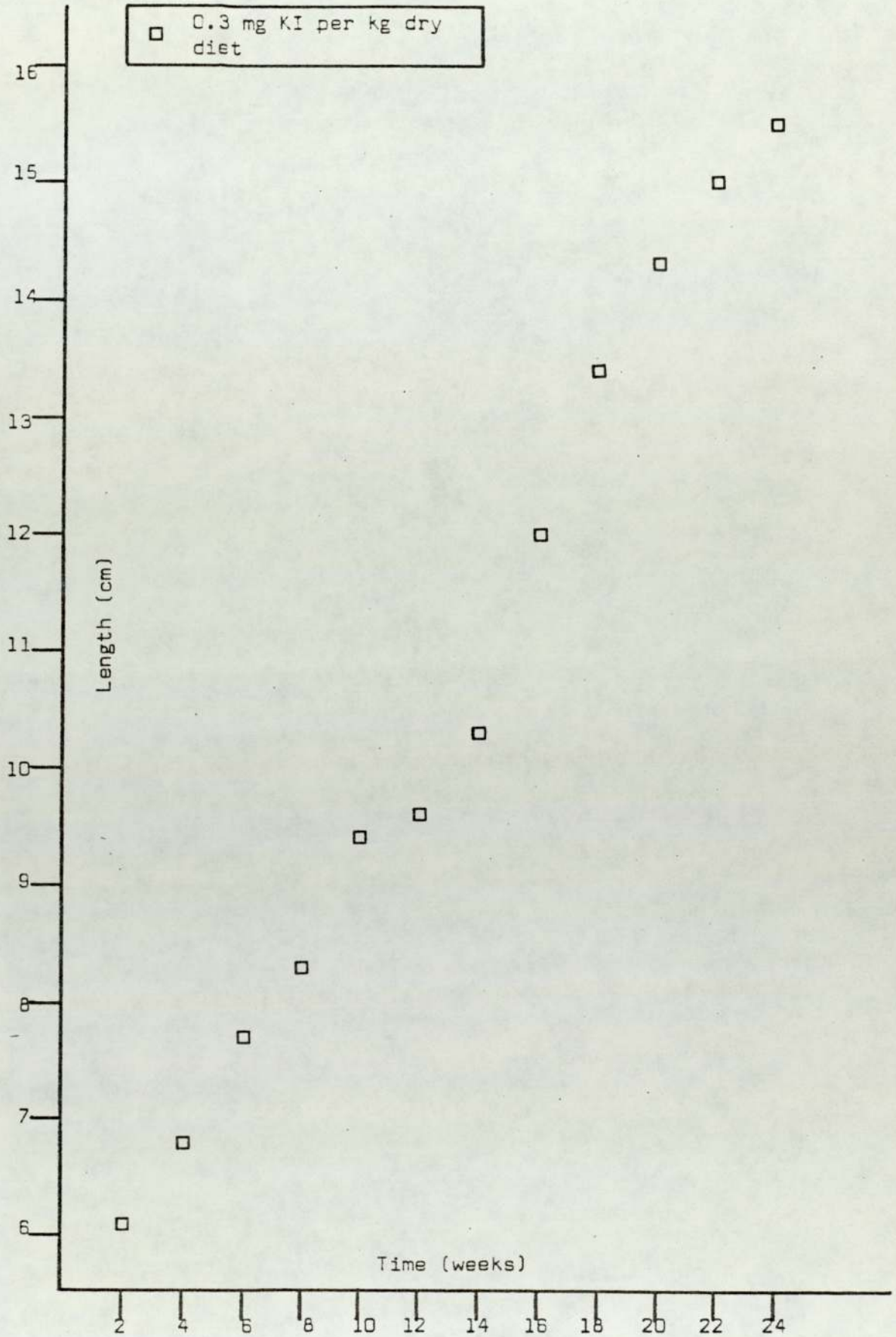


Figure 8.2.3. Effect of Potassium Iodide on Body Length of carp.

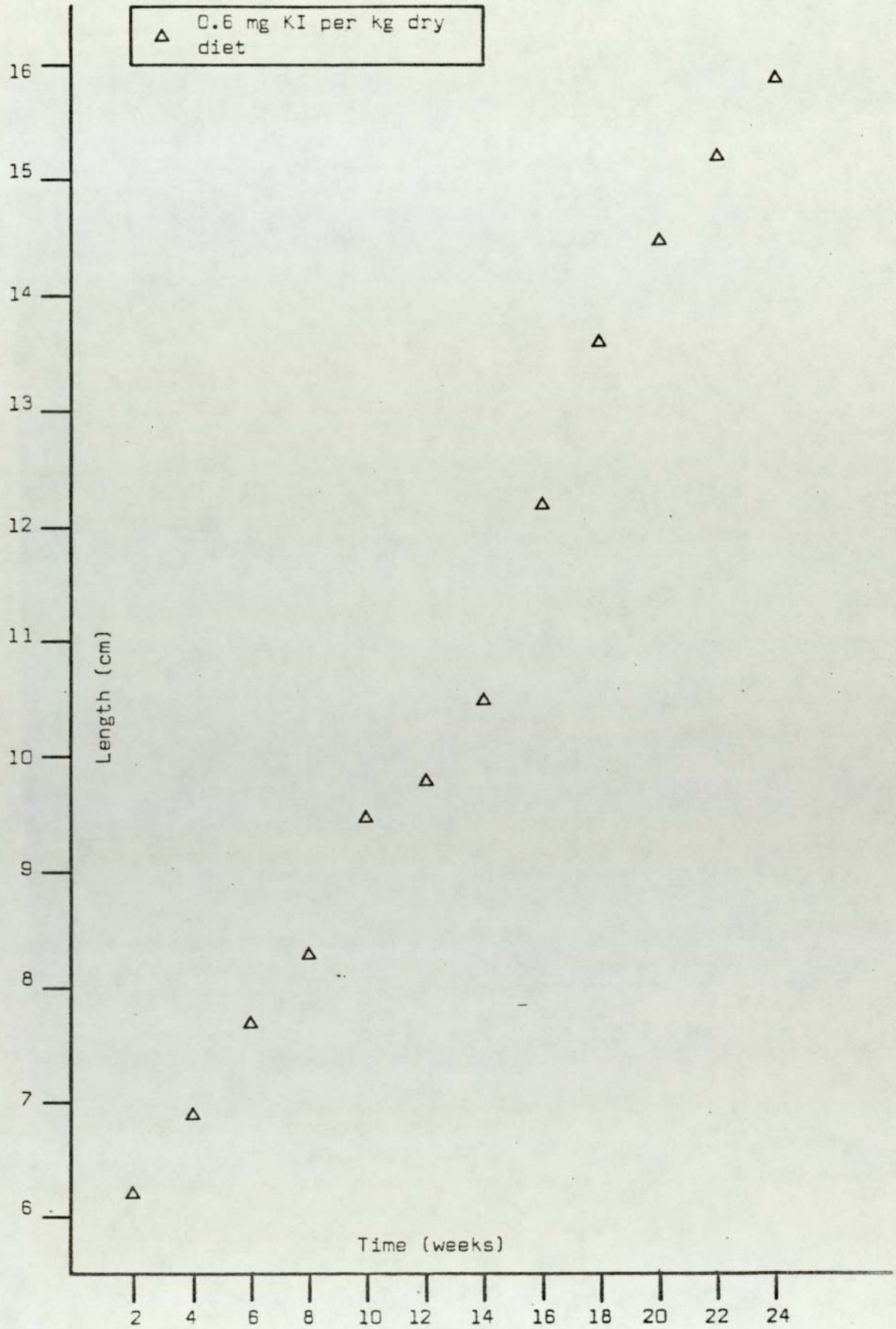


Figure 8.2.4. Effect of Potassium Iodide on Body Length of carp.

