THE EFFECT OF NON-STEROID GROWTH PROMOTANTS ON THE GROWTH OF COMMON CARP

TAGRIED SHAHAB AHMAD

Doctor of Philosophy

THE UNIVERSITY OF ASTON IN BIRMINGHAM

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SUMMARY

Feed additives are widely used in livestock production to improve animal performance. These additives include antibacterial agents (ABA), steroid hormones, minerals and vitamins. Low concentrations of antibiotics are commonly added to livestock feedstuffs in order to stimulate body growth. In fish antibiotics have not been used and even when they were applied experimentally in the past they did not seem to improve growth rate. However in the present study, for the first time, some ABAs such as virginiamycin, terramycin and payzone have been shown to increase the weight and feed conversion efficiency of the fish significantly and to a lesser extent so have other antibiotics such as tylosin and avoparcin. These feed additives were tested mainly on mirror carp *Cyprinus carpio* .

The mode of action of these antibiotics on growth were sought. This investigation included their effect on the bacterial population of the intestinal tract, on the histology of the gut and on their possible protein sparing effect. In order to study possible protein sparing ABA were added to diets containing two levels of protein (low: 25% and high: 38%).

Other substances such as zeranol, a weak hormone-like compound, and emtryl a protozocide were also examined for their effects on growth and were compared with the effects of ABA. The role of all these feed additives on the biochemical composition of the muscle of the fish and on their liver, kidney and intestine were also investigated.

The results showed that ABA that promoted growth significantly tended to increase the fat content of fish. Unlike ABA zeranol increased the protein and water content of the muscles of the treated fish. Two antibiotics virginiamycin and terramycin, were injected into carp subcutaneously to ascertain their effect by different routes of administration. The effect was negative which may give support to the hypothesis that antibiotics act on the bacterial flora of the intestine to promote growth. Finally the safety of the use of these feed additives and their residues in the edible tissues were discussed. The general conclusion of the study is that ABA can promote growth of fish but that there is no clear evidence that they promote this growth through effect as bacteriocidal agents.

Key words : Feed-additives, protein, growth, carp

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CHAPTER ONE

1. General Background And Objective :

Recent rises in the cost of red meat and poultry have pressured the consumer to seek other less expensive sources of protein. One of these, aquatic products, and fish in particular, is the fastest-growing segment of animal production. Undoubtedly the increasing demands for fresh and saltwater products have caused the world to face a bigger danger, namely the exhaustion of the aquatic food resources. This growth of the commercial catching of wild fish has led to greater attention being paid to fish culture, an ancient form of animal husbandry; records of pond culture in China stretch back as far as 2852 BC.

Since the end of the Second World War, three factors have modified considerably the image of fish cultivation. The first is the development of modern forms of transport which facilitates the transport of young fish, fry and eggs over long distances. The second factor is the growth of artificially-induced reproduction of many economically important species of fish. The third and most important factor is the intensification of fish cultivation and the use of artificial food based on concentrates distributed in a pelleted form. This food is easier to prepare, to conserve and to distribute than traditional food, and in a number of cases it can be made very economically. First used for the cultivation of salmonids, pelleted food is now used for other species, such as cyprinids, eels and catfish. The last few

years have seen a rapid development of research in aquatic nutrition, now entering a new phase in which feed and feeding methods must be further improved to make aquatic farming more economically viable. In aquaculture the cost of feeding is the largest single cost factor. Basically the nutritional requirements of fish are similar to those of mammals, with the exception of the need for a higher protein requirement and correspondingly lower energy need. These may vary from species to species. Today it is realized that those requirements need to be much further refined to maximize production, bearing in mind rearing, health and ecological requirements and conditions for a given species in a specific culture system. Nutritionists are under increasing pressure to provide more specialized and more exact rations and to look for methods which can be used to promote growth and increase weight and feed utilization of the fish. This will often mean adding growth-promoting substances to the basic food to serve these purposes.

Various substances, which are not nutrients in the classical sense of the term, have been used commercially for decades as growth-promotant additives to livestock feeds in relatively small amounts; they produce significant increases in rates of growth and/or efficiency of feed conversion in farm animals, and some of them also promote growth in fish. They include hormones, vitamins, mineral elements, and, from the 1950's, antibiotics. An antibiotic is defined as the

product of a micro-organism which, when present in very great dilution, will inhibit the growth of certain other organisms. Other similar compounds are not strictly defined as antibiotics, because they are not produced by microorganisms, but some have a similar action included by many authors under the general term of "antibiotic growth effect" (Visek 1978). To avoid confusion, the term "antibacterial agent" (ABA) will be used for all such compounds throughout this thesis.

It is logical to expect that the rate of growth of an animal will be increased when pathogenic or debilitating infection is eliminated, and this in essence is the reason for feeding ABA's. Indeed, the effects on growth and the resultant commercial and financial pressures are such that they are now added virtually universally to all kinds of animal feeds, and it is difficult or impossible to obtain them free from added antibiotic. This is causing increasing concern, that the widespread and indiscriminate use of these powerful drugs has undesirable effects, the evolution of resistant pathogens and the presence of residues in human foods.

In fish, the effects on growth of adding antibiotics to the diet have been generally inconclusive or equivocal, or reported only incidentally to other work. This thesis investigates the growth-promotant effects of various antibacterial agents either fed or administered to the common carp *Cyprinus carpio* by injection.

Injection procedures are perhaps too laborious and difficult to be applied practically on fish farms, but were chosen here for three reasons: (1) to evaluate the efficiency of the hormone-like compound zeranol, which is normally injected into livestock (2) to gain a better understanding of the mode of action of the drugs used; and (3) to compare with the dietary route of administration.

The drugs used were chosen (1) because they have previously been used in various species of fish but studies were incomplete, or their results not very clear; (2) they have been shown to be effective in other animals; and (3) to compare the effects of antibiotics and other antibacterial agents with a protozocide and a hormone-like compound. The drugs studied were tylosin, avoparcin, terramycin and virginiamycin as antibiotics, payzone as antibacterial, emtryl a protozocide, and zeranol a hormone-like compound.

The common carp *Cyprinus carpio* was chosen because of its ease of handling in the facilities available. The carp is farmed throughout Asia, the Middle East and Europe and is considered to be one of the most important food fish in the world, and is therefore a species which might benefit from being reared with a diet containing a growth promotant.

CHAPTER TWO

2. ANTIBIOTICS AND ANTIBACTERIAL AGENTS

2.1 Development Of Dietary Use Of Antibiotics :

Commercial feeds for terrestrial farm animals have been supplemented with vitamins, minerals, and hormones for decades, in order to improve growth and feed utilisation, and these additives were joined by antibiotics some 40 years ago. Many of them have positive effects also in fish.

Moore et al (1946) were among the earliest workers with antibiotics, using chicks. They noted that sulfasuxidine and streptomycin added to a basal purified diet, either singly or in combination, led to increased growth response, and stated that the observations suggested the inhibition of intestinal bacteria that were either producing toxic materials, or were rendering certain dietary components unavailable to the birds. Morehouse and Mayfield (1946) showed that 3-nitro-4-hydroxyphenyl arsonic acid, which is an antbacterial agent rather than an antibiotic, produced increased growth when added to the natural diet of chickens and turkeys. Harned et al (1948) reported that the addition of aureomycin to feed increased the growth rate of chicks.

These results with poultry triggered a widespread experimental and practical interest in other animals such as pigs and calves (e.g. Rusoff et al. 1951, Loosli et al. 1951, Braude et al 1953, Coates 1955), and during the 1950's and 60's there was much work on the use of antibiotics as growth promotants (Stockstad and Jukes 1950, Jukes and Williams

1953, Libby and Schaible 1955, Taylor 1957, White — Stevens 1957, and others), together with extensive application of antibacterial additives in livestock feeding. The general findings were that adding small quantities of ABA to the diets of young terrestrial animals not only served to control certain infections, but also produced unexpected increases in growth rate even when the animals were asymptomatic. It appeared that subclinical intestinal infections of an unsuspected and unidentified nature were commonly present and perhaps the infecting organisms merely displaced beneficial bacteria. In any event improvements in growth were noted in apparently healthy animals, however healthy disease-free animals that were fed in carefully cleaned and disinfected quarters showed insignificant growth when fed diets containing antibiotics (Hays and Speer 1960; Hays 1969; Libby and Schaible 1955), and others thought that such animals did not respond at all to antibacterial additives (Taylor 1957; Wallace 1970). Much evidence supporting these statements is available. For instance a report by Bowland (1956) showed that the response to chlortetracycline induced a 14.3% improvement in weight gain for pigs fed in an old barn while an increase of only 7.5% was observed in a new barn (cited by Coates and Harrison 1969). There is a suggestion that the lack of response to antibacterial additives in uncontaminated quarters was because of the excellent growth of the untreated animals (Jukes 1953; Francois 1962).

The antibiotics commonly used included chlortetracycline, penicillin, oxytetracycline, tylosin, and streptomycin, whose effects were reviewed by Wallace (1970). Other valuable monographs or reviews dealing with ABA were by Jukes and Williams (1953), Stockstad (1954), Francois (1962), Hays (1969), and Visek (1978). No more recent reviews have been located despite a computer aided search. Work during the past few years appears to concentrate mainly on the bacterial resistance caused by the prolonged use of antibiotics (Swann 1969; Jukes 1971; Aschbacher 1976; Silver and Mercer 1976; and FAD 1984) or on the residues in the animal body (Herman et al 1969; Ljungberg et al 1969; Salte 1982).

There have been fewer reports of ABA use in fish; this may be due to the concept that the intestine of healthy fish is nearly sterile when free from food. Snies kzo (1957) reviewed antibiotic use in salmonids, and claimed that their addition to the diet of fingerlings had no significant effects on the growth rate of the fish. It is interesting to mention here that some authors observed the presence of various strains of bacteria in Antarctic birds after several others had previously maintained that their digestive tracts were sterile (Francois 1962). This is probably because methods of isolating and identifying bacteria were not very effective at the time the observations were made.

The effects of feeding antibiotics on fish growth were somewhat negative during the 1950's. Wolf (1952) fed

Aureofac, a mixture of aureomycin and vitamin B_{12}, to brown trout at 65.7 ppm in plant protein and in fish-meal diets, and found a slight increase in growth rate in the fish on the vegetable diet, but not in those on the animal diet. Addition of 18 to 71.8 ppm terramycin to these diets did not stimulate growth. Wagner (1954) fed aureomycin, terramycin, penicillin, and chloromycin to rainbow trout at 22 ppm, and obtained negative results; it was concluded that there were significant differences in metabolism between fish and homoiotherms such as poultry and swine, and/or the action of the drugs on the microflora was different in these animals.

These negative effects led to a loss of interest in antibiotics as possible growth promotants in fish; scattered work, aimed at the use of antibiotics as chemotherapeutic agents, produced some unexpected growth changes, but the results lacked accuracy or statistical analysis (referred to later in this thesis). However, since this time there have been some reports indicating that ABA may have a positive effect on growth of fish; these are summarised in table 2.1. Sen and Chatterjee (1976) used a variety of growth promoting substances, including minerals, starch, vitamins, yeast and antibiotics (chloromycetin, entrocycline and hoestacycline) on fry of major carp. The growth was enhanced significantly with cobalt chloride and starch but the results with the antibiotics were not encouraging except entrocycline which seemed to increase the growth. Hashimoto (1953) in his

Table 2.1 : Review of antibacterial agents which gave apparent positive growth increase in fish.

Drug	Species of fish	Author and year
Aureomycin	Trout	Jukes and Williams (1953)*
Entrocycl-ine	Rohu	Sen & Chatterjee (1976)
Payzone	Estuary Grouper	Chua & Teng (1980)
Payzone	Carp	Parova et al. (1982)*
Terramycin	Carp	Korneeva (1963)*
Terramycin	Carp	Sukhoverkhov (1967)*
Terramycin	*Labeo rohita* *Catla catla* *Cirrhina mirgala*	Mitra and Ghosh (1967)*
Oxytetra-cycline	Carp	Rijkers et al.(1980)*

* No statistical analysis of data.

experiments on carp, using aureomycin alone and togther with vitamin B_{12}, showed no growth stimulation.

2.2 Effect Of Antibacterial Growth Additives On Intestinal Flora :

The first theory to explain the action of antibacterial drugs on growth promotion appeared very plausible, because the only property which various ABA have in common when used therapeutically is that they destroy harmful bacteria. To confirm that this action also applied to intestinal flora, the destruction of which induced growth, two methods were initially proposed: One was to treat antibiotics in such a way as to remove antibacterial activity and the other was to rear experimental animals devoid of any associated bacterial population. Antibiotics have been inactivated in a number of ways producing degradation products which show no antibacterial activity. Inactivated chlortetracycline has produced no growth response following oral administration either in rats (Peterson and Johnson 1953) or in pigs (Taylor 1957). Many other investigators reached the same conclusion when they used different antibiotics on different animals. However, Halama (1958) disagreed, finding that inactivated penicillin possessed a growth-promoting property for chicks. Similar results were found by Taylor and Gordon (1955) in pigs treated orally with inactivated penicillin.

Although experiments using small numbers of chicks and turkeys gave some evidence that moderate growth stimulation

could occur with antibiotic-supplemented diets under germ-free conditions (Jukes 1955, 1971; Francois 1962), studies with larger number of animals failed to show growth improvement (Coates et al. 1963 and Freeman et al. 1975). Jukes and Williams (1953) treated chick embryos, which can be considered as an animal without intestinal flora, by injecting them under antiseptic conditions with aureomycin, penicillin or streptomycin and found they did not show any increase in weight. Whitehair and Thompson (1956) reported that piglets delivered by Caesarean section and immediately isolated and reared on a synthetic diet showed no growth response to chlortetracycline.

From the above it seems that generally an antibiotic growth response does not occur following the administration of an inactivated antibiotic or in a germ-free environment, although some exception may occur such as in the experiments which showed that chickens reared in strict isolation showed some antibiotic response (Hill and Larson 1955). It seems probable in such cases that the technique of isolation permitted some degree of environmental contamination.

These results do not allow one to deduce definitely the site of action of antibacterial agents. Another method by which it was shown that the intestine must play a role in the growth increase was attempted by administering the antibacterial drug via a number of different routes and comparing the growth responses obtained. Grochke and Evans

(1950), Whitehill et al. (1950) gave low concentrations of antibiotics to chicks by intravenous and intramuscular injection, and found no increase in the growth rate. However other investigators (Oldfield and Hale 1952; Rusoff et al. 1953) claimed that both penicillin and Chlortetracycline injected into chicks and pigs will give as great a growth response as when given orally. A more recent work by Rijekers et al. (1980) showed that injecting fish with oxytetracycline diminished growth while oral administration increased growth. This apparent discrepancy is probably associated with the precise method of administration. Thus Becker et al. (1952) interestingly found that although an aqueous solution of penicillin when given as intramuscular injection on alternate days did not increase the rate of growth of chicks, a preparation of penicillin in oil which was absorbed slowly did increase growth rate.

2.3 Mode Of Action Of Antibacterial Agents :

While the beneficial effects of antibacterial drugs as growth agents have been recognized, the causative mechanism of this activity has remained obscure until recently, and it may be that the action varies from one ABA to another. However a number of theories have been proposed but unfortunatly the supporting evidence has been conflicting.

Four mechanisms were proposed in 1946 by Moore et al., and since then their ideas have been rephrased and expanded

by Jukes and Williams (1953), Taylor (1957) and Visek (1978). In summary these ideas are :-

(1) That the antibacterial agents may inhibit or destroy organisms which produce subclinical infections, that is, they suppress organisms which produce toxic reactions and cause a slowing of growth of the host animal.

(2) That antibacterial agents may produce an increase in the number or activity of organisms which synthesize certain known or unknown vitamins or growth factors which are eventually made available to the host.

(3) That antibiotics inhibit organisms which compete with the host for available nutrients.

(4) That there is enhanced efficiency of absorption and utilization of nutrient because the wall of the intestinal tract is morphologically thinner, or that physiological and biochemical mechanisms are modified.

There is insufficient evidence to support any single mode of action to the exclusion of all others. However, investigators concentrated their attention mainly on the effect of these drugs on bacterial counts and on their metabolism.

Changes in certain groups of microorganisms were found after treatment with antibacterial agents, but many of these reports have been conflicting. Sieburth et al.(1952) noted a decrease in coliforms and enterococci from feeding terramycin to poultry, while Johansson et al. (1953) reported an

increase in intestinal enterococci from feeding aureomycin to rats. Sieburth et al. (1951) showed in pigs that the count of *Cl. welchii* was reduced from 7.9 million to 10 organisms only per gram of faeces in animals receiving 15 ppm oxytetracycline!. This finding led the authors to believe that the animals benefited from the reduction in *Cl. welchii* toxin production. However in the same year Williams et al. (1951) showed that feeding *Cl. welchii* toxins to chicks did not depress growth. A similar confusing picture has appeared with other organisms. Moore et al. (1946) found a reduction in the caecal coliforms of chicks receiving streptomycin but Anderson et al. (1952) found that penicillin increased the total count of caecal coliforms. In both cases the antibiotic-fed animals showed increases in growth rate. Maska (1960) has confirmed that antibiotics increased the number of coliforms in the excreta of pigs. Jukes (1971) reviewed some examples of the variation in bacterial count obtained from different antibiotics. Increases, decreases and no change in *E. coli*, *Lactobacilli*, aerobes, anaerobes and total enterococci are seen in table 2.2.

Moore and Holdeman (1974) argued that the early work which showed increase in growth rate and which was not correlated with difference in bacterial counts was because the workers at that time had not employed the more recently developed methods needed for culturing anaerobic microorganisms, the predominant species in the gastrointestinal tract. Rizzoto

Table 2.2 : Effect of various antibiotics in increasing or decreasing various types of intestinal microorganisms in animals (Jukes 1971).

Antibiotic	Animal	Observed effect on intestinal organism#					
		1*	2*	3*	4*	5*	6*
Chlortetracycline	Pigs	+	+	+	+	+	
Penicillin	Chicks	+					
Chlortetracycline	Chicks	-	-	-			
Oxytetracycline	Chicks					+	
Penicillin	Rats	+	0		-	0	
Chlortetracycline , penicillin	Pigs						Gram-positive -
Oxytetracycline	Turkeys		+				
Oxytetracycline , bacitracin	Calves	0					Streptococci 0
Oxytetracycline	Rats				-		Proteus +,-; Streptococci +
Streptomycin	Turkeys						Yeasts +
Chlortetracycline	Rats	+	+			+	
Penicillin	Chicks					+	
Chlortetracycline	Chicks	0	0	0	0	0	
Penicillin	Chicks	+					
Penicillin	Chicks@	-	-	-	-	-	
Chlortetracycline	Ducks	-	-	-	-	-	
Penicillin	Ducks	+	-	+	+	-	
Oxytetracycline	Ducks	+	-	+	-	-	
Streptomycin	Ducks	+	0	+	-	+	
Penicillin	Poults	+	-				
Chlortetracycline	Chicks		+			+	
Penicillin	Pigs	+					
Streptomycin & Penicillin	Pigs						Proteus +
Penicillin	Chicks	+	-	-	-	-	
Various	Pigs				-		Clostridia -
Penicillin	Chicks				-		
Penicillin , Oxytetracycline	Turkeys	+		+	-	+	
Chlortetracycline	Calves		0				
Chlortetracycline	Chicks		0				
Penicillin	Chicks						*A. aerogenes* +
Various	Chicks	+	-				Proteus +

: + = an increase, - = a decrease, 0= no effect, @= In caeca
* 1 = *E.coli* , 2 = Lactobacilli, 3 = Aerobes, 4 = Anerobes, 5 = Enterococci, 6 = Other organisms

and Alford (1955) reported that addition of antibiotics to the diet of chicks always caused a decrease in the counts of *Lactobacilli*, coliforms and anaerobes in the duodenum and small intestine. Francois (1962) reviewed some work of other people which showed again little agreement between reports in dogs, rats, pigs, chickens and ducks. Assuming that the response of antibiotics and antibacterial agents is dependent on the presence of bacteria associated with the host animal, and that no consistent change can be shown in their relative numbers, it is reasonable to suppose that the response may be dependent on changes in metabolism rather than the viability of these intestinal organisms (Taylor 1957; Jukes 1970).

2.3.1 Effect Of Antibacterial Additives On Metabolism :

It was found that aureomycin supplementation to raw and heated soyabean rations increased the amount of nitrogen absorbed from each ration during its passage through the small intestine of rats (Carroll et al. 1953). The work of Sieburth et al. (1954) showed a consistent reduction in the respiration rate, as measured by oxygen consumption and carbon dioxide evolution of the microflora throughout the digestive tract, especially in the small intestine of chicks fed a diet containing 10 ppm of chlortetracycline. The author suggested that decreased use of nutrient by the microflora might permit a greater utilization of nutrients from the feed by the host animal.

Wallace (1970) suggested that either the intestinal enzyme

systems of the host animal or those of the microflora were more involved in increased metabolic action. He doubted that there was a direct metabolic effect on the general body tissues of the host animal, because effective concentrations in the feed were quite low and absorption of most additives was limited. He concluded that the beneficial effects were derived from the effect on the metabolism of the digestive tract flora. Visek et al. (1959) found that three antibacterial agents, penicillin, chlortetracycline and arsanilic acid, when fed to rats at 100 mg per kg of diet decreased *in vivo* urea hydrolysis in the gastrointestinal tissues plus content, but there was no inhibition by the direct addition of the three agents *in vitro*. Kornberg and Davies (1955) had previously shown that oral adminstration of antibiotics and sulfonamides in sufficent dosage would virtually eliminate the gastrointestinal microflora and abolish urea hydroysis *in vivo*. Chlortetracycline, penicillin, arsanilic acid, isoniazid, barbituric acid and copper were used to inhibit urease activity in the gastrointestinal tract, and all enhanced growth of animals fed a semi-purified diet (Visek 1978). It was suggested by Stokstad et al. (1953) that the growth response to antibiotic supplements is influenced by the type of carbohydrate in the diet. This was confirmed by Eyssen and Desomer (1963) when they observed that both the nutrient absorption and growth rate of chicks were improved

by antibiotics to a greater extent on sucrose diets than on starch-based diet. Harbers et al. (1963) also found that ureolytic activity and ammonia concentration in intestinal contents of chicks were lower on such diets. Suppression with antibiotics was greater with the less complex carbohydrates. Combe et al. (1976) working on rats, concluded that the presence of non-pathogenic bacterial flora in the digestive tract has to be considered as a factor having an unfavourable action on the digestion and absorption of dietary lipid. The effect of their presence on the long-chain saturated fatty acid and palmtic acid is particularly marked. Beede and Stanly (1977) surveyed the effect of sixteen antibiotics on lactate and volatile fatty acid production *in vitro* in the rumen of steers and noticed that four of these antibiotics reduced lactate production and the others eliminated it completely. The action of antibiotics on mineral metabolism and therefore on growth may be indirect, being exerted by means of vitamin D or by means of parathyroid activity. In chickens, Havermann and Hartfiel (1956) have noted by x-ray examination that calcification was improved by terramycin and was greater the higher the amount of antibiotic used. Ross and Yakowitz (1954) have shown that penicillin increases the ash content of bones of chicks, thereby indicating increased calcium retention.

2.3.2 Nutrient-Sparing Effect :

It has been known for a long time that the gastrointestinal microflora of species with simple stomachs synthesise significant quantities of water-soluble vitamins which are utilized by the host. Jukes (1971) reported that penicillin and the tetracycline group were found to lessen the requirements of the rat for several vitamins and minerals. The blood concentration of riboflavin and calcium in chicks were increased by feeding chlortetracycline. March and Biely (1952) showed that different amounts of B complex vitamin in the diet of growing chicks did not increase their body weight despite supplementary additives in their diet. In fish *(Tilapia nilotica)*, feeding 1% of the antibiotic succinylsulfathiazol significantly lowered the rate of intestinal vitamin B_{12} synthesis (Lovell and Limsuwan 1982). The addition of the antibiotic was meant to suppress the growth of the microorganisms which synthesize vitamin B_{12}.

An effect which has frequently been observed in studies of growth stimulation of animals by antibiotic feeding is the apparent sparing action of antibacterial agents on dietary protein, as reported for pigs (Cunha et al. 1950), chicks (Machlin et al. 1952) and turkeys (McGimns 1951). The work of Vijayaraghavan et al. (1952) with mice also indicated a marked growth stimulation on addition of aureomycin to diet having a shortage of essential amino acids, though it did not improve growth in rations containing relatively good quality protein, either animal or an animal-plant mixture. It was

suggested that aureomycin may promote the development of microflora which enhance the availability of essential amino acids essential for growth. The growth-stimulatory degree of aureomycin added to raw and heated soyabean meals, with or without added free methionine, was suggested to be related both to the ease of digestibility and to the amino acid composition of dietary protein (Hensley et al. 1953). The protein requirement of chicks for maximum growth during the first six weeks was 19% when aureomycin was added to the diet and 21% in its absence (Machlin etal 1952). No such differences were found by Slinger et al. (1952) but they commented that antibiotics enhanced the utilization of both protein and energy compounds rather than reducing the requirements for them. More recent reports concentrate mainly on the feed conversion efficiency rather than the sparing effect, though some have referred to the latter. Vervaeke et al. (1979) reported that virginiamycin and spiramycin resulted in sparing of a measurable quantity of glucose when they were used as growth promotors in pigs. Payzone was found to have a sparing effect on protein, fat and other organic substances (Schneider et al. 1970; Menke 1970). Terramycin incorporated in a vegetable protein diet for fish made up for the lack of animal protein. The antibiotic supposedly improved the protein metabolism (Sukhoverrkor 1967).

2.3.3 Change In The Intestinal Wall :

It has not been proved that a direct relationship exists

between the effect of antibiotics on growth and the morphology of the digestive tract and the intestine in particular. However, Gordon (1952, quoted from Francois 1962) first reported that the weight of the small intestine of chickens receiving penicillin was significantly reduced. This has been confirmed by others. Coates et al. (1955) noted that the length of the intestine was little affected by the antibiotic and that the fat and moisture content was not altered but they concluded that the antibiotic caused a thinning of the intestinal wall. Although reduction in thickness, length and weight of the small intestine have been found, histological examination has sometimes failed to reveal the nature of these changes. However, Jukes et al. (1956) reported that chickens treated with penicillin had a duodenum whose diameter was smaller than in controls. The thickness of some of the layers of the lining of the intestinal wall was also reduced, in particular reduction occurred in the tunica propria and the villi. Klaus and Fewson (1955) showed that chlortetracycline did not alter either the weight or the length or composition of the intestine of pigs. Vonk et al. (1957) found that in pigs receiving chlortetracycline, the weight of the first three metres of the small intestine and the dry weight of the mucosa were lower, but the differences were not significant. An interesting finding was reported by Hill et al. (1957) when they noticed that the weight of the intestine decreased

before the growth effect could be detected, which led them to think that it was doubtful whether there was any relationship between the growth promotion effect and the thickness of the small intestine. Some investigators suggested that in the presence of antibacterial supplements, certain nutrients may be readily absorbed from the small intestine. This was supported by Catron et al. (1953) who found that following the administration of equal quantities of glucose solution to control and chlortetracycline-fed pigs, the latter showed significantly higher blood glucose than the controls. Caroll et al. (1953) also found that chlortetracycline enhanced the absorption of cystine, leucine, lysine and methionine from the small intestine of rats. These later experiments indicated an effect of antibiotic on the physiology of intestinal mucosal absorption. Jukes (1971) and Dubos et al. (1967) suggested that certain intestinal bacteria have a mild physiological inflammatory or toxic effect which results in the thickening of the gut wall. This effect, they suggested, may be removed or suppressed when chicks are fed antibiotics or kept in a germ-free state, resulting in a thinning of the intestinal wall which improved the uptake of nutrients. Madge (1969[a,b], 1971) has studied the effect of antibacterial feed additives on the absorption of different nutrients in mice and he suggested that generally the feed additives at low concentration increased intestinal transport of different solutes, particularly sugars and amino acids, and the

intestinal wall became thinner. In a later study Madge (1973) showed that four feed additives, flavomycin, quindoxin, arsanilic acid and copper sulphate, had different effects on absorption, increase, decrease or no change. None of them significantly increased the body weight of the mice and some animals were in fact slightly lighter. The conclusion was that no relationship existed between intestinal absorption nd body weight in mice fed these compounds.

2.4 Fish Microflora :

Studies on the microbiology of fish can be categorized as either freshwater or marine, and also according to the source of the organisms, indigenous or non-indigenous. Non-indigenous organisms are the pathogens and those causing spoilage of fish carcasses. Up to the late 1960's, the literature placed most emphasis on spoilage organisms and their effects with respect to public health and food poisoning. These studies were concerned with the natural body microflora of slime, gills and mucus, and with the intestinal and faecal flora of freshly-caught fish (e.g. Tarr 1943, Liston 1956, 1957, Georgala 1958, Shewan 1961 and Colwell 1962). Lindsay (1983) criticised the methods that earlier workers used for their sampling procedures and culturing.

A number of workers have held that the internal organs, muscle, intestine and body fluids of healthy live or freshly-caught fish are sterile (Procter and Nickerson 1953; Shewan

1949 and Snieszko 1957), though numerous others have demonstrated the existence and composition of a gastrointestinal microflora in cultured, free-living, starved fish and in fish given sterile food (Trust 1975; Sakata et al. 1978 and Lesel 1979). Others have dealt with the variation in the intestinal microflora of fish reared in freshwater and transferred to seawater. Sakata et al. (1980) reported that the microflora of fresh and sea-water tilapia, *Tilapia zilli*, consists mainly of *Aeromonas sp.* or *Vibrio* and *Aeromonas* respectively. A similar study carried out by Hamid et al.(1978) found that intestinal bacterioflora of grey mullet *Mugil cephalus*, cultivated in fresh and sea water were affected by salinity. It was concluded that water conditions caused changes in the microflora; in fresh water the predominant genera were *Enterobacter* and *Bacillus* while in sea water *Pseudomonas*, *Vibrio* and *Aeromonas* were predominant.

Other work concentrated on the autochthonous (indigenous) bacteria in the body of fish generally and the intestinal tract in particular, which include obligate anaerobes, facultative anaerobes and aerobes. The intestinal tract of most mammals, including man, contains high concentrations of microbes, a large proportion of which are sensitive anaerobes (Savage et al. 1968; Drasar and Hill 1974). The gastrointestinal tract of fish appears to have a simpler microflora than that of homeotherms, and the

predominant bacteria isolated from the fish gut have been aerobes or facultative anaerobes (Trust and Sparrow 1974). However Sakata et al. (1980[a+b]) found that obligate anaerobes were commonly present in the intestine of most freshwater fish they studied, including carp *(Cyprinus carpio)* and rainbow trout *(Salmo gairdnerii)*, though the number of anaerobes in rainbow trout were negligible. Trust et al. (1979) considered their finding as the first evidence of the presence of obligate anaerobes among the resident bacterial flora of the grass carp, goldfish and rainbow trout. The mid gut was the most heavily colonized. Obligate anaerobic bacteria were identified as species of *Actinomyces*, *Bacteriodes*, *Eubacterium*, *Fusobacterium* and *Peptostreptioccus* . A wide variety of facultative anaerobic bacteria were isolated with *Aeromonas hydrophila* predominating, but the gastrointestinal tract of hatchery-cultured rainbow trout contained insignificant numbers of anaerobes.

There has been a deal of disagreement between reports concerning the effect of environment, temperature and food on the kind of bacteria present in the body of the fish and on the route by which those bacteria enter the fish. Lesel (1979) reported that there is a quantitative relationship between water microflora and the digestive tract but not all the taxonomic groups found in water are found in the digestive tract. In spite of what was usually believed,

prolonged starvation does not produce a sterile digestive tract after even 91 days. The number of bacteria were identical to those observed in fish fed daily. Trust (1975) reported that although starvation of salmon *(Oncorhynchus keta)* caused a reduction in the total numbers of viable organisms per gram wet weight of the digestive tract plus content, bacteria were still present after 75 days starvation. Similar results were obtained when the salmon were fed with sterilized food for 55 days. Trust showed a decrease in aerobic bacteria and suggested that the reduction in the total numbers represented a loss of those organisms not intimately associated with the mucus layers of the epithelium.

Campbell and Buswell (1983) found that the microflora of young stages of Dover sole *(Solea solea)* was comparable with that of tank water. In older fish, however, the bacteria in the intestine were similar to those found in the natural diet. When the fish were offered the pelleted diet there was no relationship between the pellet microflora and that found in the fish intestine. Therefore it was suggested that the indigenous intestinal microflora initially arose from the diet and became established coincident with development of the special ecological condition in the gut. Shewan (1977) also suggested that the intestinal microflora of fish is conditioned not so much by the food or external environment as by the special ecological condition existing in the

stomach and intestine such as pH, anaerobes and presence of bile salts. Trust (1975) was of the opinion that the *Aeromonas hydrophila* and *A. shigelloides* in the digestive tract of chum salmon did not originate in the diet or the water supply but may have come from the surface of the incubating eggs. The conditions in the digestive tract allowed these aeromonads to establish themselves. Recently Sugita et al. (1985) interestingly reported that the fish influenced the water they were reared in. They found that the aerobic and anaerobic bacteria which included *Aeromonas* and *Bacteriodes* type A and B, present in high concentration in the gastrointestinal tract of carp *(Cyprinus carpio)* were found in the ambient water in addition to *Enterobacteriaceae Pseudomonas, Flavobacterium* and *Cytophage.* This was no doubt due to introduction by the faeces into the water. When the fish were removed from the water these bacteria disappeared. Sugita et al. (1983) found that the gastrointestinal tract of freshwater fish contains facultative anaerobes. The *Enterobacteriaceae, Vibrio* and *Aeromonas* groups were always predominant but the obligate anaerobes *Bacteriodes* type A and B were not isolated from some fish samples. *Vibrio, Aeromonas* and *Bacteriodes* type A and B were all isolated from the sediment and aquatic plants of the environment. Thus the bacteria in the gastrointestinal tract of the wild fish were suspected to originate from the environment. Similar findings by Sugita et al. (1980,1982) were reported in

tilapia, ayu and carp. Nieto et al. (1984) compared the bacterial flora associated with fingerling rainbow trout cultivated in two different hatcheries. The examination of the bacteria present in the water samples from each hatchery indicated that the general bacterial population of apparently healthy fish was a reflection of their respective environments particularly in the case of the *Enterobacteraceae* . Nevertheless *Pseudomonas* and *Aeromonas* were the more characteristic bacteria of the freshwater fish microflora regardless of the respective location.

The effect of temperature of the water in which fish are living was also studied. Geldreich and Clarke (1966) showed that the optimum temperature for multiplication of faecal coliforms and *Streptococcus faecalis* in the fish intestine was between 10 and 20°C. These bacteria were commonly encountered in fish intestine when the ambient temperature was between 13 and 18°C. Trust et al. (1979) studied the occurrence of obligate anaerobes; they were present in significant numbers in grass carp and goldfish but not in rainbow trout and it was concluded that this difference was because of the temperature employed in the cultivation of the fish. The carp and goldfish were maintained at 18-22°C while the trout were maintained at 11°C. At this lower temperature the time of passage of material through the gut is about 12 hours which is not long enough for bacterial flora to reach the population required to establish a gut microflora before

food passes out of the gut, whereas in carp and goldfish the time required for multiplication is less at the higher temperature. The total number of coliforms and *Streptococcus* were elevated when the water temperature increased from 17°C to 26°C during the summer (Niemi and Taipalinen 1982). A change in water temperature (not specified) while carp were under feeding conditions caused a significant increase in the epiphytic bacterial counts on the skin and gill and also in the intestinal tract (Takabashi and Fujino 1984).

The role of the intestinal microflora in mammals has been widely studied, especially the contribution to the well being of the host (Scha deler 1973). In fish the role of microflora has also been studied. Kashiwada et al. (1971) reported synthesis of vitamin B_{12} and folic acid by the intestinal microflora in the carp. The synthesis of amino acid by carp *Cyprinus carpio* microflora in significant quantities, depending on age, growth rate, nutrition and microflora, was reported by Lesauskiené and Syvokiené (1977). Intestinal bacteria of carp were found to be capable of synthesizing vitamin B_{12}, especially at higher temperatures. To a lesser extent, synthesis of nicotinic acid and pantothenic acid by intestine Gram-negative bacteria can occur (Teshima and Kawashiwada ; 1967). Trust et al. (1979) reported that the facultative bacteria were active in utilizing cellobiose and have a proteolytic and lipolytic activity under anaerobic *in vitro* conditions. Limsuwan and Lovell (1981) showed that

intestinal bacteria in channel catfish fed diets adequate in cobalt (an essential component of vitamin B_{12}) could synthesize significant amounts of vitamin B_{12}. It was demonstrated that this source of the vitamin was readily absorbed from the intestinal tract of the fish but there was not enough to meet the requirement of the fish for normal growth. Similar results were found by Dupree (1966). Lovell and Limsuwan (1982) found that a significant amount of vitamin B_{12} was synthesized by microorganisms in the intestine of *Tilapia nilotica* ; dietary supplementation of this vitamin is probably unnecessary for this species, because enough was synthesised for normal growth and for maintenance of a constant liver concentration of the vitamin over a period during which the fish increased their weight eight fold. The great difference in the apparent rate of intestinal synthesis of vitamin B_{12} between tilapia and channel catfish and the difference in dietary dependence for the vitamin between fish such as common carp or *T. nilotica* and channel catfish or chinook salmon indicates that dietary requirement for various vitamins may be markedly different among different species of fishes.

2.4.1 *Aeromonas hydrophila* :

Aeromonas hydrophila has been found in the present work to be the sole species of bacteria in the intestinal tract of *Cyprinus carpio* , the fish investigated in this thesis, and it is necessary to give a short account of the biology of

this bacterium.

In Bergey's "Manual of Determinative Bacteriology" (1974) the original definition of *Aeromonas hydrophila* included its salient properties: Gram-negative, straight rods, polar flagellated or immotile, facultative anaerobes, fermenting carbohydrates with formation of acid or acid and gas, oxidase-positive and reducing nitrates to nitrites. *A. hydrophila* is recognized sometimes as an important pathogen of freshwater fishes, and also as a pathogen of warm-blooded animals including humans (Poppof and Veron 1976). Species of the genus *Aeromonas* are considered as autochthonous inhabitants of the aquatic environment. *A. hydrophila* has received particular attention because of its association with human disease. Kaper et al. (1981) found that it is ubiquitous occurring in numbers ranging from <0.3 per litre to $5x10^{3}$ per cm^{3} in estuarine waters, its concentrations were inversely related to salinity and to the concentration of dissolved oxygen. Trust et al. (1979) showed that *A. hydrophila* was a predominant species in the gastrointestinal tract of grass carp; it was present in all samples tested and comprised 42% of the isolates. In other work Trust et al.(1978) found that *A. hydrophila* was the most important facultative species in the gut of freshwater fish and in some cases was the sole bacterial species present (quoted from Trust et al. 1979).

Niemi and Taipalinen (1982) found that *A. hydrophila* was

abundant in effluent water of a farm where rainbow trout were reared. In a study of bacterial flora in the alimentary tract of five species of freshwater salmonid Trust and Sparrow (1974) found that species of *Aeromonas* comprised 13% of the isolate recovered. Other work on the bacterial flora of the digestive tract of chum salmon (*Oncorhynchus keta*) growing in fresh water under controlled culture conditions, indicated that the predominant species was *Aeromonas hydrophila* (Trust 1975). It was was not found in either the feed or tank water. *A. hydrophila* was even found in the digestive tract of starved fish and fish which consumed sterile diets. The author commented that it has also been demonstrated in the digestive tract of free living salmonids. He regarded it as indigenous in the gut of salmonid fish living in fresh water.

As yet the role of *A. hydrophila* seems not very well recognized although most reports agree that it is an opportunist pathogen of fish, aquatic animals and humans. Some other reports either deny that *A. hydrophila* is a pathogen or even claim that it had a beneficial effect on fish. Recently Kanai and Wakabayashi (1984) studied the production of protease obtained from the culture filtrate of *A. hydrophila* . This protease was injected into carp at a concentration of 200μg per 100 g body weight. The fish died suffering from extensive haemorrhage in the abdominal cavity. The authors referred to the large quantity of extracellular protease produced by *A. hydrophila* . Wakabayashi et al.

(1981) described that culture filtrate of *A. hydrophila* had lethal activity but they did not demonstrate which substances were toxic. On the other hand Sugita et al (1985) showed that *A. hydrophila* was one of the predominant bacteria in the gastrointestinal tract of carp and other freshwater fish and that this bacterium was not associated with fish disease. Nieto et al. (1984) suggested that *A. hydrophila* which was found on the body surface or in the intestinal lumen of fish was a facultative pathogen because it was ubiquitous in the aquatic environment, and that under environmental or physiological stress could produce epizootic outbreaks, but would not do so in fish populations maintained under optimal conditions. Trust et al (1979) reported that *A.hydrophila* was unable to utilize cellulose or carboxymethyl cellulose but was capable of cellobiose degradation. They concluded that the wide-ranging nutritional capabilities of this aeromonad probably explains its abundance in the gut of many species of fish.

CHAPTER THREE

3. POTENTIAL GROWTH PROMOTANTS

A short description is now given of the various drugs examined in this study for their possible growth promoting capability.