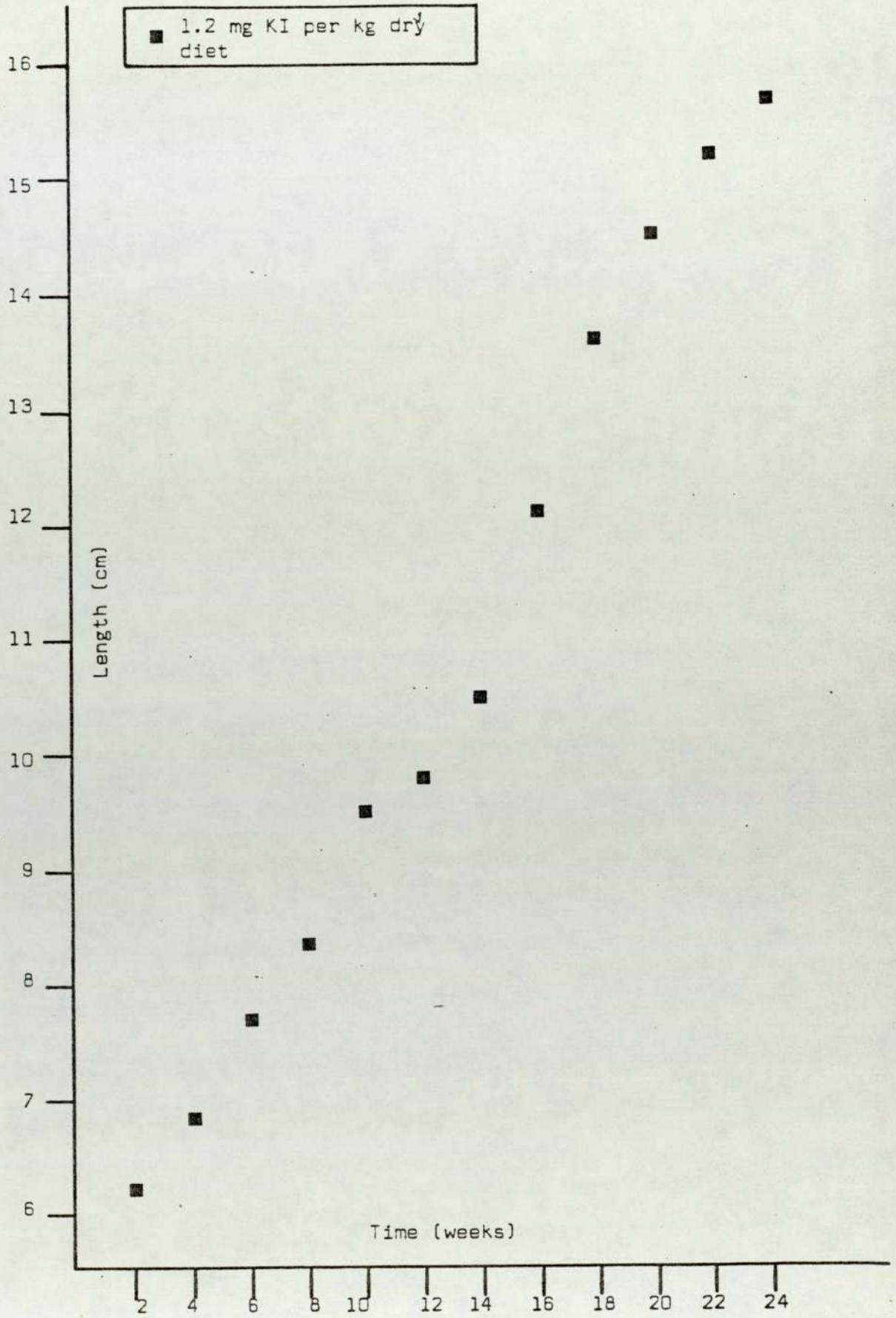


Figure 8.2.5. Effect of Potassium Iodide on Body Length of Carp.

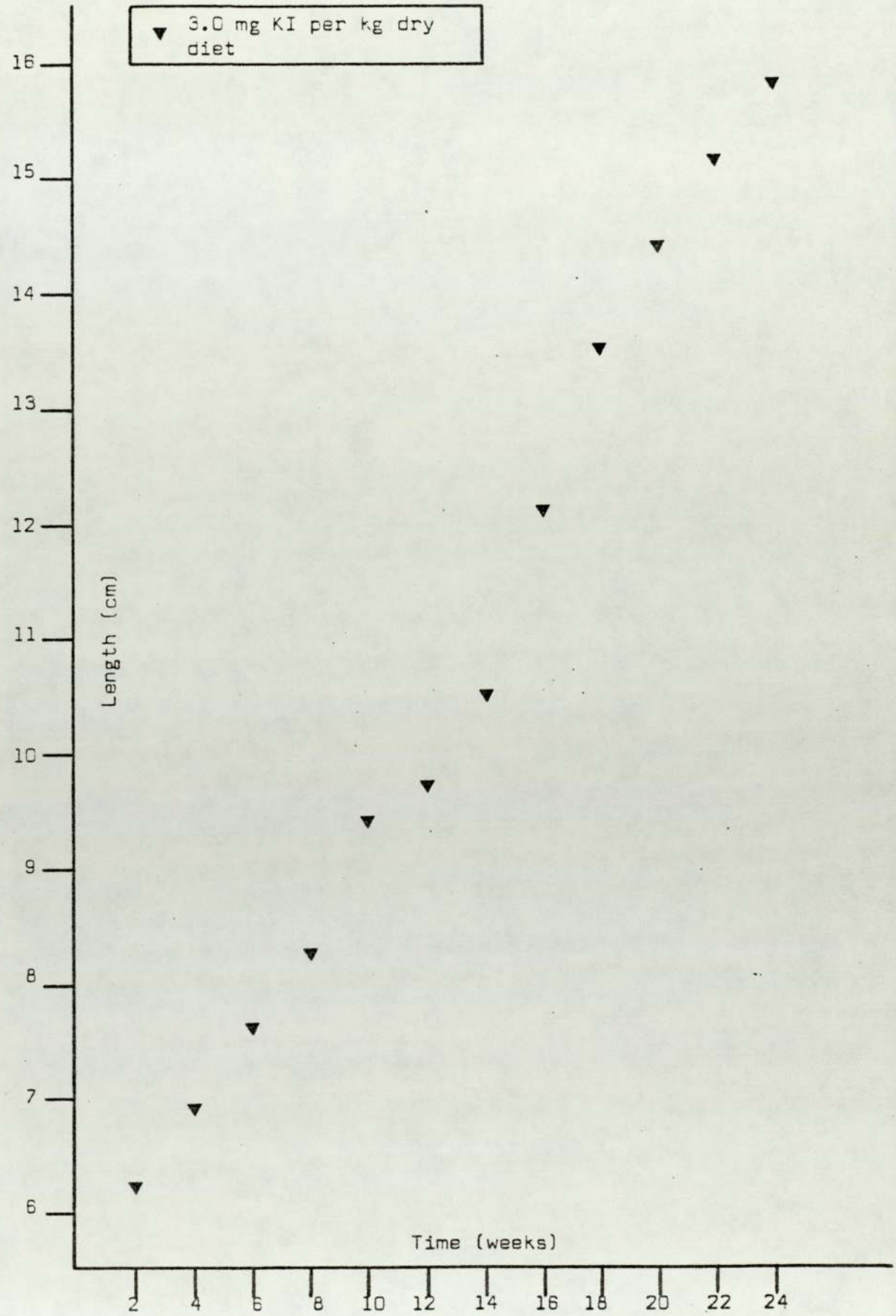


Figure 8.2.6. Effect of Potassium Iodide on Body Length of carp.

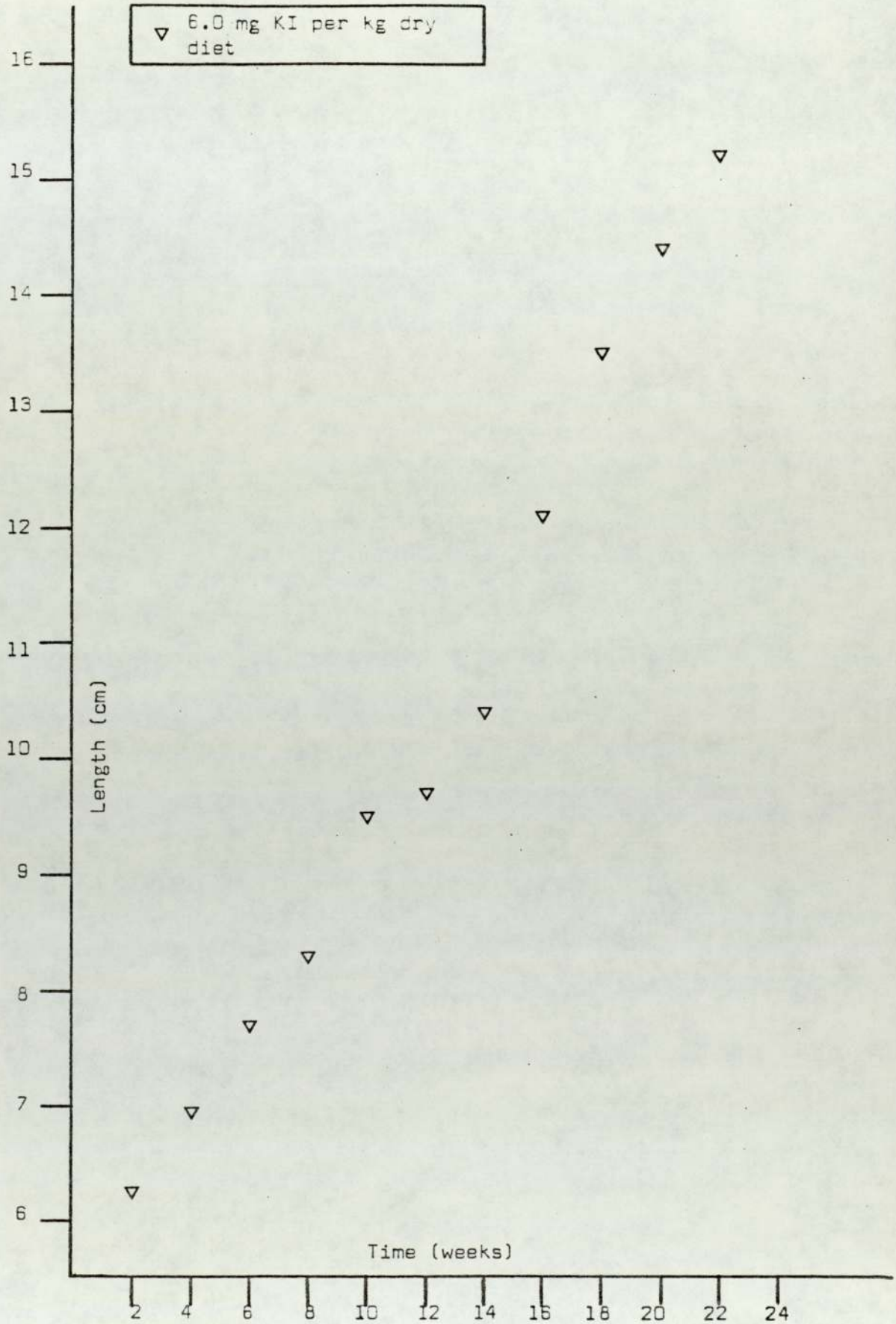


Figure 8.2.7. Effect of Potassium Iodide on Body Length of carp.