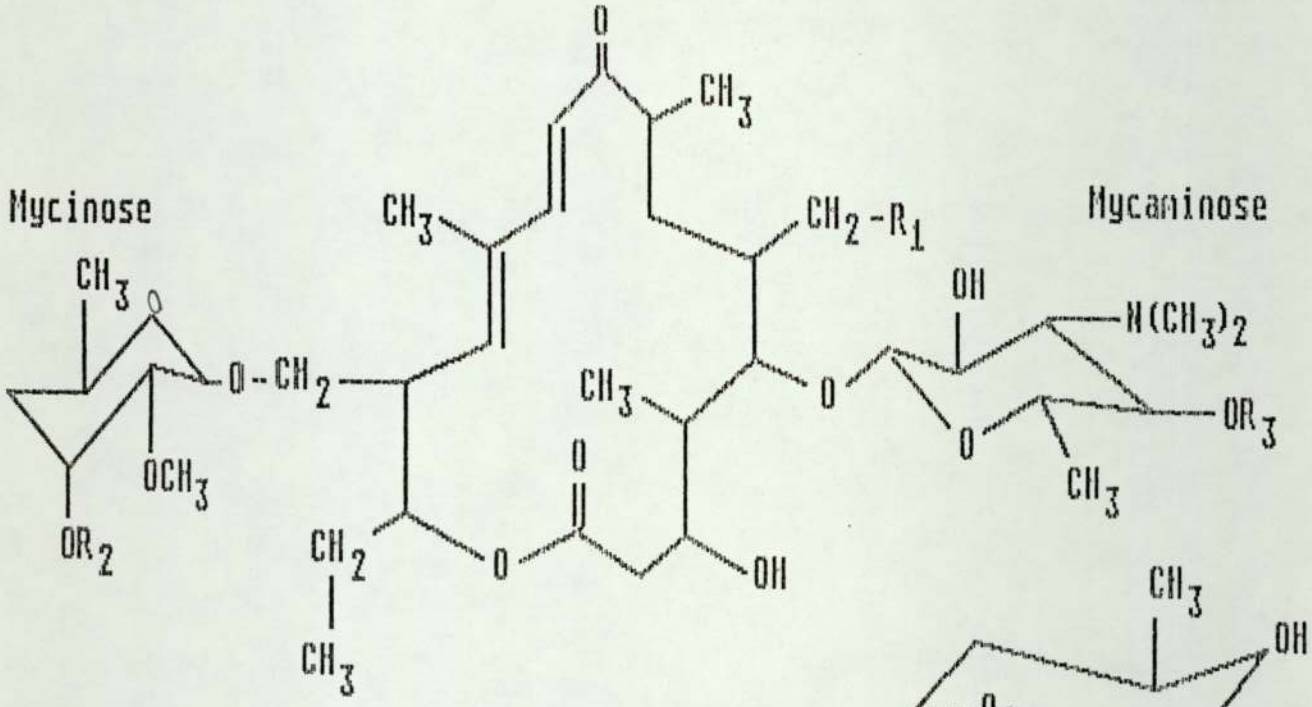
3.1 Tylosin :

3.1.1 Properties :

Tylosin is classified as a macorolide antibiotic. It has an empirical formula of $C_{46}H_{77}NO_{17}$, with a tylonolide ring and the sugars mycaminose, mycarose and mycinose (figure 3.1).

Tylosin is produced from a strain of the actinomycete *Streptomyces fradiae* isolated in 1955 from a Thailand soil sample hence the generic name tylosin (Hamill et al 1961). Antibiotic activity of tylosin was demonstrated against Gram-positive bacteria and poultry mycoplasma. Following development work, tylosin became available in the United States as tylan for the treatment of mycoplasma infections and as a growth promotant and feed efficiency improver for swine. Tylosin has not been used as an antibiotic for humans.

Physically tylosin is an amorphous colourless compound which is only very slightly soluble in water but readily soluble in organic solvents. However, highly water-soluble salts such as tylosin tartrate can be readily formed. Four factors of tylosin have been identified: Factor A, tylosin, accounts for more than 80% of the drug and desmycosin, factor



	R_1	R_2	R_3
TYLOSIN A	-CHO	-CH3	Mycarosyl
TYLOSIN B	-CHO	-CH3	-H
TYLOSIN C	-CHO	-H	Mycarosyl
TYLOSIN D	$-CH_2OH$	-CH3	Mycarosyl

Figure 3.1 The chemical structure of tylosin

B, for slightly more than 5%. The reminder consists of factor C and D (Macrosin and relomycin) as shown in figure 3.1.

3.1.2 Mode Of Action :

The antibacterial activity of tylosin is due to the inhibition of protein synthesis within the bacterial cell. Inhibition occurs at two stages in the chain of events which lead up to protein synthesis either in or on the bacterial cell ribosome on which peptide chains are built. Secondly, transfer ribonucleic acid - amino acid complexes are inhibited from attachment to the mRNA-ribosome complexes thus preventing the peptide bond formation necessary for protein synthesis (Cracknell 1983). The action of tylosin *in vitro* has been demonstrated to be both bacteriostatic and bacteriocidal (Mao and Wingard 1968; Mao and Robinshaw 1971; Pestka 1971 and Suzuki 1970). Tylosin possesses a wide spectrum of activity being especially active against Gram-positive bacteria but it is also active against Gram-negative bacteria such as *Vibrio coli* . Tylosin is not active against members of the Entero-bacteriaceae such as *Escherichia coli* and *Salmonella sp.* apparently because it cannot penetrate the cell wall of this group of bacteria.

3.1.3 Pharmacokinetics Of Tylosin :

While it is extremely important that a compound administered to food-producing animals is safe, it is also desirable that it be rapidly excreted from those tissues or

animal products (i.e. before being consumed by man). There are differences in the absorption of tylosin in different species when administered by the oral route. In chickens, peak amounts of tylosin are observed in the circulating blood some four hours after a single oral dose. Studies on rats and dogs have shown that tylosin is absorbed mainly in the intestine following oral or intraduodenal dosing, serum concentrations peaking at approximately two hours after dosing. Tylosin elimination has been generally found to be quite rapid. It is slower in newborn calves (139 minutes) than in one-week-old animals (75 minutes) or yearlings (65 minutes) (Burrows et al. 1983). No residues in chicken carcasses were found 24 hours after treatment with tylosin even at twice the recommended dosage rate of 0.5 g per litre drinking water. Pigs that have been fed 1000 g tylosin phosphate per ton of feed (which is ten times the normally recommended dose) for 105 days are free of all detectable tylosin residues 48 hours after ceasing medication. No residues were observed following feeding the normal recommended amount of 100 ppm even with a nil withdrawal time (Pankhurst 1983).

3.1.4 Tylosin As Growth Promoter :

The growth-promoting effect of low amount of tylosin in the food of growing pigs was first reported by Jordan et al.(1960). They found that the optimum quantity of tylosin was inversely related to the age group as 40g, 20g, and 10g

per ton of food was the optimum for groups of up to 20 kg, 50 kg and from 50 kg to market weight respectively. Melliere et al. (1973) surveyed the effect of feeding 11 to 22 ppm tylosin in the feed on the rate and efficiency of gain of finishing swine; in 68 experiments conducted on commercial farms, at universities and research laboratories between 1966-1971, an inverse relationship between percent gain improvement due to tylosin and gain of control was found. The authors also concluded that there was a positive relationship between improvement in feed efficiency and rate of gain in tylosin-treated swine. The data support the hypothesis that improvements in rate and efficiency of gain due to an antibiotic are inversely related to the relative performance of non-treated swine. Wachholz and Heidenrich (1970) added tylosin to rations fed in two environments one which had never been used to raise pigs compared with another area which had been used for several years. The results showed that the magnitude of response to tylosin was greater in the old than in the new environment; a significant ($P<0.05$) difference in response was noted between the environments at all phases (starter, grower and finisher). This result confirmed the concept of Braude et al. (1953) that the efficiency of a growth promoter was greatest in animals that had a poor standard of performance. Jones and Tarrant (1982) reviewed nineteen feed trials which were conducted in eight European Countries to examine the effect of tylosin at 20 mg

per kg in the food of healthy pigs under good management conditions. Average daily gain and food conversion ratio (FCR) were improved by 33 g and 0.17 respectively over those of untreated control animals, with no change in average daily food consumption. These improvements in growth performance were similar in the grower and finisher phases, and the response was not affected by sex or initial weight. Pollmann et al. (1980) found that feeding starter pigs on diets containing tylosin showed improvement in average daily gain and FCR. In finishing pigs no positive response could be detected.

Other experiments on pigs were also conducted by Shively etal. (1981) and by Adams et al. (1981). Hereford steers with an average weight of 336 kg were fed tylosin, 11 mg per kg of diet. Tylosin had no effect on feed efficiency but tended to depress intake and average daily gain (Hornton and Nicholson 1980). Hays and Muir (1979) showed that tylosin improved the weight gain and feed efficiency by 2.8% and 1% respectively in four experiments using tylosin as a growth promoter in chickens.

No work could be found on fish involving tylosin either as an antibiotic or as a growth promotant.

3.2 Payzone (Nitrovin) :

3.2.1 Properties :

Payzone is the trade name of nitrovin which is a

selective antibacterial feed additive. The active ingredient of nitrovin is a chemically synthesized guanidine derivative, with the chemical name of 1,5 bis[5-nitro-2-furyl] 1,4-pentadien-3-one amidinohydrazone hydrochloride.

The chemical formula:- C14H12N6O6.HCl

The structural formula is shown in figure 3.2.

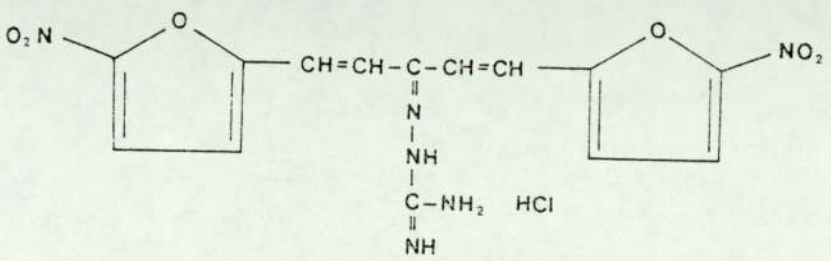


Figure 3.2. The Chemical Structure of Payzone (Nitrovin)

Nitrovin is soluble in lower alkyl alcohols, dimethylsulphoxide, dimethylformamide, pyridine and other similar organic amines. It is insoluble or very slightly soluble in cold water and ether.

3.2.2 Mode Of Action Of Payzone :

The mechanism by which dietary nitrovin improves feed conversion and weight gain is not totally understood at this

time. However, it may owe its growth-promoting effects in part to its influence on the intestinal flora. Gerlach (1970) and Gly stroff (1970) studied the effect of feeding nitrovin on the intestinal flora of chicks, and found that the diet containing nitrovin increased the population of lactobacilli. Their importance was shown by King (1968) in studies with pigs; feeding lactobacilli significantly improved weight gain and feed efficiency. Fuller (1976) has also shown their importance in maintaining a healthy microbial population in the gut. Nakamura et al. (1952) found that nitrovin possessed a high degree of activity against a wide range of bacterial species which included Escherichia coli, Salmonella and Shigella. However, the Swann committee (1969) reported no antimicrobial activity. Williams-Smith (1972) investigated the antibacterial spectrum of nitrovin in vitro and found that its activity was limited to certain Gram-positive organisms, and it did not affect Gram-negative bacteria including Salmonella spp., Shigella spp. and Escherichia coli. Studies by American Cyanamid have determined that nitrovin shows no antibacterial activity against Enterobacteriaceae including the bacteria mentioned above.

Nitrovin has some bacteriostatic activity against some Gram-positive organisms *in vitro* but not *in vivo* (Cyanamid of Great Britain Manual). Some studies indicated that nitrovin has a nutrient-sparing effect. Schnieder et

al.(1970) demonstrated that nitrovin significantly improved protein digestibility in pigs. Menke (1970) also found significant improvement in the apparent digestibility of crude protein, fat and other substances in pigs fed nitrovin.

3.2.3 Pharmacokinetics Of Payzone :

Alford (1967) stated that nitrovin is virtually unabsorbed from the gastrointestinal tract and leaves no detectable residues. Gatterdam and Plaisted (1966) using chickens as experimental animal indicated that nitrovin is poorly absorbed through the gut and that nearly all of it is excreted in the faeces within 48 hours. Further investigations by the same workers in 1967, using a radioactive C^{14} tracer in rats, showed that one day following treatment, 91.2% of an orally-administered dose of nitrovin was recovered, 99.5% was in the faeces, about 0.04% in the urine and only 0.03% in the tissues and carcase. Cyanamid of Great Britain, using a spectrophotometric method capable of detecting nitrovin down to 0.1 ppm in muscle, fat, liver or 0.2 ppm in kidney, found no detectable residues in chicken tissues after 70 days of feeding at 25 ppm. Feeding 10 ppm for 84 days (Smith and Coward 1967) and 40 ppm for 14 days (Coluvita 1966) produced no detectable residues in pigs.

4.2.4 Payzone As Growth Promoter :

The first trial with nitrovin as a feed additive was made with chicks. Further experiments with other livestock

showed that it has a very positive effect on the growth rate and feed conversion efficiency of pigs and calves (Eggert et al. 1965-1968). Many investigators found that pigs fed with nitrovin either at 80 ppm, 100 ppm, or 54 mg per head per day improved growth rate by 5.5%, 4.1% and 4.5%, and FCR by 4.2%, 5.2% and 3.1% respectively (Schneider etal 1970; Batterham and Fagan 1970 and Giess er and Kirchgessner 1971). In order to define the optimum growth-promoting dose for bacon pigs under European conditions, diets containing 5, 10, 20 and 40 ppm nitrovin were evaluated (using 163 pigs). The optimum effect taking into account both weight gain and feed conversion efficiency came from a 10 ppm inclusion of the drug (Pettersson 1974). However Simonsson (1972) found no improvement in growth rate and feed conversion ratio when nitrovin was given to 5-8 week-old weaned pigs.

In calves nitrovin has been included in the milk replacer of veal calves and milk replacer and dry feeds of rearing calves. Two experiments involving 50 calves fed 25 ppm nitrovin in the milk replacer from 1-2 weeks of age to 90 days, resulted in an increase in average daily gain of 6% and improvement in FCE of 7.7%. The daily liveweight gain and feed conversion efficiency were improved by 12.5% and 5.2% respectively from 1 to 6 weeks, and an improvement of 9.7% and 5.8% in daily growth rate and food conversion ratio up to twelve weeks. Beef calves from 1 to 10 weeks were individually fed rations containing 25 ppm nitrovin, 17% oil

and 24% crude protein. The daily liveweight gain and feed conversion efficiency were improved by 8.2% and 0.4% respectively.

There are two reports on the use of nitrovin as a growth promoter in fish. Chua and Teng (1980) found that feeding nitrovin at 1 gm per kg food to Estuary grouper *Epinephelus salmoides* kept in floating cages increased growth by an average of 62.8% over the control during a period of five months. Parovà et al. (1982) in their trial on carp (species not named) fed 15 mg and 25 mg nitrovin per kg diet for 112 days; at 25 mg per kg there was an 11.8% increase in the gain of carp biomass and a 10% decrease in the consumption of feed mixture per kg of weight gain was found. No statistical analysis was made to evaluate the significance of these findings.

3.3 Avotan 50 / Avoparcin :

3.3.1 Properties :

Avoparcin is a growth-promoting feed antibiotic and is a glycopeptide produced by fermentation in a strain of *Streptomyces candidus*. Its production, isolation and physiochemical characteristics were first described by Kunstmann et al. (1968). It is a white, hygroscopic, amorphous solid with no definite melting point. Avoparcin and its salts are soluble in water, dimethylformamide and dimethylsulphoxide, but are not soluble in common organic

solvents with the exception that the free base form is moderately soluble in methanol. It has been shown that avoparcin is stable in feed blending and pelleting processes. Its empirical formula: C, 47.26; H, 5.08; N, 5.76; Cl, 1.74

and the molecular structure is shown in figure 3.3.

3.3.2 Mode of Action Of Avoparcin :

The exact way in which avoparcin exerts its growth-promoting effect in livestock is not known, but it is believed to be related mainly to its antibacterial activity against enteric organisms that cause sub-optimal growth, since it is virtually unabsorbed from the gastrointestinal tract when administered orally.

Redin and Dornbush (1968) reported preliminary studies that showed it to be active *in vitro* against Gram-positive bacteria, but inactive against Gram-negative pathogens up to the concentration of 100 g/ml. It was effective against infections of *Staphylococcus aureus* strains and with *Diplococcus pneumoniae* and *Streptococcus pyogenes* in mice by injection parenterally but not when given orally.

In pigs, 100% of a single oral dose of avoparcin was eliminated intact within 48 hr, most in faeces and only a trace (0.7%) in the urine; similar results have been obtained in chickens, turkeys, dogs, rats and man. It was undetectable in the blood in pigs, dogs, rats and chicken, confirming lack of absorption from the gut.

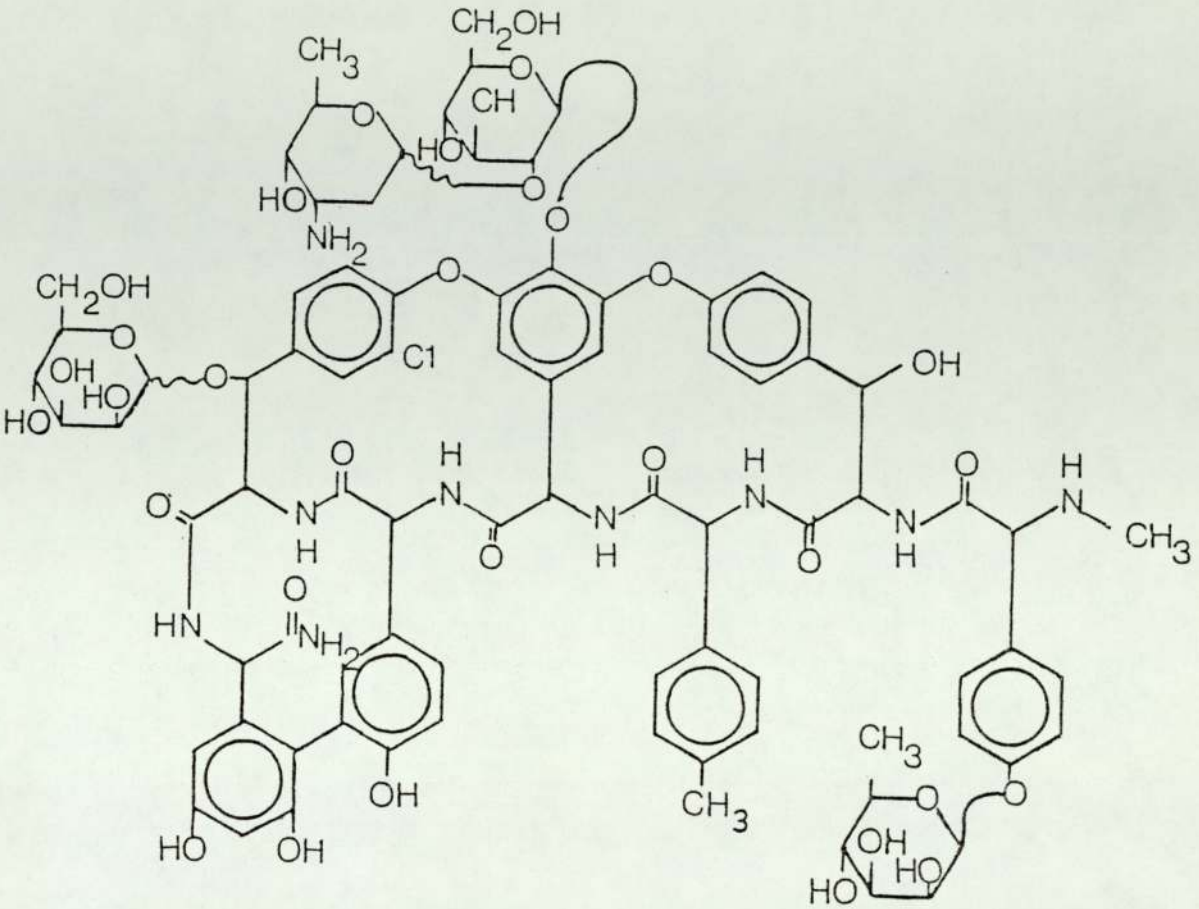


Figure 3.3 The chemical structure of Avoparcin

3.3.3 Avoparcin As Growth Promotant :

In two experiments with groups of male day-old broiler chickens in batteries, Roth-Maier and Kirchgeßner (1976) added 5, 7.5, 10, 15 and 20 ppm avoparcin-lauryl- sulphate to the all-mash feed. Weight gains were significantly increased by 5%. The relative crude nutrient contents of the body mass were similar both for the control and the avoparcin-fed animals. A dose of 5-10 ppm avoparcin was optimum for growth.

In an experiment on broiler turkeys Hulan and Proudfoot (1981) found that 10 ppm avoparcin significantlly increased the mean liveweight of older, but not of younger animals but FCR was not significantly improved in any experiment. Lesson et al. (1980) tested the effectiveness of avoparcin on growth and feed utilization of broiler chickens. Avoparcin had no significant growth-promoting effect on younger chickens fed 10 ppm. Again, older chickens fed avoparcin showed significantly ($P<0.05$) improved feed intake and body weight. Increasing the dose to 20 ppm did not result in any further improvement. Johnson et al. (1979) in an experiment on beef cattle found that feeding avoparcin at 66 ppm resulted in increased average daily gains ($P<0.05$) over the controls. The cattle fed lower doses had a weight gain similar to the control. Avoparcin at all doses improved feed efficiency. Dyer et al. (1980) compared the effect of 33,

49.5 and 66 ppm avoparcin in diet of heifers. All treated cattle consumed less feed for unit gain ($p<0.05$) than did the controls. Feed intake of the heifers fed 49.5 ppm avoparcin was lower ($P<0.05$) than the control. They concluded that 49.5 ppm was optimum for efficiency for the heifers.
No work has been reported for fish.

3.4 Terramycin (Oxytetracycline) :

3.4.1 Source and Structure :

Terramycin was discovered in 1950, its chemical structure was established in 1952 and given the name oxytetracycline. Terramycin is an antibiotic which belongs to the tetracycline group and it is a product of *Streptomyces rimosas* . The name terramycin is derived from the grey earthy appearance of the mould. The antibiotic is effective against both Gram-positive and Gram-negative bacteria and is bacteriostatic. Terramycin has the chemical structure given in figure 3.4 (Welch 1953).

3.4.2 Use As Chemotherapeutic agent :

Tetracycline groups have been used in different species of animals as chemotherapeutic agents, and have also been used as growth promotants in livestock and fish. In fact most of the early work using antibiotics for curing diseases, for example scour in calves, used tetracycline compounds.

In fish, as in any other animals, terramycin is very effective against various infections and pathogens such as

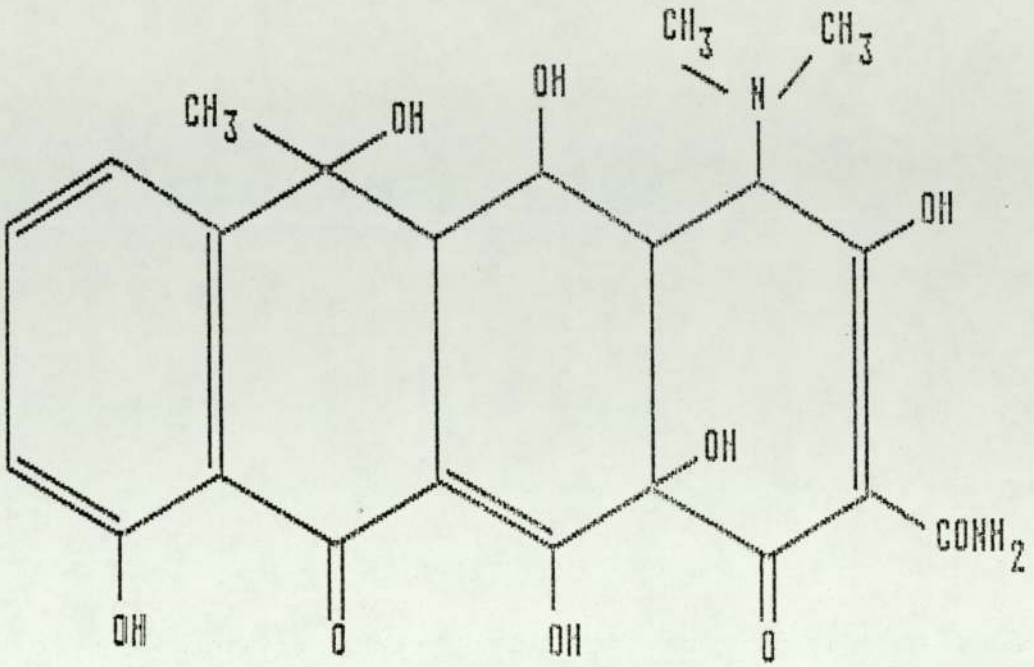


Figure 3.4 The chemical structure of Terramycin

furunculosis, abdominal dropsy, *Aeromonas salmonicida* and putrefactive disease of catfish (Blasiola et al.1980). Pal and Tribathi (1978) found that tail rot, dropsy and reddish blotches amongst carp and loss of barbels in catfish were successfully controlled by feeding terramycin to the diseased fish. However Blasiola et al (1980) found that terramycin was not very effective if used as a bath for treating systemic infections. This is in line with results obtained by Trust (1972) who concluded from his work that the bath method has doubtful therapeutic value when using dosages of specific antibacterials recommended by many manufacturers for the control of fish diseases in aquaria. Terramycin has also proved a successful prophylactic agent against *Vibrio* in crustaceans and bacterial diseases of reptiles (Corliss 1979). Different routes for the administration of terramycin to fish have been used: it has been incorporated into the food and given orally, has been administered by injection or has been added to the water. Advantages and disadvantages are associated with each method.

3.4.3 Terramycin As Growth Promotant :

Jukes and Williams (1953) reviewed the role of the tetracycline group as a growth promotant on calves, chickens, pigs and lambs. Calves showed good growth. Aschbacher (1976) presented another review of the use of antibiotics as growth promotant (including terramycin) in chickens, turkeys, swine and calves. The effective quantities for growth promotion are

from 5 to 50 mg per ton of food or 0.005-0.1 mg per pound (2.2 kg) body weight per day.

Using terramycin as a growth promoter has been recognized as a possibility for fish as with livestock. Leonov (1963) indicated that antibiotic preparations favourably affected the growth of carp (quoted from Sukhoveskho 1967). The latter author used a grain-based food containing terramycin for carp, since it had already been shown to have growth-stimulating effects on other animals. Terramycin was mixed with the diet at dosages ranging from 2500 to 40000 units per kg of food. The doses of 5000 to 10000 units per kg of food appeared to be the best. A further experiment compared continuous use with periodical additions of terramycin, but the results were equivocal. Mitra and Ghosh (1967) similarly reported the effect of feeding terramycin to a number of freshwater fishes, *Labeo rohita*, *Catla catla* and *Cirrhina mrigala* . The experiments were carried out for three months on a small number of fish with initial weight ranging between 35.5 and 41.6 gm. The results showed that growth was enhanced by 0.89, 0.9 and 0.9 gm in the three species respectively. Again no statistical analysis was made nor was the dosage given. Korneeva (1963), using carp, incorporated 10000 units of terramycin per kg of food in a ration fed equivalent to 10% fish body weight per day and observed an increase of about 30% in growth. Again no statistical analysis was made. Two size groups of brown

shrimp *(Penaeus aztecus)* were fed formulated food containing 100, 1000 or 5000 mg of oxytetracycline per kg of food for three weeks. Growth and survival rate were then measured. The results showed that at all three concentrations of oxytetracycline, the small shrimp group (mean initial weight 143.4 mg) consumed one-third the amount of food of controls. Growth, however, was more rapid with diets containing 100 and 1000 mg of oxytetracycline per kg food than with the control diet. The large shrimp group (mean initial weight 458.1 mg) receiving oxytetracycline consumed one-fourth the feed of shrimp on the control diet. Some growth inhibition was apparent in these groups of shrimp and the FCR were lower in the shrimp receiving the diet containing oxytetracycline (Corliss et al. 1977). Oxytetracycline has also been fed in formulated feed to juvenile white shrimp *(Penaeus setiferus)* at concentrations of 1, 5 and 10 g per kg of food for three weeks. Although the main purpose of the experiment was to determine protection of the shrimp against *Vibrio* , it seemed from the results that the treated shrimp gained more weight than the controls (Corliss 1979). In a trial aiming to treat chinook salmon *(Oncorhynchus kitsutch)* against *Vibrio anguillarum*, an unexpected increase in growth occurred in fish treated with terramycin incorporated in the diet at a concentration of 10 mg per 100 kg fish for 10 days (Sawyer and Strout 1977). Again no statistical analysis was made. Novotony (1975) obtained similar results with chinook salmon

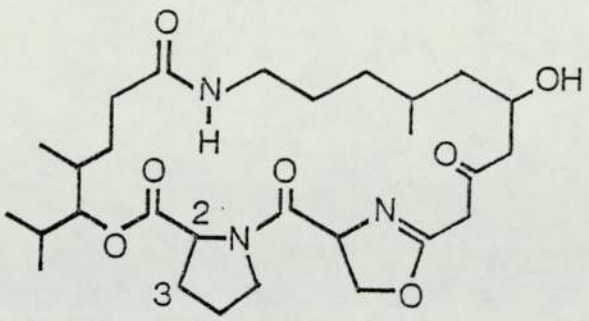
(Oncorhynchus kitsutch) finding that terramycin counteracted the suppression of growth by *Vibriosis* . Rijekers et al. (1980) studied the immune system of cyprinid fish. They found that oral administration of oxytetracycline at 2000 ppm in *Cyprinus carpio* (30-84 g) had a significant growth-promoting effect ($P<0.05$), but injection with 50 mg oxytetracycline per kg of fish significantly diminished growth, higher doses giving greater growth inhibition.

Numerous other workers (e.g. Irwin 1959; Silven et al. 1968; Amend and Ross 1970; Stradine and McBirde 1979; Salte 1982 and Keck et al. 1984) have investigated tissues concentrations of terramycin after oral or bath administration. These studies will be discussed later in this thesis.

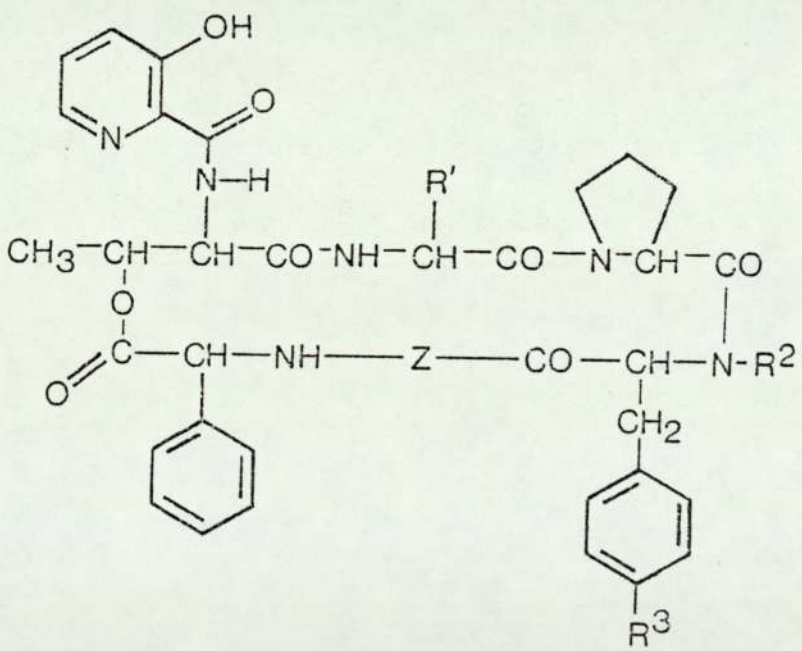
3.5 Virginiamycin :

3.5.1 Source and Structure :

Virginiamycin is a composite antibiotic produced by *Streptomyces virginiae* which was isolated from Belgian soil. There are two chief components in virginiamycin, factor M and factor S. Factor M is present in the larger amount and is mainly active against *Micrococcus aureus* , while factor S is mainly active against *Bacillus subtilis* . Together both components act synergistically in exhibiting a wide range of activity against Gram-positive organisms. The chemical structure is shown in figure 3.5.



Factor M



Factor S

Figure 3.5 The chemical structure of Virginiamycin

3.5.2 Mode Of Action Of Virginiamycin :

Virginiamycin is bacteriocidal rather than bacteriostatic. It does not simply slow down or inhibit growth of microorganisms, but acts through its ability to kill bacteria. It was reported that it exhibits a bacteriopause, where bacteria which come into contact with it for a short time lose their ability to multiply for a considerable time after withdrawal of the drug (Garnett 1974). Virginiamycin is thought to inhibit protein synthesis in microorganisms, and thereby destroy them by interfering with the translation step.

Although the process is not completely understood, evidence supports the theory that virginiamycin binds irreversibly to an acceptor site on the ribosomal subunit. The synergism of factors M and S in virginiamycin can be accounted for by the fact that both have the same primary target, i.e., peptide chain formation (Cocito 1969).

Young healthy pigs kept in hygienic conditions were fed an artificial milk diet and the quantitative effect of virginiamycin (50 ppm) on bacterial flora, biochemical metabolites and physiological aspects of the intestinal tract were examined (Vervaeke 1978). A positive nutritional effect resulted in 10% increased growth and 7% improved FCR. It was suggested that the growth improvement may have been due to :

a) changes in the composition and topographical distribution of the intestinal flora;

b) inhibition of the breakdown of glucose and other useful nutrients;

c) decrease in the microbial production of lactic acid, volatile fatty acids and ammonia;

d) reduction in the rate of passage through the intestine accompanied by improved absorption.

Using mice Madge (1971) found that oral administration of virginiamycin at 0.01, 1.0 and 5.0 mg per 30 gm mouse per 14 days resulted in an improvement in intestinal absorption of D-glucose, D-galactose, L-histidine and L-arginine. The improvement in absorption, however, was accompanied by loss in weight of the mice, the higher the dosage the greater the loss. Also, the wet weight of the small intestine was decreased likewise. The author concluded that oral administration of virginiamycin resulted in a semi-starvation state, a condition which is known to increase intestinal absorption in mice.

3.5.3 Tissue Residues :

Roberfroid and Dumont (1972) found that virginiamycin was poorly absorbed when orally administered at 100 mg per kg body weight of pigs. No drug was found in blood samples taken at intervals up to 30 hours after dosing, and only trace amounts in urine samples taken at intervals over 48 hour after dosing. The same authors determined tissue residues in pigs fed virginiamycin at 155 g per ton of food daily for 18

weeks. Half the animals were killed on the last day of feeding, and the rest after a 24 hour withdrawal period. No residues (less than 0.1 ppm) could be detected in the muscle, liver, kidneys, fat and skin even from pigs killed on the last day of feeding.

A similar absence of tissue residues was found in chickens fed virginiamycin in doses from 20 to 2,500 mg per kg of feed for three to nine weeks; the tissues analysed were muscle, liver, kidneys, fat, skin, gizzard and serum. In experiments on laying hens fed 5 to 100 gm virginiamycin per ton feed, residues of virginiamycin in the eggs were not detectable (Mulder et al 1976).

3.5.4 Virginiamycin As Growth Promotant :

The use of virginiamycin as a growth promotant has been widely demonstrated in pigs. In an experiment on the efficiency of growth promotion of swine, Harpege et al (1981) added virginiamycin to the diet at 1 mg per kg. In three trials out of four on starter pigs, daily weight gain, daily feed intake and feed efficiency were not significantly different between the treatment and the control, but in the fourth trial pigs grew faster when fed virginiamycin. Jewell and Veum (1981) found that virginiamycin increased the average daily gain in the starter phase of pigs fed 27.5 mg per kg food. Adams et al (1981) found that, using a diet containing 18% crude protein fed to pigs of an average weight of 7 kg, virginiamycin increased average daily gain and

average daily feed consumption with no significant differences in gain per feed. The response was greater in the younger pigs. Similarly Veum et al. (1981) found that virginiamycin significantly increased average daily gain in starter but not in grower pigs. During the finishing stage virginiamycin increased average daily gain on the low protein but not on the high protein diet. Other experiments have also been conducted on pigs (Kennedy et al. 1981; Aviotti et al. 1980 and Powley et al. 1981). Hays and Muir (1979) reviewed the use of virginiamycin as a growth promoter in chickens. The results showed that weight gain was improved by 16% in ten experiments and feed efficiency improved by 9% in four experiments.

The use of virginiamycin in fish has not yet been studied. However, in an unpublished experiment, Smith-Kline incorporated 0, 20 and 40 ppm of virginiamycin in the feed of trout for three months. After the positive results found in this experiment, a complementary trial on trout was conducted using 80 ppm dosage. The details of these experiments will be discussed later on this study.

3.6 Emtryl (Dimetridazole) :

Emtryl is a protozocide with the chemical structure of 1, 2-dimethyl-5nitromidazol. Its chemical structure is given in figure 3.6.

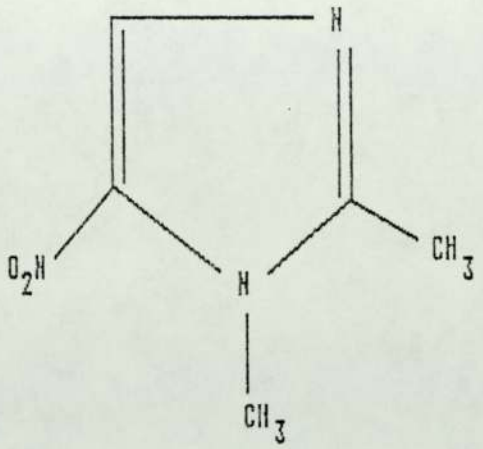


Figure 3.6The Chemical Structure of Emtryl.

It is a white crystalline powder, slightly soluble in water but it is soluble in dilute mineral acids and aqueous and organic acid. Not much work has been done on emtryl. Hofman and Meyer (1974) recommmended dimetridazole at 0.15% in feed for treatment of hexamitiasis. Some fish feed manufacturers have mixed emtryl premix in the feed of fish (May and Baker 1983, personal communication). It was used in the present study for comparing its effect with the other drugs.

3.7 Zeranol :

3.7.1 Source and Stracture :

Several investigators found that animals feeding on mouldy corn or spoiled grain showed evidence of estrogenic stimulation. Following such an occurrence in swine, Stob et al (1962) set out to isolate from the mould a substance responsible for the estrogenic effect. From cultures of the fungus *Gibberella zeae (Fusarium graminearum)* , the authors

isolated a crystalline compound which when fed to castrated mice elicited a uterotropic response that is characteristic of estrogens. The substance was found to be a resorcyclic acid lactone and was given the name Zearalenone. Reduction of the double bond and a ketone group in the lactone ring converted the compound to zearalanol or zeranol which also possesssed a uterotropic (oestrogenic) activity. Its chemical structure is given in figure 3.7.

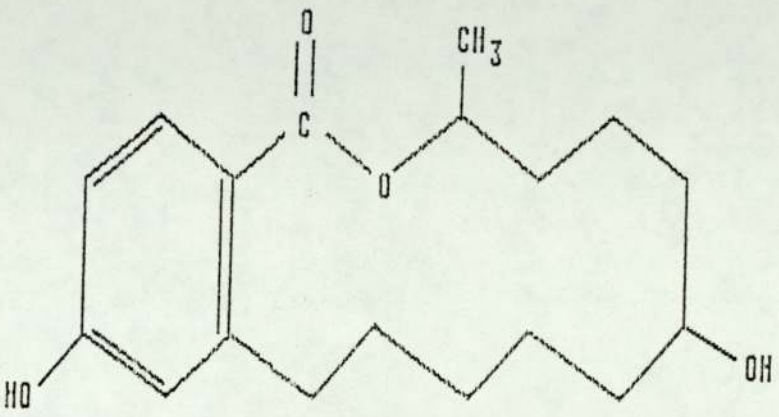


Figure 3.7 The Chemical Structure of Zeranol.

3.7.2 Mode Of Action :

The mechanism by which zeranol produces its anabolic effect has not been conclusively determined. However the possible mode of action of anabolic agents including zeranol has been reviewed by different investigators. Heitzman (1979) reported that "the common action of all anabolic agents is to increase N-retention". N-balance studies confirmed that

zeranol given orally to castrated pigs increased N-retention (Berende et al 1980). Similar results were found by (Chan et al. (1975) when they studied parenterally-administered anabolic agents with sex-steroid like activity. N-retention was increased but did not alter absorption or metabolism of the alimentary tract. Estrogens are thought to exert their primary effect on chromatin transcription and gene expression in cell nuclei of particular target tissues, which include the uterus, liver and chick oviduct. Estrogens probably act together with growth hormone (GH) and insulin. The combination of increased GH and insulin in the muscle cell is thought to increase protein accretion. Injection of GH increases live weight gain in pigs and young cattle (Heitzman 1979; Preston 1975 and Buttery et al 1978). Zeranol implantation (12 mg) in sheep significantly increased the concentration of serum insulin (Wangsness et al 1981), possibly as a result of increased release of GH; pituitary weight was also increased. Injections of GH in sheep have been shown to increase plasma insulin concentration (Davis et al 1970). However, although serum GH was significantly increased, insulin was not significantly greater when cattle were implanted subcutaneously at the base of the ear with 36 mg zeranol (Borger et al 1973). They attributed the non-significant increase in insulin to the time of taking the blood sample from the implanted steers. They also found no change in pituitary weight. Peck and Chesworth (1977) studied

the effect of zeranol in ewes using uterine binding proteins *in vitro* . The result indicated that although zeranol was apparently estrogenic *in vitro* it had little activity when injected into the intact animal.

3.7.3 Tissue Residue :

Sharp and Dyer (1972) implanted five steers at the base of the ear with 72 mg of tritium-labelled zeranol and then monitored excretion until slaughter 65-125 days later. Radioactivity could be detected in the excreta for approximately 90 days after implantation, but was found in only 6 of 55 blood samples analyzed, of which 5 were taken 8-10 days after implantation. Radioactivity was not detectable in tissues at time of the sacrifice except in the bile and in one of the pancreas samples. Under the American FDA regulations a withdrawal period of 65 days is stipulated, and 70 days in UK. At that time tissues are found to be free from residues, using a test method which is sensitive down to 10^{-4} ppm (Brown 1972).

3.7.4 Zeranol As A Growth Promoter :

Steers implanted with 36 mg zeranol and fed with 9.5%, 11% and 12.5% protein in the diet showed 7.8% faster gain than the controls. The amount of protein had no significant effect on daily weight gain, though the implanted steers on 9.5% protein consumed more feed than the control, while those on 11% and 12.5% protein consumed less (Borger et al. 1973).