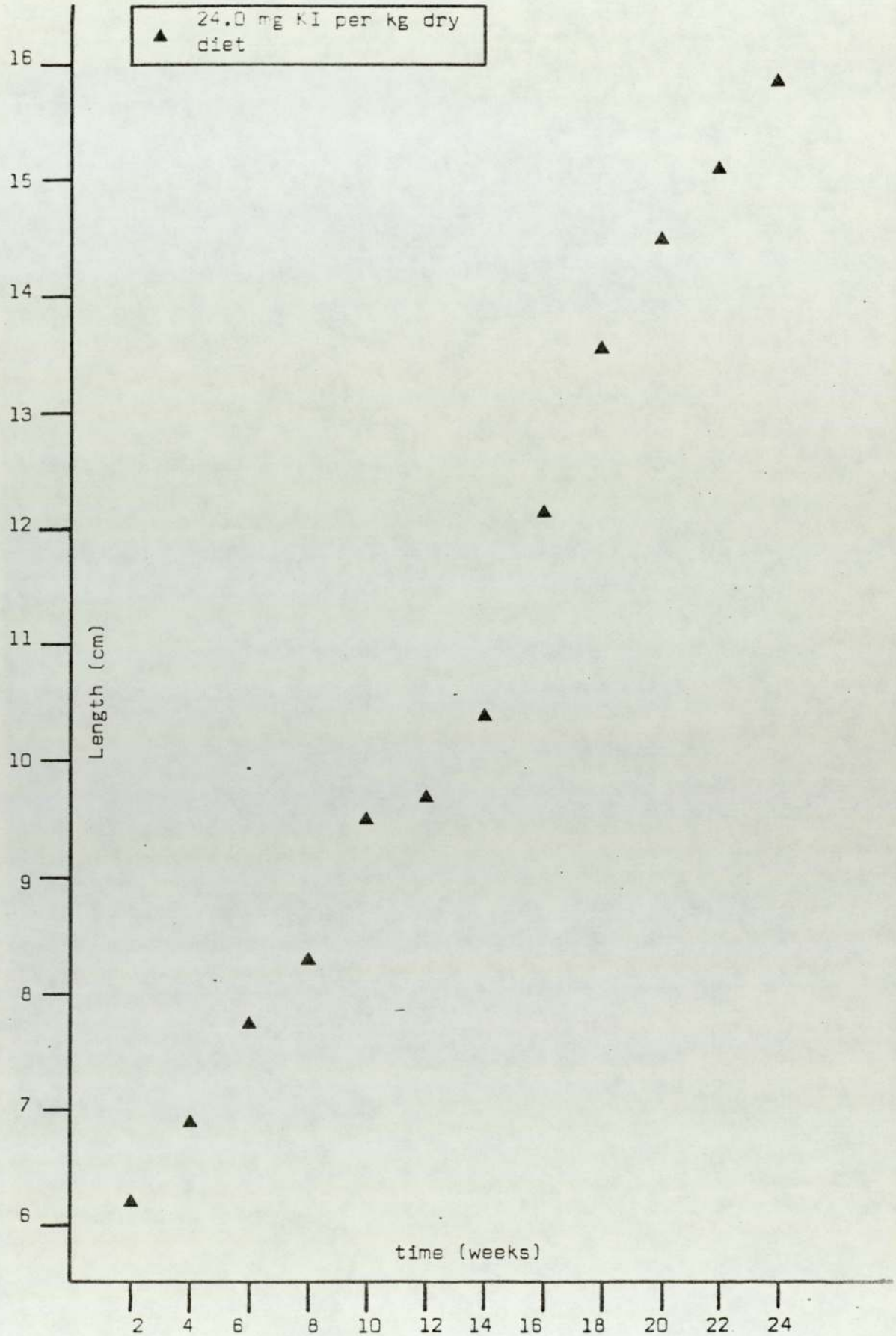


Figure 8.2.8. Effect of Potassium Iodide on Body Length of carp.

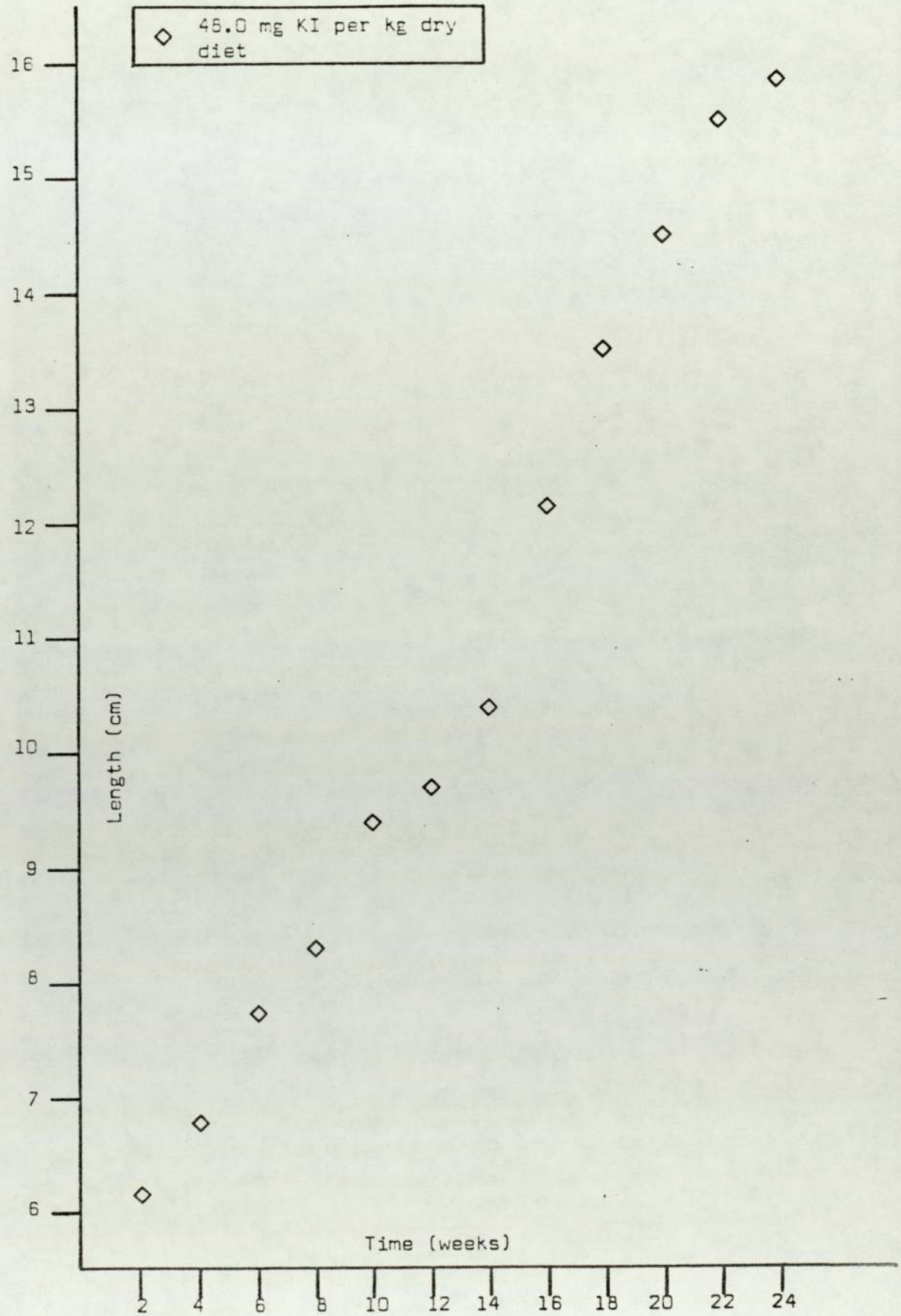


Figure 8.2.9. Effect of Potassium Iodide on Body Length of carp.

Table 8.5

Effect of Oral Administration of Potassium Iodide on Daily Increase of Body Weight of Carp

Period in weeks	Daily increase of mean Body Weight, g per 100 g initial weight								
	Concentration of Potassium Iodide in the diet, mg/kg dry diet								
	Control	0.1	0.3	0.6	1.2	3.0	6.0	24.0	48.0
0 - 4	4.9	5.1	5.2	5.4	5.6	5.2	5.2	4.9	5.0
4 - 8	3.4	3.5	3.5	3.9	3.9	3.6	3.6	3.4	3.4
8 - 12	4.8	5.0	5.0	5.1	5.2	4.9	4.9	4.7	5.0
12 - 16	5.4	5.7	5.7	5.7	5.6	5.5	5.5	5.3	5.3
16 - 20	5.6	6.4	6.4	6.4	6.3	6.2	6.2	6.0	6.0
20 - 24	5.7	6.9	6.9	6.9	6.9	6.7	6.7	6.6	6.6

Table 8.6

Effect of Oral Administration of Potassium Iodide on Daily Increase of Body Length of Carp

Period in weeks	Daily increase of Mean Body Length, cm per 100 cm initial length								
	Concentration of Potassium Iodide in the diet, mg per kg dry diet								
	Control	0.1	0.3	0.6	1.2	3.0	6.0	24.0	48.0
0 - 4	0.4	0.4	0.5	0.5	0.5	0.5	0.5	0.5	0.5
4 - 8	0.6	0.6	0.7	0.7	0.7	0.7	0.6	0.7	0.7
8 - 12	0.6	0.6	0.7	0.7	0.7	0.7	0.7	0.6	0.6
12 - 16	0.8	0.8	0.9	0.8	0.9	0.8	0.8	0.9	0.9
16 - 20	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
20 - 24	0.8	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9

Table 8.7

Effect of Oral Administration of Potassium Iodide on the Food Utilization Efficiency of Carp

Period in weeks	Food Utilization Efficiency, weight gain/food given								
	Concentration of Potassium Iodide in the diet, mg/kg dry diet								
	Control	0.1	0.3	0.6	1.2	3.0	6.0	24.0	48.0
0 - 4	0.76	0.80	0.82	0.84	0.85	0.80	0.81	0.78	0.84
4 - 8	0.52	0.51	0.54	0.54	0.53	0.51	0.52	0.51	0.52
8 - 12	0.81	0.83	0.80	0.80	0.82	0.81	0.81	0.81	0.84
12 - 16	0.72	0.72	0.72	0.72	0.70	0.71	0.70	0.71	0.72
16 - 20	0.75	0.75	0.78	0.78	0.77	0.78	0.78	0.78	0.78
20 - 24	0.69	0.75	0.74	0.74	0.79	0.73	0.73	0.73	0.73

utilization efficiency between the groups receiving iodide in their diet, when there was a decline in this value for the controls. This decrease was more pronounced in the last 12 weeks of the experiment.