Utley et al.(1976) also showed significant weight gain when heifers were implanted with zeranol. Similar findings were reported by Wilson and Burdette (1973) and by Kores et al (1974). Bennett et al (1974) implanted zeranol in cattle at 36 mg once or twice; the results showed a 10 to 14% improvement in growth rate and 9% in food conversion, combined with a reduction of up to 31 days in time to slaughter. Similar results were obtained when zeranol was implanted in intensively-cultivated farm animals. Sucking calves implanted with 12, 24 and 36 mg zeranol showed similar daily gain in weight in the implanted and control during the period from birth to weaning (Lamm et al. 1980). Calves implanted more than once showed better daily weight gain than calves implanted only once. Berende et al (1980) studied the effect of 64 ppm zeranol given orally on the performance of castrated male pigs over 16 weeks. The mean weight gain during the 6 weeks was 32 kg for control and 33.9 kg for the treated pigs, and the FCR was 2.94 and 2.78 respectively. Similar effects were seen ten weeks after ceasing treament. Wiggins et al (1979) implanted wether lambs with 12 mg zeranol for 46 days, finding that the average daily weight gain, total gain and final weight were significantly greater, although the increase in the final weight of the implanted lambs appeared to be due to heavier gastrointestinal tracts. No significant difference in feed conversion occurred.

No work on fish has been carried out with zeranol; this

may be due to the inconvenience of implanting fish and a possibly different endocrine system. Zeranol would probably not be a suitable drug for use in aquaculture except perhaps to obtain rapid growth of breeding stock.

CHAPTER FOUR

4. MATERIALS AND METHODS GENERAL

4.1 The Aquarium Systems :

Three types of aquarium systems were built in order to achieve two basic requirements for the experiments. The first was for the maintenance of constant environmental conditions, in particular, temperature and dissolved oxygen. The second was to maintain a healthy stock of fish. If fish are held in a restricted volume of water, changes in water quality occur which must be corrected. These changes include, increase in the concentration of dissolved ammonia, dissolved organic material, solid faecal matter, dissolved carbon dioxide and a decrease in the dissolved oxygen in the water. Two recirculating systems were used in this study for most of the experiments. A third system, a flow through system was used for one carp experiment only.

4.1.1 System One :

This system was an enclosed continuous circulating aquarium which was situated in the main aquarium room. This type of system was used in all experiments except in experiment 5 and 7 and it is shown in figure 4.1.

The water was circulated from a header tank (304 litre capacity) into eight separate fish holding tanks (experimental tanks) and back to the header tank. Water flowed from the header tank by gravity to enter the experimental tanks (96 litre capacity and water volume was

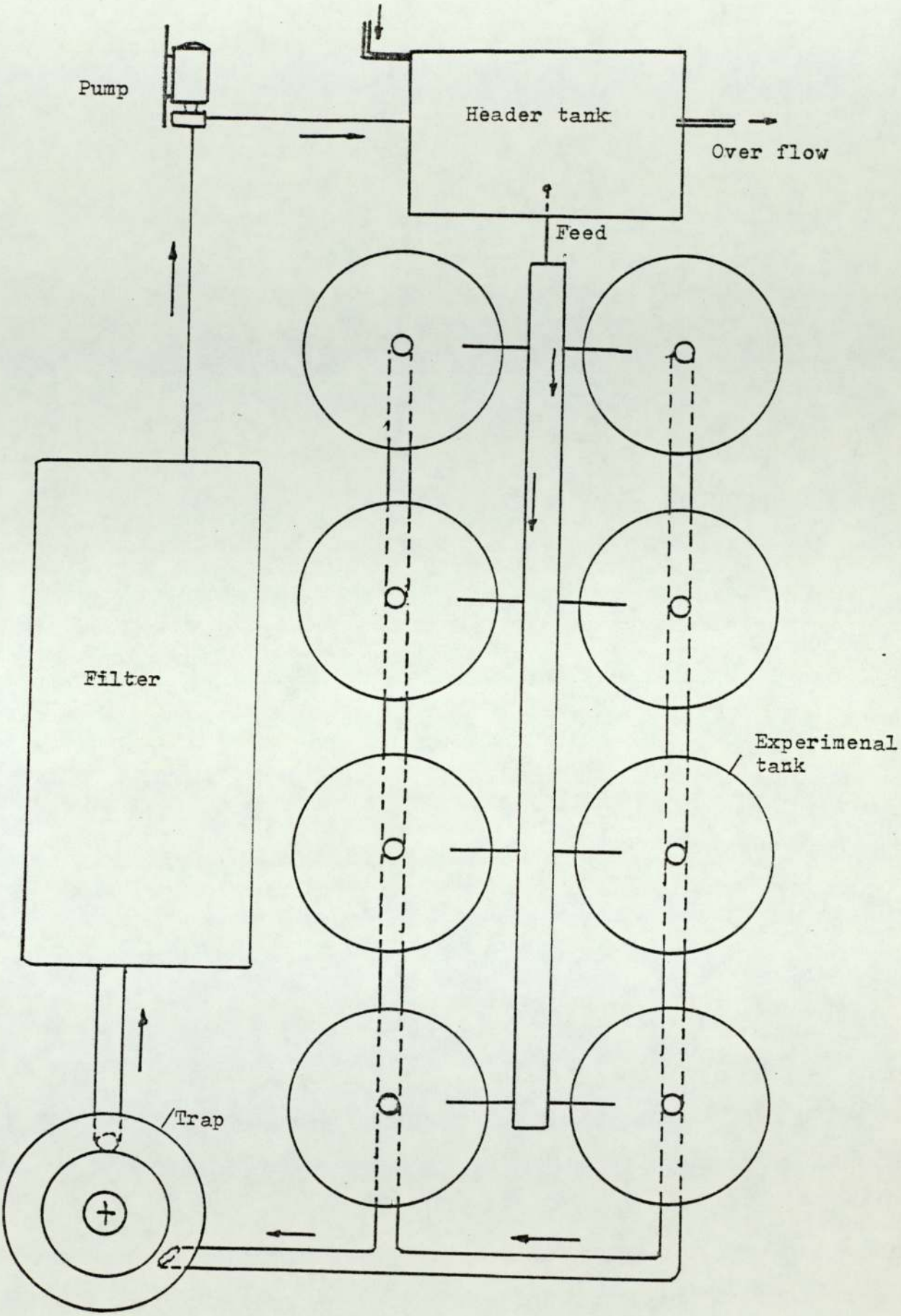


Figure 4.1 A diagrammatic representation of system 1

kept at 60 litre) by aid of a pipe which was divided into two branches, each of which was further subdivided into four smaller with holes in them. The water flow was regulated by a valve and was directed at the surface of the water tangentially. This created a circulation of water within the tank. This with a centrally placed drain (2 cm. dia.) ensured both efficient changeover of the water in the tanks and clearance of faeces from the bottom of the tank. A wider pipe (4 cm. dia.) was covered by a piece of plastic mesh and placed on the drain preventing small fish from becoming wedged in the drain. Water and waste left the experimental tank via a constant-level pipe to a faecal trap and then to the filter. The trap was a semiconical plastic container the shape of which allowed faeces to precipitate in the bottom of the trap. Waste matter was cleaned from the top at regular intervals. The water from the trap left by a 10 cm. dia. pipe and flowed onto the surface of the filter. The filter was an open-topped box (575 litre capacity) inside which was gravel supported on a corrugated perforated plastic sheet which was in turn supported on house-bricks. Water entered the filter at the top surface and was drawn down through the gravel by suction of a water pump (James Beresford and Sons) drawing water from under the corrugated plastic sheet. Suspended solids were regularly removed from the surface of the gravel filter with the aid of a suction cleaner. The pumped water was sprayed onto the surface of the water in the header tank.

The water in this tank was maintained at a constant level by a stand pipe inside it. Excess water in the header tank was returned to the filter by gravity with a spraying action, this affecting reoxygenation of the water. The water entered the experimental tanks at rate of 1.1 litre per minute. Make-up water was continuously added to the header tank to compensate for loss through evaporation and to keep ammonia within an acceptable limit. A heater connected to a thermostate was installed in the header tank to maintain water temperature at 25±1°C. 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

Parameter	Value
Temperature	25±1°C
Dissolved oxygen	8.2-9.5 ppm
Total ammonia	Less than 0.2 ppm
pH	6.9-7.1

4.1.2 System Two :

This system was used in experiment 7. The system was similar to system 1 except in three points :

1) A cooler instead of a heater was installed to maintain the water temperature at 14±1°C. The cooler was situated beside

the trap to cool the water before entering the header tank.

2) The valve regulating the water coming from the header tank to the experimental tank was fully opened to vigorously circulate the water in the tank. This was done to meet the habit of trout of swimming against the water current. The water entered the experimental tanks at the rate of 1.9 litre/min.

3) There were four experimental tanks instead of eight [illegible]. 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

Parameter	Value
Temperature	14±1°C
Dissolved oxygen	9.2-10.3 ppm
Total ammonia	Less than 0.3 ppm
pH	6.8-7.0

4.1.3 System Three :

This system was used in experiment 5. The system was an open system where the water was supplied directly from the city mains supplies. The system consisted of four plastic tanks (15 gallon (68 litre) capacity). Tap water filled the experimental tanks by normal water pressure. Water from four

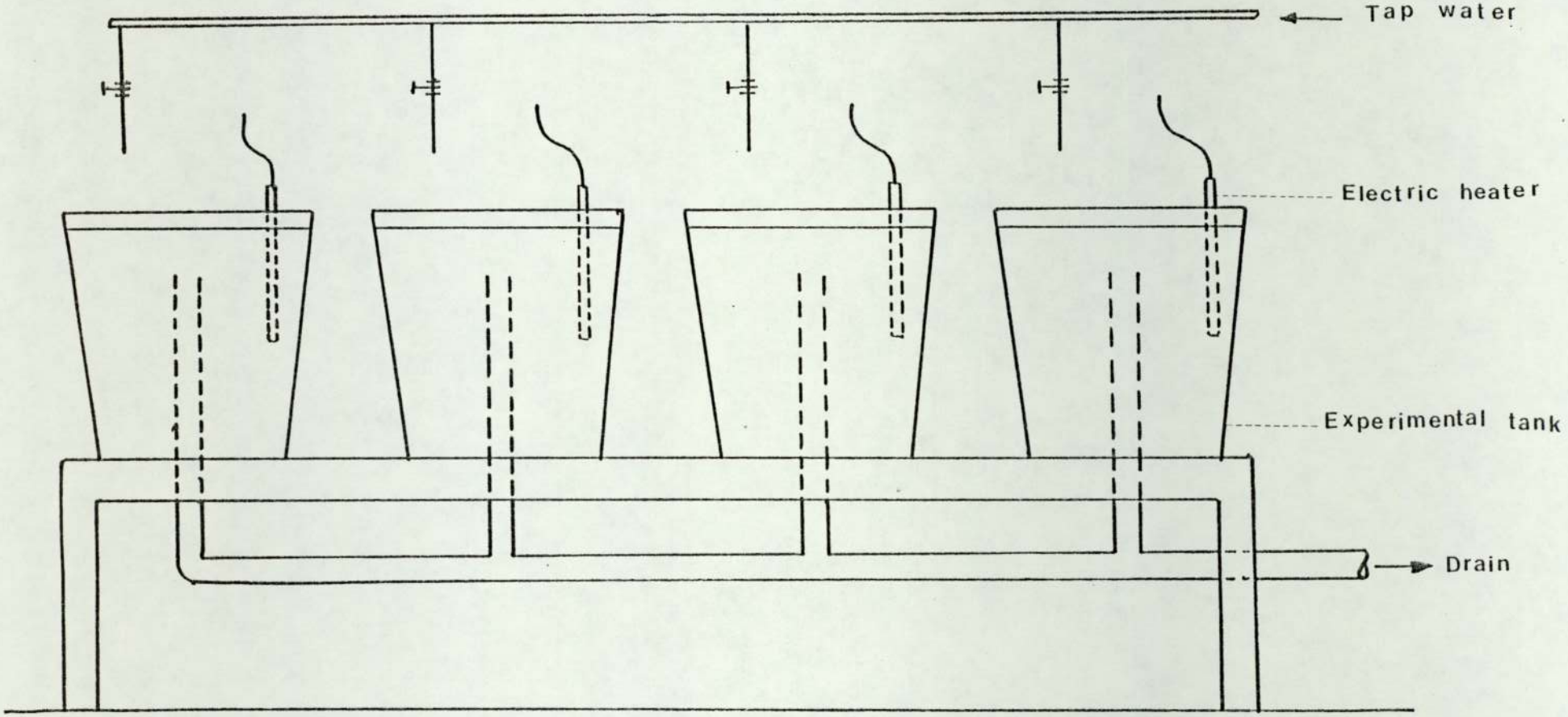


Figure 4.2 A diagrammatic representation of system 2.

outlets flowed onto the surface water of the tanks. The tanks were aerated using an air pump (Rena 301 type) connected to an air stone for each experimental tank. The water was maintained at a constant level by aid of clip type valves. A pipe terminating 2 cm away from the bottom of the experimental tanks siphoned out the waste and excess water to the drain pipe. The temperature of the water was maintained at 25±1°C by an adjustable heater in each tank. The arrangement of system 3 is shown in fig.4.2. The tanks were kept in a small room and the ambient room temperature was maintained by using a thermostatically controlled electric fan heater to further reduce fluctuation of water temperature. Water quality parameters were measured and the values recorded (average of reading, twice a week) are shown in table 4.3.

Table 4.3 : Water quality criteria in system 3

Parameter	Value
Temperature	25±1°C
Dissolved oxygen	8.2-9.5 ppm
Total ammonia	Less than 0.3 ppm
pH	7.0-7.2

4.1.4 Cleaning and Disinfecting the Aquarium System :

The whole system was cleaned and disinfected prior to each experiment to eliminate any fungal or bacterial

infection. The gravel, corrugated plastic sheet and bricks were removed from the filter and washed thoroughly by hosing.

The header tank, experimental tanks, trap and filter were cleaned carefully with a hard brush to remove any dirt attached to them. Sodium hydroxide (commercial) pellets were applied to each tank. The water then was allowed to circulate to dissolve away any trace of sodium hydroxide. All tanks then were sprayed with 2% disinfectant FAM solution (200 ml iodophors in 20 litres of water). Gloves, goggles and mask were used during this operation. After cleaning the filter was reassembled and the water was allowed to circulate for several days before introducing the fish. The experimental tanks, filters and traps were cleaned by brush and water alone while the fish were removed for the fortnightly weighing routine.

4.2 The Fish :

Common carp *Cyprinus carpio* (mirror carp type) and rainbow trout *Salmo gardnerii* were used in this study. The carp were purchased from Newhay Fisheries Ltd, Yorkshire. The trout were purchased from Burwarton Fish Farm, Shropshire. The fish were either dispatched by train in bags filled with oxygen and hermetically sealed, or were collected from the farm by ourselves. The carp purchased from Newhay Fisheries Ltd originated from Germany (Jaffa and Cotwell 1983).

The fish were in good health on arrival but

unfortunately during the summer because of the high temperature some losses of fish occurred. Dead fish did not show any external or internal disease symptoms when examined. This was confirmed by the report on fish samples which were sent to Fish Diseases Laboratories, Weymouth (Ministry of Agriculture, Fish and Food). For this reason, the number of fish varied from one experiment to another. Although the fish purchased were of the same age and from the same farm, individual variations in size were high.

4.3 Quarantine Procedure :

After arrival each batch of fish was placed in prepared clean and disinfected tanks. A green house was used as a quarantine area which was separated from the main fish culture laboratory, where all experiments were carried out. During the period that the fish were in quarantine they were fed a commercial trout diet. No antibiotics was added to the food during this period in order to avoid any possible interaction between the antibiotics used for quarantine purpose and the drugs which were to be investigated.

On the second and third day after arrival, the fish were bathed in 150 ppm formalin for one hour. Three days after, they were further treated twice on successive days by immersing in 2 ppm of malachite green for one hour in a strongly aerated tank. These treatments with malachite green and formalin were used to eliminate external parasites. The Roberts + Shepherd 1979 fish were kept in quarantine for two weeks and in the mean

time were checked for any external parasite or signs of abnormality. After two weeks the fish were transferred to the main laboratory. The temperature of the water containing the fish was increased from ambient by 3°C per day until it reached the required temperature. The fish continued to feed on commercial trout diet during the acclimation period. The manufacturers proximate analysis of this diet (Edward Baker Ltd Omega Trout And Salmon Foods) is seen in table 4.4.

4.4 Diet Formulation :

The optimum amount of protein in the diet of carp required in order to achieve maximum growth has been broadly investigated, and is generally considered to be between 31 and 45%. Different factors affecting this requirement include the age of the fish, the temperature, dietary energy, source of protein and oxygen.

In this study two concentrations of protein were chosen, 40% which was considered as a high-protein diet (HP) and 25% which was considered as a low-protein diet (LP). The higher value was chosen as it was a mean of results obtained from the literature (Ogino and Satio 1970, Ogino et al. 1976, Sen et al. 1978, Takeuchi et al. 1979 and Gooligh and Adlem n 1984). The lower amount was used as it appeared lowest investigated by previous workers (Attack et al. 1979, Wohlfarth 1978 and Jauncey 1982).

Table 4.4 : trout diet proximate analysis as applied by the manufacturer (Edward Baker Ltd Omega Trout And Salmon Foods)

Diet Composition	% or unit
Protein	47
Fat	8
Fibre	4.5
Vitamin A	18000 IU/kg
Vitamin D	2000 IU/kg
Vitamin E	120 IU/kg
Selenium	0.1 mg/kg

4.5 Diet Preparation :

The amount of diet required for each experiment was estimated from the starting weight of the fish and the expected maximum growth rate. An additional 10% was added to this, to allow for losses during pelleting, drying and sieving. Dietary ingredients are shown in table 4.5. Some of these ingredients were sieved to particle size of 2 mm$^{=}$ prior to weighing. The fishmeal and wheat middlings were used in two different ratios to reach the high and the low percentage of protein. The dry ingredients weighed according to the formulation, placed in a Hobart mixer and thoroughly blended with oil for 4-5 minutes to produce a homogenized mixture. Drugs were incorporated at this stage at three different concentrations. Water was added to make dough, which was extruded through a Hobart mincer attachment. The resulting spaghetti-like strings were laid on trays and air dried in a heated cabinet at 40°C; when almost dry to the touch, the strings were broken into pieces 3-4 mm in length then were further dried. The dry diets were passed through a sieve to remove fines, and were stored at -20°C until required. The diet for the trout experiment was prepared by placing the commercial dry trout pellets into a food blender (Kenwood type) for 4-5 minutes in order to produce a fine mixture. Drugs were then incorporated into the mixture and well mixed in the Hobart mixer for 3-4 minutes. The procedure then continued as for the carp diet. Overheating of the mixture,

Table 4.5 : The ingredients of the carp diets.

Ingredient	Percentage content	
	HP diet	LP diet
Fishmeal[a]	47	21
Wheat middlings	43	69
Oil[b]	5	5
Minerals & vitamins premix[c]	2.5	2.5
CMC[d]	2	2
Chromic oxide[e]	0.5	0.5

[a]: Edward Baker

[b]: Mixture 50% corn oil and 50% cod liver oil

[c]: Edward Baker

[d]: Carboxymethyl cellulose (binder)

[e]: Indigestible indicator

which may cause degradation, was avoided.

4.6 Feeding :

The fish were fed 2-5% of their body weight per day depending on their starting weight. The food was taken out of the freezer and left to reach the room temperature before feeding to the fish. The food was broadcasted by hand to the fish over a period of fifteen to twenty minutes to ensure the maximum consumption. The fish were fed three times a day at 0900, 1400 and 1800 except on Sundays when they were fed twice a day at 1000 and 1700. The amount of food given was adjusted after the weighing of fish fortnightly. When the fish did not consume the ration during a 45-60 minutes period, the uneaten diet was collected, dried and deduced from the amount given.

4.7 Anaesthesia :

If the fish were to be weighed, marked for individual identification or injected they were first anaesthetized, using Benzocaine (diamino benzene) at a concentration of 0.5 g per litre. This was prepared by dissolving 0.5 g of Benzocaine into a minimal amount of acetone, and this solution then mixed into ten litres of water. 5-7 fish at a time were placed in a bucket containing the anaesthetic for 5-10 minutes or until anaesthetized. The degree of anaesthesia is seen by removing a fish from the water and holding it on its side. If the eye is seen to look down from

the fish's point of view the fish is still conscious. In anaesthetized fish this reflex is lost and the eye remains as if the fish were upright. Fish took 10-15 minutes to regain consciousness. No mortality occurred as a result of anaesthesia.

4.8 Weighing :

In all experiments the fish were weighed individually, just before starting the experiment, every two weeks and at the end of the experiment. The fish were first anaesthetized and allowed to drain of water in a small net for 5-10 seconds. Excess water was then removed with an absorbent paper towel. Fish were weighed on a tared top pan balance (Sartorius-Wertce type 3719) to the nearest 0.01 g. The total length of each fish was measured to the nearest 0.1 cm. The fish were then returned to the aquarium. No food was offered on the weighing day.

4.9 Marking The Fish :

Three methods were used in this study for marking of the fish for individual identification and statistical comparisons.

1) The first method was to brand the fish by using liquid nitrogen. Two branding irons were made from 2.5 mm single strand copper wire, each with a different coloured insulation. The colours enabled the appropriate brand to be selected when the ends were submerged in the liquid nitrogen

and the insulation protected the user's hand from burning. The fish were anaesthetized and lightly blotted on a paper towel and laid on the side with the head to the left. Fish were branded by placing the cold ends of the brands against the skin, just above the lateral line and behind the operculum, for about 4-7 seconds. The fish were appropriately numbered. The fish then were weighed and measured for length and returned to tanks prior to starting experiment on the next day (fig.4.3).

2) Marking the fish by using a tagging gun was carried out in most of the experiments except when the fish were too small. The tags were made out of 5 mm wide Dymo tape trimmed to an oval shape 1 cm in length. One end was embossed by letters and numbers to achieve various combinations for identification of individual fish. At the other end a 2-mm hole was made with a hot iron. The fish were anaesthetized and the tag attached to the fish by inserting the hole of the tag in the hollow needle of the gun. By pressing the trigger a plastic string of 2.5 cm length was released which attached the tag to the fish. The tag was placed just in front of and below the dorsal fin (fig.4.4).

3) The third method was used in Experiment 2, 3, 4 and 6 because the fish were very small. This method was similar to the second method but the tags were commercially purchased. The tags were 5 mm dia. numbered plastic discs with a 1-mm hole at the top. Fish were anaesthetized and after removal

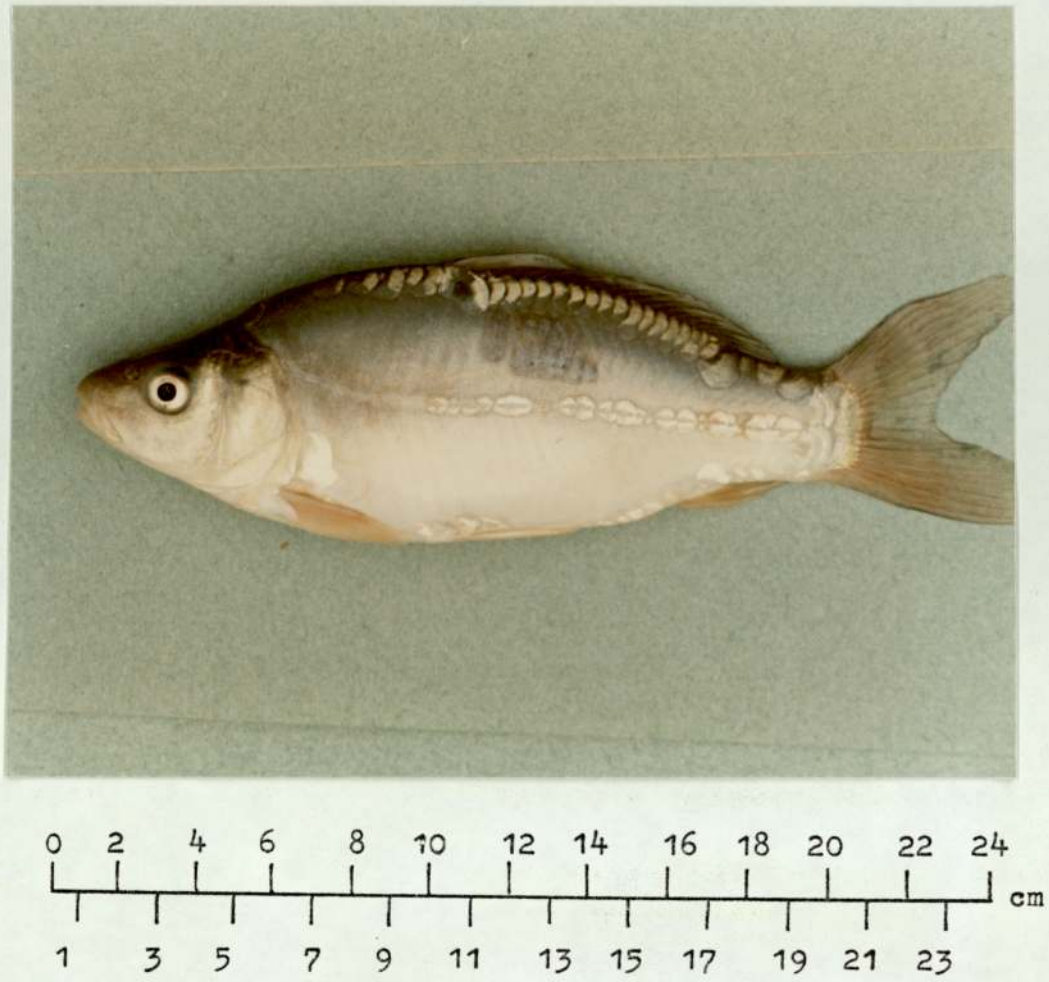


Figure 4.3. Cold branding method

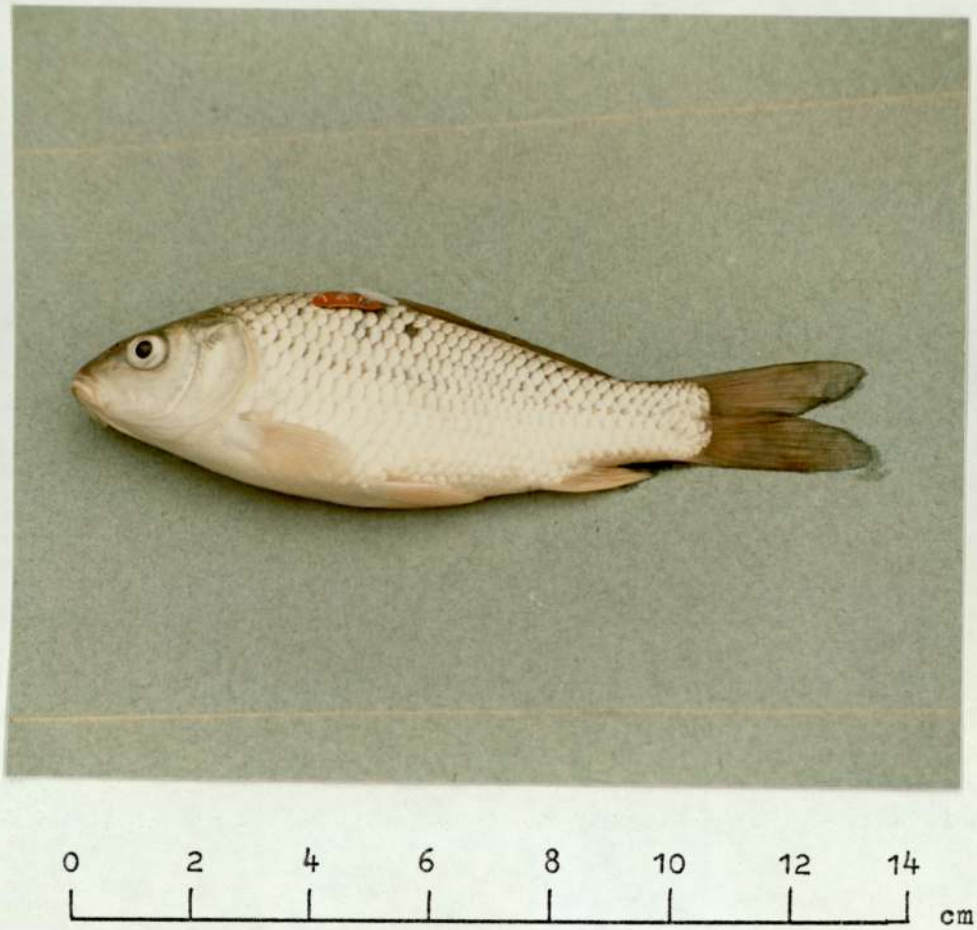


Figure 4.4. The tagging gun method

from anaesthetice a 0.5 mm dia. hypodermic needle was inserted through the fish just in front and below the dorsal fin. One end of a 7-cm length of nylon fishing line was inserted into the needle and drawn through the fish. The tag was then threaded on to the nylon string allowing a generous loop for growth. The free ends of the nylon string were then knotted several times to securely attached the tag (fig.4.5).

4.10 Temperature And Light :

The carp in all experiments were kept in the main laboratory, the temperature of which was kept at 25±1°C. For the trout experiment, the water was cooled to 14±1°C. Photoperiod was set to 12 hours day and 12 hours night. Fluorescent lights were used.

4.11 Proximate Analysis :

Proximate analysis of the two diets, the dietary ingredients and muscle of fish were carried out by the following procedures :

a) Crude protein : Microkjeldahl method for total nitrogen (AOAC methods 1970); protein taken as N x 6.25.

b) Crude lipid (ether extract) : Petroleum ether (40-60°C boiling range) extraction in a Soxhlet apparatus.

c) Ash : Incineration at 550°C in a muffle furnace for 24 hours.

d) Fibre : Treating the sample with 1.4% sulphuric acid and 1.4% sodium hydroxide (AOAC 1970).

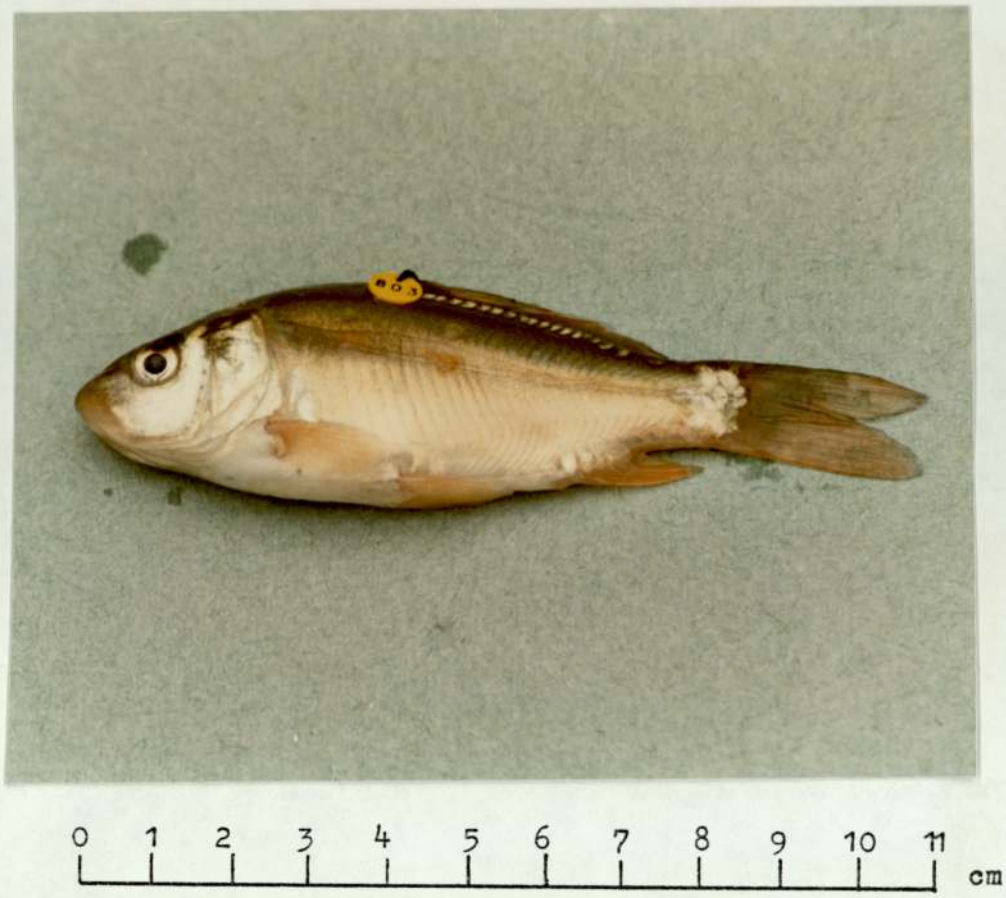


Figure 4.5. The disc tagging method

e) Moisture : A weighed sample was placed in a preweighed jar and left in an oven at 105°C for 24 hours or until a constant weight was obtained.

f) Nitrogen free extract (NFE) : By difference :

NFE (soluble carbohydrate) = 100 - (protein + moisture + lipid + fibre + ash)

All figures being expressed as percentage. The results of the analysis of the diets are shown in table 4.6.

4.12 Digestibility :

For digestibility studies fish were removed from tanks and anaesthetized and faeces stripped from them, by stroking firmly down the belly from the ventral fin to the anus using much the same technique as for stripping eggs from ripe fish. Because of the small quantities, the faeces from 3-4 fish were pooled, weighed wet, and dried for 24 hours at 60°C, then kept in tightly cover vials for analysis. Samples from HP and LP diets were also dried in the same way for analysis.

The method described by Lied et al. (1982) for determining protein digestibility with a chromic oxide indicator was used, based on using an inert indigestible unabsorbable material which passes through the digestive tract at a uniform rate and is easily identified. 0.5% chromic oxide, Cr_2O_3, was mixed with the diet.

4.13 Gross Energy :

The gross energies of HP and LP diets were measured. The

Table 4.6 : Proximate analysis of diets A and B

Constituent	Percentage content	
	HP diet	LP diet
Protein	40.0	25.0
Fat	9.5	9.3
Moisture	16.8	16.5
Fibre	4.7	6.2
Nitrogen-free extract	19.4	33.9
Ash	9.6	9.1
Gross energy	4.136(cal/g)	4.187(cal/g)

conventional ballistic bomb calorimeter was first used but because of uncertainty of results a chemical method was adopted. This depends on measuring the calorific value by oxidation with a solution of potassium dichromate in sulphuric acid (O'shea and Maguire 1962). Energy values were obtained by dividing the amount of 1.5 N dichromate used to oxidize 1 g of food material by a factor depending on the protein content. The results are shown in table 4.6.

4.14 Histological Technique :

Samples of fish from each treatment group which were taken for a general study of gut histology were selected on the basis of average body weight. The average body weight of the sample closely approximated the average body weight of the entire lot from which the sample was drawn. After killing the fish in an excess amount of anaesthetic (Benzocaine), the whole intestine was removed. The method described by Warren (1936) and Gordon and Bruckner-Kardoss (1961) was used for fixing and cutting samples from the anterior and medial part of the gut with some modification. The whole gut from the oesophagus to the anus was emptied of most of its content by pressing very gently with a forceps from the top to the end. The remaining material was washed out completely under a pressure of 60 cm, using a 3-litre jar filled with 10% formalin and situated at 60 cm height, as pressure reservoir. After 5 minutes the lower part of the intestine was closed by tying it with a piece of thread and the whole intestine allowed to distend under the

pressure of the flow of the formalin solution. When distension reached its limit, the clip valve connected to the jar was turned off and the upper end of the intestine was tied off. The intestine was then laid in a 12 inch trough full of Bouin's solution and left overnight for fixation. The gut was divided into three parts, Oesophageal (anterior), medial and recatal (posterior), as described by Al-Hussaini (1949). Pieces of 3-4 mm were cut from the anterior and medial part and placed in 70% alcohol to wash out the Bouin's solution. The general method of washing, hardening and embedding in paraffin wax (56°C m.p.) was used. Serial of transverse sections were cut at 5μm (Kent Cambridge type microtome), stained with Haematoxylin and Eosin, or in some cases with Mallory, and mounted in a BDH mounting medium (Bancroft & Stevens 1982).

4.14.1 Microscopy And Photograpy :

Stained sections examined on a Leitz Wetzlar Microsystem-70 microscope. Photomicrographs were taken for colour pictures on Agfachrome CT18 film. The photomicrographs were developed and printed professionally.

4.14.2 Quantitative Histology :

The measurement of the thickness of the wall of the intestine was made by using micrometer eyepiece. The circumference of the stained intestinal section (fig. 4.6) and the length of the folds (fig. 4.7) were measured. The average of ten different readings of each was taken for each

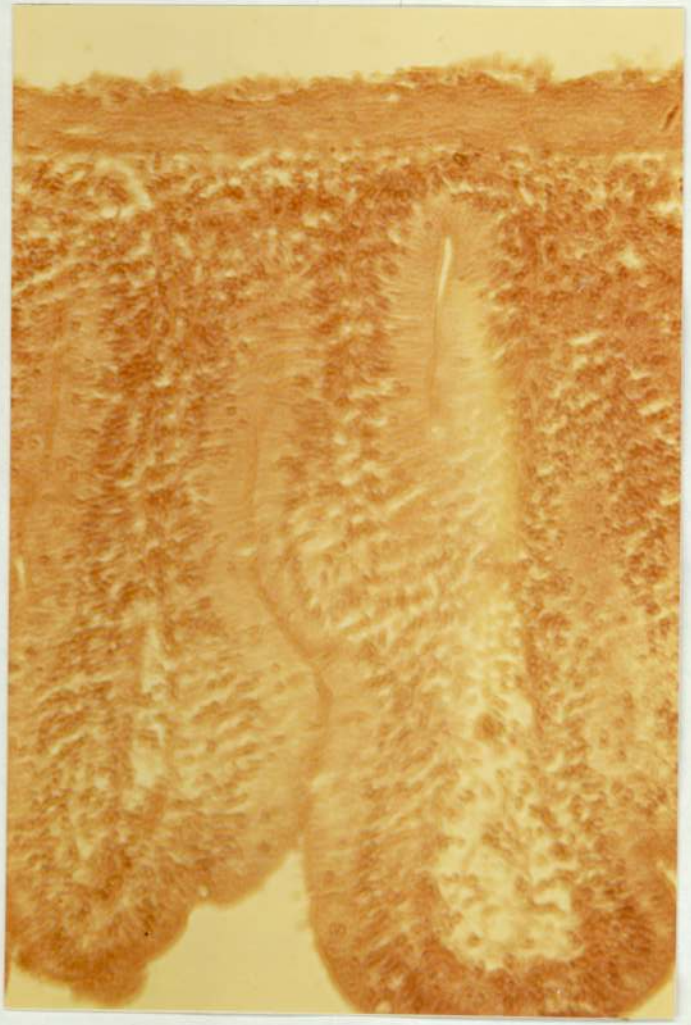


Figure 4.7 The fold H&E X 512

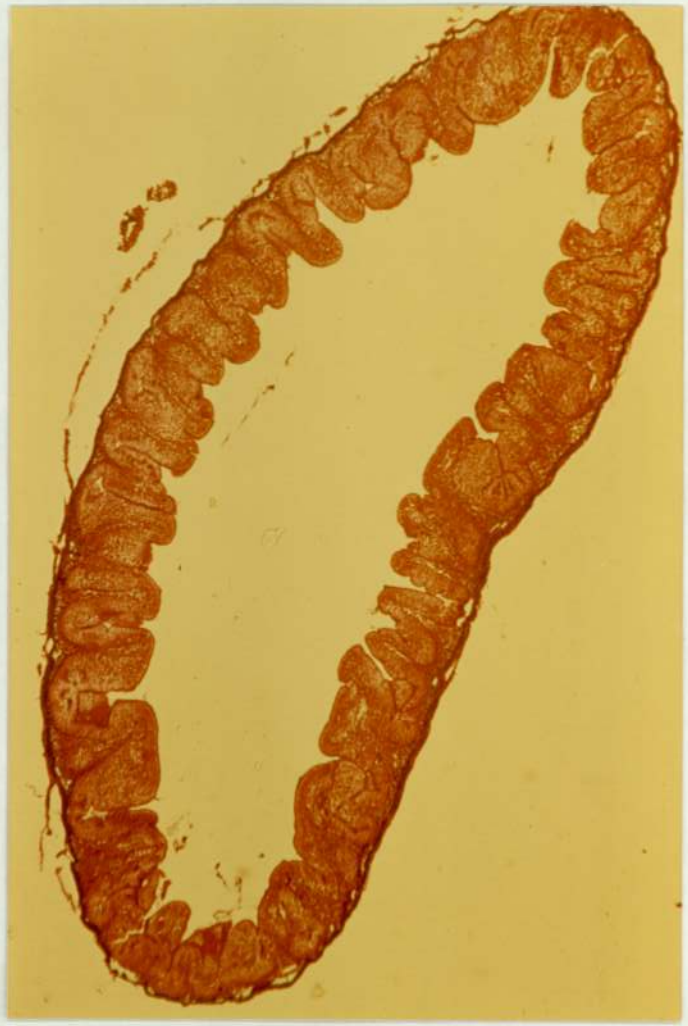


Figure 4.6 Section of the medial intestine H&E X 80

measurement. The ratios between the two averages were then counted.

4.15 Bacteriological Methods :

4.15.1 Media :

Five different media were used in this study :

1) Nutrient Agar (NA) : This medium was purchased commercially from oxoid; its formula is :

Lab-Lemco powder	1.0 g
yeast extract	2.0 g
Peptone	5.0 g
Sodium chloride	5.0 g
Agar No.3	15.0 g
pH	7.4

2) Blood Agar (BA) : The blood base agar was purchased from oxoid and the blood (discarded human blood) was provided by Birmingham Blood Transfusion Center. The formula for the blood base agar is :

Lab-Lemco powder	10 g
Peptone	10 g
Agar No.3	15 g
Sodium chloride	5 g
pH	7.3

3) MacConkey Agar : This was purchased from Oxoid :

Peptone	20 g

Lactose	10 g
Bile salts	5 g
Sodium chloride	5 g
Neutral red	0.75 g
pH	7.4

4) Meat Yeast Peptone Agar (MYPA) : This was prepared in the laboratory from the following formula (Lindsay 1983) :

Meat extract	3 g
Yeast extract	3 g
Peptone (bacteriological)	10 g
Agar	15 g

5) NBGT-1/3S Agar (selective medium) : This was prepared in the laboratory to isolate obligate anaerobic bacteria. The formula used was as follows (Sakata et al. 1980) :

Lab-Lemco powder	2.4 g
Proteose peptone	10.0 g
Yeast extract	5.0 g
Na_2HPO_4	4.0 g
Glucose	1.5 g
Soluble starch	0.5 g
L-Cystine	0.2 g
Antifoam B(10% sol.)	5.0 g
L-Cysteine $HCL H_2O$	0.5 g
Tween-80	1.0 g

Agar	15.0 g
Human blood	50.0 ml
NBGT additive-solution (1/3S)	20 ml
N-taurocholate	1.5 mg/100 ml
Brilliant green	1.5 mg/100 ml
Fradiomycin sulfate	350 mg/100 ml

4.15.2 Preparation Of Media :

1) Nutrient agar : 28 g of nutrient agar was dissolved in 1000 ml distilled water on a hot plate and autoclaved for 15 minutes at 15 lb pressure (121°C).

2) Blood agar : On arrival the blood was divided into 25 ml filling sterilized universals. The base blood agar was prepared by dissolving 40 g of the base medium in 1000 ml distilled water and autoclaved for 15 minutes at 15 lb pressure (121°C). The medium was placed in a water bath (50°C) to cool. The blood was mixed with the cooled agar aseptically at 10 percent. The medium was then poured into sterilized plastic petri dishes, and was allowed to set and dry, either closed at 37°C over night or open at 50°C for 1 hour.

3) MacConkey agar : The agar was dissolved in distilled water to a concentration of 40 g/l and autoclaved at 15 lb (120°C) for 15 minutes and poured as for blood agar.

4) MYPA : The ingredients were mixed together and

dissolved in 1000 ml distilled water and the procedure was carried out as above.

5) NBGT 1/3S (Skata 1980) : All ingredients were mixed together except the antibiotic and dissolved in 1000 ml distilled water and autoclaved as above. The antibiotic (fradiomycin sulfate) was sterilized by seitz filteration and mixed with the other ingredients and poured as above.

4.15.3 Fish Bacteriological Preparation :

Samples of fish were taken from each treatment and killed in an excess amount of anaesthetic (Benzocaine). Immediately, the ventral surface of the fish was swabbed with 70% ethanol. An incision was made from the gill to the anus and the gut was removed aseptically. The intestine was then divided into three parts; the content of the middle part was pooled and placed in a preweighed sterilized vial and weighed. In some cases the mid gut with its content was weighed and placed in a sterilized petri dish. This was then homogenized for 30-60 secondes at intervals to avoid overheating which may kill the bacteria. For all samples a suitable amount of diluent (1/3 strength Ringer solution) was added to effect tenfold dilution. These samples then were diluted serially and placed onto five different media (NA, BA, MacConkey, MYPA and NBGT 1/3S) incubated aerobically and anaerobically (except the last, which was incubated anaerobically only) for 3-5 days at 25°C. Anaerobiosis was established by evacuating the atmosphere of an anaerobic jar

(Gaspak BBL) containing steel wool with disposable hydrogen-carbon dioxide generator envelopes. Disposable anaerobic idicators (changes from white to blue in the presence of oxygen) were used to ensure complete evacuation. In the trout experiment (Experiment 6) the same procedure was performed on samples of the content of the stomach or the intestine.

4.15.4 Qualitative Bacteriological Examination :

After incubation the colonies were studied carefully for two points. Firstly the colonial morphology was studied, including size, shape and colour. The bacterial colonies on blood agar were further examined for haemolysis. Secondly, microscopic appearance of the different types of colony was studied using Gram-stain. A representative of each colony type appearing on plates was selected. The isolates were then purified and maintained by weekly transfusion on MYPA or NA agar. The API strip method (API Laboratory product Ltd) was used for identification of the isolates by consulting the API computerized profile index. API 20E was used for identifying Enterobacteriacea and other Gram-negative bacteria. API 20B was used for identifying Gram-positive bacteria. These systems are simply explained as follows : API kits have the same construction. The individual test consists of dehydrated chemical in a set of plastic cupuls (moulded to a strip of plastic) which are inoculated with a bacterial suspension. API is designed for the performance of 23 standard

biochemical test from a single colony of bacteria on the plating medium (Holmes et al. 1978).

4.15.5 Qualitative Study Of *A. hydrophila* :

When it became clear that *A. hydrophla* was the sole bacterium isolated from the gut of common carp(*Cyprinus carpio*) the general counting method described by Cruickshank (1972) was used. The method included taking samples of the gut content and diluting serially. Inocula of 0.1 ml were taken from each dilution and inoculated on MYPA agar and incubated at 25°C for 48 hours. The plate, which contained 30-300 colonies, was counted.

4.16 Testing The Sensitivity Of *A. hydrophila* To Antibiotics *in vitro* :

The method described by Cruickshank (1972) was used with slight modification to determine the effect of terramycin and virginiamycin on *A. hydrophila* . The principle of the method is a follows : A concentration gradient of the antibiotic is prepared in a medium fully adequate to support the growth of the test organism, which is added uniformaly to the mixture. Following incubation, growth in the medium is measured. The modification was that instead of a concentration gradient, different concentrations of the drug were prepared separately and poured in separate petri dishes, one for each concentration plus one for the control (without drug), all being set up in duplicate. The bacteria were isolated and

checked for identification by the API method. After incubation for different periods of time, the plates were compared with the control.

4.17 Analysis Of Experimental Data :

The growth data were measured by two-way analysis of variance and means were compared by the "t" test. Where valid, correlation coefficient methods were also used. Significance between means was taken at $P<0.05$ (Sokal + Rohlf 1961)

Specific growth rate (SGR) : a measure of the percentage weight or length gain per day, daily gain (DG), was determined from the formula :

$$G = \frac{\log_e YT - \log_e Yt}{T-t} \times 100$$ (Weatherley 1972)

Where YT = the final size (at time T)

Yt = the initial size (at time t)

t = starting time

T = finishing time

T - t = time in days

e = the base of natural logarithms

G = specific growth rate

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

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

Where C = condition factor

W = weight in grams

L = length in centimetres

The gains in weight or length over the controls were calculated by

$W_n - w_n = g_n$ gain in size of treated fish (mean)

$W_c - w_c = g_c$ gain in size of control fish (mean)

$$g\% = \frac{g_n - g_c}{g_c}$$

Where g% = percentage gain in size of treated fish

Food utilization efficiency (FUE) : was calculated from the formula

$$FUE = \frac{\text{wet weight (g)}}{\text{Food given (g)}}$$

The hepato-somatic index (HSI), reno-somatic index (RSI) and viscero-somatic index : were calculated as follow

$$\left.\begin{matrix} HSI \\ RSI \\ VSI \end{matrix}\right\} = \frac{\text{weight of the organ}}{\text{weight of body}} \times 100$$

Digestibility coefficient : was calculated as :

$$\text{Digestibility} = 100 - 100 \times \frac{Cr_2O_3 \text{ in feed (\%)}}{Cr_2O_3 \text{ in faeces (\%)}} \times \frac{\text{nutrient [protein in faeces (\%)]}}{\text{nutrient [protein in feed (\%)]}}$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{weight gain (g)}}{\text{protein given (g)}}$$

$$\text{Net protein retention (NPR)} = 100 \times \frac{\text{increase in carcass protein}}{\text{protein in feed}}$$

4.18 Experimental Material And Method :

4.18.1 Tylosin Fed To Carp (Experiment 1) :

4.18.1.1 Aquarium System : System 1 as described in 4.1.1.

4.18.1.2 The Fish : Mirror carp *Cyprinus carpio* with initial mean weight of 11.9 g and initial length of 9.6 cm. The fish were subject to quarantine procedure 4.3 prior to the experiment.

4.18.1.3 Diet : HP and LP diets were prepared as described in 4.5 and tylosin was mixed at concentrations of 0, 50, 100 and 150 mg per kg diet. The diet was offered at 5% of the fish body weight at the start of the experiment and this amount was reduced to 2.5% as the fish grew bigger as mentioned in 4.6.

4.18.1.4 Marking The Fish : The fish were anaesthetized as described in 4.7 and marked by the branding method 4.9 and weighed as in 4.8.

4.18.1.5 Temperature And Light : The fish were kept in tanks in which the ambient temperature was 25±1°C and the photoperiod schedule as described in 4.10.

4.18.1.6 Proximate Analysis : Was carried out as described in 4.11.

4.18.1.7 Histological Technique : Intestinal sections were cut and stained as in 4.14.

4.18.1.8 Statistical Analysis : Two way analysis of variance was applied to analyse the results.

4.18.2 Payzone Fed To Carp (Experiment 2) :

4.18.2.1 Aquarium System : System 1 as described in 4.1.1.

4.18.2.2 The Fish : Mirror carp *Cyprinus carpio* with initial mean weight of 5.1 g and initial length of 6.8 cm. The fish were subject to quarantine procedure 4.3 prior to the experiment.

4.18.2.3 Diet : HP and LP diets were prepared as described in 4.5 and payzone was mixed at concentrations of 0, 25, 50 and 100 mg per kg diet. The diet was offered at 5% of the fish body weight at the start of the experiment and this amount was reduced to 3% as the fish grew bigger as mentioned in 4.6.

4.18.2.4 Marking The Fish : The fish were anaesthetized as described in 4.7 and marked by method No. 3 as described in 4.9 and weighed as in 4.8.

4.18.2.5 Temperature And Light : The fish were kept in tanks in which the ambient temperature was 25±1°C and the photoperiod schedule as described in 4.10.

4.18.2.6 Proximate Analysis : Was carried out as described in 4.11.

4.18.2.7 Histological Technique : Intestinal sections were cut and stained as in 4.14.

4.18.2.8 Statistical Analysis : Two way analysis of variance was applied to analyse the results.

4.18.3 Avoparcin Fed To Carp (Experiment 3) :

4.18.3.1 Aquarium System : System 1 as described in 4.1.1.

4.18.3.2 The Fish : Mirror carp *Cyprinus carpio* with initial mean weight of 4.8 g and initial length of 6.7 cm. The fish were subject to quarantine procedure 4.3 prior to the experiment.

4.18.3.3 Diet : HP and LP diets were prepared as described in 4.5 and avoparcin was mixed at concentrations of 0, 10, 20 and 40 mg per kg diet. The diet was offered at 5% of the fish body weight at the start of the experiment and this amount was reduced to 2.5% as the fish grew bigger as mentioned in 4.6.

4.18.3.4 Marking The Fish : The fish were anaesthetized as described in 4.7 and marked by method No. 3 as described in 4.9 and weighed as in 4.8.

4.18.3.5 Temperature And Light : The fish were kept in tanks in which the ambient temperature was 25±1°C and the photoperiod schedule as described in 4.10.

4.18.3.6 Proximate Analysis : Was carried out as described in 4.11.

4.18.3.7 Histological Technique : Intestinal sections were

cut and stained as in 4.14.

4.18.3.8 Statistical Analysis : Two way analysis of variance was applied to analyse the results.

4.18.4 Terramycin Fed To Carp (Experiment 4) :

4.18.4.1 Aquarium System : System 1 as described in 4.1.1.

4.18.4.2 The Fish : Mirror carp *Cyprinus carpio* with initial mean weight of 20.36 g and initial length of 11.0 cm. The fish were subject to quarantine procedure 4.3 prior to the experiment.

4.18.4.3 Diet : HP and LP diets were prepared as described in 4.5 and terramycin was mixed at concentrations of 0, 10, 50 and 100 mg per kg diet. The diet was offered at 3% of the fish body weight at the start of the experiment and this amount was reduced to 2.5% as the fish grew bigger as mentioned in 4.6.

4.18.4.4 Marking The Fish : The fish were anaesthetized as described in 4.7 and marked by the tagging gun method as described in 4.9 and weighed as in 4.8.

4.18.4.5 Temperature And Light : The fish were kept in tanks in which the ambient temperature was 25±1°C and the photoperiod schedule as described in 4.10.

4.18.4.6 Proximate Analysis : Was carried out as described in 4.11.

4.18.4.7 Histological Technique : Intestinal sections were cut and stained as in 4.14.

4.18.4.8 Fish Bacteriological Preparation : As described in

4.15.3.

4.18.4.9 *In Vitro* Testing Of The Drug : As described in 4.16.

4.18.4.10 Statistical Analysis : Two way analysis of variance was applied to analyse the results.

4.18.5 Virginiamycin Fed To Carp (Experiment 5) :

4.18.5.1 Aquarium System : System 3 as described in 4.1.3.

4.18.5.2 The Fish : Mirror carp *Cyprinus carpio* with initial mean weight of 17.4 g and initial length of 10.9 cm. The fish were subject to quarantine procedure 4.3 prior to the experiment.

4.18.5.3 Diet : Diet A and B were prepared as described in 4.5 and virginiamycin was mixed at concentrations of 0, 40, 80 and 100 mg per kg diet. The diet was offered at 3% of the fish body weight at the start of the experiment and this amount was reduced to 2.5% as the fish grew bigger as mentioned in 4.6.

4.18.5.4 Marking The Fish : The fish were anaesthetized as described in 4.7 and marked by the tagging gun method as described in 4.9 and weighed as in 4.8.

4.18.5.5 Temperature And Light : The fish were kept in tanks in which the ambient temperature was 25±1°C and the photoperiod schedule as described in 4.10.

4.18.5.6 Proximate Analysis : Was carried out as described in 4.11.

4.18.5.7 Histological Technique : Intestinal sections were cut and stained as in 4.14.

4.18.5.8 Fish Bacteriological Preparation : As described in 4.15.3.

4.18.5.9 Statistical Analysis : One way analysis of variance was applied to analyse the results.

4.18.6 Virginiamycin Fed To Carp (Experiment 6) :

4.18.6.1 Aquarium System : System 1 as described in 4.1.1.

4.18.6.2 The Fish : Mirror carp *Cyprinus carpio* with initial mean weight of 4.3 g and initial length of 6.5 cm. The fish were subject to quarantine procedure 4.3 prior to the experiment.

4.18.6.3 Diet : HP and LP diets were prepared as described in 4.5 and virginiamycin was mixed at concentrations of 0, 40, 80 and 100 mg per kg diet. The diet was offered at 5% of the fish body weight as mentioned in 4.6.

4.18.6.4 Marking The Fish : The fish were anaesthetized as described in 4.7 and marked by method No. 3 as described in 4.9 and weighed as in 4.8.

4.18.6.5 Temperature And Light : The fish were kept in tanks in which the ambient temperature was 25±1°C and the photoperiod schedule as described in 4.10.

4.18.6.6 Proximate Analysis : Was carried out as described in 4.11.

4.18.6.7 Histological Technique : Intestinal sections were cut and stained as in 4.14.

4.18.6.8 Fish Bacteriological Preparation : As described in 4.15.3.

4.18.6.9 In Vitro Testing Of The Drug : As described in 4.16.

4.18.6.10 Statistical Analysis : Two way analysis of variance was applied to analyse the results.

4.18.7 Virginiamycin Fed To Rainbow Trout (Experiment 7) :

4.18.7.1 Aquarium System : System 2 as described in 4.1.2.

4.18.7.2 The Fish : Rainbow trout *Salmo gairdneri* with initial mean weight of 35.4 g and initial length of 15 cm. The fish were subject to quarantine procedure 4.3 prior to the experiment.

4.18.7.3 Diet : Commercial trout diet as described in 4.5 and virginiamycin was mixed at concentrations of 0, 40, 80 and 100 mg per kg diet. The diet was offered at 3% of the fish body weight as mentioned in 4.6.

4.18.7.4 Marking The Fish : The fish were anaesthetized as described in 4.7 and marked by method No. 3 as described in 4.9 and weighed as in 4.8.

4.18.7.5 Temperature And Light : The fish were kept in tanks in which the ambient temperature was 25±1°C and the photoperiod schedule as described in 4.10.

4.18.7.6 Proximate Analysis : Was carried out as described in 4.11.

4.18.7.7 Histological Technique : Intestinal sections were

cut and stained as in 4.14.

4.18.7.8 Fish Bacteriological Preparation : As described in 4.15.3.

4.18.7.7 Statistical Analysis : "t" test was applied to analyse the results.

4.18.8 Emtryl Fed To Carp (Experiment 8) :

4.18.8.1 Aquarium System : System 1 as described in 4.1.1.

4.18.8.2 The Fish : Mirror carp *Cyprinus carpio* with initial mean weight of 16.3 g and initial length of 9.8 cm. The fish were subject to quarantine procedure 4.3 prior to the experiment.

4.18.8.3 Diet : Only HP diet was used and emtryl was mixed at concentrations of 0, 50, 100 and 150 mg per kg diet. The diet was offered at 5% of the fish body weight at the start of the experiment and reduced to 4% as mentioned in 4.6.

4.18.8.4 Marking The Fish : The fish were anaesthetized as described in 4.7 and marked by method No. 3 as described in 4.9 and weighed as in 4.8.

4.18.8.5 Temperature And Light : The fish were kept in tanks in which the ambient temperature was 25±1°C and the photoperiod schedule as described in 4.10.

4.18.8.6 Proximate Analysis : Was carried out as described in 4.11.

4.18.8.7 Histological Technique : Intestinal sections were cut and stained as in 4.14.

4.18.8.8 Fish Bacteriological Preparation : As described in

4.15.3.

4.18.8.9 Statistical Analysis : One way analysis of variance was applied to analyse the results.

4.18.9 Zeranol Injected Into Carp (Experiment 9) : The fish were injected with 0.8 mg zeranol per week for eight weeks.

4.18.9.1 Aquarium System : System 1 as described in 4.1.1.

4.18.9.2 The Fish : Mirror carp *Cyprinus carpio* with initial mean weight of 79.6 g and initial length of 16.5 cm. The fish were subject to quarantine procedure 4.3 prior to the experiment.

4.18.9.3 Diet : Commercial trout diet (Edward Baker) was given to the fish at 2% of their body weight through out the experiment.

4.18.9.4 Marking The Fish : The fish were anaesthetized as described in 4.7 and marked by method No. 3 as described in 4.9 and weighed as in 4.8.

4.18.9.5 Temperature And Light : The fish were kept in tanks in which the ambient temperature was 25±1°C and the photoperiod schedule as described in 4.10.

4.18.9.6 Proximate Analysis : Was carried out as described in 4.11.

4.18.9.7 Statistical Analysis : "t" test was applied to analyse the results.

4.18.10 Virginiamycin And Terramycin Injection Into Carp (Experiment 10) : 36 fish were divided into three groups of

12, first group was injected with 13 mg (10% active material) virginiamycin, the second group was injected with 2 mg terramycin and the third group was injected with the saline for four weeks.

4.18.10.1 Aquarium System : Three tanks of system 1 as described in 4.1.1.

4.18.10.2 The Fish : Mirror carp *Cyprinus carpio* with initial mean weight of 56.73 g and initial length of 15.2 cm. The fish were subject to quarantine procedure 4.3 prior to the experiment.

4.18.10.3 Diet : Commercial trout diet (Edward Baker) was given to the fish *ad libitum* .

4.18.10.4 Marking The Fish : The fish were anaesthetized as described in 4.7 and marked by the tagging gun method as described in 4.9 and weighed as in 4.8.

4.18.10.5 Temperature And Light : The fish were kept in tanks in which the ambient temperature was 25±1°C and the photoperiod schedule as described in 4.10.

4.18.10.6 Statistical Analysis : The "t" test was applied to analyse the results.

CHAPTER FIVE

5. RESULTS

5.1 Effect Of Feeding Tylosin To Carp (Experiment 1) :

The antibiotic tylosin was added to the diet of carp at a concentration of 100 ppm, as was recommended by the manufacturer; 50 ppm above and below this dosage was also used. Tylosin was incorporated into high and low protein diets. The experiment was carried out for only 8 weeks because by the end of the eighth week the brand symbol of the fish was becoming too difficult to read.

5.1.1 Acceleration Of Growth:

The fish fed the high protein (HP) diet gained significantly greater weight and length than the fish on the low protein (LP) diet. When tylosin was added to the HP diet at concentrations of 0, 50, 100 and 150 ppm the percentage increases in weight were 187%, 195%, 218.3% and 213% respectively and 39.2%, 42%, 49.5% and 44.4% in length. These differences were not significant ($P>0.05$). The percentage increases in weight of the fish fed the LP diet supplemented with tylosin at the above dosages were 157%, 176.8%, 176% and 169.6% respectively. The increases in length were 30.6%, 34,8%, 33.8% and 33.3% respectively. The results are shown in tables 5.1 and 5.2 and figures 5.1, 5.2, 5.3 and 5.4.

5.1.2 Specific Growth Rate (Daily Gain):

During the first two weeks the daily gain (DG) in weight was higher in the control than in the treated fish especially

Table 5.1 : Change in weight of carp *(Cyprinus carpio)* fed on diets containing tylosin for a period of eight weeks at 25±1°c. Numbers given are mean of 20 fish ±S.E.

(a) High Protein Diet

Duration in Weeks	Concentration of tylosin (mg/kg food)			
	150	100	50	control
0	11.93±0.4	11.93±0.5	11.8±0.53	12.21±0.47
2	17.03±0.7 (42.7%)	16.66±0.78 (39.6%)	16.0±0.64 (35.6%)	17.98±0.73 (47.3%)
4	24.12±1.2 (102.2%)	24.25±1.2 (103.3%)	22.82±0.86 (93.4%)	25.88±1.2 (112.0%)
6	29.62±1.3 (148.3%)	29.23±1.29 (145.0%)	28.2±0.97 (139.0%)	29.8±1.5 (144.1%)
8	37.35±1.4 (213.1%)	37.97±1.46 (218.3%)	34.82±1.5 (195.1%)	35.06±1.84 (187.1%)

Table 5.1 (cont'd.)

(b) Low Protein Diet

Duration in Weeks	Concentration of tylosin (mg/kg food)			
	150	100	50	control
0	11.71±0.46	11.84±0.54	11.71±0.51	12.28±0.47
2	16.69±0.61 (42.5%)	17.0±0.89 (43.6%)	17.2±0.86 (46.9%)	18.11±0.7 (42.5%)
4	23.21±0.9 (98.2%)	23.75±1.4 (100.6%)	24.52±1.4 (109.4%)	25.82±1.03 (110.0%)
6	26.58±1.06 (127.0%)	27.28±1.5 (108.6%)	28.11±1.8 (140.0%)	28.3±1.07 (130.5%)
8	31.57±1.2 (169.6%)	32.57±1.7 (175.0%)	32.41±1.8 (176.8%)	31.64±1.3 (157.7%)

Table 5.2 : Change in length of carp *(Cyprinus carpio)* fed on diets containing tylosin for a period of eight weeks at 25±1°c. Numbers given are mean of 20 fish ±S.E.

(a) High Protein Diet

Duration in Weeks	Concentration of tylosin (mg/kg food)			
	150	100	50	control
0	9.7±0.13	9.5±0.16	9.5±0.15	9.7±0.16
2	10.4±0.17 (8.0%)	10.4±0.17 (9.6%)	10.3±0.14 (8.4%)	10.7±0.18 (11.1%)
4	11.7±0.18 (21.3%)	11.6±0.2 (22.1%)	11.4±0.15 (20.4%)	11.9±0.25 (23.6%)
6	12.9±0.2 (33.4%)	12.8±0.22 (34.7%)	12.2±0.15 (28.7%)	12.9±0.23 (33.0%)
8	14.0±0.23 (44.4%)	14.2±0.2 (49.5%)	13.5±0.16 (42.0%)	13.5±0.23 (39.2%)

Table 5.2 (cont'd.)

(b) Low Protein Diet

Duration in Weeks	Concentration of tylosin (mg/kg food)			
	150	100	50	control
0	9.7±0.15	9.6±0.17	9.6±0.16	9.8±0.17
2	10.4±0.13 (7.7%)	10.4±0.21 (8.2%)	10.5±0.18 (9.0%)	10.8±0.15 (9.7%)
4	11.5±0.15 (19.2%)	11.5±0.25 (20.0%)	11.6±0.21 (20.9%)	11.9±0.15 (21.0%)
6	12.2±0.17 (26.7%)	12.3±0.26 (27.6%)	12.4±0.21 (29.2%)	12.5±0.17 (28.0%)
8	12.9±0.18 (33.3%)	12.9±0.27 (33.8%)	12.9±0.24 (34.8%)	12.8±0.16 (30.6%)

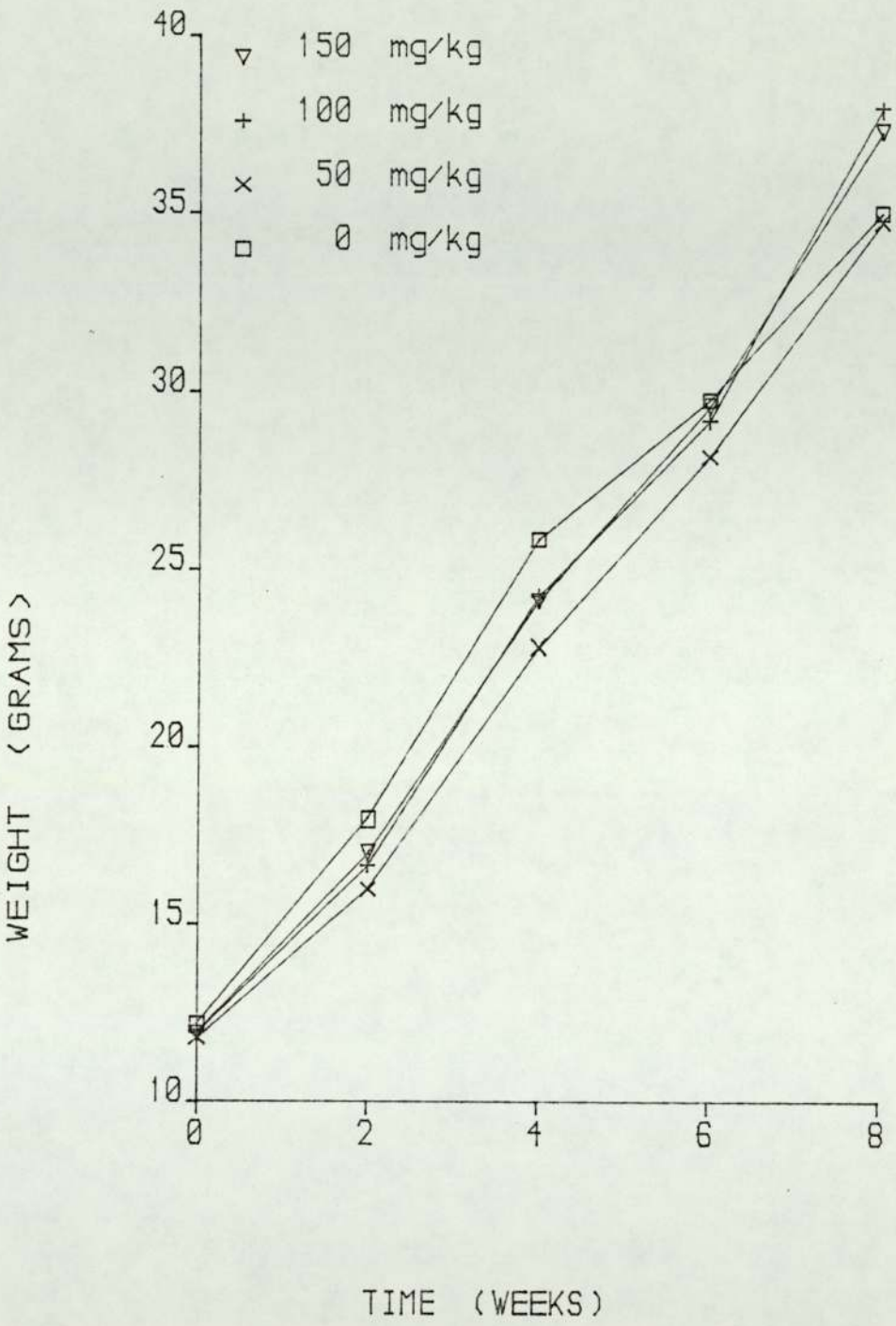


Figure 5.1 Effect of tylosin on the weight of carp fed HP diet

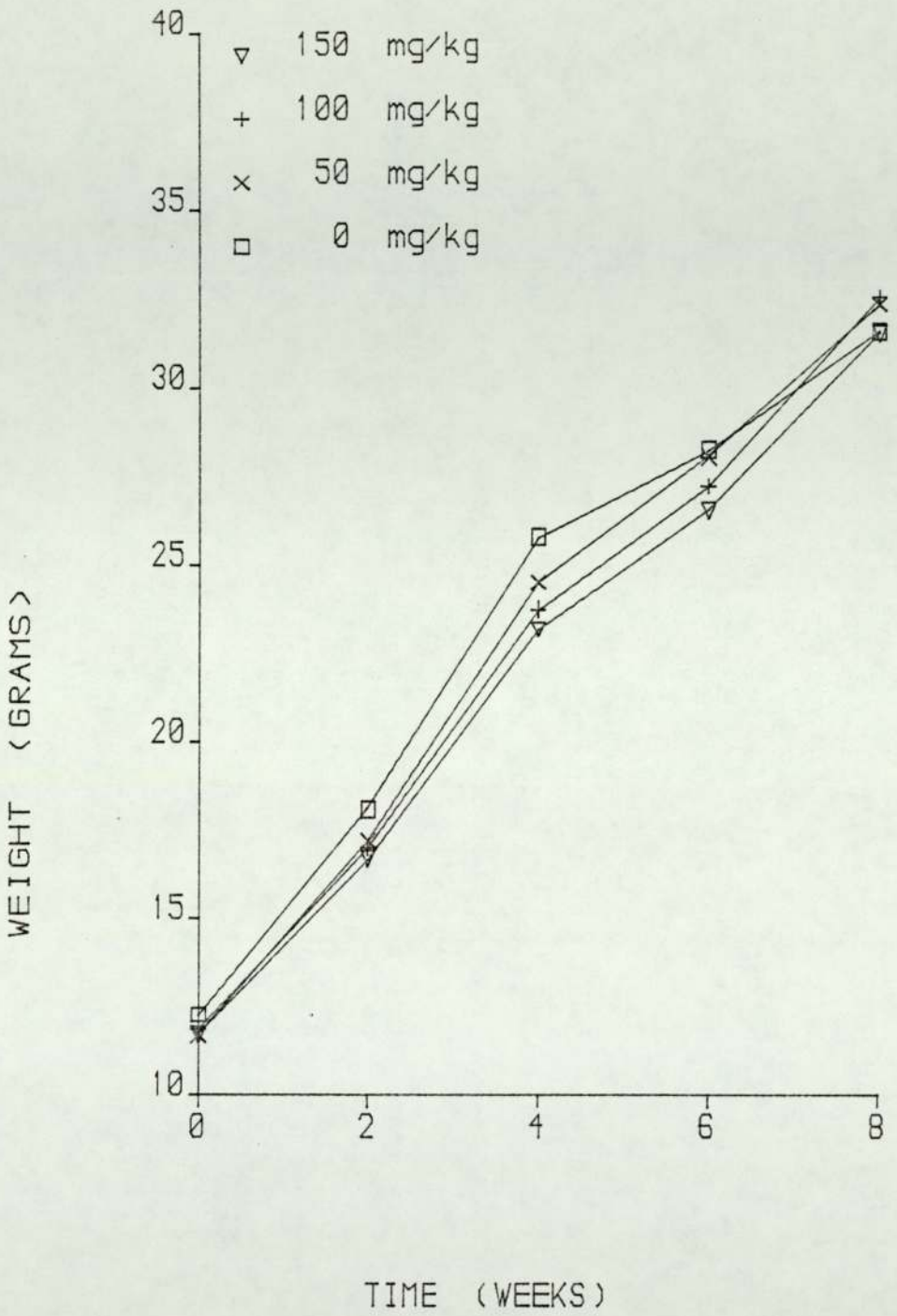


Figure 5.2 Effect of tylosin on the weight of carp fed LP diet

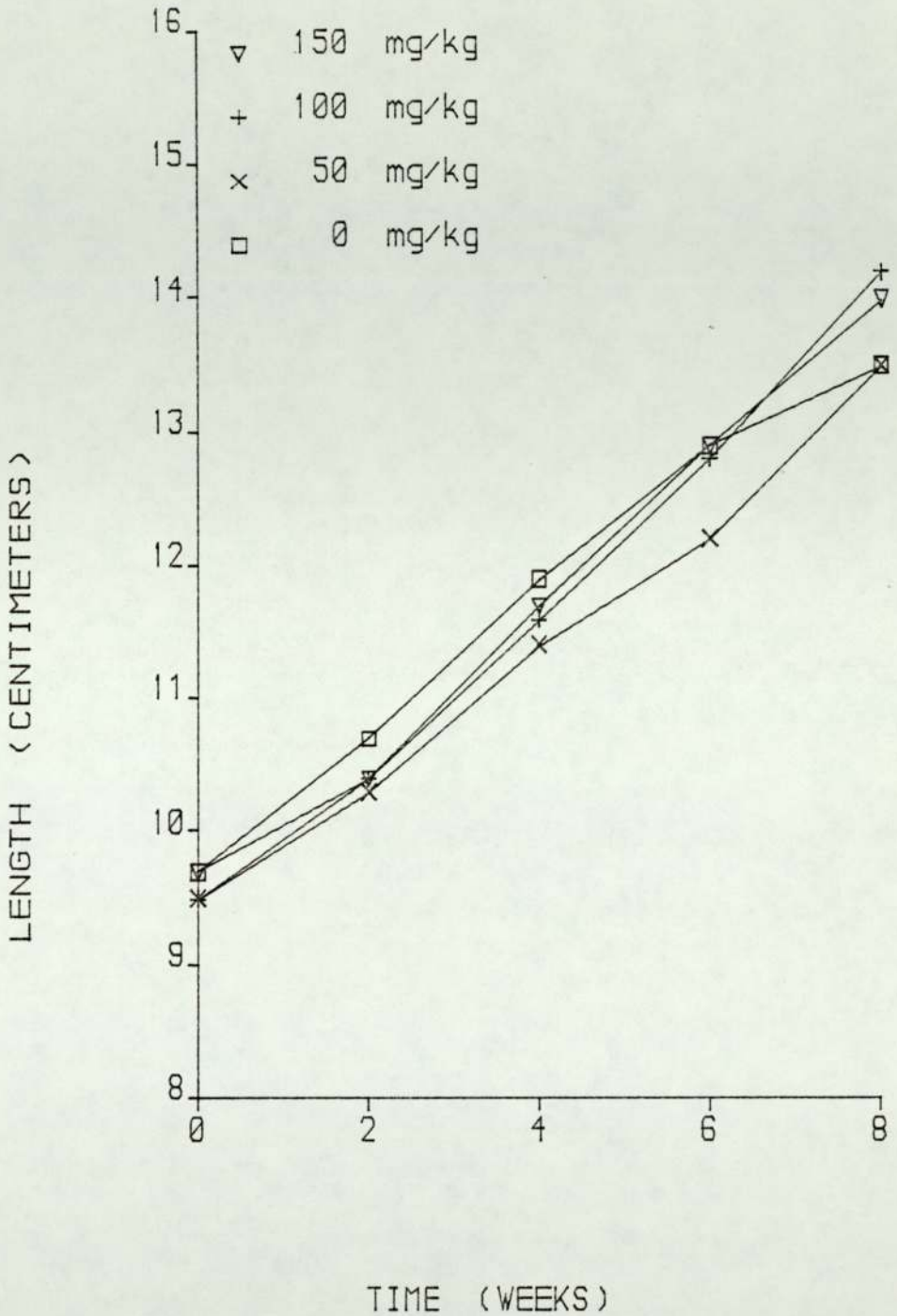


Figure 5.3 Effect of tylosin on the length of carp fed HP diet

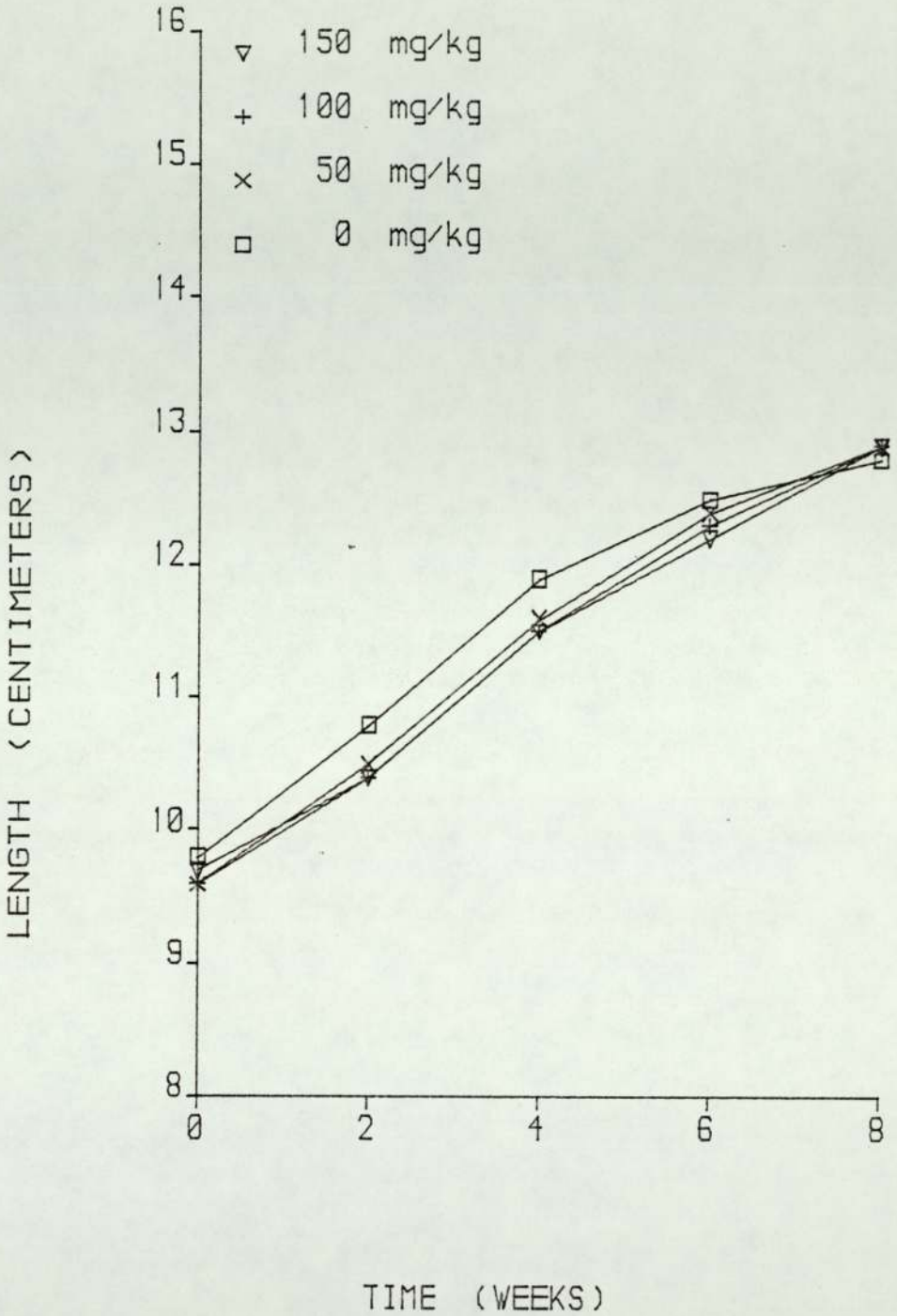


Figure 5.4 Effect of tylosin on the length of carp fed LP diet

on the LP diet. The fish fed HP diet and treated with tylosin continued to increase the DG over that of the controls until the end of the experiment. With the LP diet the treated fish tended to increase more than the control in the second half of the experiment. Although the DG was higher in the treated fish than in the controls the differences were not significant. The controls on both diets had higher length DG than the treated fish during the first two weeks too. The improvement in the length DG was clear from the second period (2-4 weeks) in all the treated fish. The overall increase in length DG was not significantly higher in the treated fish than in the controls. Results are shown in tables 5.3 and 5.4 and figures 5.5 and 5.6.

5.1.3 Feed Utilization Efficiency (FUE):

The ratios of the gain in weight to the food given (FUE) were lower in the treated fish than in the controls during the first four weeks in both diets. The FUE increased to be higher in the treated fish than in the controls during the second four weeks. However the overall FUE was higher in the fish receiving tylosin-medicated diets as is seen in table 5.5.

5.1.4 Proximate Analysis of the Body Compotion:

The muscle of the treated and of the control fish was subjected to biochemical analysis. No differences in the water and protein content were caused by either of the two

Table 5.3 : Effect of feeding tylosin on the specific growth rate (SGR) of carp (weight).

Period in Weeks	Concentration of tylosin (mg/kg food)							
	High protein diet				Low protein diet			
	150	100	50	0	0	50	100	150
0-2	2.7	2.6	2.3	2.8	3.0	2.9	2.8	2.7
2-4	2.7	2.9	2.7	2.6	2.7	2.7	2.6	2.5
4-6	1.6	1.4	1.6	1.5	0.81	1.1	1.1	1.0
6-8	1.8	2.1	1.6	1.2	0.98	1.3	1.4	1.3
0-8	2.2	2.25	2.0	2.0	1.87	2.0	1.98	1.9

Table 5.4 : Effect of feeding tylosin on the specific growth rate (SGR) of carp (length).