The highest value was observed in the group receiving 1.2 mg iodide per kg dry diet, throughout the experiment.

Condition Factor

At the start of this experiment the mean value of the condition factor which relates the fish weight to its length was 0.012 - 0.013 among all the groups. For the first 4 weeks of the experiment all the groups including the controls showed a rise in the condition factor values, which was more pronounced in the group receiving 1.2 mg iodide per kg dry diet. After this initial rise the condition factor declined in all the groups including the controls for the next 4 weeks; thereafter, it rose again in all the groups for the next 4 weeks. After the 12th week of the experiment the condition factor started to decline in all the treated groups as well as the controls until the experiment terminated.

The highest values of this factor were observed in the group receiving 0.1 mg iodide per kg dry diet throughout the experiment (Table 8.8).

8.3.2 SURVIVAL RATE

There were no mortalities in the experimental groups for nearly 4 weeks from the start of this experiment. Mortality in the control group could be considered normal for carp under the experimental conditions during this period. A persistent daily

Table 8.8

Effect of Oral Administration of Potassium Iodide on the Condition Factor of Carp. Values given are the mean ± S.E.

Duration in weeks	Condition Factor								
	Concentration of Potassium Iodide in the diet, mg/kg dry diet								
	Control	0.1	0.3	0.6	1.2	3.0	6.0	24.0	48.0
0	0.012	0.012	0.012	0.012	0.012	0.013	0.012	0.013	0.012
4	0.021	0.021	0.021	0.021	0.022	0.021	0.021	0.021	0.021
8	0.015	0.014	0.014	0.015	0.015	0.015	0.015	0.015	0.014
12	0.016	0.017	0.016	0.015	0.016	0.016	0.015	0.016	0.016
16	0.012	0.012	0.012	0.012	0.011	0.012	0.012	0.011	0.011
20	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009
24	0.009	0.010	0.009	0.009	0.009	0.009	0.009	0.009	0.009

mortality occurred in the controls and the 0.1 and 0.3 mg iodide per kg dry diet groups in the last 8 weeks. Within 2 months over one-half of the fish in the control group had died, when the mortality in the treated groups mentioned above were 65% and 60% respectively.

There was no evidence of goitre noted on gross examination in the fish in treated groups or in controls.

Table 8.9 illustrates the mortality rate of the treated groups and the controls during this experiment.

8.3.3 CRANIO-SOMATIC, HEPATO-SOMATIC, RENO-SOMATIC AND VISCERO-SOMATIC INDICES

The ratios of different body organs to body weight are given in Tables 8.10 and 8.11 over a period of 24 weeks of growth. There were no differences in these ratios between the treated groups throughout the experiment; although some differences were observed between the controls and treated groups, they were not significant. The ratios of each body organ to the weight of the fish for each group over a period of 24 weeks are given in Figures 8.3 - 8.6.

8.3.4 BODY COMPOSITION

Proximate analysis of intermediate (12 weeks) and terminal (24 weeks) samples showed no significant differences in gross body composition (moisture; protein; fat; ash) between any groups including the controls. However, the highest protein and fat contents of the body were observed in the group that received 1.2 mg iodide per kg dry diet, and the highest ash content in the group that received 48.0 mg iodide per kg dry diet. Table 8.12 shows the proximate body analysis over a period of 24 weeks of feeding.

Table 8.9

Survival Rate of Carp fed diet supplemented with Potassium Iodide for 24 weeks

Duration in weeks	Concentration of Potassium Iodide in the diet/mg per kg dry diet																	
	Control		0.1		0.3		0.6		1.2		3.0		6.0		24.0		48.0	
	No. of fish	%	No. of fish	%	No. of fish	%	No. of fish	%	No. of fish	%	No. of fish	%	No. of fish	%	No. of fish	%	No. of fish	%
0	160	100	40	100	40	100	40	100	40	100	40	100	40	100	40	100	40	100
2	160	100	40	100	40	100	40	100	40	100	40	100	40	100	40	100	40	100
4	158	98.8	40	100	39	97.5	40	100	40	100	40	100	40	100	40	100	40	100
6	158	98.8	39	97.5	39	97.5	40	100	40	100	40	100	39	97.5	40	100	40	100
8	149	93.1	39	97.5	38	95	39	97.5	39	97.5	39	97.5	39	97.5	39	97.5	39	97.5
10	149	93.1	38	96	38	95	39	97.5	39	97.5	39	97.5	39	97.5	38	95	38	95
12	144	90	38	96	38	95	38	95	38	95	38	95	39	97.5	37	92.5	38	95
14	136	85	36	90	36	90	38	95	38	95	38	95	39	97.5	36	90	38	95
16	128	80	34	85	32	80	38	95	37	92.5	38	95	37	92.5	34	85	38	95
18	115	72	30	75	30	75	38	95	37	92.5	37	92.5	36	90	34	85	36	90
20	96	60	28	70	26	65	37	92.5	37	92.5	37	92.5	36	90	34	85	34	85
22	88	55	28	69	24	60	37	92.5	37	92.5	36	90	36	90	34	85	34	85
24	72	45	26	65	24	60	36	90	37	92.5	36	90	36	90	34	85	34	85

Table 8.10

Cranio somatic, Hepato somatic, Reno somatic and Viscero somatic indices of carp fed potassium iodide supplemented diet over a period of 12 weeks

	Concentration of Potassium Iodide in the diet, mg/kg dry diet								
	Control	0.1	0.3	0.6	1.2	3.0	6.0	24.0	48.0
No. of samples	3	3	3	3	3	3	3	3	3
Weight of fish, mean ± S.E.	13.50 ± 0.75	14.10 ± 0.25	14.15 ± 0.30	14.20 ± 0.50	15.00 ± 0.35	14.00 ± 0.40	14.50 ± 0.33	14.10 ± 0.45	14.10 ± 0.60
Brain weight (g); CSI	0.09 0.64	0.10 0.70	0.10 0.70	0.10 0.69	0.11 0.73	0.10 0.69	0.10 0.68	0.09 0.63	0.09 0.63
Liver weight (g); HSI	0.29 2.07	0.30 2.11	0.31 2.17	0.31 2.15	0.32 2.13	0.30 2.06	0.31 2.11	0.33 2.29	0.30 2.10
Kidney weight (g); RSI	0.06 0.43	0.07 0.49	0.07 0.49	0.07 0.48	0.08 0.53	0.07 0.48	0.08 0.54	0.09 0.62	0.07 0.49
Viscera weight* (g); VSI	0.57 4.07	0.59 4.14	0.60 4.20	0.61 4.22	0.63 4.20	0.60 4.14	0.61 4.15	0.61 4.23	0.59 4.13

*gut, swim bladder and heart

Table 8.11

Cranio somatic, Hepato somatic, Reno somatic, and Viscero somatic indices of carp fed potassium iodide supplemented diet over a period of 24 weeks

	Concentration of Potassium Iodide in the diet, mg/kg dry diet								
	Control	0.1	0.3	0.6	1.2	3.0	6.0	24.0	48.0
No. of samples	3	3	3	3	3	3	3	3	3
Weight of fish, mean ± S.E.	30.00 ± 0.95	35.00 ± 1.60	35.10 ± 1.55	35.10 ± 1.30	35.50 ± 2.00	35.10 ± 1.50	35.30 ± 1.45	35.20 ± 1.35	34.80 ± 1.20
Brain weight (g); CSI	0.15 0.50	0.18 0.51	0.19 0.54	0.19 0.54	0.20 0.56	0.21 0.60	0.20 0.57	0.19 0.54	0.18 0.51
Liver weight (g); HSI	0.50 1.67	0.65 1.86	0.64 1.82	0.65 1.85	0.66 1.86	0.65 1.84	0.65 1.83	0.64 1.84	0.63 1.80
Kidney weight (g); RSI	0.10 0.33	0.12 0.34	0.11 0.31	0.13 0.37	0.14 0.39	0.14 0.39	0.13 0.38	0.15 0.43	0.14 0.40
Viscera weight* (g); VSI	1.10 3.67	1.48 4.09	1.41 4.02	1.44 4.10	1.46 4.11	1.45 4.11	1.44 4.08	1.43 4.06	1.42 4.06