Period in Weeks	Concentration of tylosin (mg/kg food)							
	High protein diet				Low protein diet			
	150	100	50	0	0	50	100	150
0-2	0.59	0.70	0.62	0.81	0.71	0.67	0.61	0.57
2-4	0.90	0.83	0.81	0.82	0.76	0.80	0.79	0.78
4-6	0.73	0.76	0.51	0.56	0.43	0.51	0.47	0.47
6-8	0.61	0.80	0.76	0.36	0.17	0.33	0.37	0.39
0-8	0.71	0.77	0.68	0.63	0.51	0.58	0.56	0.55

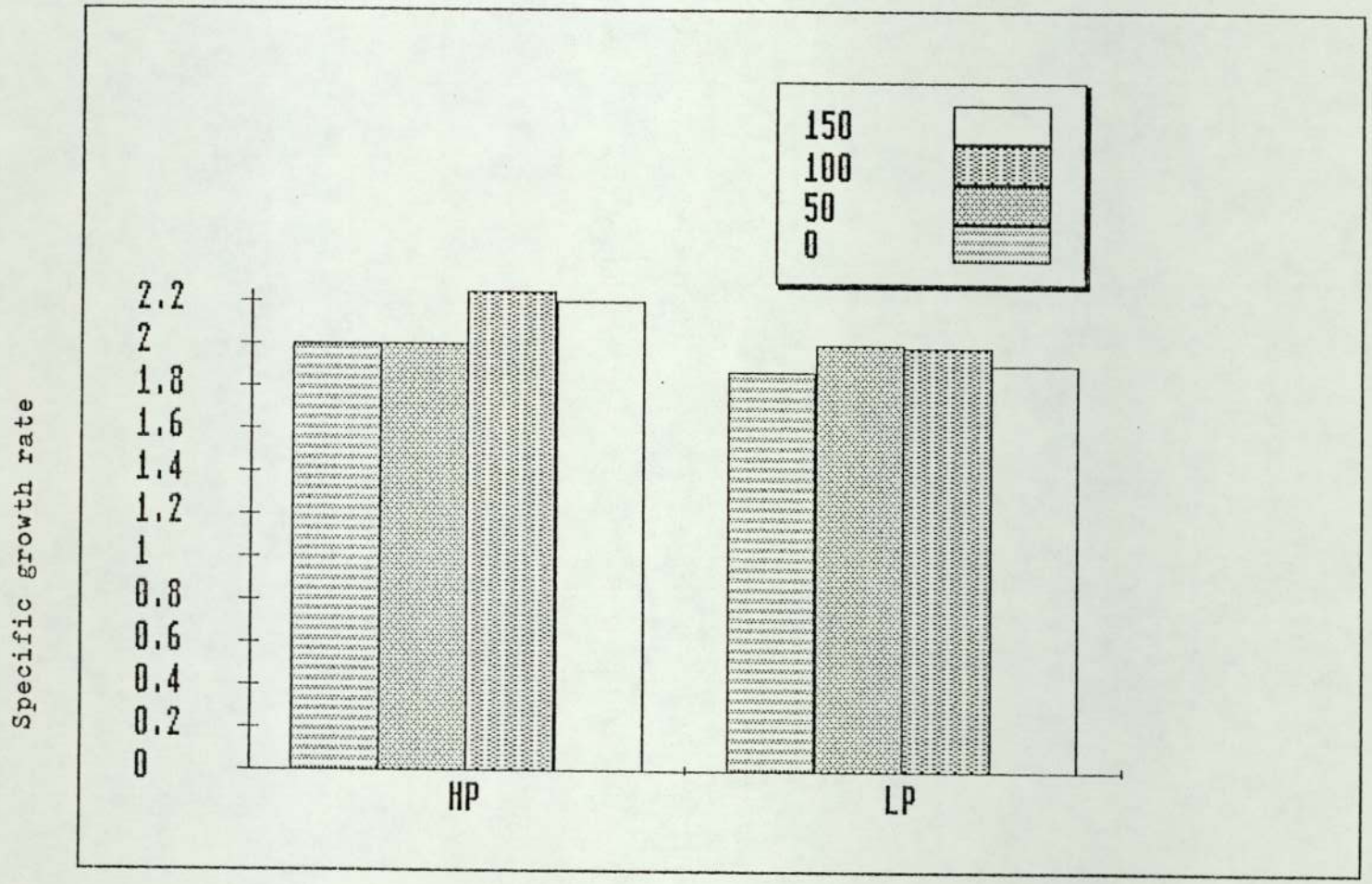


Figure 5.5 Effect of tylosin on the specific growth rate (weight) of carp

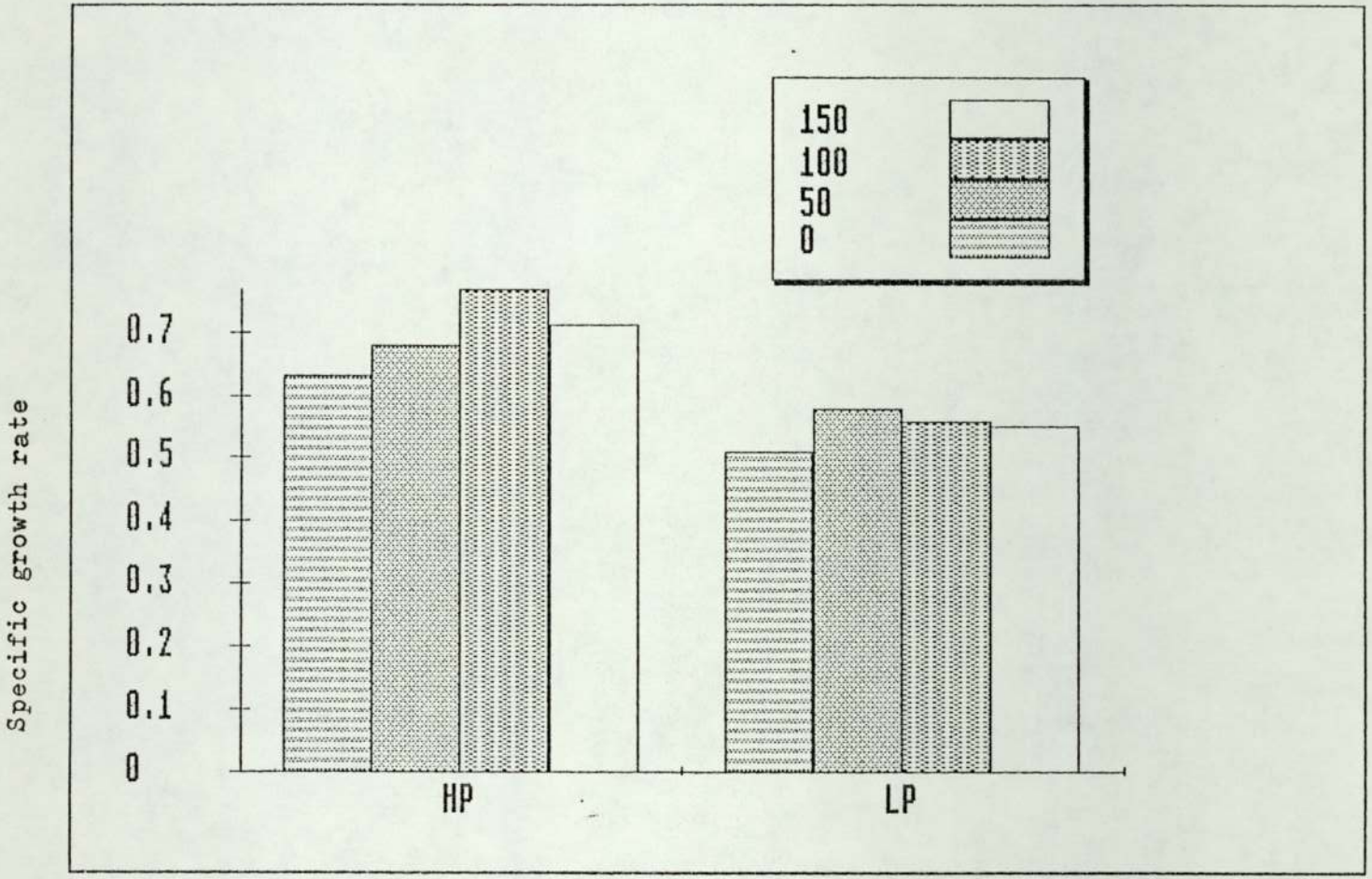


Figure 5.6 Effect of tylosin on the specific growth rate (length) of carp

Table 5.5 : Effect of feeding tylosin on food utilization efficiency of carp.

Period in weeks	Concentration of tylosin (mg/kg food)							
	High protein diet				Low protein diet			
	150	100	50	0	0	50	100	150
0-2	0.65	0.61	0.54	0.74	0.75	0.72	0.67	0.66
2-4	0.64	0.70	0.66	0.73	0.65	0.66	0.61	0.61
4-6	0.65	0.59	0.68	0.48	0.30	0.44	0.43	0.42
6-8	0.80	0.92	0.75	0.56	0.37	0.47	0.60	0.58
0-8	0.69	0.71	0.67	0.63	0.52	0.58	0.58	0.57

factors, the diet protein percentage or the drug. The protein content of the diet apparently increased the fat content of the muscle. However by using the "t" test to compare the means of the two controls the differences were not significant. This contradiction may be due to the significant effect of the interaction between the protein content of the diet and the drug. This interaction increased the fat content of the muscle of the treated fish on the HP diet. Results are seen in table 5.6.

5.1.5 Somatic Indices:

The hepato-somatic indices and the viscero-somatic indices (the ratio of the of the viscera apart from the liver) were measured in fish from each tank. No significant effect of the drug or the protein content was observed (table 5.7).

5.1.6 Digestibility coefficient :

The digestibility coefficient of the HP diet was between 90-91% and between 89-89.7% for the LP diet.

5.1.7 Histological Examination :

The microscopical examination of the intestinal sections of the treated and the control fish showed that there was no apparent effect of either tylosin or the protein content of the diet.

Summary of the performance of carp fed diet containing

Table 5.6 : Proximate analysis of body composition of carp fed diet containing tylosin. Number of samples eight fish ±S.E.

Body composition	Concentration of tylosin (mg/kg food)							
	High protein diet				Low protein diet			
	150	100	50	0	0	50	100	150
Water	78.7 ±0.20	78.2 ±0.31	78.0 ±0.50	79.5 ±0.20	79.4 ±0.23	78.6 ±0.24	77.7 ±1.00	79.2 ±1.10
Protein	72.4 ±1.40	72.6 ±0.70	73.4 ±1.30	74.2 ±1.50	72.8 ±1.50	71.8 ±1.30	74.3 ±1.30	72.0 ±1.50
Fat	19.9 ±1.00	19.8 ±0.80	19.8 ±1.10	15.4 ±1.20	15.8 ±0.48	14.6 ±1.50	14.3 ±1.60	16.8 ±1.70
PER	1.8	1.86	1.77	1.65	2.07	2.27	2.24	2.24
NPR	27.2	30.2	28.6	24.8	31.5	33.2	36.7	33.0

Table 5.7 : Hepato-somatic and viscero-somatic indices of fish fed diet containing tylosin. Number of samples eight fish ±S.E.

Tissue indices	Concentration of tylosin (mg/kg food)							
	High protein diet				Low protein diet			
	150	100	50	0	0	50	100	150
Hepato-somatic indices (HSI)	1.17 ±0.14	1.35 ±0.22	1.08 ±0.08	1.14 ±0.09	1.30 ±0.10	1.03 ±0.10	1.06 ±0.09	0.97 ±0.09
Viscero-somatic indices (VSI)*	16.7 ±1.06	17.3 ±0.42	16.8 ±0.60	17.8 ±0.50	18.0 ±0.99	17.4 ±0.78	17.3 ±0.95	15.7 ±1.05

* All organs apart from liver, intestine and heart.

tylosin over the controls is shown in table 5.8.

5.2 Effect OF Feeding Payzone (Nitrovin) To Carp(Experiment 2):

The non-antibiotic payzone was added to the diet of carp at the concentration recommended by the manufacturer, 25 mg per kg diet. Two other concentrations were also used, 50 and 100 mg per kg. These three dosages were mixed with HP and LP diets and were fed to the carp for ten weeks. Twenty fish were used per treatment.

5.2.1 Acceleration Of Growth :

The fish receiving the HP diet were significantly heavier and longer than those on the LP diet ($P<0.001$). The percentage increases in weight and length of the first group were 314% and 174% while the second group gained 53.7% and 30% respectively. When payzone was added to the HP diet at concentrations of 25, 50 and 100 mg per kg the percentage increases in weight were 397%, 244.1% and 244.4%, and in length were 61.2%, 51.5% and 47.8%. When payzone was added to the LP diet at the above concentrations, the increases in weight were 204%, 114.7% and 117.7%, and in length were 36.4%, 32.8% and 29% respectively. The increase in growth was only significant ($P<0.05$) at the 25 mg dosage with the HP diet. The other two groups showed less increase in weight and length than the control. The 25 mg dosage with the LP diet did not increase significantly over the control; fish on the

Table 5.8 : Percentage increases in body weight and length, specific growth rate for weight and length and feed utilization efficiency of carp fed diet containing tylosin over the controls.

	Concentration of tylosin (mg/kg food)		
	150	100	50
a. HP diet :			
weight	11.2	14.0	-
length	13.2	23.7	5.5
SGR (weight)	10.0	12.5	-
SGR (length)	12.7	22.2	7.9
FUE	9.5	12.7	6.3
b. LP diet :			
weight	2.6	7.1	6.9
length	6.7	10.0	10.0
SGR (weight)	9.9	6.6	6.6
SGR (length)	7.8	9.8	8.9
FUE	9.6	9.3	11.5

other two dosages were lighter but longer than the control. Results are given in tables 5.9 and 5.10 and figures 5.7, 5.8, 5.9 and 5.10.

5.2.2 Specific Growth Rate (Daily Gain):

During the first two weeks all fish including the control on the HP diet had the same values of weight and length DG, though for the rest of the experiment only the group receiving the 25 mg per kg had generally higher values. The group receiving the same concentration on the LP diet had greater overall weight DG than the control . The length DG of the fish fed the LP diet was different as the fish receiving the 50 mg dosage had sometimes higher DG than that of the control. The 25 mg group had higher values during the first six weeks. The results are shown in tables 5.11 and 5.12 and figures 5.11 and 5.12.

5.2.3 Feed Utilization Efficiency (FUE) :

The ratio of the weight gain to the food given was similar in all the groups of the fish fed the HP diet during the first two weeks. Only the group receiving the 25 mg/kg had higher FUE than the control after the second week and until the end of the experiment. The groups receiving the higher dosages had either lower or similar FUE to the control throughout the experiment. The 25 mg group on the LP diet always had a higher FUE value than that of the control but the other two groups had either lower or equal values to the

Table 5.9 : Change in weight of carp *(Cyprinus carpio)* fed on diets containing payzone for a period of ten weeks at 25±1°c. Numbers given are mean of 20 fish ±S.E.

(a) High Protein Diet

Duration in Weeks	Concentration of payzone (mg/kg food)			
	100	50	25	control
0	5.00±0.38	5.08±0.34	5.00±0.35	5.08±0.36
2	6.49±0.53 (29.8%)	6.58±0.48 (29.5%)	6.60±0.47 (32.0%)	6.53±0.54 (28.5%)
4	8.60±0.84 (72.0%)	9.20±0.73 (81.0%)	9.54±0.78 (90.8%)	9.22±0.89 (81.5%)
6	11.80±1.1 (136.0%)	12.30±1.0 (142.1%)	12.89±1.3 (157.8%)	12.10±1.3 (138.2%)
8	14.64±1.4 (192.8%)	15.06±1.2 (196.5%)	17.50±1.6 (250.0%)	16.07±1.8 (216.3%)
10	17.22±1.55 (244.4%)	17.48±1.3 (244.1%)	23.95±1.8 (379.0%)	21.03±2.0 (314.0%)

Table 5.9 (cont'd.)

(b) Low Protein Diet

Duration in Weeks	Concentration of payzone (mg/kg food)			
	100	50	25	control
0	5.35±0.42	5.03±0.32	5.00±0.44	5.47±0.35
2	6.07±0.52 (13.5%)	5.64±0.36 (12.1%)	7.20±0.56 (20.0%)	6.28±0.43 (14.8%)
4	7.66±0.7 (43.2%)	7.00±0.49 (39.2%)	7.70±0.79 (54.0%)	8.00±0.6 (46.2%)
6	9.22±0.9 (72.0%)	8.30±0.63 (66.0%)	9.28±1.0 (85.6%)	9.47±0.7 (73.2%)
8	10.30±1.0 (92.5%)	9.40±0.76 (88.0%)	11.78±1.4 (135.6%)	11.99±0.98 (119.2%)
10	11.65±1.1 (117.7%)	10.80±0.9 (114.7%)	15.20±1.6 (204.0%)	15.00±1.0 (174.2%)

Table 5.10 : Change in length of carp *(Cyprinus carpio)* fed on diets containing payzone for a period of ten weeks at 25±1°c. Numbers given are mean of 20 fish ±S.E.

(a) High Protein Diet

Duration in Weeks	Concentration of payzone (mg/kg food)			
	100	50	25	control
0	6.7±0.77	6.8±0.64	6.7±0.68	6.7±0.73
2	7.3±0.91 (9.0%)	7.4±0.8 (8.8%)	7.3±0.82 (9.0%)	7.3±0.85 (9.0%)
4	8.0±1.52 (19.4%)	8.2±0.95 (20.6%)	8.3±1.17 (23.9%)	8.2±1.08 (22.4%)
6	9.0±1.33 (34.3%)	9.1±1.09 (33.8%)	9.2±1.31 (37.3%)	9.0±1.36 (33.8%)
8	9.6±1.4 (43.3%)	9.8±1.28 (44.1%)	10.0±1.41 (49.2%)	9.7±1.48 (44.8%)
10	9.9±1.5 (47.8%)	10.3±1.35 (51.5%)	10.8±1.59 (61.2%)	10.3±1.64 (53.7%)

Table 5.10 (cont'd.)

(b) Low Protein Diet

Duration in Weeks	Concentration of payzone (mg/kg food)			
	100	50	25	control
0	6.9±0.83	6.7±0.76	6.6±0.94	7.0±0.75
2	7.2±0.93 (4.3%)	7.1±0.79 (6.0%)	7.1±1.0 (7.6%)	7.3±0.79 (4.3%)
4	7.7±1.0 (11.6%)	7.6±0.89 (13.4%)	7.7±1.23 (16.7%)	7.9±0.95 (12.9%)
6	8.2±1.25 (18.8%)	8.1±1.04 (20.9%)	8.3±1.5 (25.8%)	8.4±1.11 (20.0%)
8	8.6±1.43 (24.6%)	8.5±1.18 (26.9%)	8.7±1.7 (31.8%)	8.8±1.18 (25.7%)
10	8.9±1.59 (29.0%)	8.9±1.22 (32.8%)	9.0±1.81 (36.4%)	9.1±1.3 (30.0%)

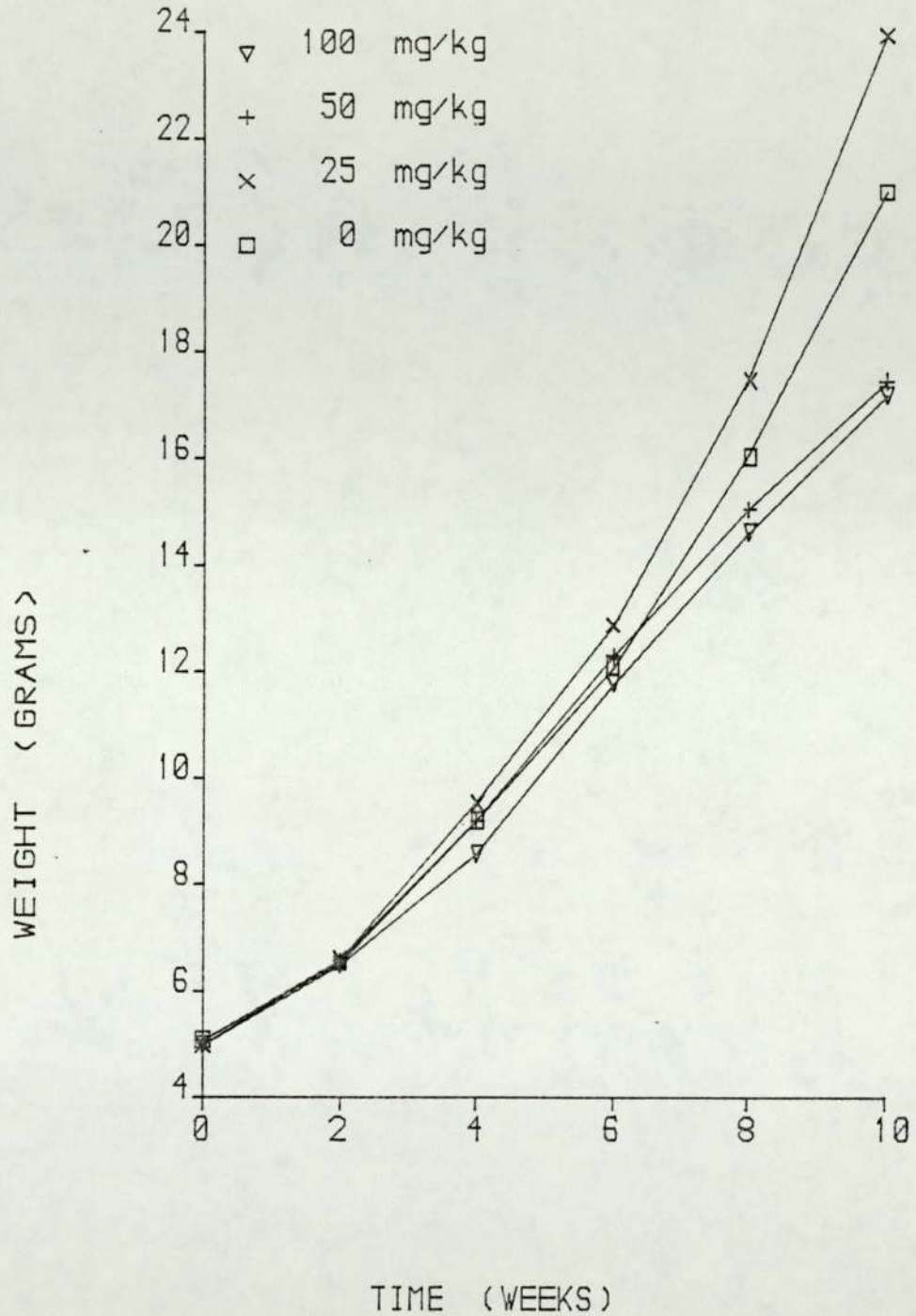


Figure 5.7 Effect of payzone on the weight of carp fed HP diet

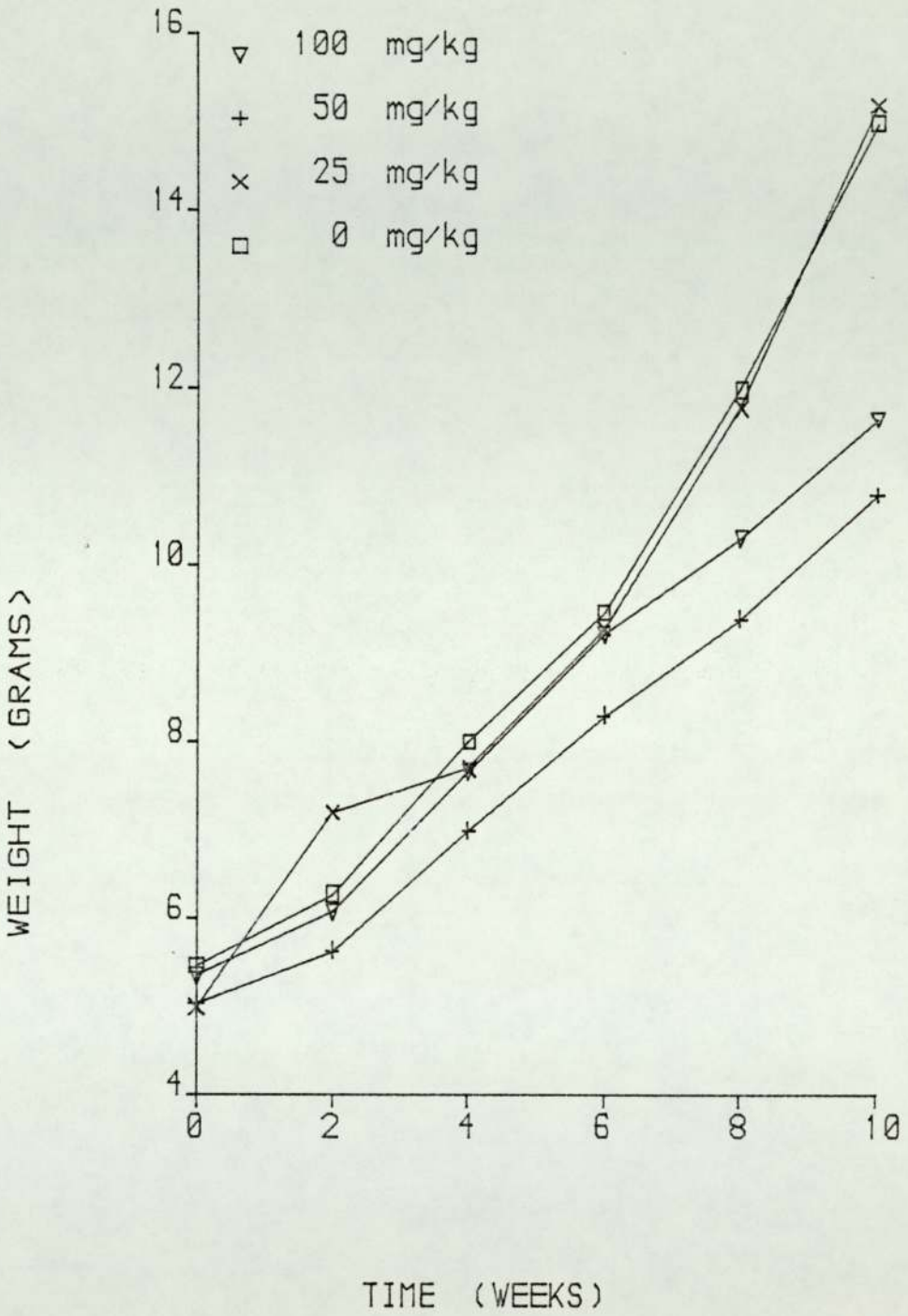


Figure 5.8 Effect of payzone on the weight of carp fed LP diet

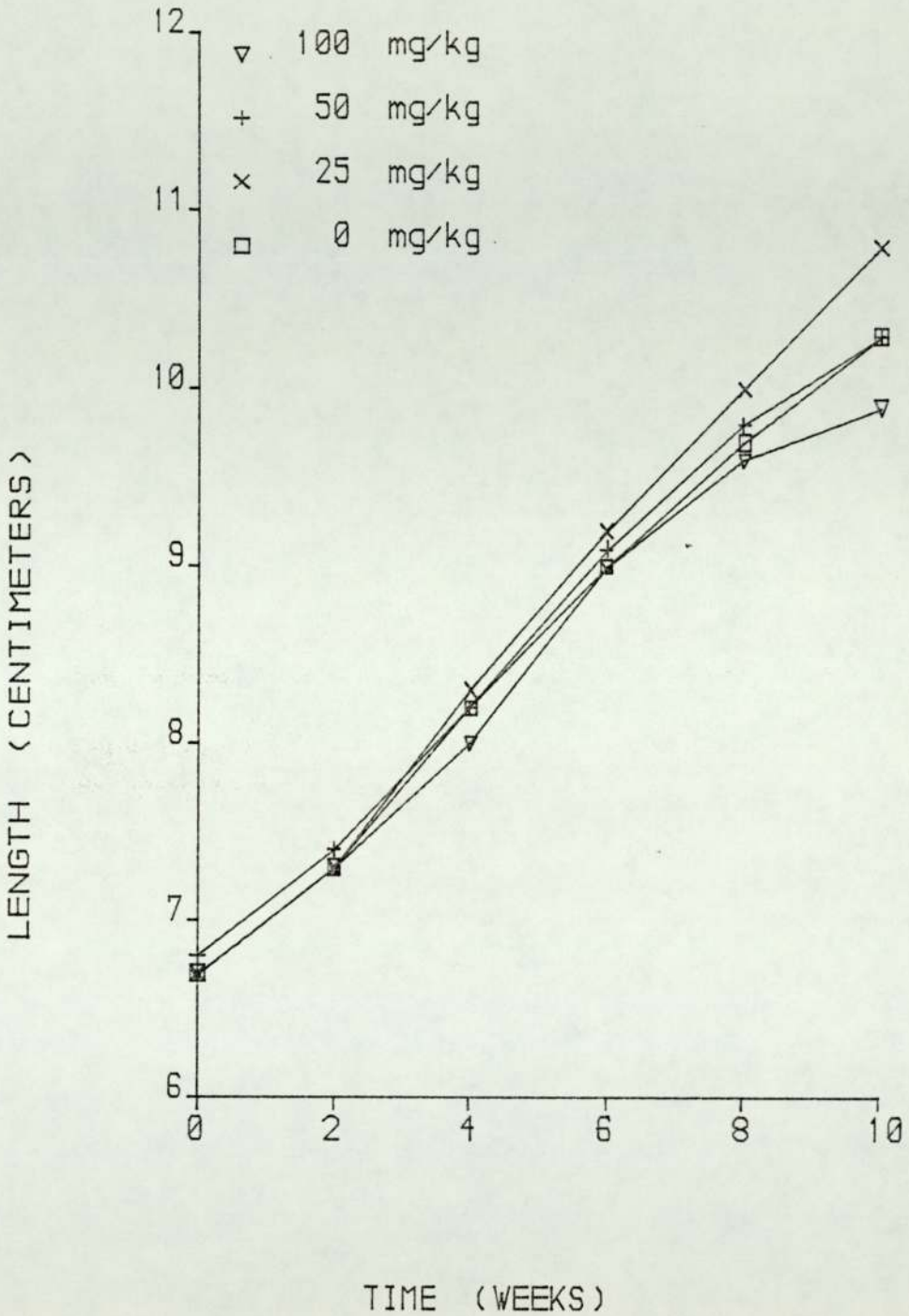


Figure 5.9 Effect of payzone on the length of carp fed HP diet

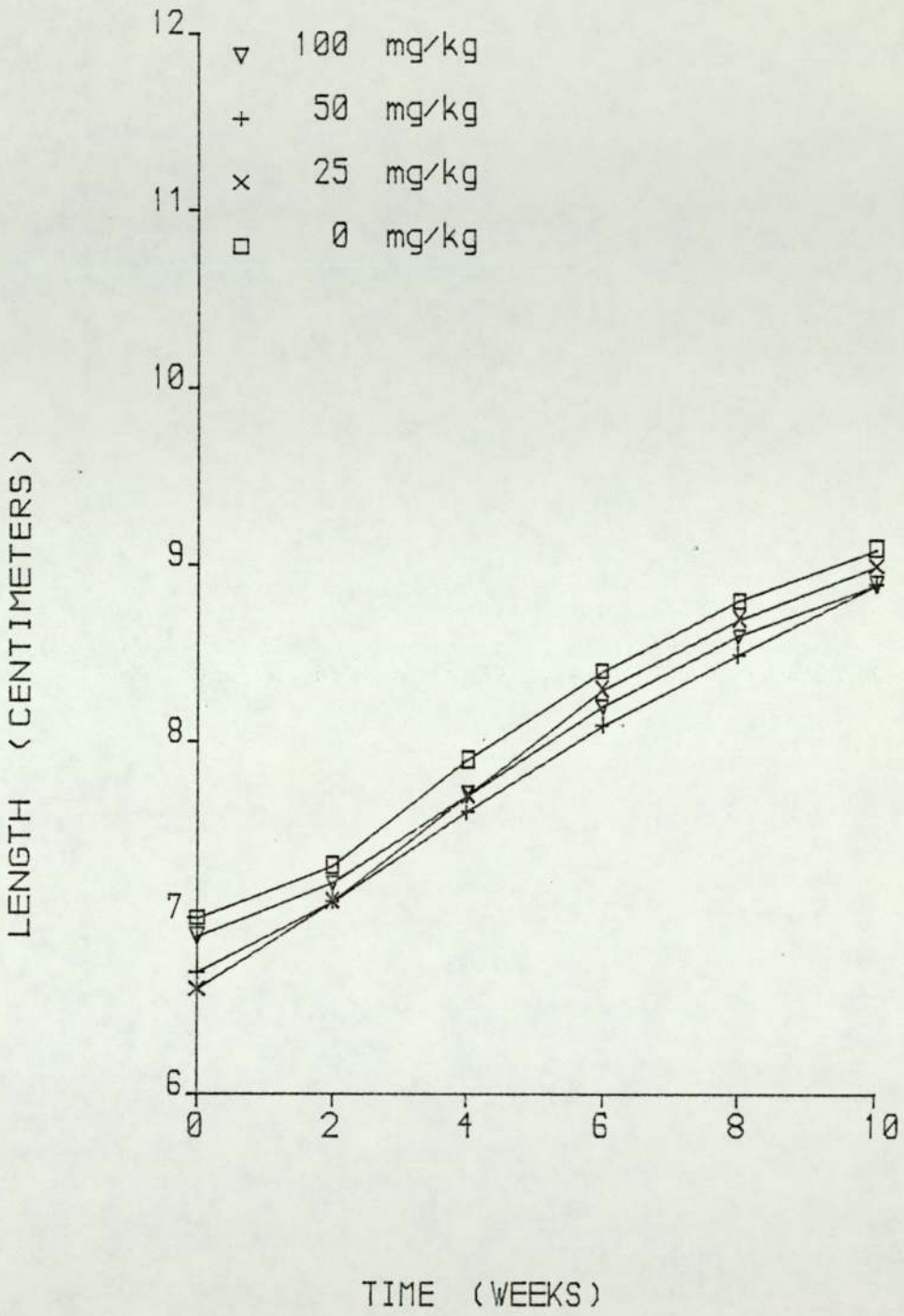


Figure 5.10 Effect of payzone on the length of carp fed LP diet

Table 5.11 : Effect of feeding payzone on the specific growth rate (SGR) of carp (weight).

Period in Weeks	Concentration of payzone (mg/kg food)							
	High protein diet				Low protein diet			
	100	50	25	0	0	25	50	100
0-2	2.0	2.0	2.0	2.0	1.06	1.4	0.88	0.97
2-4	2.2	2.6	2.8	2.7	1.9	1.9	1.7	1.8
4-6	2.4	2.2	2.3	2.1	1.3	1.4	1.3	1.4
6-8	1.7	1.6	2.4	2.4	1.8	1.8	0.96	0.85
8-10	1.3	1.15	2.4	2.07	1.72	1.96	1.07	0.95
0-10	1.91	1.92	2.4	2.2	1.55	1.7	1.18	1.19

Table 5.12 : Effect of feeding payzone on the specific growth rate (SGR) of carp (length).

Period in Weeks	Concentration of payzone (mg/kg food)							
	High protein diet				Low protein diet			
	100	50	25	0	0	25	50	100
0-2	0.66	0.65	0.66	0.66	0.32	0.56	0.45	0.33
2-4	0.7	0.79	0.99	0.89	0.61	0.62	0.52	0.51
4-6	0.91	0.8	0.79	0.72	0.47	0.58	0.49	0.48
6-8	0.5	0.57	0.64	0.58	0.36	0.36	0.37	0.37
8-10	0.24	0.38	0.59	0.46	0.26	0.26	0.35	0.26
0-10	0.6	0.64	0.73	0.66	0.4	0.48	0.44	0.39

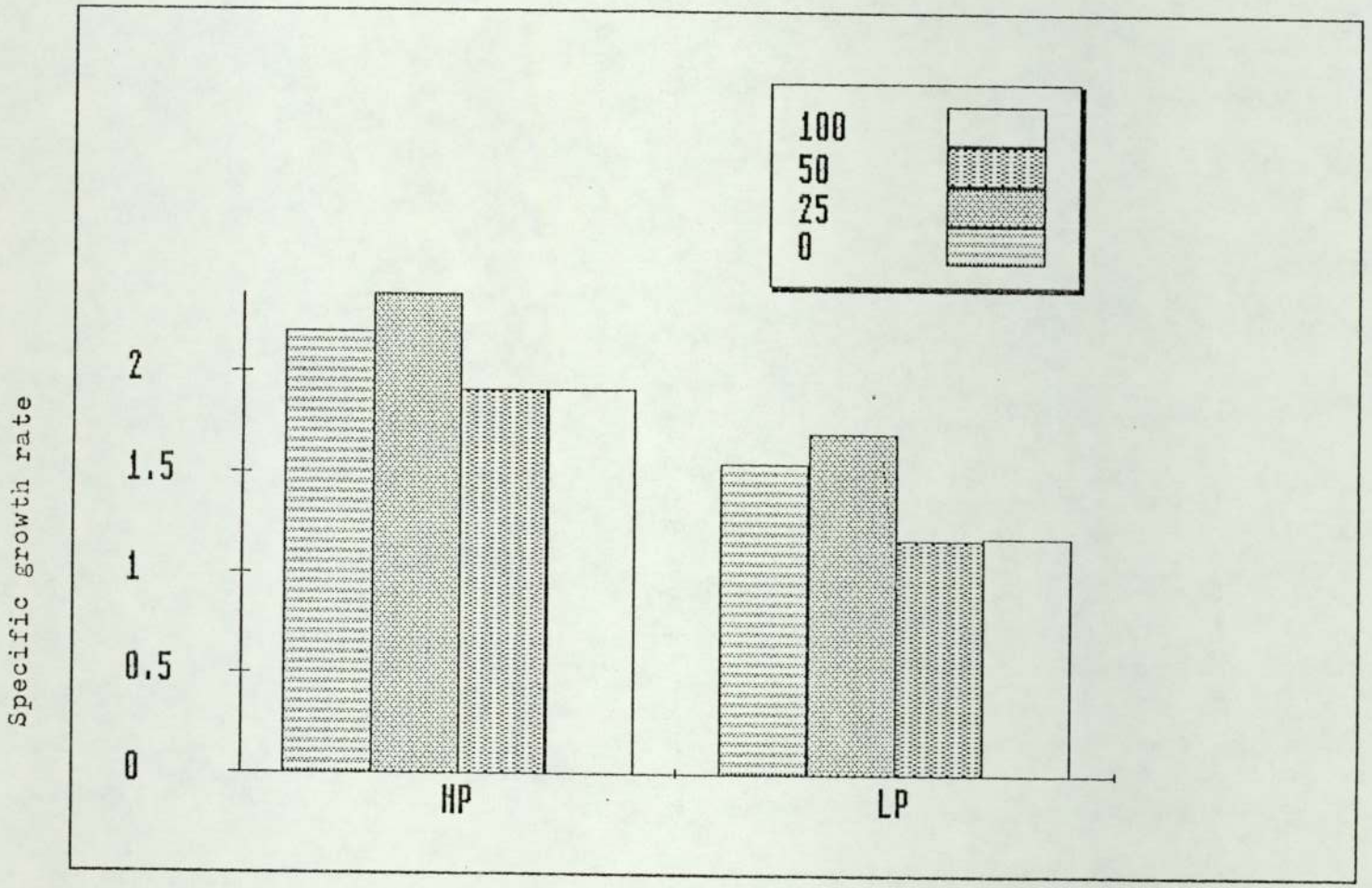


Figure 5.11 Effect of payzone on the specific growth rate (weight) of carp

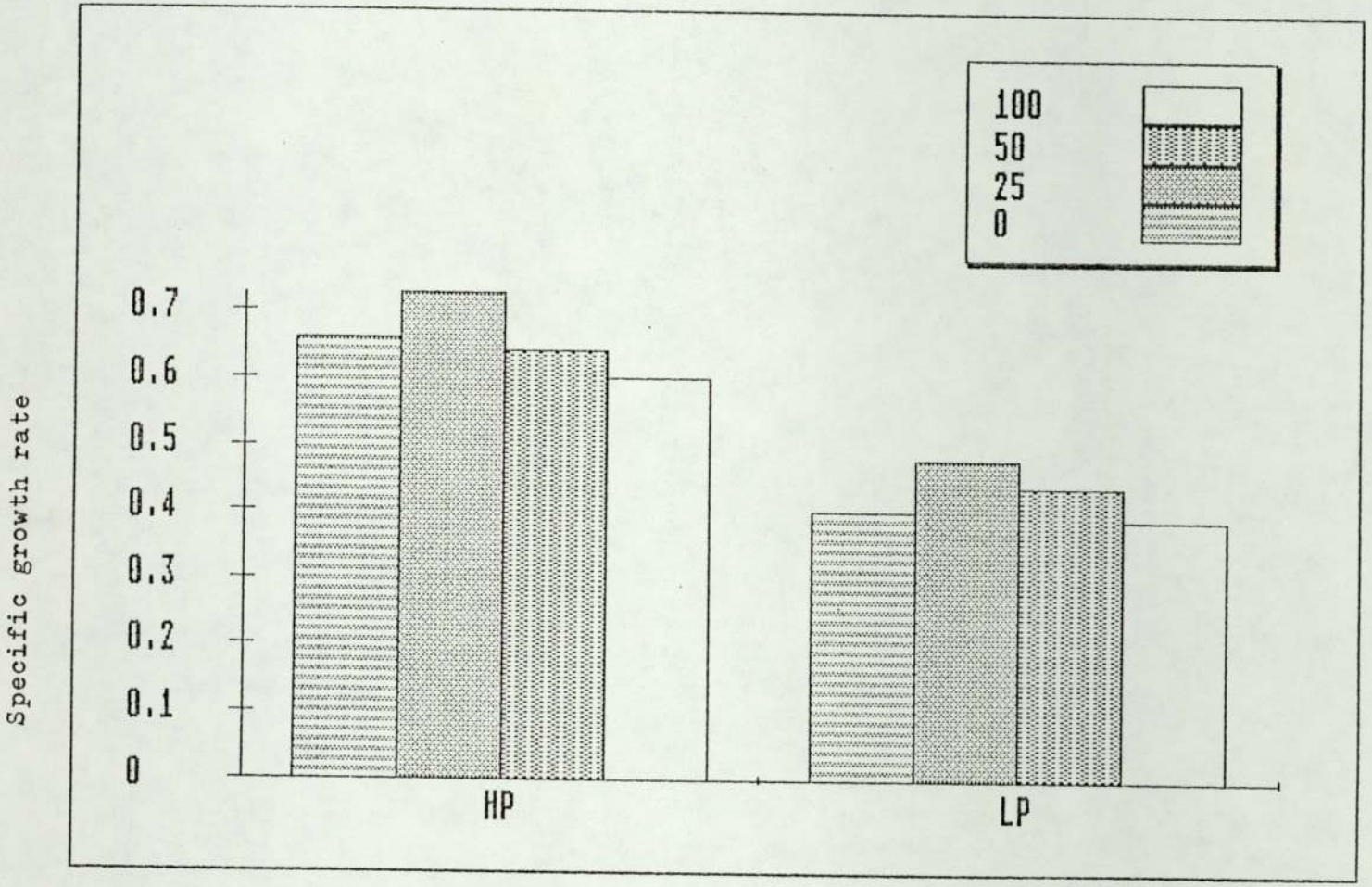


Figure 5.12 Effect of payzone on the specific growth rate (length) of carp

control throughout the experiment. Results are in table 5.13.

5.2.4 Proximate Analysis Of The Body Composition :

The content of the muscle of the fish treated with 25 mg payzone per kg HP diet which showed the best growth rate was compared with the control and the group receiving the identical dosage on the LP diet. The results showed no differences in protein, water and ash content. The payzone-treated fish on the HP diet had significantly more fat in their muscle than the control. The protein of the diet had no effect on any of these parameters. Results are in table 5.14.