*gut, swim bladder and heart

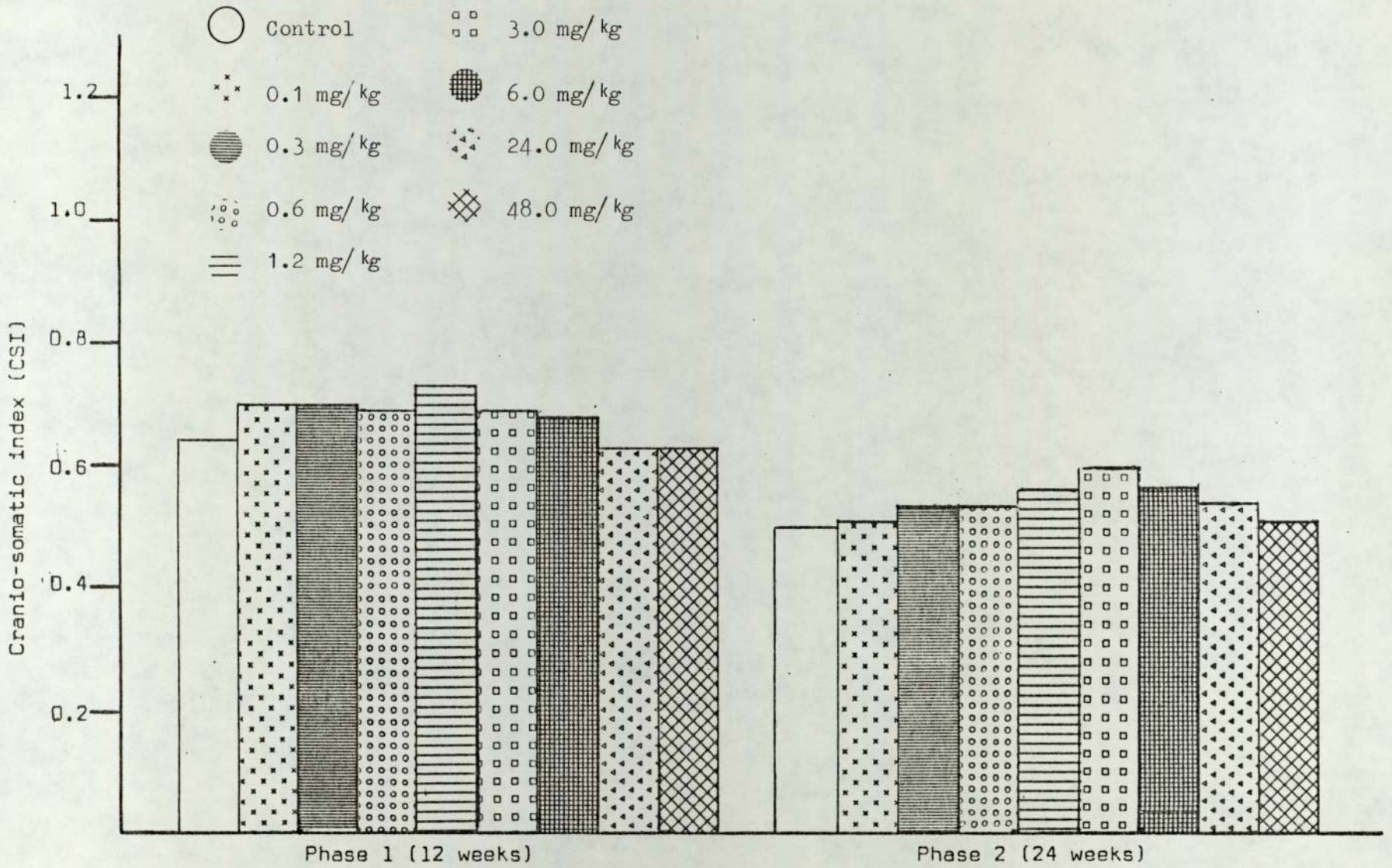


Figure 8.3. Effect of Potassium Iodide on the CSI of carp

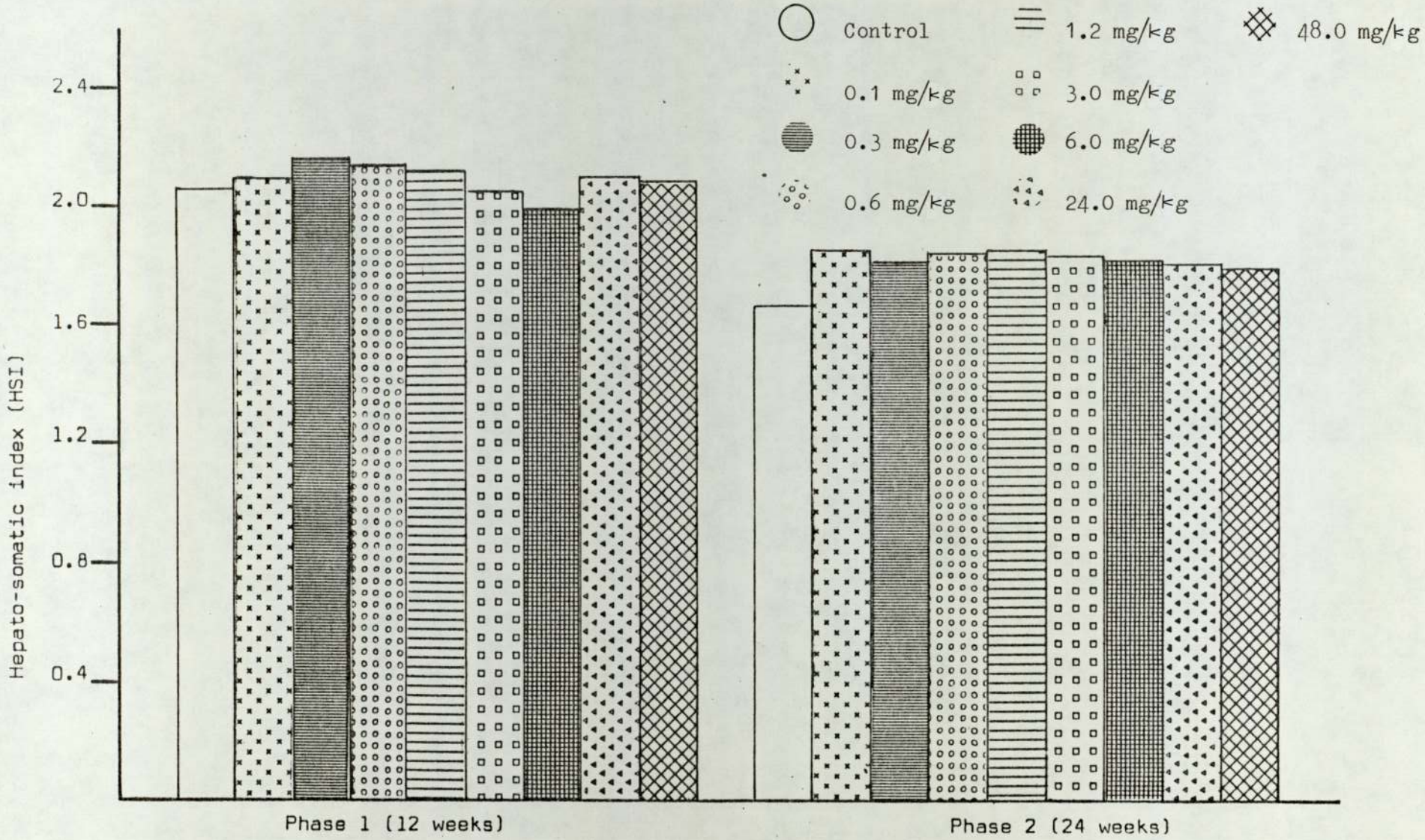


Figure 8.4. Effect of Potassium Iodide on the HSI of carp.

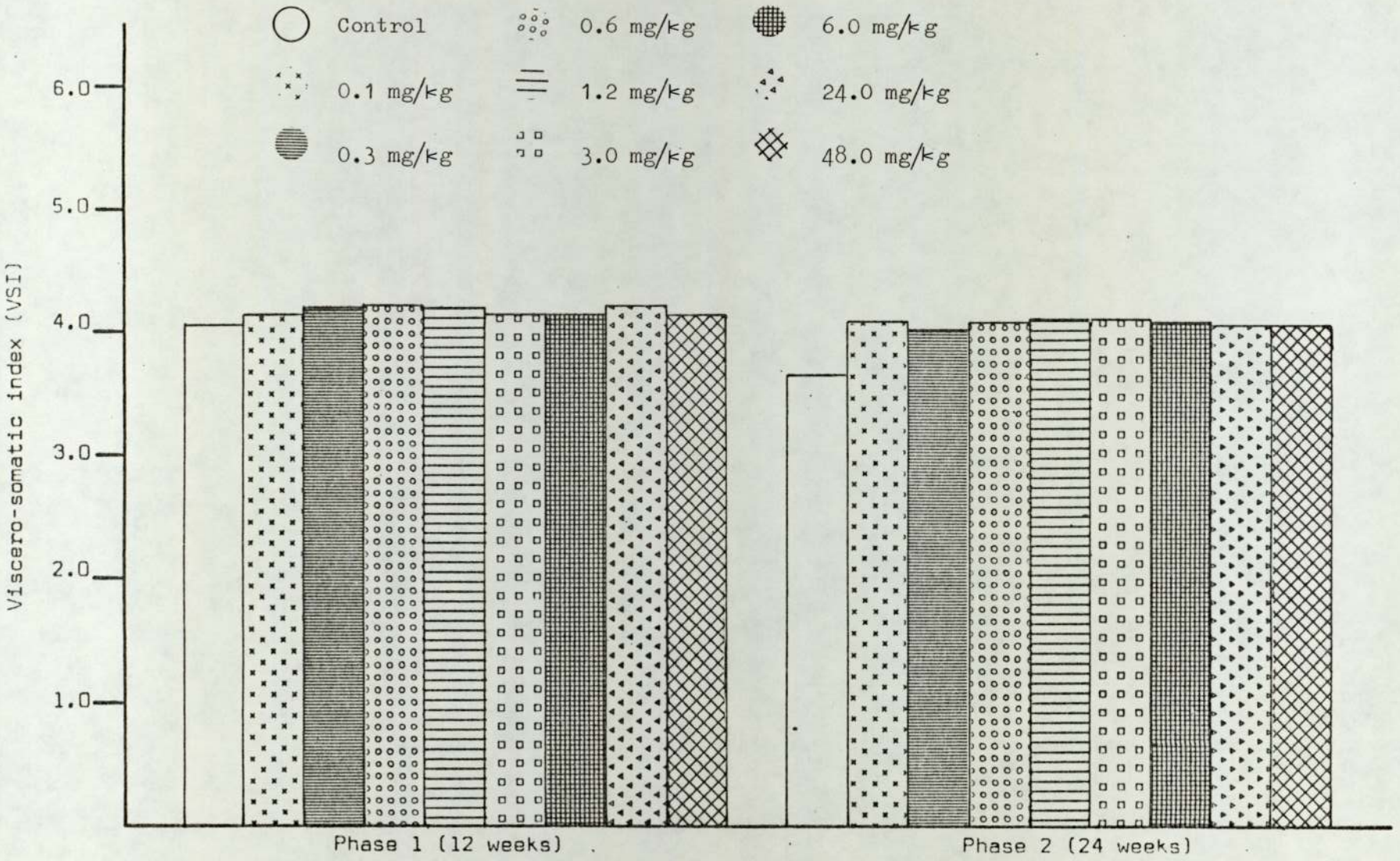


Figure 8.5. Effect of Potassium Iodide on the VSI of carp.