5.2.5 Somatic Indices :

The ratio of the weight of the body organs (liver, kidney and intestine) to the fish body weight were calculated. The results showed that the protein in the diet had no effects on these parameters. The HP fed fish the 25 mg payzone had lower HSI than the control. Results are in table 5.15.

5.2.6 Digestibility Coefficient :

The HP diet had values between 90 and 92% and the LP diet 83.5%, irrespective of the drug concentration.

5.2.7 Histological Examination :

The thickness of the intestine of the treated fish was identical to that of the control fish.

Table 5.13 : Effect of feeding payzone on feed utilization efficiency of carp.

Period in weeks	Concentration of payzone (mg/kg food)							
	High protein diet				Low protein diet			
	100	50	25	0	0	25	50	100
0-2	0.46	0.47	0.47	0.47	0.22	0.31	0.19	0.21
2-4	0.5	0.61	0.69	0.63	0.42	0.43	0.38	0.41
4-6	0.57	0.52	0.54	0.48	0.28	0.31	0.28	0.31
6-8	0.62	0.57	0.92	0.92	0.67	0.69	0.34	0.31
8-10	0.45	0.41	0.95	0.77	0.64	0.74	0.38	0.33
0-10	0.52	0.51	0.74	0.65	0.45	0.5	0.31	0.32

Table 5.14 : Proximate analysis of body composition of carp fed diet containing payzone. Number of samples eight fish ±S.E.

Body composition[a]	Concentration of payzone (mg/kg food)			
	High protein diet		Low protein diet	
	25	0	0	25
Water	78.90±0.3	79.8±0.3	79.6±0.3	79.6±0.2
Protein	71.7±1.7	71.8±0.68	71.7±1.2	72.8±0.4
Fat	10.4±1.0	9.1±1.1	9.6±0.56	9.4±0.79
Ash	7.3±0.21	7.2±0.22	7.6±0.19	7.4±0.3
PER[b]	1.94	1.69	1.97	1.79
NPR[c]	28.7	23.8	24.4	28.1

[a] : calculated on dry basis

[b] : protein efficiency ratio

[c] : net protein retention

Table 5.15 : Hepato-somatic, Reno-somatic and viscero-somatic indices of carp fed diet containing payzone. Number of samples eight fish ±S.E.

Tissue indices	Concentration of payzone (mg/kg food)							
	High protein diet				Low protein diet			
	100	50	25	0	0	25	50	100
Hepato-somatic indices (HSI)	1.21 ±0.04	1.22 ±0.02	0.95 ±0.3	1.18 ±0.01	1.19 ±0.02	1.17 ±0.02	1.20 ±0.01	1.21 ±0.01
Reno-somatic indices (RSI)	0.33 ±0.01	0.31 ±0.02	0.29 ±0.02	0.30 ±0.01	0.31 ±0.04	0.30 ±0.01	0.28 ±0.04	0.33 ±0.01
Viscero-somatic indices (VSI)	2.99 ±0.1	3.14 ±0.09	3.18 ±0.08	3.10 ±0.13	3.11 ±0.09	2.97 ±0.14	3.03 ±0.1	3.12 ±0.08

Summary of the performance of carp fed diet containing payzone over the controls is seen in table 5.16.

5.3 Effect Of Feeding Avoparcin To Carp (Experiment 3) :

The antibiotic avoparcin was added to the diet of carp at concentrations recommended by the manufacturer, 10, 20, and 40 ppm per kg, to HP and LP diets for ten weeks. Twenty fish were used for each treatment.

5.3.1 Acceleration Of Growth :

The increase in weight of fish fed the HP diet, 270% was significantly greater than that of the fish fed the LP diet, 157.1% ($P<0.001$). When 10 and 20 ppm avoparcin was added to the HP diet, the increases in weight, 257.9% and 264.3%, were less than those of the control. The increase in weight of the 40 ppm group was 305.5%. None of these differences were significant. The fish fed the supplemented LP diet showed increases of 155.6%, 166% and 174.2% and again these were not significant.

The fish fed the HP diet were longer than the fish fed the LP diet. The percentage increases in length of the first group were 52.4%, 50.6%, 54.9% and 57.1% for the carp receiving avoparcin at dosages of 0, 10, 20 and 40 ppm respectively. The fish fed the LP diet gained 37.3%, 36.7%, 39.4% and 40.3% . The addition of the drug did not improve the length significantly. Results are in tables 5.17 and 5.18 and figures 5.13, 5.14, 5.15 and 5.16.

Table 5.16 : Percentage increases in body weight and length, specific growth rate for weight and length and feed utilization efficiency of carp fed diet containing payzone over the controls.

	Concentration of payzone (mg/kg food)		
	100	50	25
a. HP diet :			
weight	-	-	18.8*
length	-	-	13.9*
SGR (weight)	-	-	9.1*
SGR (length)	-	-	10.6*
FUE	-	-	13.8
b. LP diet :			
weight	-	-	7.0
length	-	4.8	14.3*
SGR (weight)	-	-	9.7
SGR (length)	-	-	20.0
FUE	-	-	11.1

* = ($P<0.05$)

Table 5.17 : Change in weight of carp *(Cyprinus carpio)* fed on diets containing avoparcin for a period of ten weeks at 25±1°c. Numbers given are mean of 20 fish ±S.E.

(a) High Protein Diet

Duration in Weeks	Concentration of avoparcin (mg/kg food)			
	40	20	10	control
0	4.75±0.29	4.90±0.36	4.75±0.34	4.77±0.38
2	7.00±0.44 (47.0%)	7.15±0.43 (45.9%)	6.90±0.48 (45.3%)	6.70±0.57 (40.5%)
4	9.40±0.65 (97.9%)	9.40±0.58 (91.8%)	9.10±0.7 (91.6%)	9.00±0.81 (88.7%)
6	11.70±0.81 (146.3%)	11.40±0.68 (132.7%)	11.10±0.89 (133.7%)	11.00±0.96 (130.6%)
8	15.10±1.0 (217.9%)	14.60±0.88 (198.0%)	14.20±1.1 (198.9%)	14.3±1.2 (199.8%)
10	19.26±1.27 (305.5%)	17.85±1.0 (264.3%)	17.00±1.3 (257.9%)	17.65±1.5 (270.0%)

Table 5.17 (cont'd.)

(b) Low Protein Diet

Duration in Weeks	Concentration of avoparcin (mg/kg food)			
	40	20	10	control
0	4.80±0.25	4.80±0.34	4.80±0.34	4.80±0.30
2	6.30±0.3 (31.3%)	6.30±0.45 (31.3%)	6.12±0.2 (27.5%)	6.20±0.4 (29.2%)
4	7.70±0.41 (60.4%)	7.70±0.6 (60.4%)	7.30±0.55 (52.0%)	7.30±0.53 (52.0%)
6	8.90±0.5 (85.4%)	9.00±0.73 (87.5%)	8.60±0.7 (79.2%)	8.40±0.64 (75.0%)
8	10.80±0.6 (125.0%)	10.80±0.9 (125.0%)	10.40±0.89 (116.7%)	10.20±0.80 (112.5%)
10	13.16±0.8 (174.2%)	12.77±1.03 (166.0%)	12.27±1.07 (155.6%)	12.34±0.93 (157.1%)

Table 5.18 : Change in length of carp *(Cyprinus carpio)* fed on diets containing avoparcin for a period of ten weeks at 25±1°c. Numbers given are mean of 20 fish ±S.E.

(a) High Protein Diet

Duration in Weeks	Concentration of avoparcin (mg/kg food)			
	40	20	10	control
0	6.6±0.13	6.7±0.14	6.7±0.16	6.6±0.17
2	7.6±0.17 (15.0%)	7.6±0.15 (13.4%)	7.4±0.18 (10.4%)	7.4±0.2 (12.0%)
4	8.3±0.19 (25.8%)	8.4±0.18 (25.4%)	8.1±0.23 (20.9%)	8.1±0.23 (22.7%)
6	9.1±0.21 (37.9%)	9.1±0.17 (35.8%)	8.8±0.25 (31.3%)	8.8±0.24 (33.0%)
8	9.8±0.23 (48.5%)	9.8±0.12 (46.3%)	9.6±0.39 (43.3%)	9.5±0.26 (43.9%)
10	10.4±0.23 (57.1%)	10.4±0.18 (54.9%)	10.1±0.3 (50.6%)	10.1±0.24 (52.4%)

Table (5.18 cont'd.)

protein level	Low Protein Diet			
Duration in Weeks	Concentration of avoparcin (mg/kg food)			
	40	20	10	control
0	6.7±0.12	6.6±0.15	6.7±0.15	6.6±0.14
2	7.4±0.14 (10.4%)	7.3±0.18 (10.6%)	7.3±0.17 (9.0%)	7.3±0.17 (10.6%)
4	7.9±0.14 (17.9%)	7.9±0.2 (19.7%)	7.7±0.18 (14.9%)	7.7±0.2 (16.7%)
6	8.4±0.17 (25.4%)	8.3±0.21 (25.8%)	8.2±0.22 (22.4%)	8.1±0.21 (22.7%)
8	8.9±0.24 (32.8%)	8.9±0.25 (34.8%)	8.8±0.27 (31.3%)	8.6±0.23 (30.0%)
10	9.4±0.21 (40.3%)	9.2±0.25 (39.4%)	9.2±0.25 (36.7%)	9.1±0.24 (37.3%)

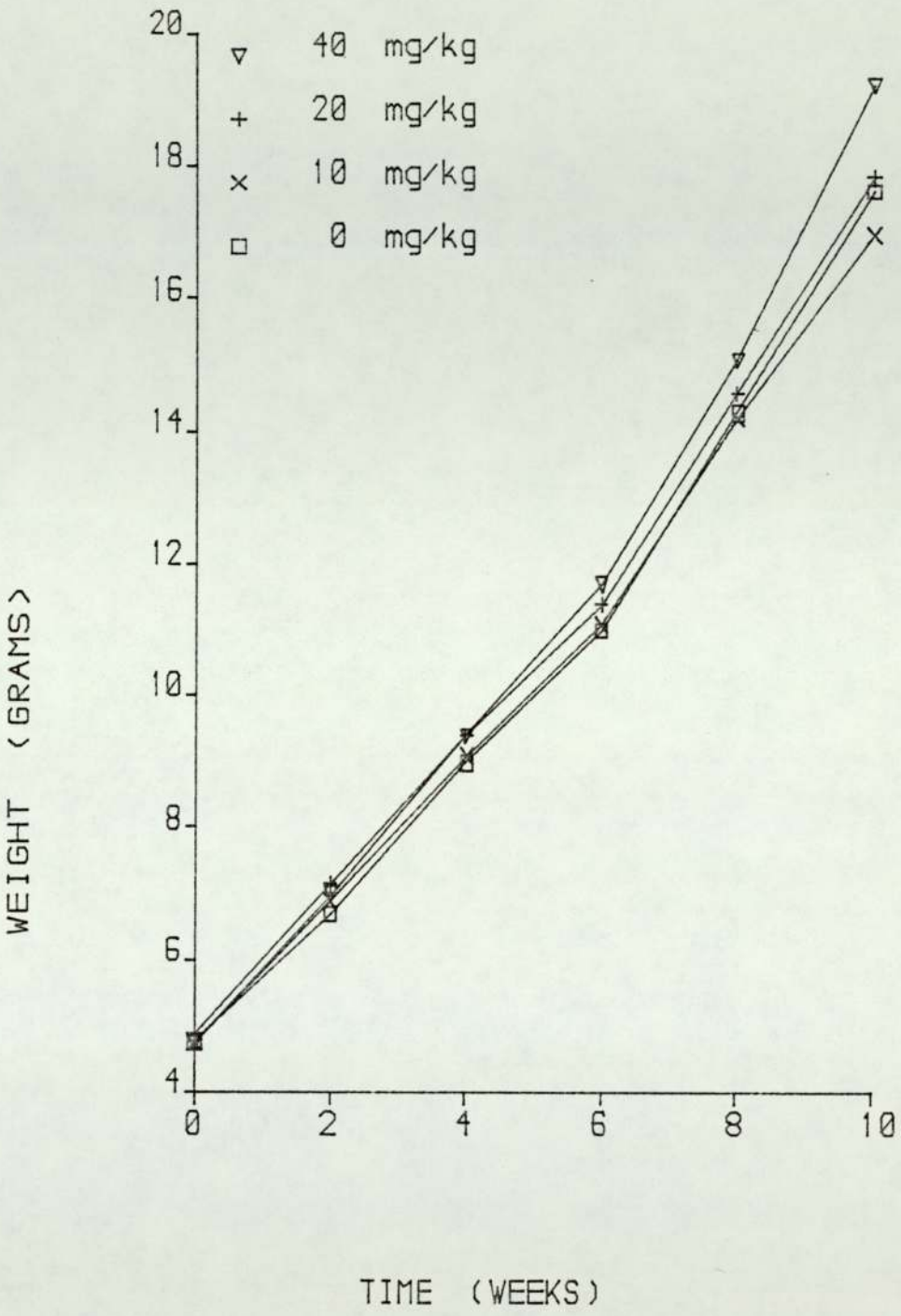


Figure 5.13 Effect of avoparcin on the weight of carp fed HP diet

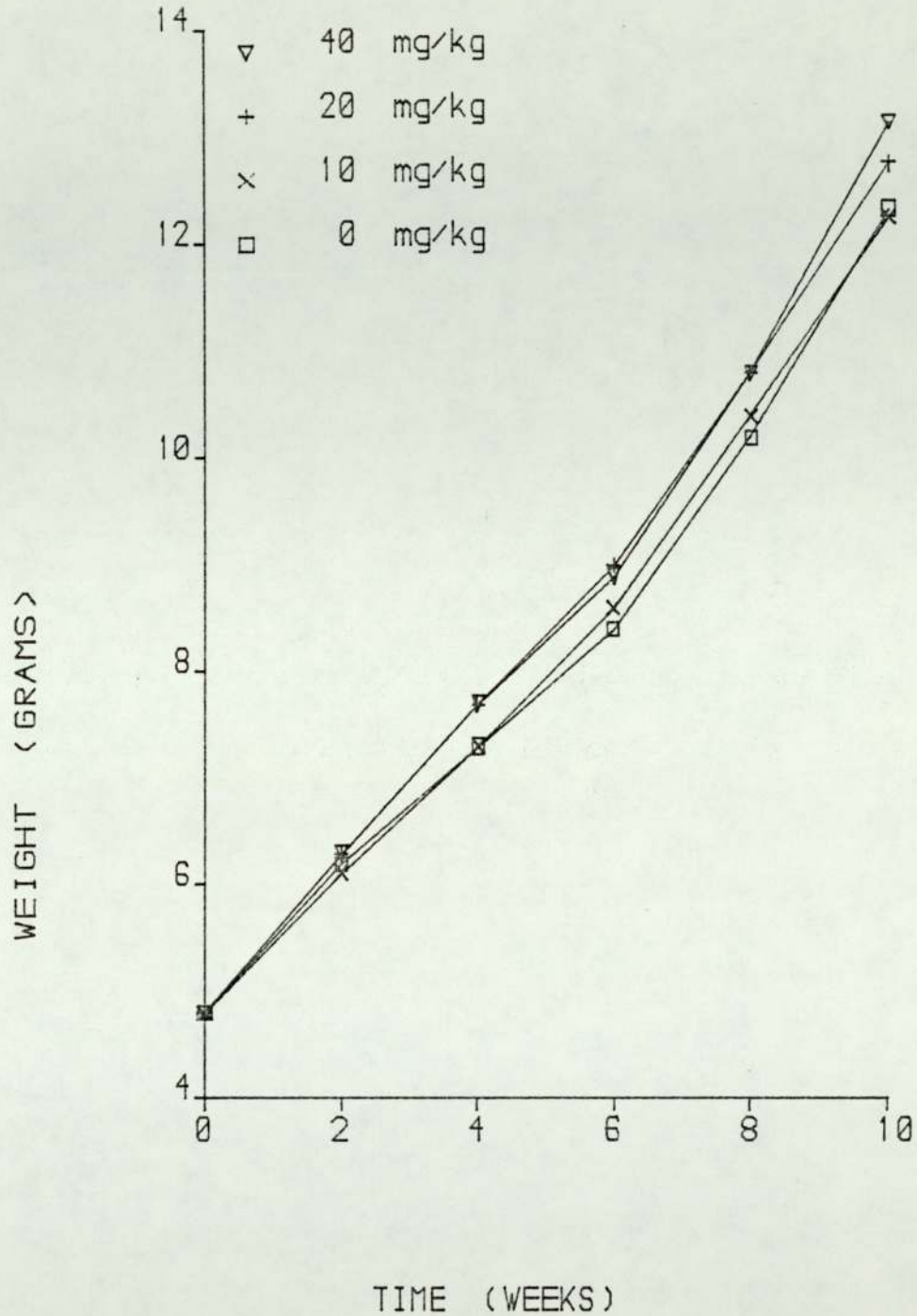


Figure 5.14 Effect of avoparcin on the weight of carp fed LP diet

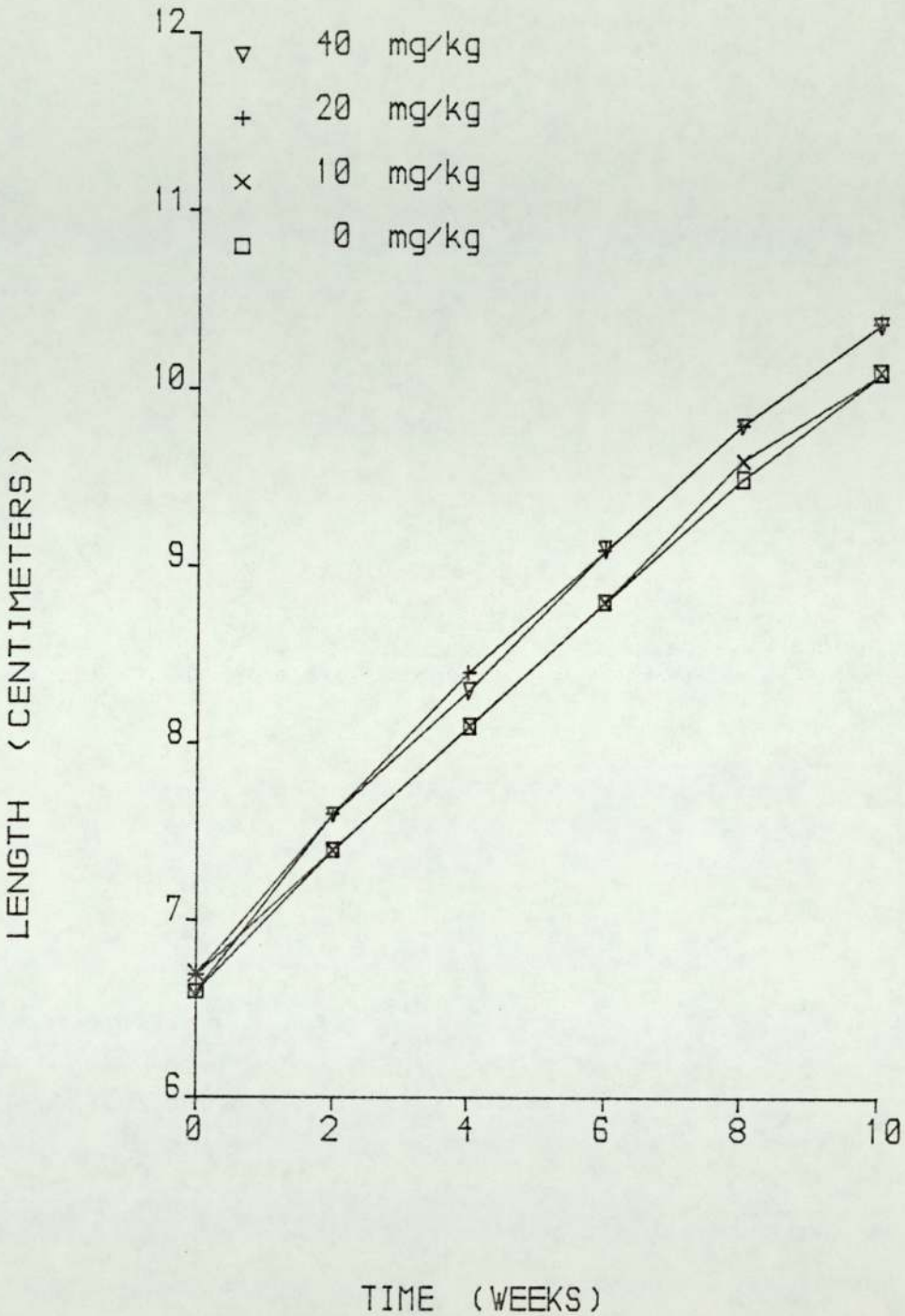


Figure 5.15 Effect of avoparcin on the length of carp fed HP diet

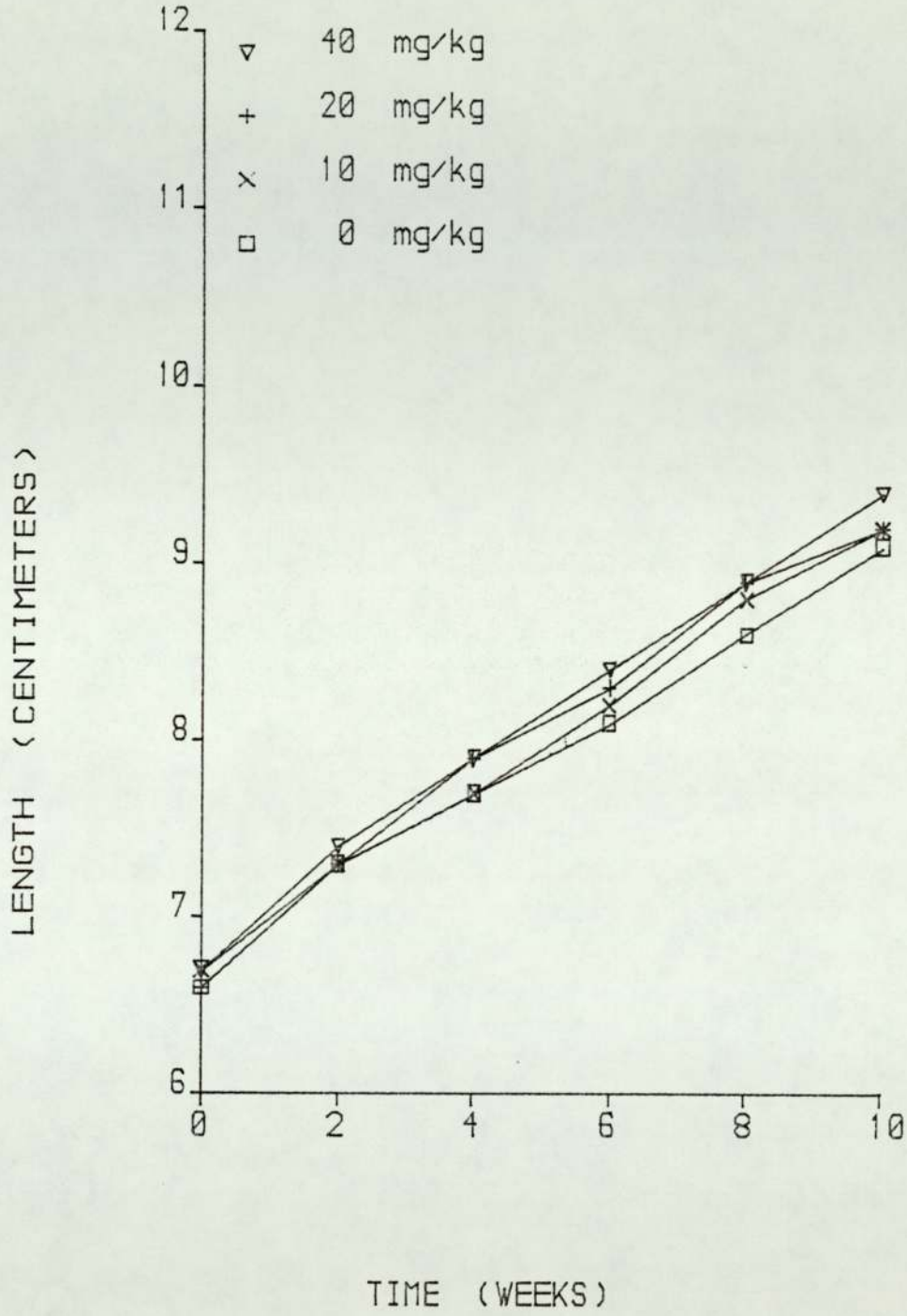


Figure 5.16 Effect of avoparcin on the length of carp fed LP diet

5.3.2 Specific Growth Rate (Daily Gain) :

The daily gain in weight of all the groups of fish was highest during the first two weeks of the experiment, and then decreased towards the end of the experiment. The DG of the fish was significantly affected by the protein content of the diet. The addition of avoparcin to the HP and LP diets apparently improved the DG in weight; however the "t" test between means was not significant (P>0.05). These results are shown in tables 5.19 and 5.20 and figures 5.17 and 5.18.

5.3.3 Feed Utilization Efficiencey (FUE) :

During the first two weeks of the experiment the FUE values were high in all HP groups and the treated fish had higher values than those of the controls. These values declined during the second period (2nd-4th week), then rose towards the end of the experiment with higher values for the controls except for the 40 ppm group. The overall FUE of the fish fed the HP diet was higher than that of the fish fed the LP diet. Adding avoparcin to these diets only improved the FUE of the carp receiving the 40 ppm dosage. Results are in table 5.21.

5.3.4 Proximate Analysis Of The Body Composition :

The dried muscle of all groups of fish was analysed for water, protein, fat and ash. Neither the protein in the diet nor avoparcin had any significant effect on changing any

Table 5.19 : Effect of feeding avoparcin on the specific growth rate (SGR) of carp (weight).

Period in Weeks	Concentration of avoparcin (mg/kg food)							
	High protein diet				Low protein diet			
	40	20	10	0	0	10	20	40
0-2	3.0	2.9	2.9	2.6	2.0	1.9	2.1	2.1
2-4	2.3	2.1	2.1	2.3	1.2	1.4	1.5	1.5
4-6	1.7	1.5	1.5	1.5	1.1	1.3	1.2	1.1
6-8	2.0	1.9	1.9	2.0	1.5	1.5	1.4	1.5
8-10	1.9	1.5	1.4	1.6	1.46	1.3	1.3	1.52
0-10	2.2	1.97	1.96	2.0	1.45	1.48	1.5	1.54

Table 5.20 : Effect of feeding avoparcin on the specific growth rate (SGR) of carp (length).

Period in Weeks	Concentration of avoparcin (mg/kg food)							
	High protein diet				Low protein diet			
	40	20	10	0	0	10	20	40
0-2	1.09	0.97	0.76	0.88	0.77	0.66	0.77	0.76
2-4	0.68	0.77	0.70	0.70	0.41	0.41	0.61	0.51
4-6	0.71	0.62	0.64	0.64	0.39	0.48	0.38	0.47
6-8	0.57	0.57	0.67	0.59	0.46	0.54	0.53	0.44
8-10	0.43	0.44	0.38	0.44	0.40	0.31	0.26	0.26
0-10	0.70	0.67	0.63	0.65	0.49	0.48	0.51	0.49

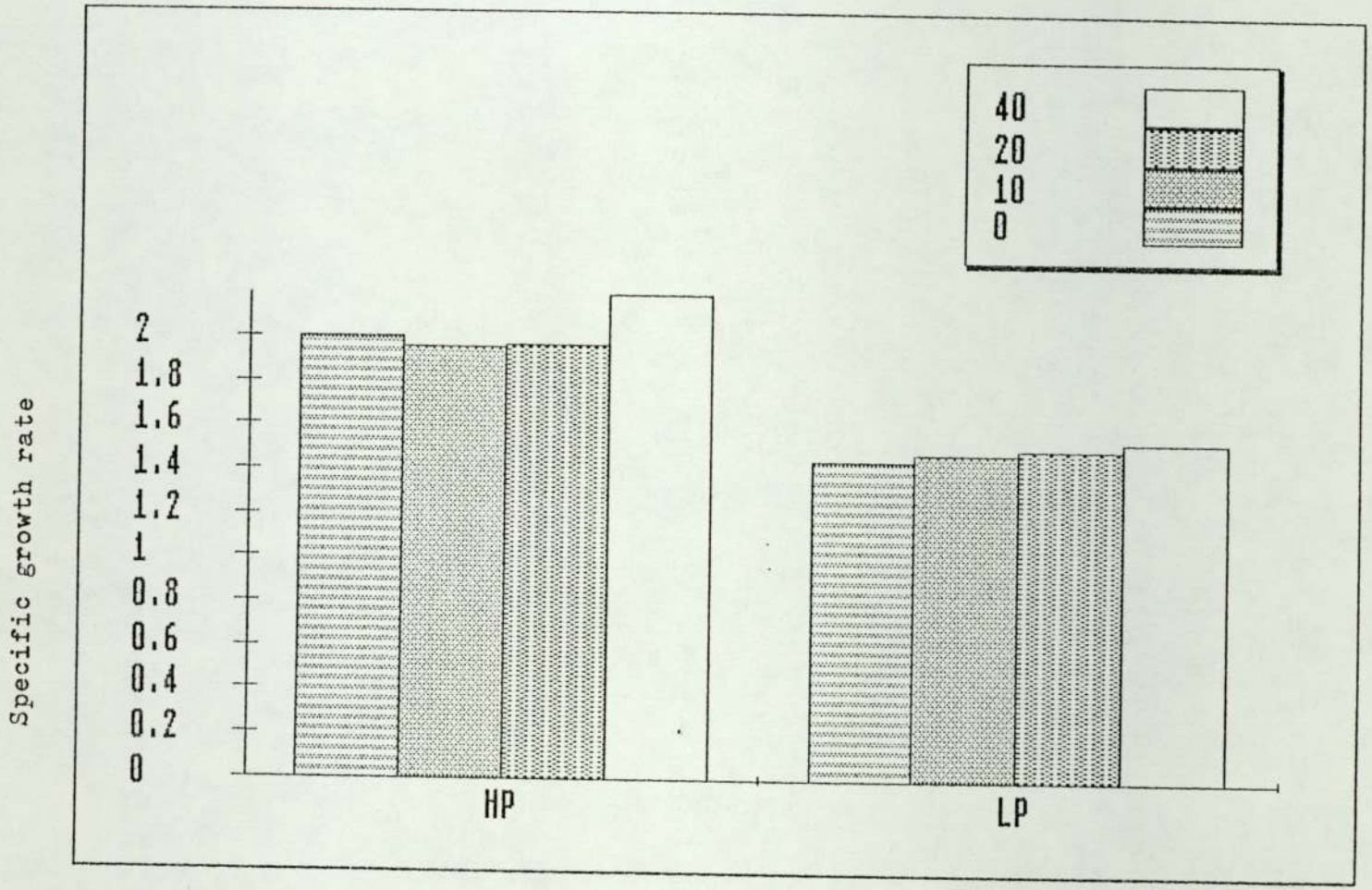


Figure 5.17 Effect of avoparcin on the specific growth rate (weight) of carp

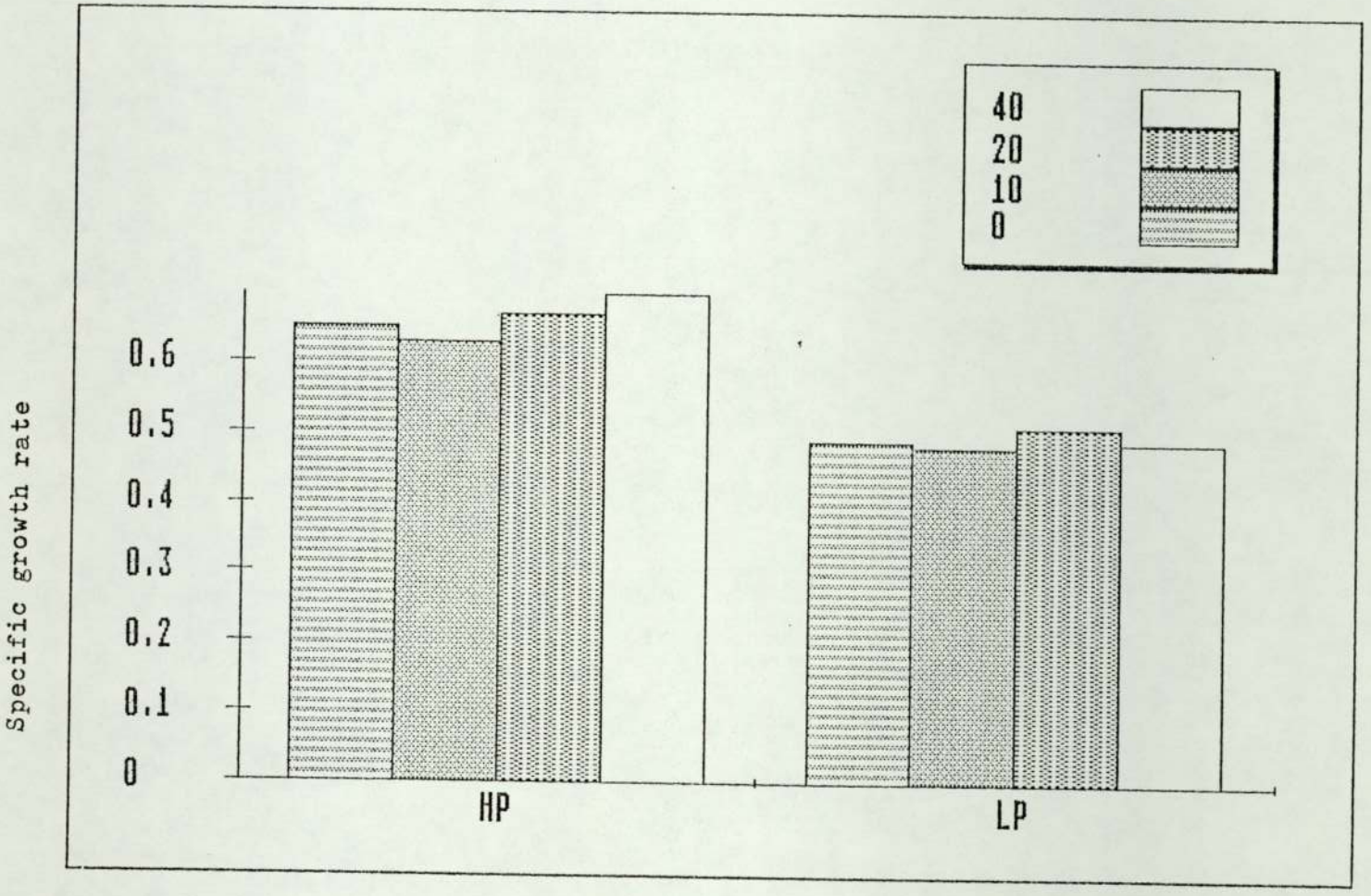


Figure 5.18 Effect of avoparcin on the specific growth rate (length) of carp

Table 5.21 : Effect of feeding avoparcin on feed utilization efficiency of carp.

Period in weeks	Concentration of avoparcin (mg/kg food)							
	High protein diet				Low protein diet			
	40	20	10	0	0	10	20	40
0-2	0.72	0.69	0.69	0.61	0.45	0.41	0.48	0.48
2-4	0.53	0.51	0.49	0.53	0.27	0.31	0.34	0.34
4-6	0.62	0.55	0.56	0.57	0.38	0.45	0.40	0.40
6-8	0.75	0.72	0.71	0.77	0.55	0.54	0.51	0.54
8-10	0.85	0.71	0.62	0.72	0.64	0.55	0.59	0.67
0-10	0.69	0.64	0.61	0.64	0.45	0.45	0.46	0.49

of these constituents. Results are in table 5.22.

5.3.5 Somatic Indices :

The ratio of the weight of the liver, kidney and intestine to the body weight was calculated from samples of all groups. These ratios were similar in the treated and the controls irrespective of either of the two factors. Results are in table 5.23.

5.3.6 Digestibility Coefficient :

The digestibility coefficient of the HP diet was between 90-93% and of the LP was between 89-90% irrespective of the drug addition.

5.3.7 Histological Examination :

No apparent effect on the thickness of the intestine was noticed as a result of feeding avoparcin.

Summary of the performance of carp fed avoparcin over the controls is seen in table 5.24.

5.4 Effect of Feeding Terramycin To Carp (Experiment 4) :

The antibiotic terramycin was supplemented to HP and LP diets of juvenile carp at concentrations of 10, 50, and 100 mg per kg diet for ten weeks. The number of fish was 16 for each treatment.

5.4.1 Acceleration Of Growth :

The protein concentration of the diet had a significant effect on the weight gain of the fish ($P<0.001$). The fish

Table 5.22 : Proximate analysis of body composition of carp fed diet containing avoparcin. Number of samples eight fish ±S.E.

Body composition[a]	Concentration of avoparcin (mg/kg food)							
	High protein diet				Low protein diet			
	40	20	10	0	0	10	20	40
Water	78.8 ±0.37	79.3 ±0.20	78.9 ±0.20	79.5 ±0.35	79.4 ±0.30	78.7 ±0.17	79.1 ±0.50	78.9 ±0.30
Protein	68.4 ±1.80	70.4 ±1.00	69.0 ±0.50	71.6 ±1.50	70.5 ±0.94	71.6 ±0.38	69.5 ±1.50	70.3 ±0.77
Fat	14.2 ±1.10	13.5 ±0.40	15.0 ±1.00	13.0 ±1.50	13.8 ±1.40	14.3 ±0.50	13.8 ±1.90	14.2 ±0.90
Ash	7.3 ±0.30	7.7 ±0.20	6.9 ±0.23	7.2 ±0.40	7.1 ±0.09	7.6 ±0.08	7.8 ±0.30	7.1 ±0.20
PER[b]	1.80	1.67	1.60	1.69	1.79	1.80	1.84	1.90
NPR[c]	25.1	23.7	23.4	24.4	24.7	26.8	25.4	27.6

[a] : calculated on dry basis

[b] : protein efficiency ratio

[c] : net protein retention

Table 5.23 : Hepato-somatic, Reno-somatic and viscero-somatic indices of carp fed diet containing avoparcin. Number of samples eight fish ±S.E.

Tissue indices	Concentration of avoparcin (mg/kg food)							
	High protein diet				Low protein diet			
	40	20	10	0	0	10	20	40
Hepato-somatic indices (HSI)	1.95 ±0.90	1.89 ±0.70	2.0 ±1.1	1.99 ±0.83	1.91 ±0.67	1.85 ±0.13	1.97 ±0.90	2.0 ±1.2
Reno-somatic indices (RSI)	0.35 ±0.03	0.32 ±0.05	0.36 ±0.02	0.33 ±0.30	0.29 ±0.01	0.31 ±0.02	0.33 ±0.02	0.34 ±0.06
Viscero-somatic indices (VSI)	2.39 ±1.0	2.44 ±1.3	2.8 ±0.4	2.25 ±0.7	2.13 ±1.3	2.2 ±0.3	2.1 ±0.7	2.3 ±0.1

Table 5.24 : Percentage increases in body weight and length, specific growth rate for weight and length and feed utilization efficiency of carp fed diet containing avoparcin over the controls.

	Concentration of avoparcin (mg/kg food)		
	10	20	40
a. HP diet :			
weight	-	-	12.7
length	-	-	8.60
SGR (weight)	-	-	10.0
SGR (length)	-	-	7.70
FUE	-	-	7.80
b. LP diet :			
weight	-	-	10.9
length	-	-	8.0
SGR (weight)	-	-	6.20
SGR (length)	-	-	
FUE	-	-	8.90

fed the HP diet gained more than the LP groups irrespective of the drug concentration. Terramycin also had a significant effect on increasing the weight of the treated fish ($P<0.001$). The carp receiving 0, 10, 50 and 100 mg terramycin per kg HP diet gained 89.9%, 124%, 139.5% and 122%, Carp fed the LP diet gained 58.9%, 73.9%, 74.3% and 86% respectively. Weight gain of all HP-fed groups was significantly higher than the control. The fish fed the HP diet were also longer than the fish fed the LP diet. When terramycin was added at the above dosages to the HP diet the gains in length were 22.7%, 30.9%, 33.6% and 29% respectively. The "t" test showed that the differences between the treated fish and the control were significant in all the groups ($P<0.001$). The fish fed the LP diet gained in length 18.2%, 20%, 22% and 24.8% respectively. The differences were significant only in the LP group receiving the 100 mg dosage ($P<0.05$). Results are shown in tables 5.25 and 5.26 and figures 5.19 and 5.20, 5.21 and 5.22.

5.4.2 Specific Growth Rate (Daily Gain) :

The calculation of the daily gain in weight of the fish fed the HP supplemented diet showed much the same pattern as the increase in the absolute weight. All showed significant increases compared to the control. The fish fed the LP diet also had significantly greater daily gains in weight. The DG in length showed a similar pattern to the gain in total

Table 5.25 : Change in weight of carp *(Cyprinus carpio)* fed on diets containing terramycin for a period of ten weeks at 25±1°c. Numbers given are mean of 16 fish ±S.E.

(a) High Protein Diet

Duration in Weeks	Concentration of terramycin (mg/kg food)			
	100	50	10	control
0	20.43±0.72	20.38±0.69	20.28±0.64	20.38±0.65
2	23.52±0.81 (15.0%)	25.05±0.72 (22.9%)	24.69±0.65 (21.3%)	24.26±0.83 (19.0%)
4	26.56±0.97 (30.0%)	29.04±0.83 (42.5%)	27.71±0.71 (36.2%)	26.53±0.95 (30.2%)
6	31.09±1.2 (52.2%)	33.24±0.82 (63.1%)	31.53±0.78 (54.9%)	29.76±1.1 (46.0%)
8	37.66±1.7 (84.0%)	39.96±1.16 (96.0%)	37.73±0.97 (85.0%)	33.9±1.4 (66.0%)
10	45.38±2.1 (122.0%)	48.81±1.7 (139.5%)	45.59±1.3 (124.0%)	38.7±1.6 (89.9%)

Table (5.25 cont'd.)