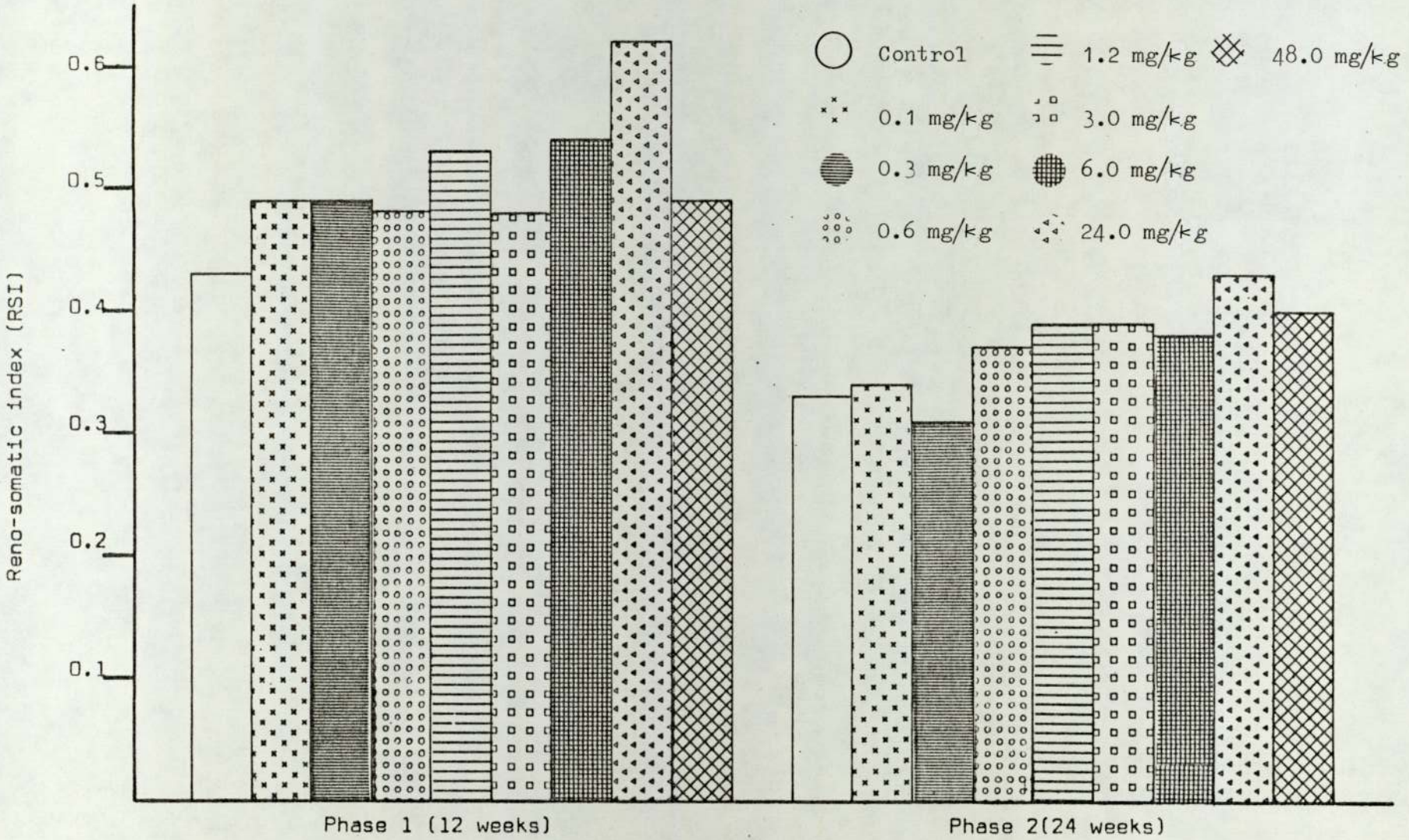


Figure 8.6. Effect of Potassium Iodide on the RSI of carp.

Table 8.12

Body Composition of Carp Fed Potassium Iodide Supplemented diet for a period of 24 weeks

Body Composition of carp %	Concentration of Potassium Iodide in the diet, mg/kg dry diet								
	Control	0.1	0.3	0.6	1.2	3.0	6.0	24.0	48.0
Moisture	77.50	77.50	77.30	77.60	76.00	76.50	76.80	76.70	76.90
Crude Protein	11.50	11.45	11.40	11.10	12.10	11.50	11.70	11.60	11.80
Total Fat	4.30	4.20	4.30	4.40	4.80	4.60	4.56	4.70	4.50
Ash	2.50	2.50	2.60	2.55	2.60	2.70	2.80	2.90	3.10
Nitrogen Free* extract + fibre	4.20	4.35	4.40	4.35	4.50	4.70	4.14	4.10	3.70
Total	100	100	100	100	100	100	100	100	100

*NFE + fibre were determined by the formula:

NFE + fibre = 100 - (% moisture + % crude protein + % total fat + % ash)

8.3.5 IODINE CONTENT OF THE BODY

Iodine stored in the body as thyroidal iodide in carp was estimated as described in Chapter 4 (4.8) by removing the thyroid-containing area consisting of the floor of the mouth from the tongue to the last gill arch; after removing, this was freed from the excess peripheral tissues, weighed, wrapped in a plastic film and quick frozen for subsequent micro-iodine determination. Tables 8.13 and 8.14 show the results obtained from this experiment over a period of 24 weeks. The thyroid iodine storage in the intermediate samples showed that the fish in the control and the treated groups which received 0.1 and 0.3 mg iodide per ^{k}g dry diet contained significantly ($P < 0.01$) less iodine than the fish in all other treated groups (Figure 8.7). At the termination of the experiment, the same result was obtained (Figure 8.8). Less than 4.7 µg iodine was stored in the thyroid area in the control, 0.1 and 0.3 mg/^{k}g groups compared with more than 9.1 µg thyroidal iodine in the other treated groups.

8.3.6 DEFICIENCY SYMPTOMS

Careful examination disclosed no observable deficiency symptoms during the first 12 weeks of the feeding trial, although evidence of simple goitre was looked for externally. At the termination of the experiment there was no evidence of goitre noted on gross examination on fish. However, some of the fish (10%) receiving 24.0 and 48.0 mg iodide per ^{k}g dry diet exhibited protruding eyes which may indicate an effect of high intake of iodine by these two groups only.

8.4 DISCUSSION AND CONCLUSIONS

Our results showed that iodide intakes which were insufficient

Table 8.13

Effect of feeding diet containing potassium iodide on

iodine stored in the body over a period of 12 weeks

Iodine stored over a period of 12 week of feeding; micrograms thyroidal iodine per 100 g body weight	Concentration of Potassium Iodide in the diet, mg/kg dry diet								
	Control	0.1	0.3	0.6	1.2	3.0	6.0	24.0	48.0
	1.1 ± 0.11	3.0 ± 0.07*	2.8 ± 0.20*	5.1 ± 0.12	5.5 ± 0.15	5.7 ± 0.17	5.7 ± 0.13	5.9 ± 0.10	5.9 ± 0.12

* = $P < 0.01$

Table 8.14

Effect of feeding diets containing potassium iodide on iodine stored in the body over a period of 24 weeks

Iodine stored over a period of 24 weeks of feeding; micrograms thyroidal iodine per 100 g body weight	Concentration of Potassium Iodide in the diet, mg/kg dry diet								
	Control	0.1	0.3	0.6	1.2	3.0	6.0	24.0	48.0
	3.3 ± 0.14*	4.5 ± 0.15*	4.7 ± 0.17*	9.1 ± 0.22	10.5 ± 0.19	10.8 ± 0.31	11.1 ± 0.27	11.4 ± 0.53	11.9 ± 0.41

* = P <0.01

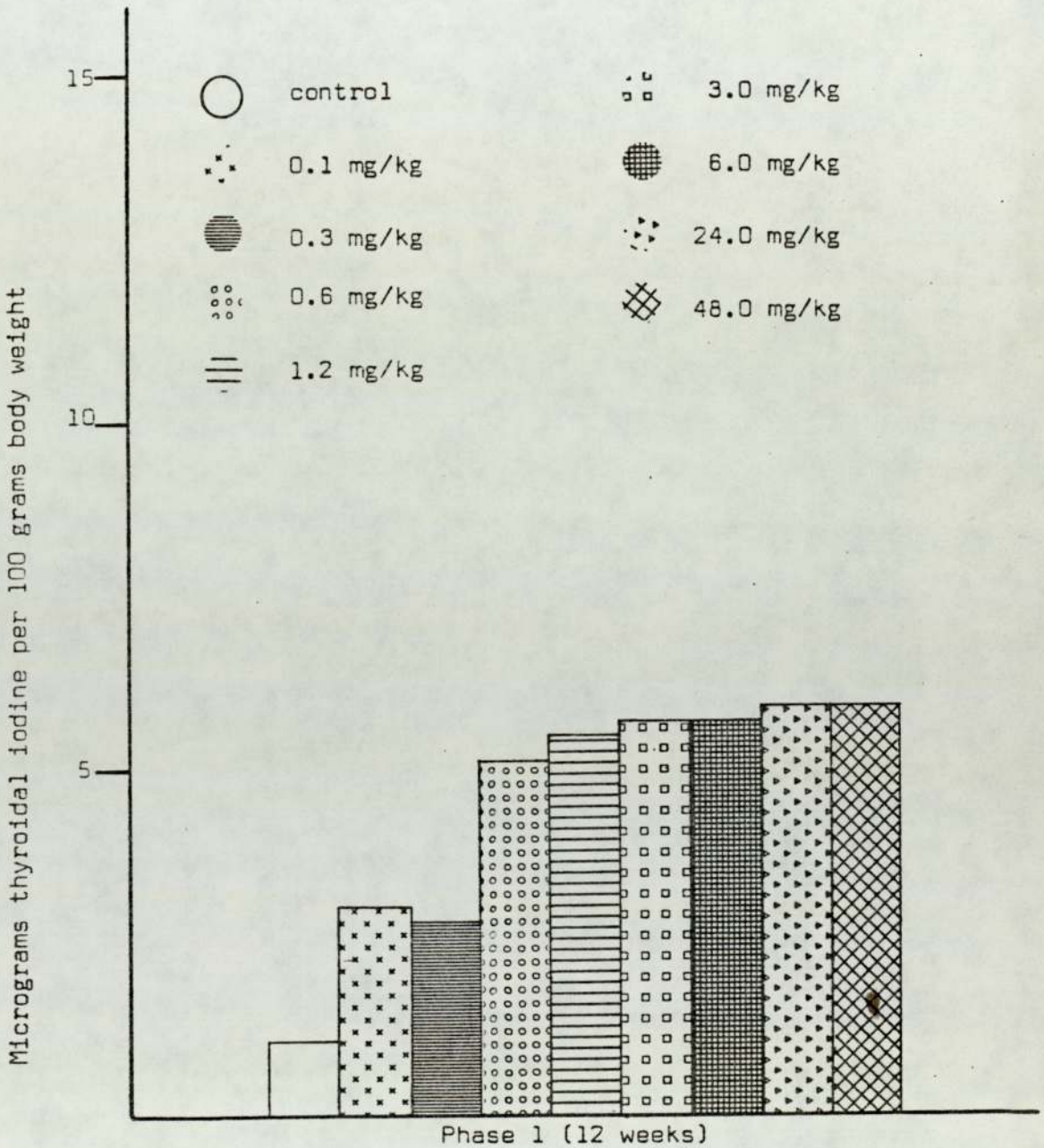


Figure 8.7. Iodine stored in carp body over a period of 12 weeks.

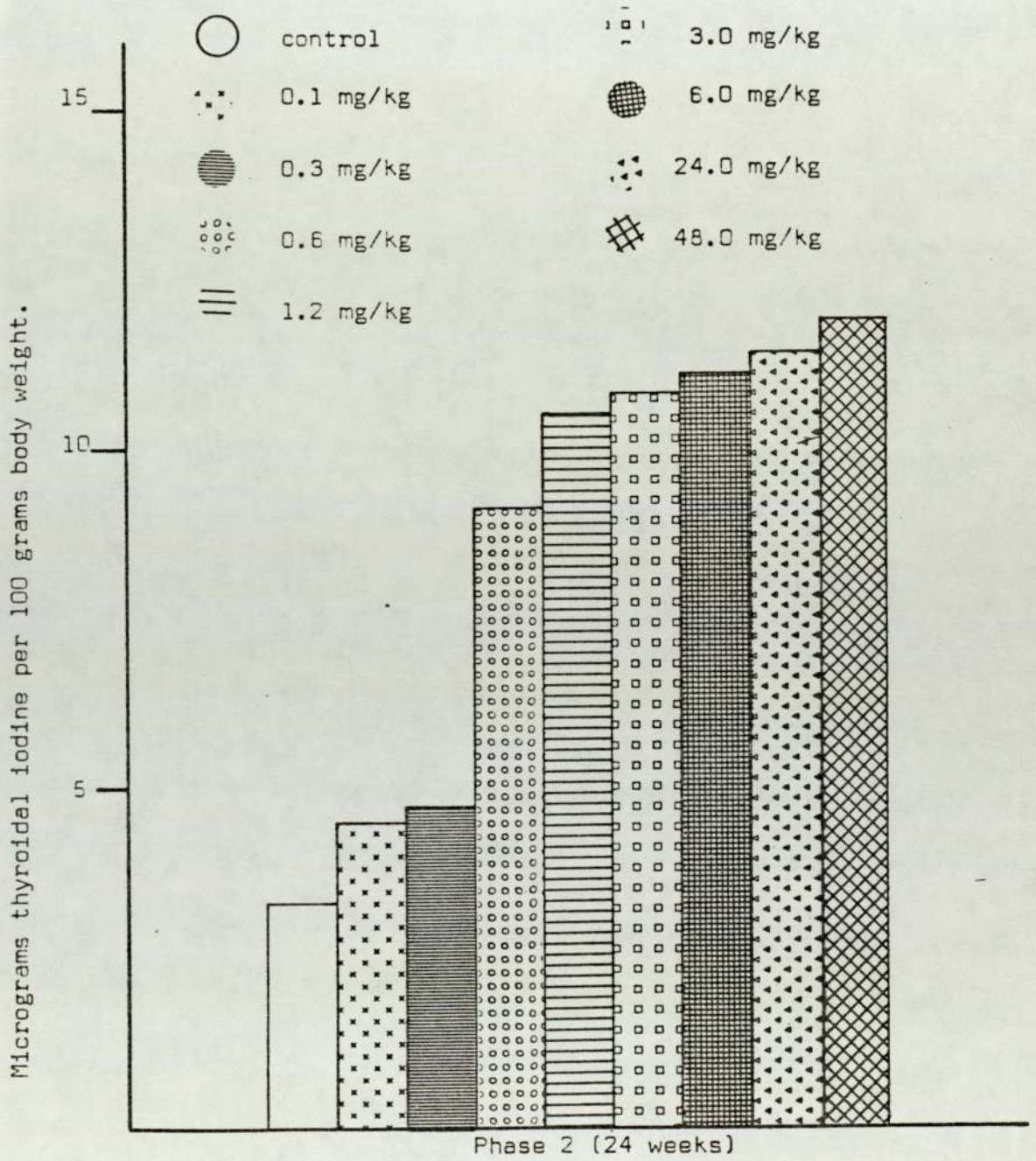


Figure 8.8. Iodine stored in carp body over a period of 24 weeks.

to maintain adequate thyroid iodide storage in carp did not interfere with the normal growth of the fish. This response is consistent with the results of comparable experiments reported for many other animals.

Levine et al., 1933, reported no growth difference in rats fed levels of iodide from 15 - 400 micrograms per kg of diet. Scott et al., 1960, reported that pheasants or quail fed a low iodide diet (0.2 mg per kg of diet) showed no growth response to added iodide supplements. LaRoche and Leblond, 1952, compared the growth rate and histological appearance of thyroid glands of Atlantic Salmon, Salmo salar, which were fed an iodine-deficient diet, beef liver, in untreated and iodide-enriched water. It was shown that the iodide treatment slightly reduced growth rate; LaRoche and Leblond, 1954, reported that the growth rate of thyioidectomized and control Atlantic Salmon did not differ significantly for 10 months following the administration of I^{131} for the thyroid destruction. Barrington et al., 1961, observed that the addition of thyroid powder to a diet of liver caused increased growth in rainbow trout, Salmo gairdnerii; Robertson and Chaney, 1953, reported that rainbow trout living under conditions of low iodide intake did not develop thyroid hyperplasia.

Although the significance of increased numbers of mortalities in the fish fed the lower levels of iodide during the latter part of the feeding trial cannot be fully understood, sufficient corraborative evidence exists to suggest an explanation. (Hoar 1952); suggests than an anadromous teleost making a prolonged stay in fresh water is under added osmotic stress which demands additional thyroid hormones. Baker-Cohen, 1959, reported that

heterotopic thyroid tissue developes as a compensatory device when iodine demands of the animal exceed the available supply of this essential component of thyroid hormones.

Based on the maintenance of the maximal thyroid iodine storage the evidence presented indicates that, for the experimental conditions described, the minimal iodide requirement of carp fingerlings is 0.6 mg/kg dry diet and the optimum iodide level in the diet for carp at that size is 1.2 mg/kg dry diet.

CHAPTER NINE

GENERAL CONCLUSIONS

9. GENERAL CONCLUSIONS

It may be concluded that carp, Cyprinus carpio, definitely respond to cobalt chloride treatment by improved growth and surivival rates, and that the optimum cobalt requirement for carp is 3 mg per kg dry diet (Chapters 5 and 6). However, when the dose of cobalt was increased to 30 mg per kg dry diet both the growth and the survival rates declined and all fish finally died.