(b) Low Protein Diet

Duration in Weeks	Concentration of terramycin (mg/kg food)			
	100	50	10	control
0	20.36±0.63	20.36±0.69	20.32±0.63	20.36±0.67
2	23.22±0.66 (14.9%)	22.73±0.82 (11.6%)	22.30±0.65 (9.70%)	21.96±0.69 (7.9%)
4	25.67±0.84 (26.0%)	24.74±0.96 (21.5%)	24.26±0.73 (19.4%)	23.90±0.74 (17.4%)
6	28.13±0.9 (38.2%)	26.67±1.0 (31.0%)	26.30±0.85 (29.4%)	25.64±0.8 (25.9%)
8	32.10±1.10 (57.7%)	30.10±1.20 (47.8%)	29.83±1.08 (46.8%)	28.74±0.97 (41.2%)
10	37.86±1.2 (86.0%)	35.49±1.5 (74.3%)	35.34±1.7 (73.9%)	32.35±1.2 (58.9%)

Table 5.26 : Change in length of carp *(Cyprinus carpio)* fed on diets containing terramycin for a period of ten weeks at 25±1°c. Numbers given are mean of 16 fish ±S.E.

(a) High Protein Diet

Duration in Weeks	Concentration of terramycin (mg/kg food)			
	100	50	10	control
0	10.9±0.55	10.9±0.56	11.0±0.49	11.0±0.53
2	11.4±0.59 (4.3%)	11.8±0.53 (8.0%)	11.8±0.48 (7.3%)	11.6±0.54 (5.5%)
4	11.9±0.59 (8.9%)	12.3±0.59 (12.8%)	12.2±0.42 (11.1%)	12.0±0.54 (9.0%)
6	12.3±0.64 (12.8%)	13.0±0.51 (18.9%)	12.8±0.46 (16.2%)	12.5±0.65 (13.6%)
8	13.4±0.74 (22.6%)	13.7±1.02 (25.0%)	13.6±0.5 (23.5%)	13.0±0.72 (18.2%)
10	14.1±0.91 (29.0%)	14.6±0.73 (33.7%)	14.4±0.63 (30.9%)	13.5±0.71 (22.7%)

Table 5.26 (cont'd.)

(b) Low Protein Diet

Duration in Weeks	Concentration of terramycin (mg/kg food)			
	100	50	10	control
0	10.9±0.53	10.9±0.49	11.0±0.52	11.0±0.50
2	11.4±0.58 (4.8%)	11.5±0.52 (5.0%)	11.4±0.52 (3.9%)	11.4±0.51 (4.0%)
4	11.8±0.69 (8.3%)	11.8±0.66 (8.0%)	11.7±0.53 (6.5%)	11.8±0.46 (6.8%)
6	12.1±0.64 (12.8%)	12.3±0.61 (12.8%)	12.2±0.64 (10.9%)	12.1±0.54 (10.0%)
8	13.0±0.65 (19.3%)	12.7±0.77 (16.3%)	12.7±0.70 (15.4%)	12.6±0.63 (14.5%)
10	13.6±0.72 (24.8%)	13.3±0.88 (22.0%)	13.2±0.71 (20.0%)	13.0±1.1 (18.2%)

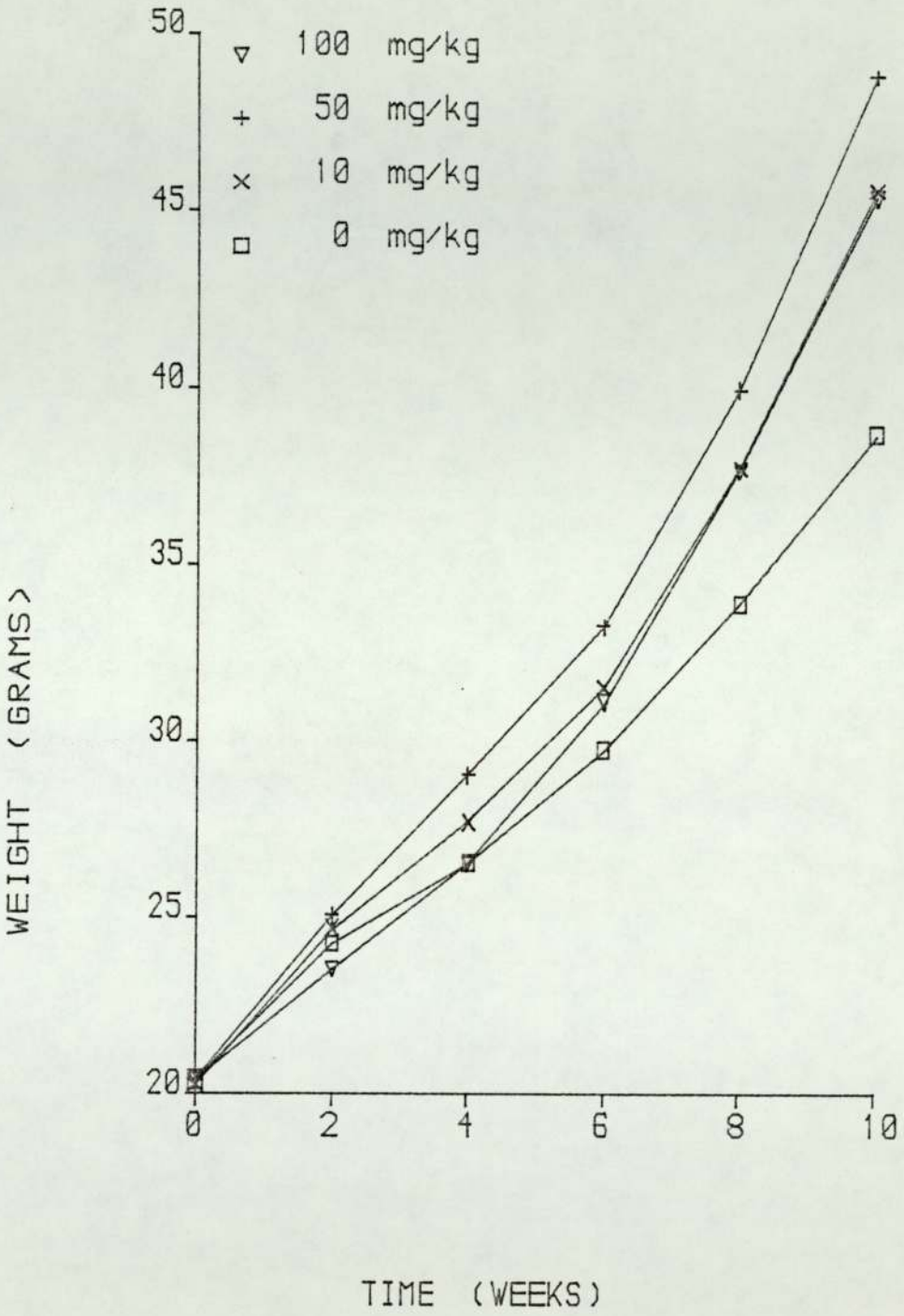


Figure 5.19 Effect of terramycin on the weight of carp fed HP diet

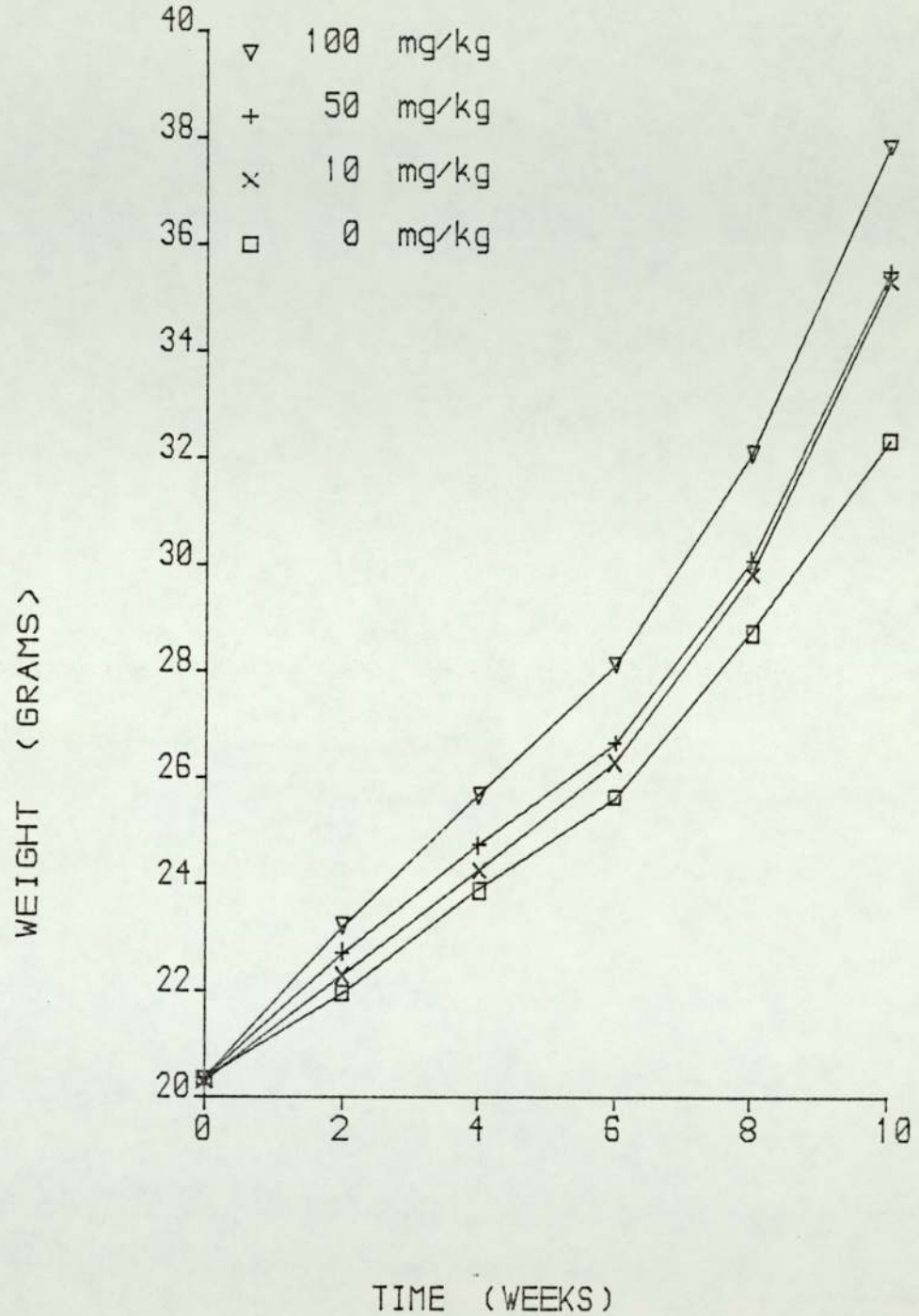


Figure 5.20 Effect of terramycin on the weight of carp fed LP diet

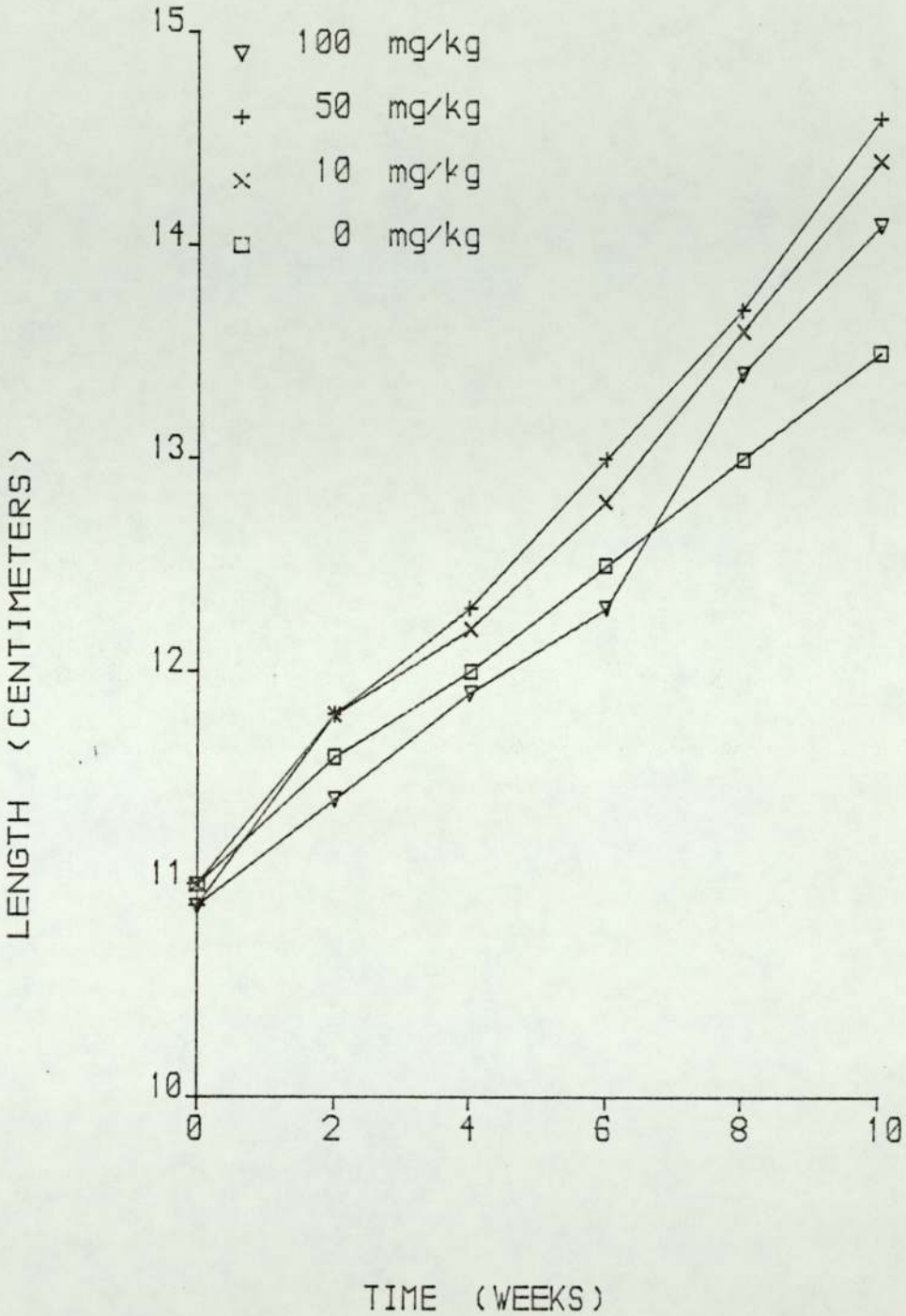


Figure 5.21 Effect of terramycin on the length of carp fed HP diet

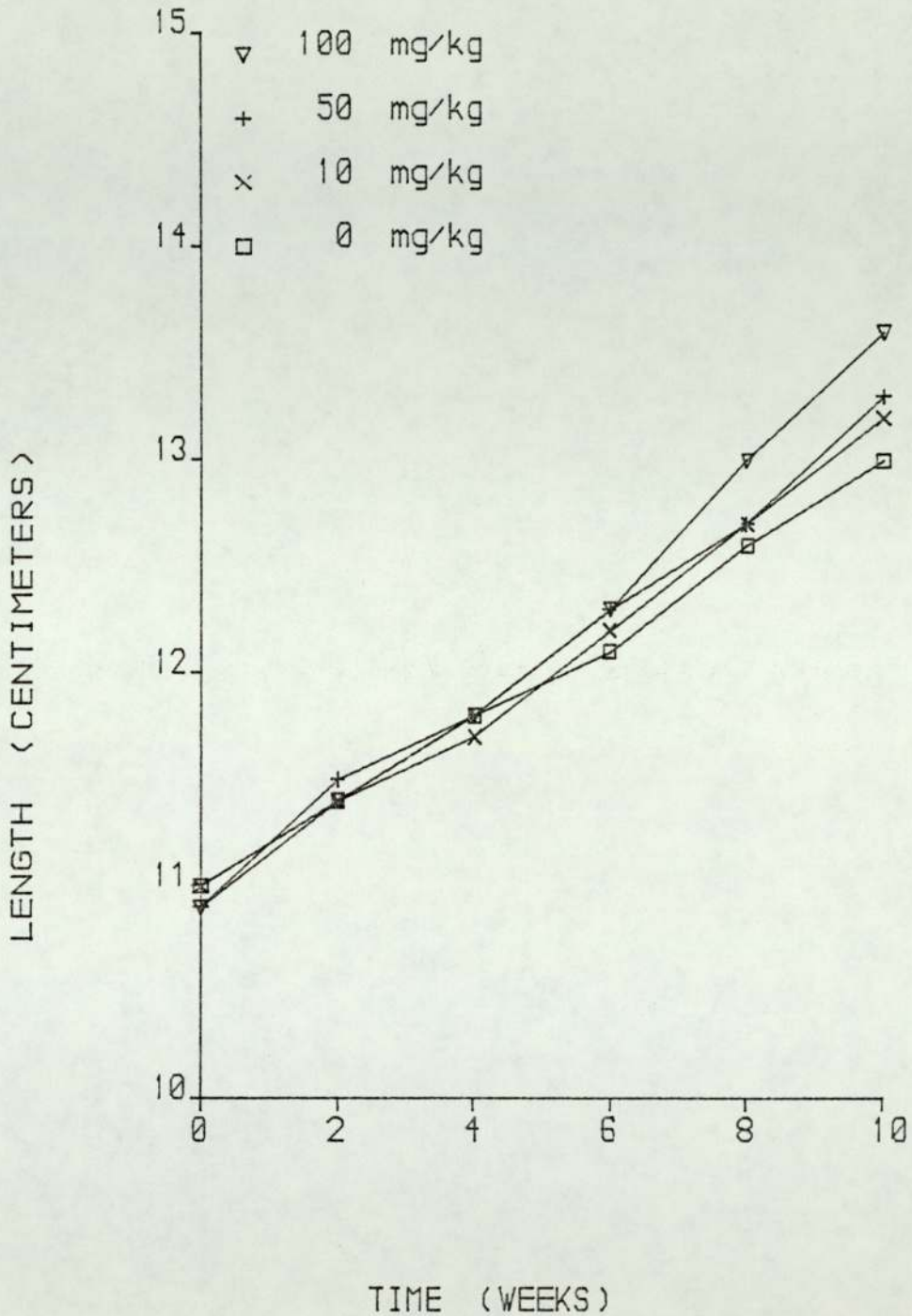


Figure 5.22 Effect of terramycin on the length of carp fed LP diet

length on both diets. Results are given in tables 5.27 and 5.28 and figures 5.23 and 5.24.

5.4.3 The Condition Factor :

The index of leanness or fatness (C) was measured. At the start of the experiment the condition factor of all the fish had the value of approximately 1.5. The fish fed the HP diet demonstrated the same value throughout the experiment, with the exception of the group receiving the 100 mg terramycin which had a value of 1.6 at the end of the experiment. Of the fish fed the LP diet, the groups receiving 50 mg drug and the control showed a decrease in the condition factor while the other two groups showed the same values throughout the experiment. Results are in table 5.29.

5.4.4 Feed Utilization Efficiency (FUE) :

The fish fed the HP terramycin-supplemented diet utilized their food more efficiently than the control most of the time throughout the experiment. The fish fed the LP supplemented diet had a smaller FUE than the fish fed the HP diet but they showed the same pattern. Results are in table 5.30.

5.4.5 Proximate Analysis Of The Body Composition :

The statistical analysis showed that neither terramycin nor the protein in the diet had a significant effect on the water, protein and ash content of the muscles of the fish. However the fat content of the muscle was affected by the

Table 5.27 : Effect of feeding terramycin on the specific growth rate (SGR) of carp (weight).

Period in Weeks	Concentration of terramycin (mg/kg food)							
	High protein diet				Low protein diet			
	100	50	10	0	0	10	50	100
0-2	1.08	1.59	1.51	1.34	0.58	0.72	0.85	1.01
2-4	0.94	1.14	0.89	0.69	0.65	0.65	0.65	0.77
4-6	1.21	1.04	0.99	0.88	0.54	0.62	0.58	0.70
6-8	1.47	1.42	1.38	1.00	0.88	0.97	0.93	1.02
8-10	1.43	1.54	1.45	1.02	0.91	1.30	1.27	1.27
0-10	1.23	1.34	1.25	0.98	0.71	0.85	0.85	0.95

Table 5.28 : Effect of feeding terramycin on the specific growth rate (SGR) of carp (length).

Period in Weeks	Concentration of terramycin (mg/kg food)							
	High protein diet				Low protein diet			
	100	50	10	0	0	10	50	100
0-2	0.34	0.61	0.54	0.41	0.27	0.27	0.41	0.36
2-4	0.33	0.32	0.26	0.26	0.27	0.20	0.20	0.25
4-6	0.25	0.43	0.37	0.31	0.19	0.32	0.32	0.31
6-8	0.66	0.40	0.47	0.30	0.31	0.31	0.25	0.40
8-10	0.39	0.49	0.49	0.29	0.24	0.30	0.35	0.35
0-10	0.39	0.45	0.43	0.31	0.26	0.28	0.31	0.34

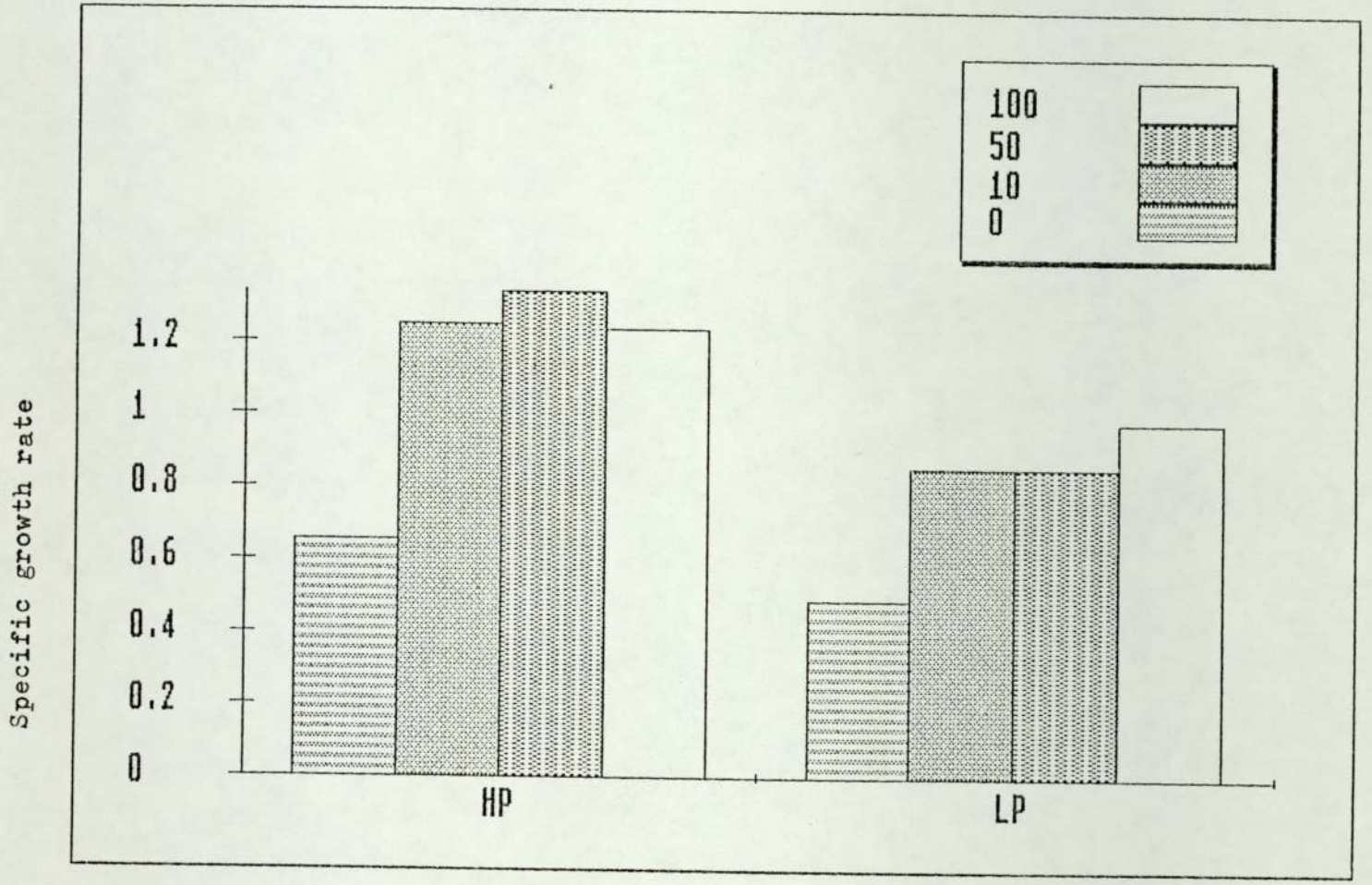


Figure 5.23 Effect of terramycin on the specific growth rate (weight) of carp

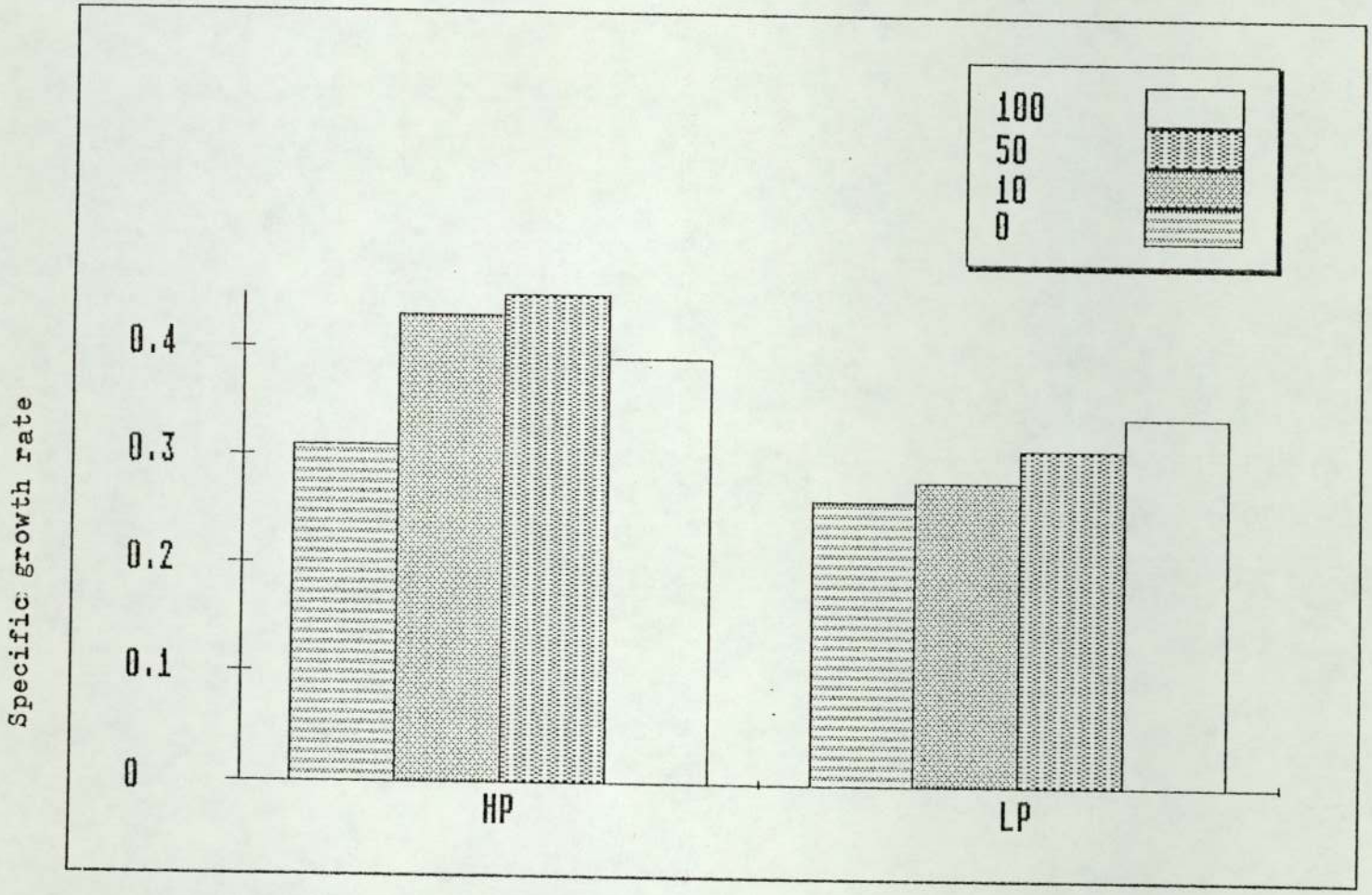


Figure 5.24 Effect of terramycin on the specific growth rate (length) of carp

Table 5.29 : Effect of feeding terramycin on the condition factor of carp. Values given are mean of 16 fish ±S.E.

(a) High Protein Diet

Duration in Weeks	Concentration of terramycin (mg/kg food)			
	100	50	10	control
0	1.53±0.012	1.51±0.014	1.52±0.010	1.51±0.014
2	1.58±0.019	1.52±0.024	1.50±0.023	1.54±0.015
4	1.57±0.020	1.55±0.029	1.51±0.014	1.52±0.16
6	1.58±0.023	1.52±0.023	1.50±0.014	1.53±0.018
8	1.55±0.018	1.50±0.018	1.52±0.021	1.53±0.016
10	1.61±0.09	1.55±0.08	1.55±0.08	1.50±0.10

Table 5.29 (cont'd.)

(b) Low Protein Diet

Duration in Weeks	Concentration of terramycin (mg/kg food)			
	100	50	10	control
0	1.52±0.012	1.53±0.01	1.51±0.014	1.53±0.013
2	1.55±0.02	1.51±0.026	1.50±0.022	1.43±0.02
4	1.55±0.03	1.50±0.02	1.50±0.027	1.46±0.015
6	1.51±0.021	1.45±0.02	1.45±0.02	1.43±0.022
8	1.45±0.021	1.47±0.017	1.45±0.026	1.43±0.015
10	1.50±0.08	1.45±0.07	1.50±0.08	1.43±0.08

Table 5.30 : Effect of feeding terramycin on feed utilization efficiency of carp.

Period in weeks	Concentration of terramycin (mg/kg food)							
	High protein diet				Low protein diet			
	100	50	10	0	0	10	50	100
0-2	0.39	0.59	0.55	0.49	0.20	0.25	0.30	0.36
2-4	0.40	0.49	0.38	0.28	0.27	0.27	0.27	0.32
4-6	0.53	0.44	0.43	0.37	0.22	0.26	0.24	0.30
6-8	0.65	0.62	0.60	0.42	0.37	0.41	0.40	0.44
8-10	0.63	0.68	0.64	0.42	0.29	0.56	0.55	0.55
0-10	0.52	0.56	0.52	0.40	0.27	0.35	0.35	0.39

addition of terramycin. Fish fed the HP supplemented diet had significantly more fat in their muscle than their control. Terramycin had no effect on the fat content of the fish fed the LP diet. Results are in table 5.31.

5.4.6 Somatic Indices :

No effect of either protein or terramycin on the ratio of the weight of the liver, kidney and intestine to the body weight was seen. The ratio of the length of the intestine to the body length was similar in the treated fish and the controls. Results are in table 5.32.

5.4.7. Bacterial Count :

Although many investigators have reported that the intestinal microflora of carp harbours different species of bacteria, as described in chapter 2, the sole bacterium found in the gut of the carp in this experiment and the following ones was the Gram-negative rod *Aeromonas hydrophila* . The viable numbers of this bacterium found in 1 g of mid-gut content of the fish fed the 50 mg terramycin per kg HP diet and of fish fed the 100 mg drug per kg LP diet, those groups which showed the highest increase in growth rate, were compared with their controls. The "t" test was used for statistical comparison. Feeding terramycin resulted in a change in bacterial count; the first group seemed to have more bacteria in their intestinal content but the difference was not significant ($P>0.05$). The medicated fish on the LP

Table 5.31 : Proximate analysis of body composition of carp fed diet containing terramycin. Number of samples eight fish ±S.E.

Body composition[a]	Concentration of terramycin (mg/kg food)							
	High protein diet				Low protein diet			
	100	50	10	0	0	10	50	100
Water	77.9 ±0.16	78.3 ±0.23	78.2 ±0.34	77.8 ±0.35	78.1 ±0.14	78.5 ±0.22	79.2 ±0.36	79.3 ±0.14
Protein	76.3 ±0.82	78.3 ±1.30	78.1 ±1.30	77.0 ±0.80	76.9 ±0.76	78.4 ±0.77	76.2 ±0.24	78.6 ±0.14
Fat	8.38 ±0.92	7.90 ±0.71	8.0 ±0.95	6.20 ±0.59	5.70 ±0.61	6.00 ±0.8	5.30 ±0.47	6.4 ±0.76
Ash	8.8 ±0.34	8.70 ±0.29	8.80 ±0.20	1.94 ±0.06	8.90 ±0.36	8.80 ±0.23	8.80 ±0.35	8.70 ±0.33
PER[b]	1.38	1.50	1.40	1.04	1.16	1.43	1.43	1.56
NPR[c]	23.8	26.6	25.1	18.5	20.1	24.6	21.2	26.6

[a] : calculated on dry basis

[b] : protein efficiency ratio

[c] : net protein retention

Table 5.32 : Hepato-somatic, Reno-somatic and vi scero-somatic indices of carp fed diet containing terramycin. Number of samples eight fish ±S.E.

Tissue indices	Concentration of terramycin (mg/kg food)							
	High protein diet				Low protein diet			
	100	50	10	0	0	10	50	100
Hepato-somatic indices (HSI)	1.94 ±0.12	1.73 ±0.09	1.87 ±0.08	2.00 ±0.13	1.83 ±0.11	1.96 ±0.16	1.82 ±0.11	1.86 ±0.06
Reno-somatic indices (RSI)	0.40 ±0.03	0.39 ±0.05	0.43 ±0.04	0.42 ±0.03	0.35 ±0.03	0.39 ±0.02	0.37 ±0.04	0.36 ±0.01
Viscero-somatic indices (VSI)	3.80 ±0.27	3.43 ±0.23	3.33 ±0.14	3.32 ±0.14	3.50 ±0.08	3.57 ±0.09	3.60 ±0.15	3.37 ±0.21
G/L*	1.60 ±0.03	1.65 ±0.03	1.64 ±0.02	1.61 ±0.03	1.66 ±0.04	1.62 ±0.02	1.66 ±0.03	1.63 ±0.03

* : Ratio of length of the gut to the total body length.

diet, on the other hand, had a significant increase in the number of bacteria in their gut content ($P<0.05$). There were no differences between the controls. Results are shown in table 5.33 and figure 5.25.

5.4.8 Testing The Sensitivity Of *A. hydrophila* To The Drug (Terramycin) *in vitro* :

The test showed that *Aeromonas hydrophila* was unaffected by the presence of terramycin, as the number of this bacterium was similar in the terramycin incorporated plates and the control plates.

5.4.9. Digestibility Coefficient :

The digestibility coefficient for the HP diet was between 90.8-91.6% and for the LP diet was between 89.4- 90%.

5.4.10 Histological Examination :

The thickness of the intestine of the treated fish seemed very similar and not changed as a result of feeding terramycin in both diets.

Summary of the performance of carp fed diet containing terramycin over the controls is seen in table 5.34.

5.5 Effect Of Feeding Virginiamycin To Carp (Experiment 5) :

Virginiamycin was mixed with a high protein diet only at concentrations of 40, 80 and 100 ppm and fed to juvenile carp for ten weeks. The number of fish for each treatment was 7.

5.5.1 Acceleration Of Growth :

Table 5.33 : Numbers of *A.hydrophila* expressed as millions in one gram of mid gut content of carp fed diet containing terramycin for ten weeks. Number of samples are 8 ±S.E.

Concentration of terramycin (mg/kg food)			
High protein		Low protein	
50	0	0	100
3.98±0.73	2.37±0.89	3.08±0.88	5.11±0.81

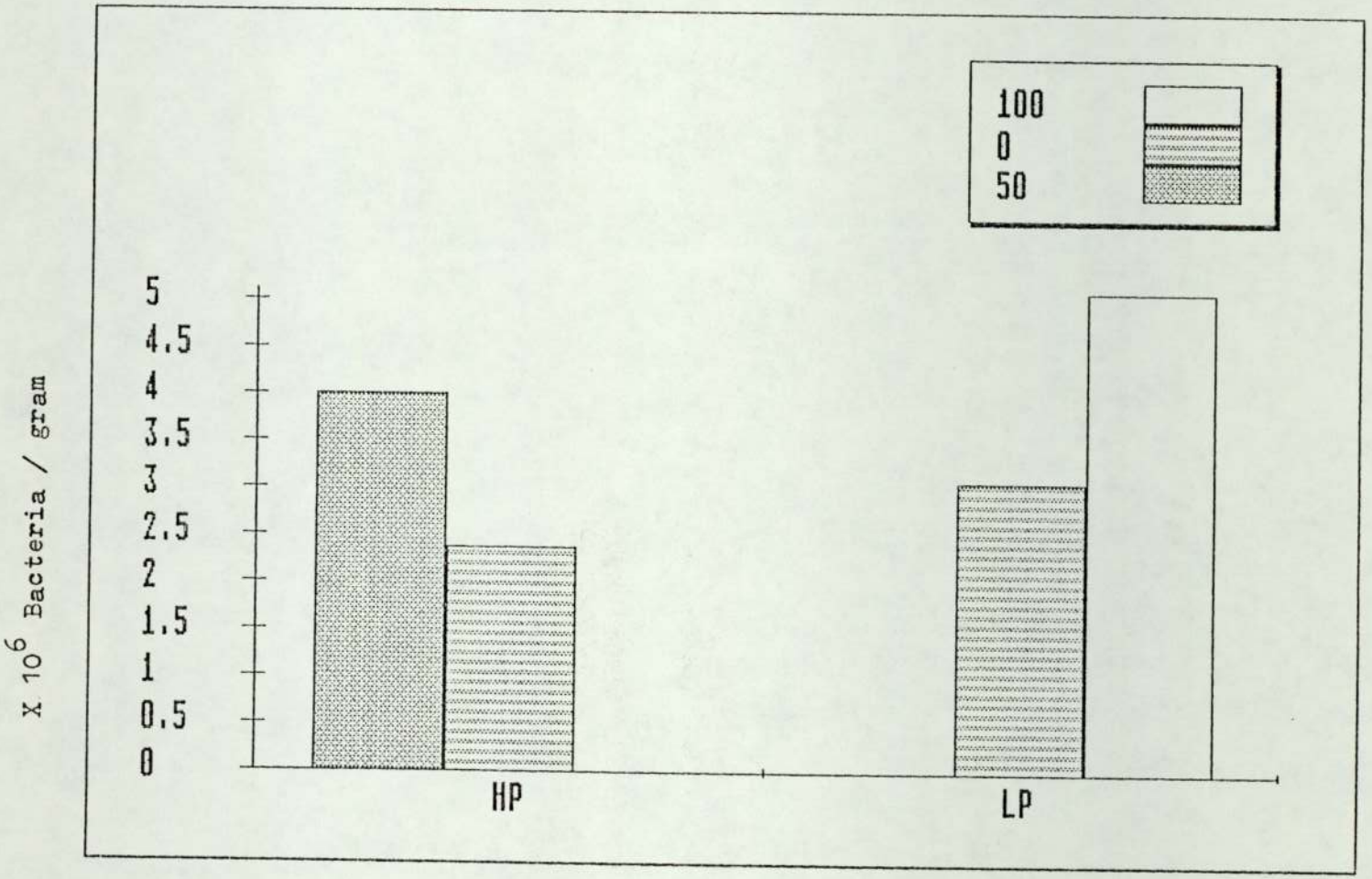


Figure 5.25 Effect of terramycin on the bacterial count of the mid-gut content of carp

Table 5.34 : Percentage increases in body weight and length, specific growth rate for weight and length and feed utilization efficiency of carp fed diet containing terramycin over the controls.

	Concentration of terramycin (mg/kg food)		
	100	50	10
a. HP diet :			
weight	36.2***	55.2***	38.2***
length	28.0**	48.0***	36.0***
SGR (weight)	25.5**	36.7***	27.6***
SGR (length)	25.8**	45.7***	38.7***
FUE	30.0	40.0	30.0
b. LP diet :			
weight	46.0**	26.2	26.8
length	35.0	20.0	10.0
SGR (weight)	33.8***	19.7*	19.0*
SGR (length)	30.8*	19.2	7.7
FUE	44.4	29.6	29.6

* = ($P<0.05$)

** = ($P<0.01$)

*** = ($P<0.001$)

When virginiamycin was added to the diet the percentage increases in the weight of fish receiving 0, 40, 80, and 100 ppm drug were 220%, 330%, 335.1% and 293.7% respectively. Statistical analysis showed that the increases were significant only at drug concentrations of 40 and 80 ppm. The percentage increases in length of the fish treated with virginiamycin at the above dosages were 42.7%, 54.1% 54.6% and 48% respectively. All differences were significant. Results are in tables 5.35 and 5.36 and figures 5.26 and 5.27.

5.5.2 Specific Growth Rate (Daily Gain) :

The fish receiving 40 and 80ppm virginiamycin generally had greater weight and length daily gain than the control throughout the experiment. The group fed 100 ppm drug had less weight daily gain in the first two weeks then gained more than the control for the rest of the experiment. Results are given in tables 5.37 and 5.38 and figures 5.28 and 5.29.

5.5.3 Feed Utilization Efficiency (FUE) :

The fish treated with 40 and 80 ppm virginiamycin utilized the food consumed more efficiently than the control throughout the experiment. The third group showed higher FUE except during the first two weeks. Results are shown in table 5.39.

5.5.4 Proximate Analysis Of The Body Composition :

Table 5.35 : Change in weight of carp *(Cyprinus carpio)* fed diets containing virginiamycin for a period of ten weeks at 25±1°c. Numbers given are mean of 7 fish ±S.E.

Duration in Weeks	Concentration of virginiamycin (mg/kg food)			
	100	80	40	control
0	17.4±0.84	17.4±1.2	17.3±1.1	17.4±0.74
2	21.2±1.1 (21.8%)	23.9±1.7 (37.4%)	22.3±1.4 (28.9%)	22.0±0.60 (26.4%)
4	29.0±1.9 (66.7%)	33.2±2.9 (90.8%)	31.2±1.6 (80.3%)	29.2±0.70 (67.8%)
6	38.3±2.8 (120.0%)	44.9±4.2 (158.0%)	41.4±2.1 (139.3%)	37.9±0.70 (117.8%)
8	52.4±4.4 (201.1%)	60.6±6.6 (248.3%)	56.1±4.9 (284.3%)	49.2±1.1 (182.8%)
10	68.5±6.2 (293.7%)	75.7±8.0 (335.1%)	74.5±3.7 (330.6%)	55.7±1.3 (220.0%)

Table 5.36 : Change in length of carp *(Cyprinus carpio)* fed diets containing virginiamycin for a period of ten weeks at 25±1°c. Numbers given are mean of 7 fish ±S.E.