Comparing the results of feeding diet containing no vitamin B_{12} (Chapter 5, section 3), and diet containing 25 micrograms vitamin B_{12} per kg diet (Chapter 6, section 3), some general conclusions may be drawn:

1. The total weight and length in a period of 24 weeks was higher in carp fed cobalt with vitamin B_{12} than those fed cobalt salt alone; there was a significant increase in the weight and length of the controls in the carp fed vitamin B_{12} (Chapters 5 and 6). This increase may not give us a good indication about the effect of the salt with or without the vitamin since the initial weight of the fish is different in the two experiments (Chapter 5 and 6). Thus, to have a clear picture about the effect of the salt (cobalt) either without or with the 25 microgram vitamin B_{12} per kg dry diet, we compare the weight and length gain of fish having almost the same initial length and weight. Table 9.1 shows that there was a significant increase in length of the control when the diet was supplemented with vitamin B_{12}, when there was no significant increase in the length between the treated groups. As for weight, there was a considerable increase in the weight of the fish receiving cobalt alone and those with the vitamin (Figure 9.1). This difference may be due to the different starting weights of the experiments (in experiment (1), Chapter 5, the values given in

Table 9.1

Weight and Length Gain in Carp Fed Diet Supplemented Either Without or With Vitamin B_{12} for 8 Weeks

Concentration of Cobalt in the diet mg/kg dry diet	Weight Gain g		Length Gain cm	
	without B_{12}	with B_{12}	without B_{12}	with B_{12}
Control	10.52**	6.00	1.50	2.20**
3	22.00***	15.00	2.30	3.60
6	18.30**	14.60	2.05	2.50
30*	13.50**	10.50	1.00*	2.30

* Samples taken after 21 weeks of feeding

** $P < 0.01$

*** $P < 0.05$

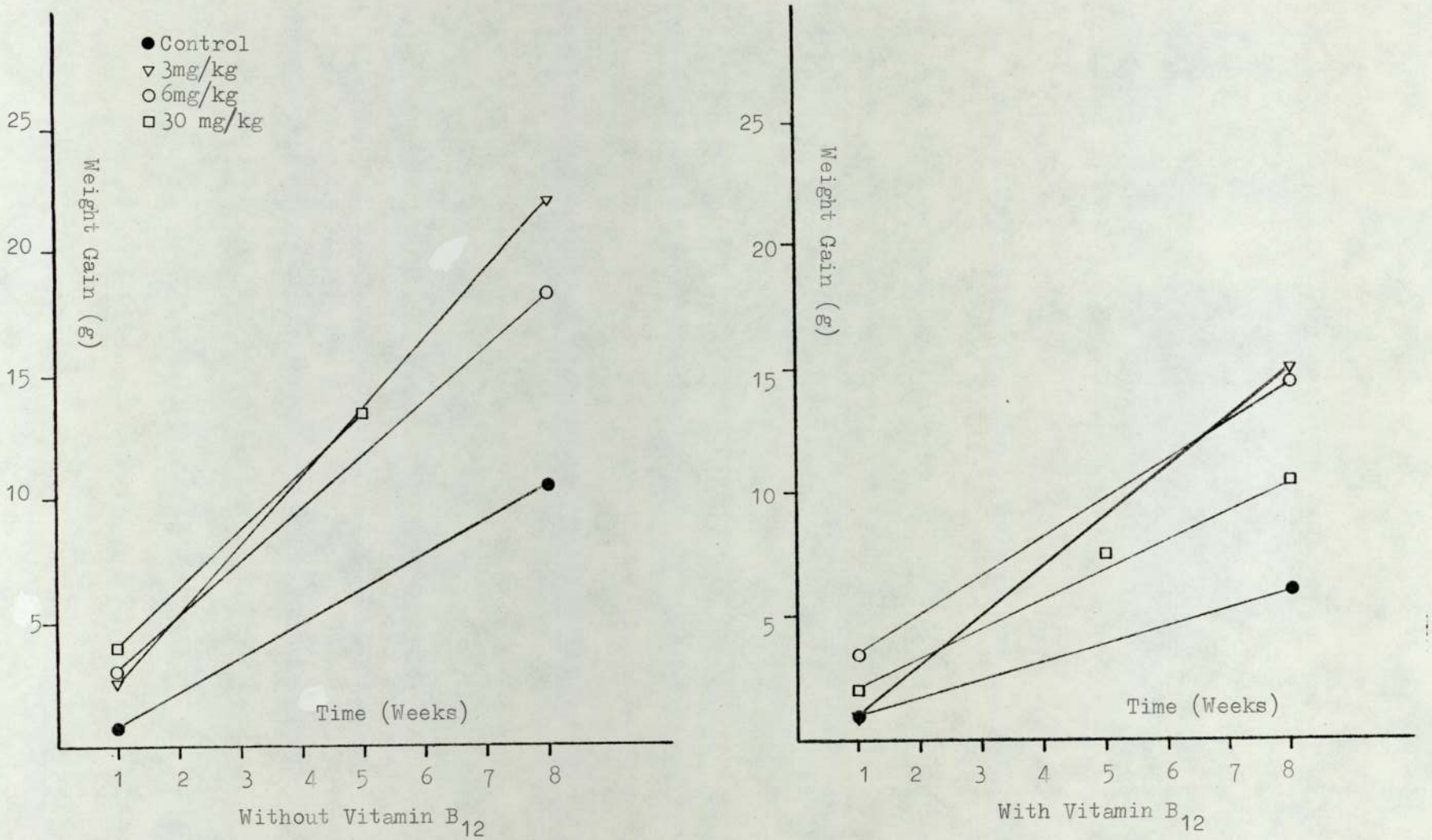


Figure 9.1. Effect of Diet Supplemented With Cobalt Either Without or With Vitamin B_{12} on Net Weight Gain in Carp .

Table 9.1 are from 16 - 24 weeks of growth, and in the second experiment the same values were from 0 - 8 weeks of growth). Thus these differences may be a result of the reason mentioned above.

2. The survival rate was higher in the groups fed cobalt with vitamin B_{12} than those without the vitamin including the controls (Table 9.2).

3. When the dose was increased to 30 mg cobalt per kg dry diet, with or without vitamin B_{12}, high mortality occurred as a result of this high dose with no significant difference between the results of the two experiments.

4. The haematological characteristics were improved more in the fish given cobalt chloride with vitamin B_{12} than those given cobalt alone (Table 9.3).

5. There was no significant differences between the experiments in the total cobalt accumulation in the body and the distribution of cobalt within the fish organs.

6. Adding vitamin B_{12} to the diet alone failed to prevent the deficiency symptoms occurring in the fish fed diet without cobalt as cobalt chloride, but the percentage of the fish that developed malformation in their tail and head was less in the group receiving the vitamin in their diet than those without the vitamin; this may possibly be due to the amount of cobalt present in the vitamin itself.

As far as the effect of adding cobalt to the diet on the resistance to wintering (low water temperatures) is concerned one may conclude that the supplementation of the diet with a dose of 3 mg cobalt per kg dry diet is the optimal dose for carp. Both growth and survival rates under different water temperatures (5 - 30^{o}C), were higher than those in other treated groups and

Table 9.2

Survival Rate of Carp Fed Diet Supplemented with Cobalt Either Without or With Vitamin B_{12} for 24 Weeks

Concentration of Cobalt in the Diet mg per Kg dry diet	Survival Rate (%)	
	Without B_{12}	With B_{12}
Control (0)	50	70
3	85	90
6	80	80
30	10	10

Table 9.3

Haematological Characteristics of Carp Fed Diet Supplemented With Cobalt Either Without Vitamin B_{12} or With Vitamin B_{12} for 24 Weeks

Concentration of Cobalt in the Diet mg per Kg dry diet	Haematological Characteristics		
		Without B_{12}	With B_{12}
Control (0)	Hemoglobin %	31	32
	Erythrocyte[1]	838	1,221
	Leucocyte[2]	26	42
3	Hemoglobin %	35	36
	Erythrocyte[1]	1,537	1,812
	Leucocyte[2]	21	23
6	Hemoglobin %	35	36
	Erythrocyte[1]	1,800	1,786
	Leucocyte[2]	20	21
30	Hemoglobin %	34	35
	Erythrocyte[1]	1,783	1,780
	Leucocyte[2]	19	17

1 Erythrocyte counts in thousands per cm^3.

2 Leucocyte counts in thousands per cm^3.

controls. These findings are in agreement with those of Sukhoverkhov, 1967; Ghosh, 1968; Sen, 1972 and Sen et al., 1976, who observed that the survival and growth of the fish (trout and carp) can be enhanced significantly by the addition of cobalt salts (0.5 mg/10 g fish weight), to the diet.

Shabalina, 1963, studied the index of fatness in trout grown on food enriched with cobalt, and found that fat in the liver and the initial melting point of fat deposit in the experimental trout were considerably lower than the controls; he concluded that trout receiving cobalt produced more unsaturated fatty acids than those in the controls and the presence of these fatty acids allows the fish to withstand low temperatures during wintering. The same results were obtained by Karpanin and Ushakov, 1961, who found a winter mortality of 80% in the controls, while for carp which received cobalt the figures were 16 and 20%.

Our results show that there was a general increase in survival rate in groups receiving cobalt as cobalt chloride in their diet (3 mg/kg dry diet), and the winter mortality (under laboratory conditions) occurring in the group mentioned above was 10% compared with 30 cortality occurring in the control.

As for iodine requirement in carp, our results showed that iodide intakes which were insufficient to maintain adequate thyroid iodide storage did not interfere with the animals' normal growth. This response in consistent with the results of comparable experiments reported earlier in Chapter 8 (8.4). It may be concluded that carp fingerlings responded to iodide treatment by improved growth and survival and that the optimal iodide requirement for this size is within the range of 0.6 - 1.2 mg per kg dry diet.

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