Duration in Weeks	Concentration of virginiamycin (mg/kg food)			
	100	80	40	control
0	10.8±0.20	10.8±0.20	10.9±0.20	11.0±0.15
2	11.1±0.20 (2.8%)	11.5±0.30 (6.5%)	11.2±0.30 (2.8%)	11.4±0.13 (3.6%)
4	12.3±0.30 (13.9%)	12.9±0.40 (19.4%)	12.7±0.30 (16.9%)	12.3±0.12 (11.8%)
6	13.5±0.40 (25.0%)	14.1±0.40 (30.5%)	13.8±0.30 (26.6%)	13.7±0.13 (24.5%)
8	15.0±0.50 (38.9%)	15.6±0.50 (44.4%)	15.2±0.30 (39.4%)	14.4±0.16 (30.9%)
10	16.0±0.50 (48.0%)	16.7±0.4 (54.6%)	16.8±0.40 (54.1%)	15.7±0.20 (42.7%)

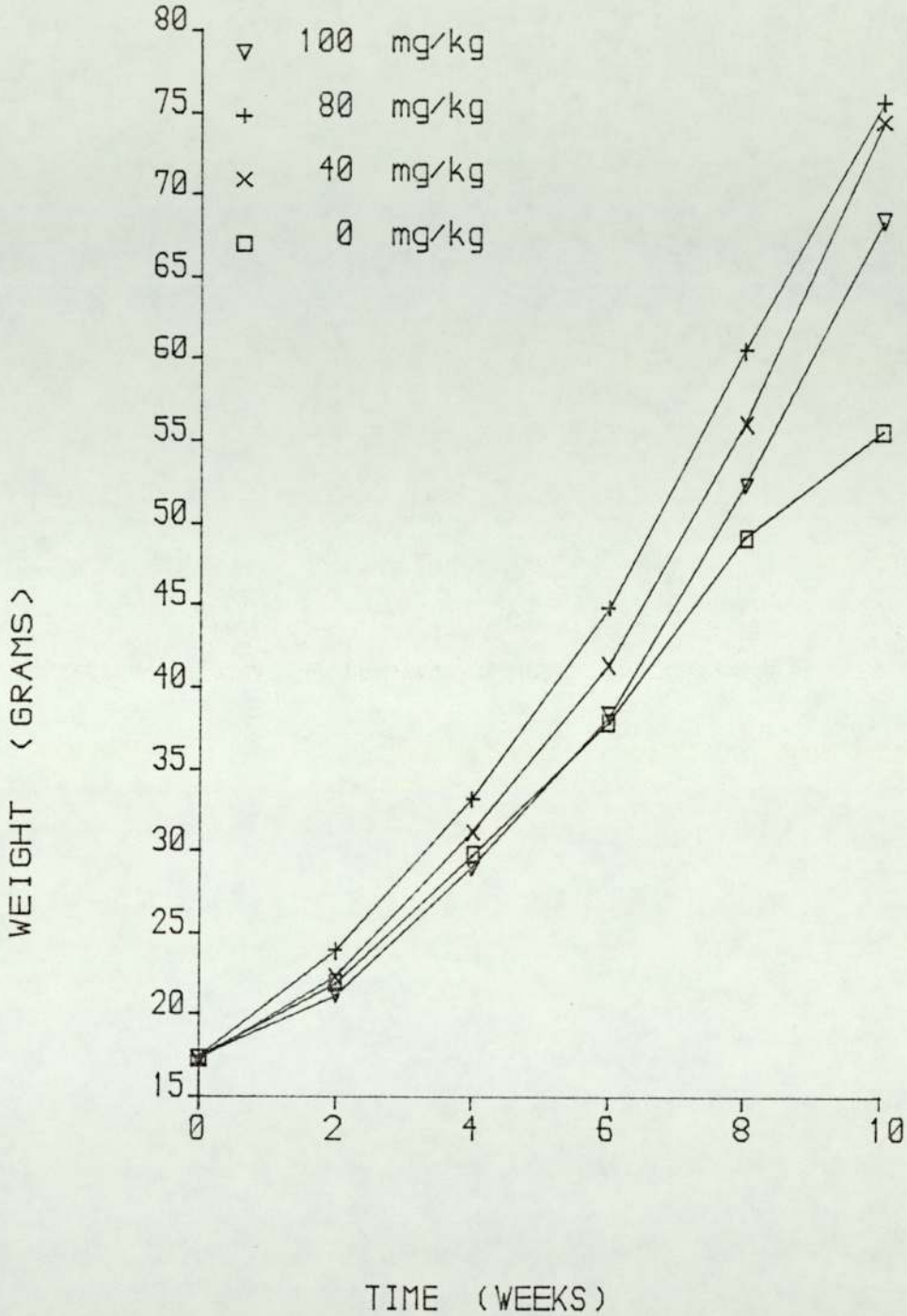


Figure 5.26 Effect of virginiamycin on the weight of carp

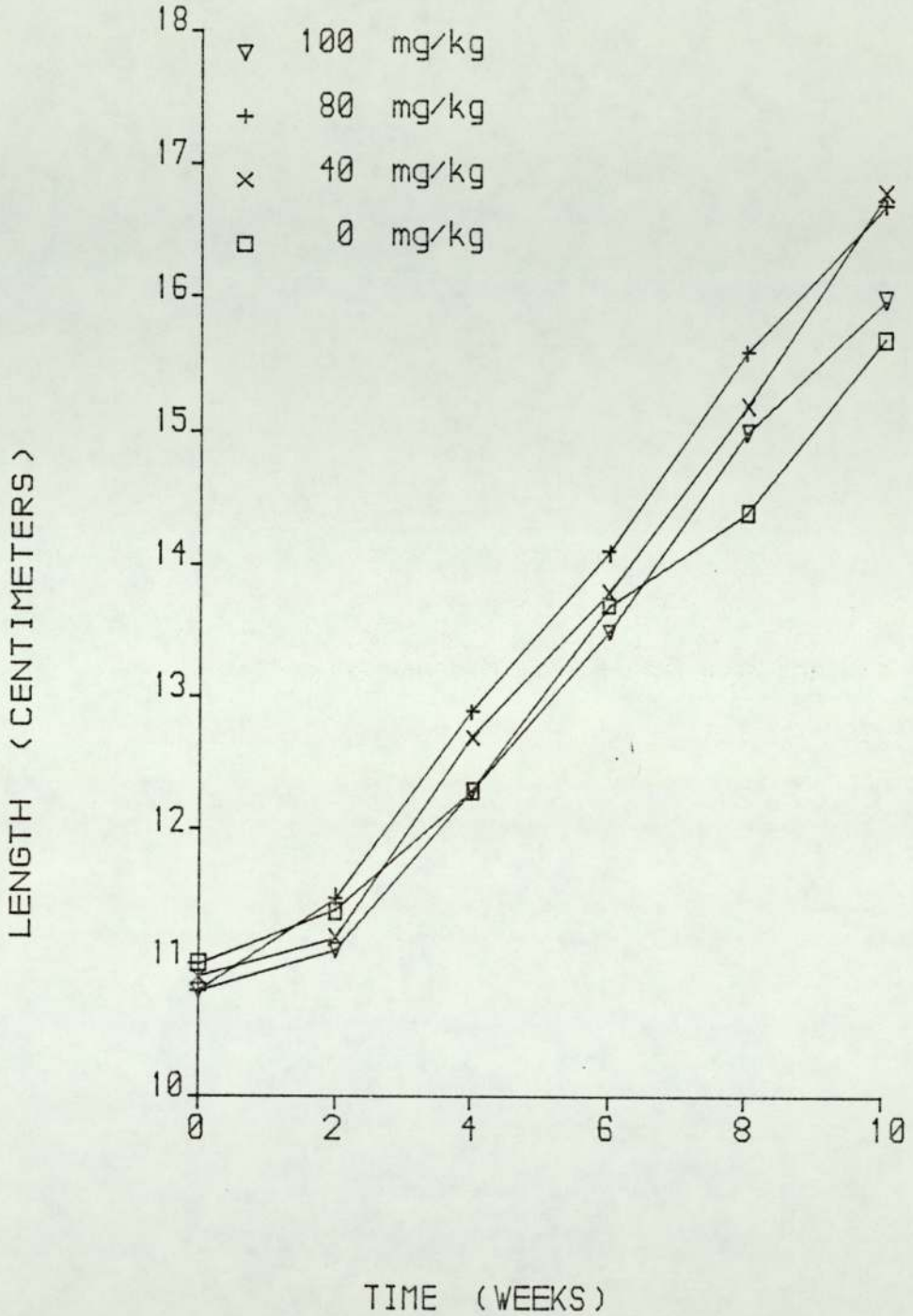


Figure 5.27 Effect of virginiamycin on the length of carp

Table 5.37 : Effect of feeding virginiamycin on the specific growth rate (SGR) of carp (weight).

Duration in Weeks	Concentration of virginiamycin (mg/kg food)			
	100	80	40	control
0-2	1.5	2.4	2.0	1.8
2-4	2.4	2.5	2.6	2.2
4-6	2.1	2.3	2.2	2.0
6-8	2.4	2.3	2.3	2.0
8-10	2.1	1.7	2.2	1.0
0-10	2.1	2.2	2.3	1.8

Table 5.38 : Effect of feeding virginiamycin on the specific growth rate (SGR) of carp (length).

Duration in Weeks	Concentration of virginiamycin (mg/kg food)			
	100	80	40	control
0-2	0.21	0.48	0.21	0.27
2-4	0.79	0.88	0.97	0.58
4-6	0.72	0.68	0.64	0.83
6-8	0.81	0.78	0.74	0.38
8-10	0.50	0.52	0.77	0.66
0-10	0.61	0.67	0.67	0.54

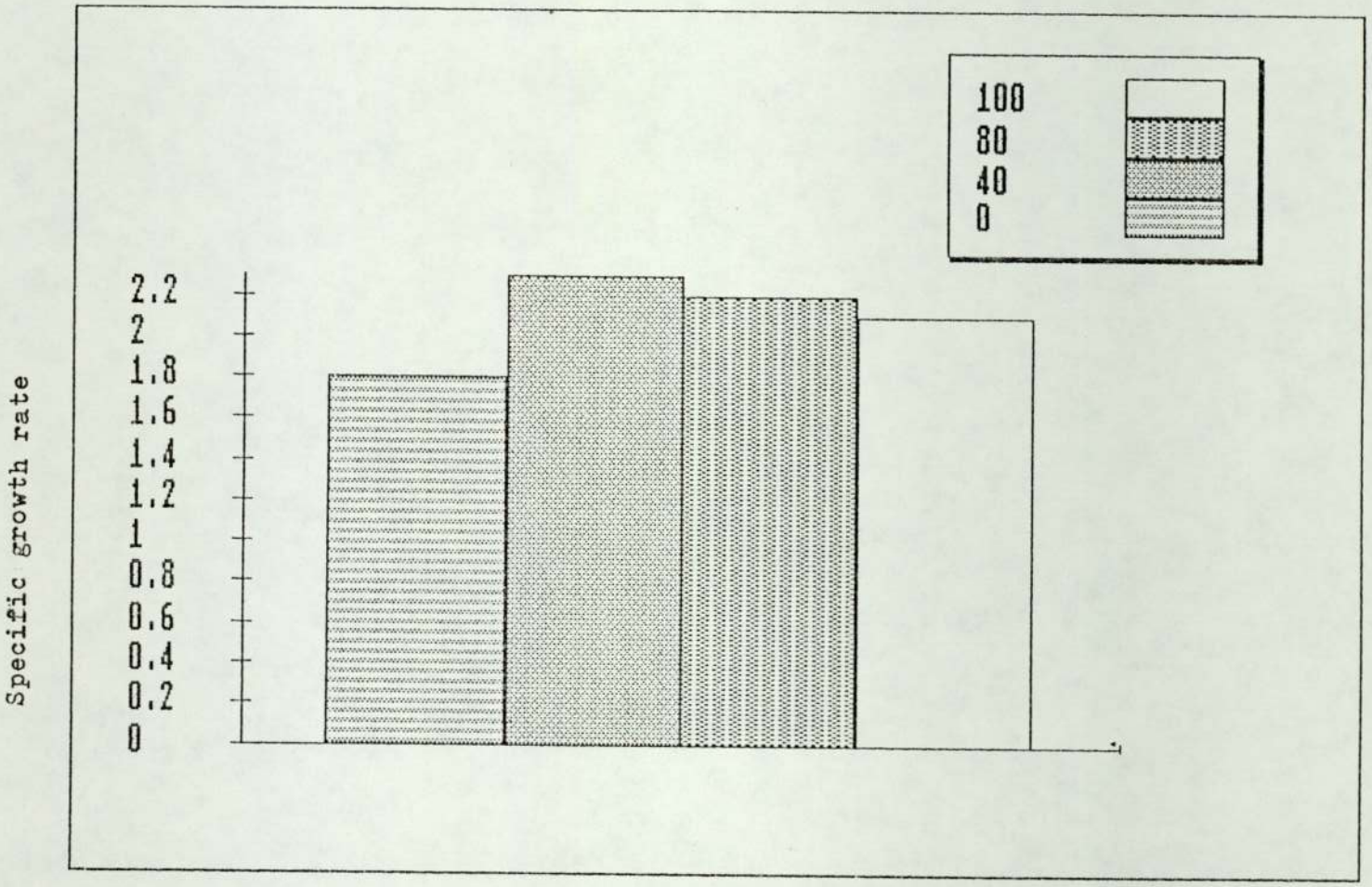


Figure 5.28 Effect of virginiamycin on the specific growth rate (weight) of carp

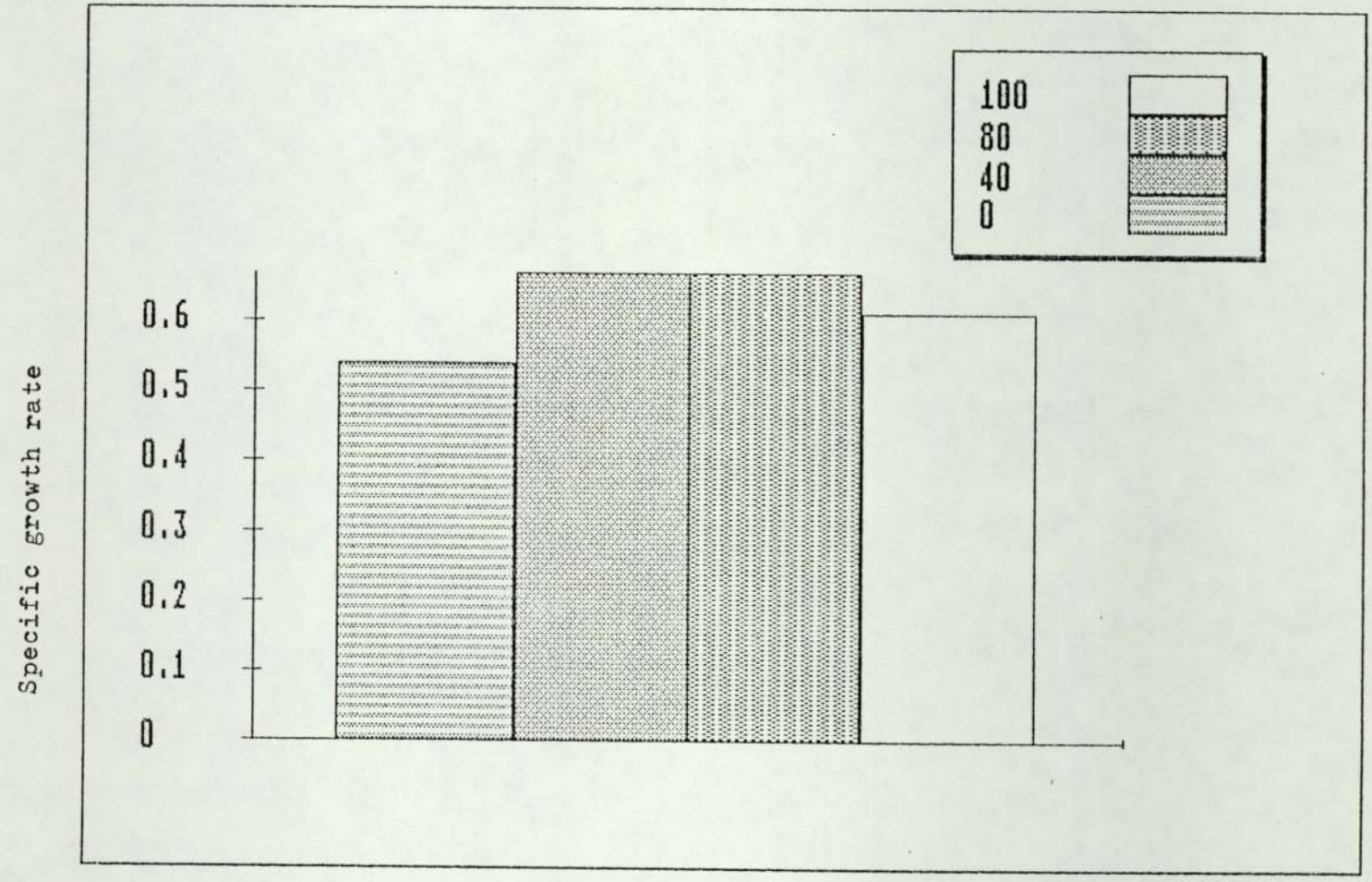


Figure 5.29 Effect of virginiamycin on the specific growth rate (length) of carp

Table 5.39 : Effect of feeding virginiamycin on feed utilization efficiency of carp.

Duration in Weeks	Concentration of virginiamycin (mg/kg food)			
	100	80	40	control
0-2	0.57	0.97	0.75	0.67
2-4	0.95	1.02	1.00	0.88
4-6	0.83	0.90	0.84	0.76
6-8	0.95	0.90	0.91	0.76
8-10	0.91	0.77	1.01	0.41
0-10	0.85	0.91	0.90	0.70

The water, protein and ash content of the muscle of the virginiamycin-treated fish was similar to the control. The drug, however, increased the fat content of the treated fish. The PER and NPE were higher in all treated carp than in the controls. Results are given in table 5.40.

5.5.5 Somatic Indices :

There was no effect of virginiamycin on the hepato-somatic and reno-somatic indices, through the drug decreased the VSI significantly. Results are in table 5.41.

5.5.6 Bacterial Count :

When one gram of mid-gut content of the control and treated fish were examined *Aeromonas hydrophila* was the sole Gram-negative rod bacterium that could be isolated. Adding virginiamycin to the diet caused a significant increase in the count of this bacterium. The differences were significant in the number of bacteria found in the 40 and 100 ppm drug treated group but not in the 80 ppm group. Table 5.42 and figure 5.30 show the results.

5.5.7 Digestibility Coefficient :

The digestibility coefficient of the HP diet was between 92.3-93.7 and of the LP was betwen 87.9-89.5.

5.5.8 Histological Examination :

Although virginiamycin reduced the weight of the intestine, it did not seem to have any effect on the

Table 5.40 : Proximate analysis of body composition of carp fed diet containing virginiamycin. Number of samples four fish ±S.E.

Body composition[a]	Concentration of virginiamycin (mg/kg food)			
	100	80	40	control
Water	77.7±0.20	78.7±0.20	78.4±0.28	78.7±0.10
Protein	73.1±0.50	75.0±0.70	74.2±0.50	75.1±0.50
Fat	11.9±0.60	10.3±0.30	9.7±0.30	7.6±0.20
Ash	7.6±0.40	7.8±0.15	7.5±0.10	7.6±0.15
PER[b]	2.29	2.34	2.41	1.75
NPR[c]	37.5	36.5	38.3	27.6

[a] : calculated on dry basis

[b] : protein efficiency ratio

[c] : net protein retention

Table 5.41 : Hepato-somatic, Reno-somatic and viscero-somatic indices of carp fed diet containing virginiamycin. Number of samples four fish ±S.E.

Tissue indices	Concentration of virginiamycin (mg/kg food)			
	100	80	40	control
Hepato-somatic indices (HSI)	2.1±0.13	1.86±0.07	1.79±0.40	2.0±0.30
Reno-somatic indices (RSI)	0.48±0.05	0.43±0.04	0.48±0.03	0.45±0.02
Viscero-somatic indices (VSI)	4.02±0.17	4.06±0.20	3.90±0.24	5.70±0.36

Table 5.42 : Number of *A.hydrophila* (millions) per gram of mid gut content of carp fed diet containing virginiamycin for ten weeks. Number of samples are 3 ±S.E.

Concentration of virginiamycin (mg/kg food)			
100	80	40	control
6.50±0.80	3.23±0.08	5.50±1.60	2.22±0.03

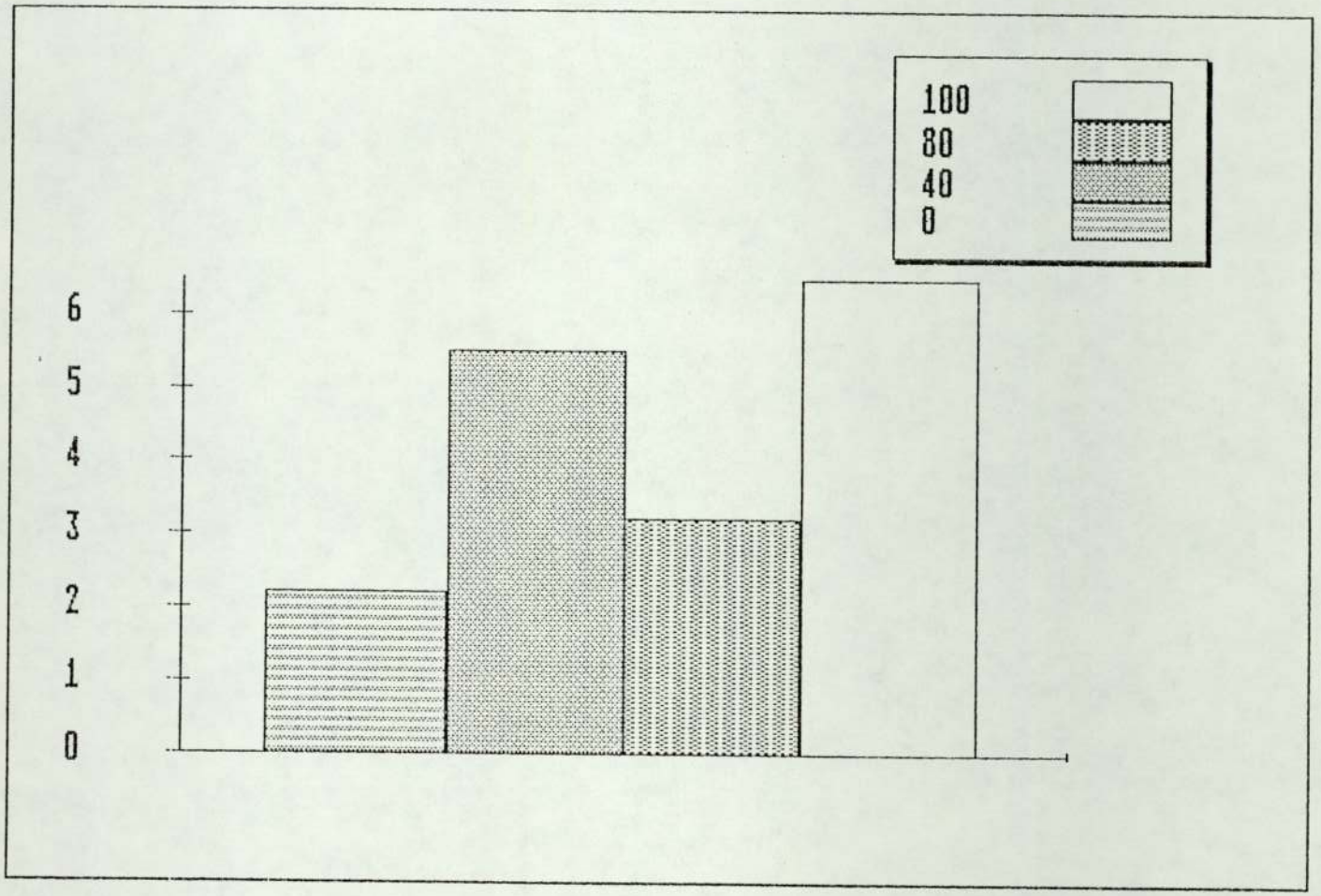


Figure 5.30 Effect of virginiamycin on the bacterial count of the mid-gut content of carp

histological structure.

Summary of the performance of carp fed diet containing virginiamycin over the control is seen in table 5.43.

5.6 Effect Of Feeding Virginiamycin To Carp (Experiment 6) :

Virginiamycin was again incorporated in the feed of carp. In this experiment it was added to high and low protein diets, HP and LP, and a larger number of fish per treatment (16) was used. The experiment was carried out for ten weeks.

5.6.1 Acceleration Of Growth :

The fish fed the HP diet were heavier and longer than the fish fed the LP diet. When the drug was added to the HP diet at concentrations of 0, 40, 80 and 100 ppm the increases in weight were 150.7%, 215.2%, 322.3% and 192.6%, when the drug was mixed with the LP diet the increases were 110%, 116.3%, 129.9% and 135% respectively. The increases in length on the HP diet were 38.6%, 49.4%, 63% and 48.4%, and for the fish fed the LP diet were 28.6%, 32.6%, 34% and 35.9% respectively. The effect of virginiamycin was significant in increasing the weight and length of the HP fish but it was not significant in increasing the weight and length of the LP diet group. The results are shown in tables 5.44 and 5.45 and figures 5.31, 5.32, 5.33 and 5.34.

5.6.2 Specific Growth Rate (Daily Gain) :

The daily weight gains for fish given the HP diet in all the treated groups were greater than the control except that

Table 5.43 : Percentage increases in body weight and length, specific growth rate for weight and length and feed utilization efficiency of carp fed diet containing virginiamycin over the controls.

	Concentration of virginiamycin (mg/kg food)		
	100	80	40
weight	33.4*	52.2**	49.3*
length	10.6*	25.5*	25.5*
SGR (weight)	16.7	22.2*	27.8*
SGR (length)	13.0	24.1*	24.1*
FUE	21.4	30.0	28.6

* = ($P<0.05$)

** = ($P<0.01$)

Table 5.44 : Change in weight of carp *(Cyprinus carpio)* fed on diets containing virginiamycin for a period of ten weeks at 25±1°c. Numbers given are mean of 16 fish ±S.E.

(a) High Protein Diet

Duration in Weeks	Concentration of virginiamycin (mg/kg food)			
	100	80	40	control
0	4.47±0.20	4.31±0.20	4.22±0.20	4.36±0.20
2	5.30±0.31 (18.6%)	5.70±0.30 (32.3%)	4.85±0.30 (14.9%)	4.85±0.30 (11.2%)
4	6.61±0.40 (47.9%)	7.50±0.40 (74.0%)	6.05±0.36 (43.4%)	5.7±0.30 (30.7%)
6	8.21±0.60 (83.7%)	9.76±0.60 (126.5%)	7.6±0.50 (80.1%)	6.75±0.50 (54.8%)
8	10.60±0.51 (137.1%)	13.50±0.90 (213.2%)	10.19±0.70 (141.5%)	8.50±0.60 (95.0%)
10	13.08±0.90 (192.6%)	18.20±1.2 (322.2%)	13.30±1.0 (215.2%)	10.93±0.80 (150.7%)

Table 5.44 (cont'd.)

(b) Low Protein Diet

Duration in Weeks	Concentration of virginiamycin (mg/kg food)			
	100	80	40	control
0	4.05±0.16	4.32±0.20	4.16±0.20	4.46±0.20
2	4.81±0.20 (18.8%)	4.90±0.20 (13.4%)	4.73±0.40 (13.7%)	4.79±0.30 (11.2%)
4	5.63±0.40 (44.0%)	5.80±0.30 (34.3%)	5.55±0.50 (33.4%)	5.60±0.40 (25.6%)
6	6.30±0.41 (55.6%)	6.61±0.43 (53.0%)	6.20±0.66 (49.0%)	6.30±0.50 (41.0%)
8	7.50±0.60 (85.2%)	7.81±0.50 (80.0%)	7.30±0.90 (75.5%)	7.20±0.80 (61.0%)
10	9.53±0.70 (135.3%)	9.93±0.80 (129.9%)	9.00±1.1 (116.3%)	9.38±1.0 (110.3%)

Table 5.45 : Change in length of carp *(Cyprinus carpio)* fed on diets containing virginiamycin for a period of ten weeks at 25±1°c. Numbers given are mean of 16 fish ±S.E.

(a) High Protein Diet

Duration in Weeks	Concentration of virginiamycin (mg/kg food)			
	100	80	40	control
0	6.50±0.13	6.50±0.10	6.50±0.11	6.50±0.11
2	7.00±0.15 (7.6%)	7.20±0.13 (10.8%)	6.90±0.12 (6.0%)	6.80±0.15 (4.9%)
4	7.50±0.18 (16.0%)	7.80±0.16 (20.0%)	7.3±0.15 (12.7%)	7.20±0.14 (10.8%)
6	8.20±0.20 (25.8%)	8.60±0.20 (32.4%)	7.90±0.19 (21.1%)	7.80±0.16 (21.0%)
8	8.80±0.22 (36.0%)	9.60±0.21 (48.0%)	8.70±0.23 (35.0%)	8.20±0.22 (27.0%)
10	9.60±1.06 (48.4%)	10.60±0.92 (63.1%)	9.70±1.08 (49.4%)	9.00±1.06 (38.6%)

Table 5.45 (cont'd.)

(b) Low Protein Diet

Duration in Weeks	Concentration of virginiamycin (mg/kg food)			
	100	80	40	control
0	6.40±0.08	6.60±0.09	6.40±0.10	6.50±0.11
2	6.90±0.13 (8.1%)	6.90±0.11 (5.3%)	6.70±0.15 (4.5%)	6.60±0.15 (3.3%)
4	7.20±0.12 (12.6%)	7.20±0.14 (9.9%)	7.10±0.19 (10.0%)	7.10±0.18 (10.1%)
6	7.60±0.16 (18.7%)	7.70±0.23 (12.3%)	7.50±0.23 (16.0%)	7.40±0.22 (14.8%)
8	8.20±0.20 (27.9%)	8.30±0.18 (27.2%)	8.00±0.27 (23.9%)	7.90±0.28 (20.2%)
10	8.70±0.20 (35.9%)	8.80±0.21 (34.0%)	8.50±1.26 (32.6%)	8.50±1.22 (30.8%)

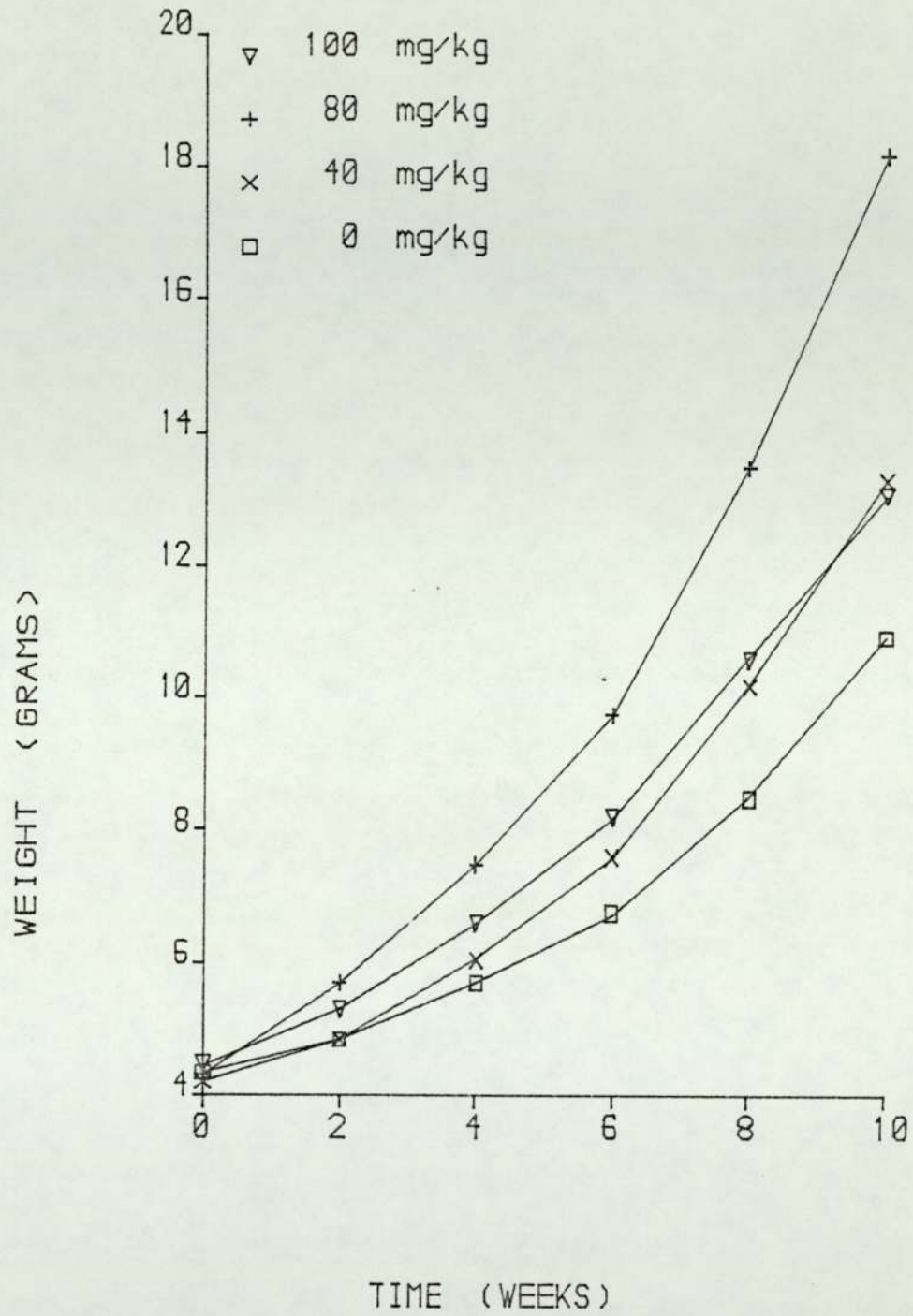


Figure 5.31 Effect of virginiamycin on the weight of carp fed HP diet

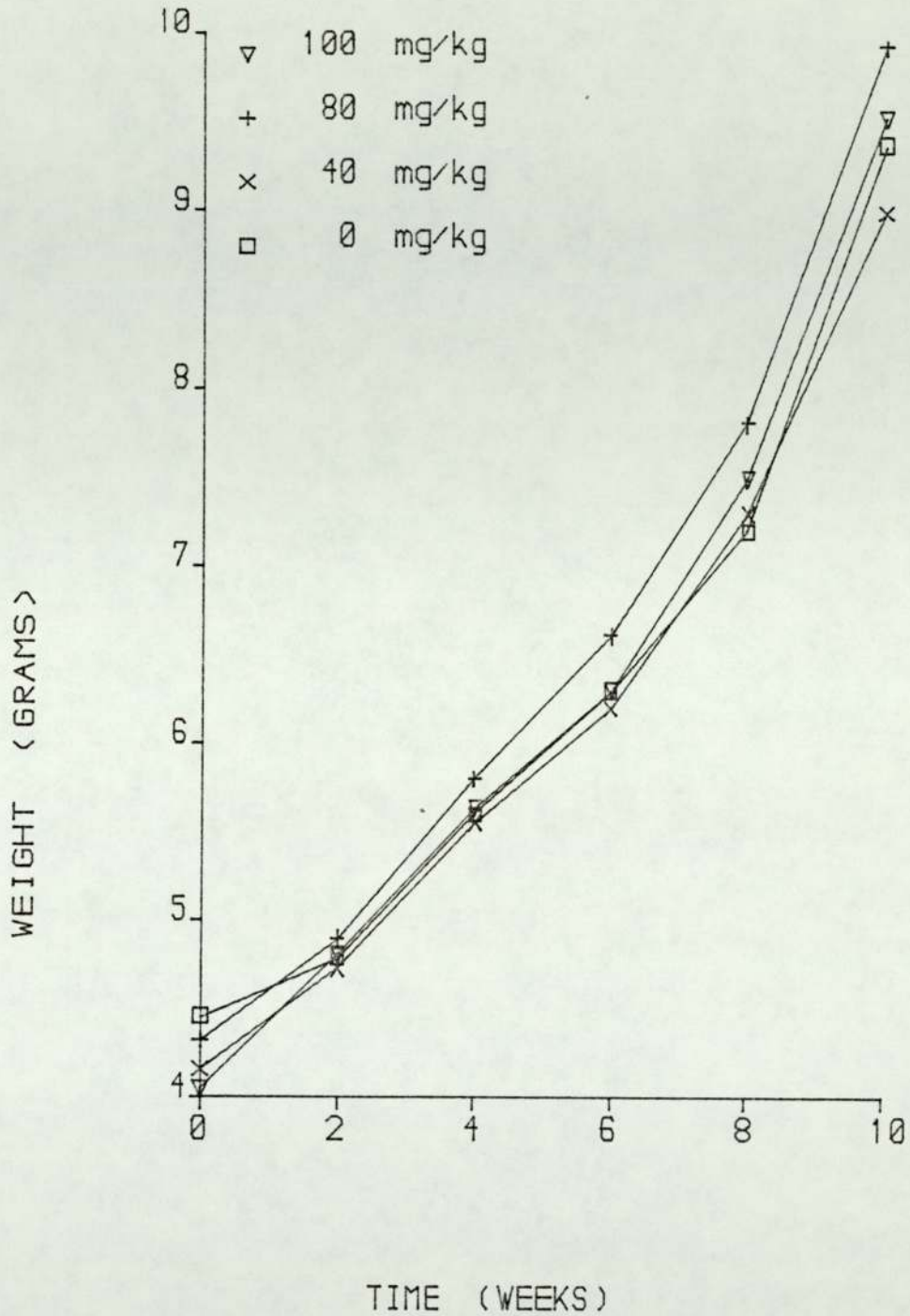


Figure 5.32 Effect of virginiamycin on the weight of carp fed LP diet

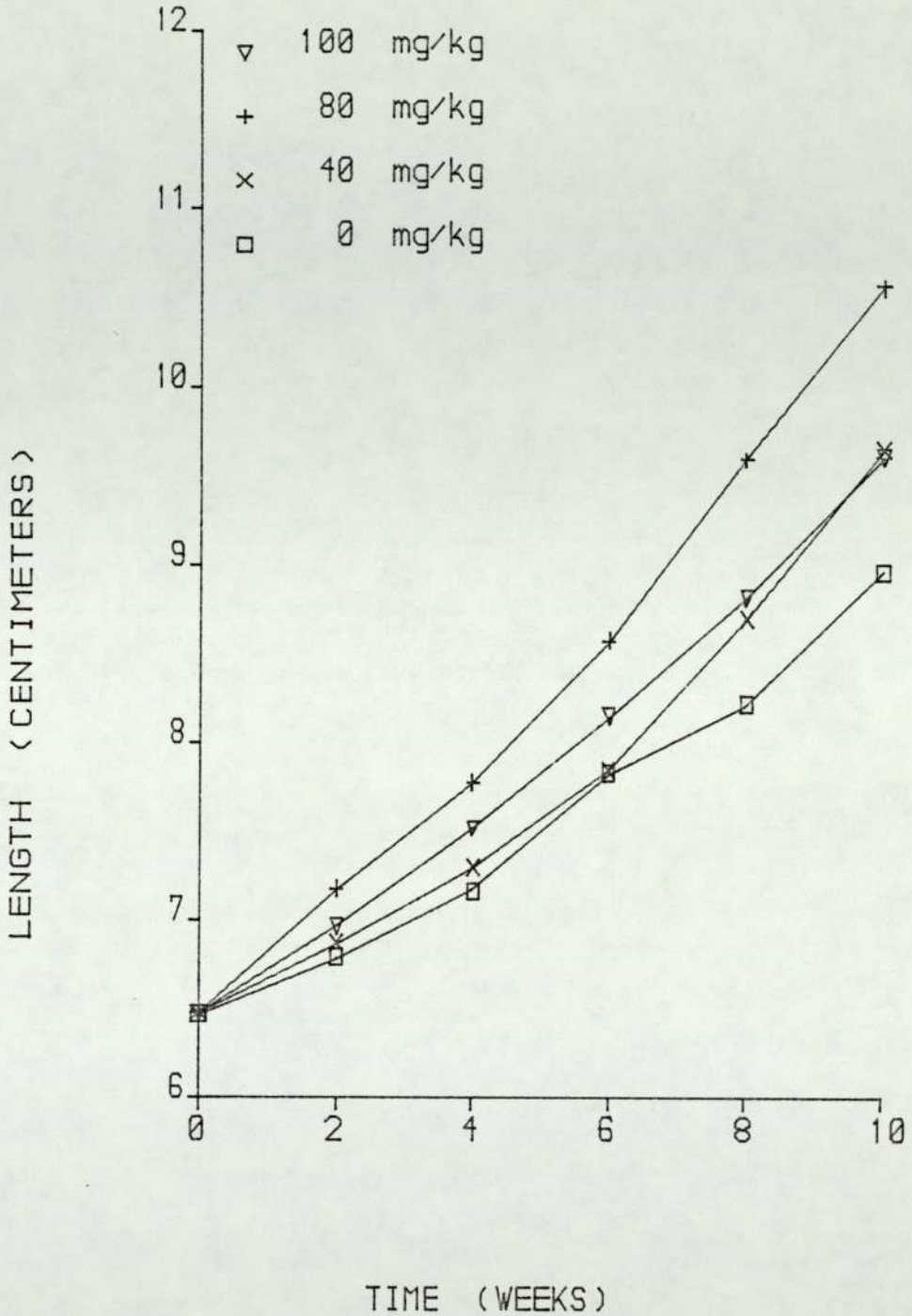


Figure 5.33 Effect of virginiamycin on the length of carp fed HP diet

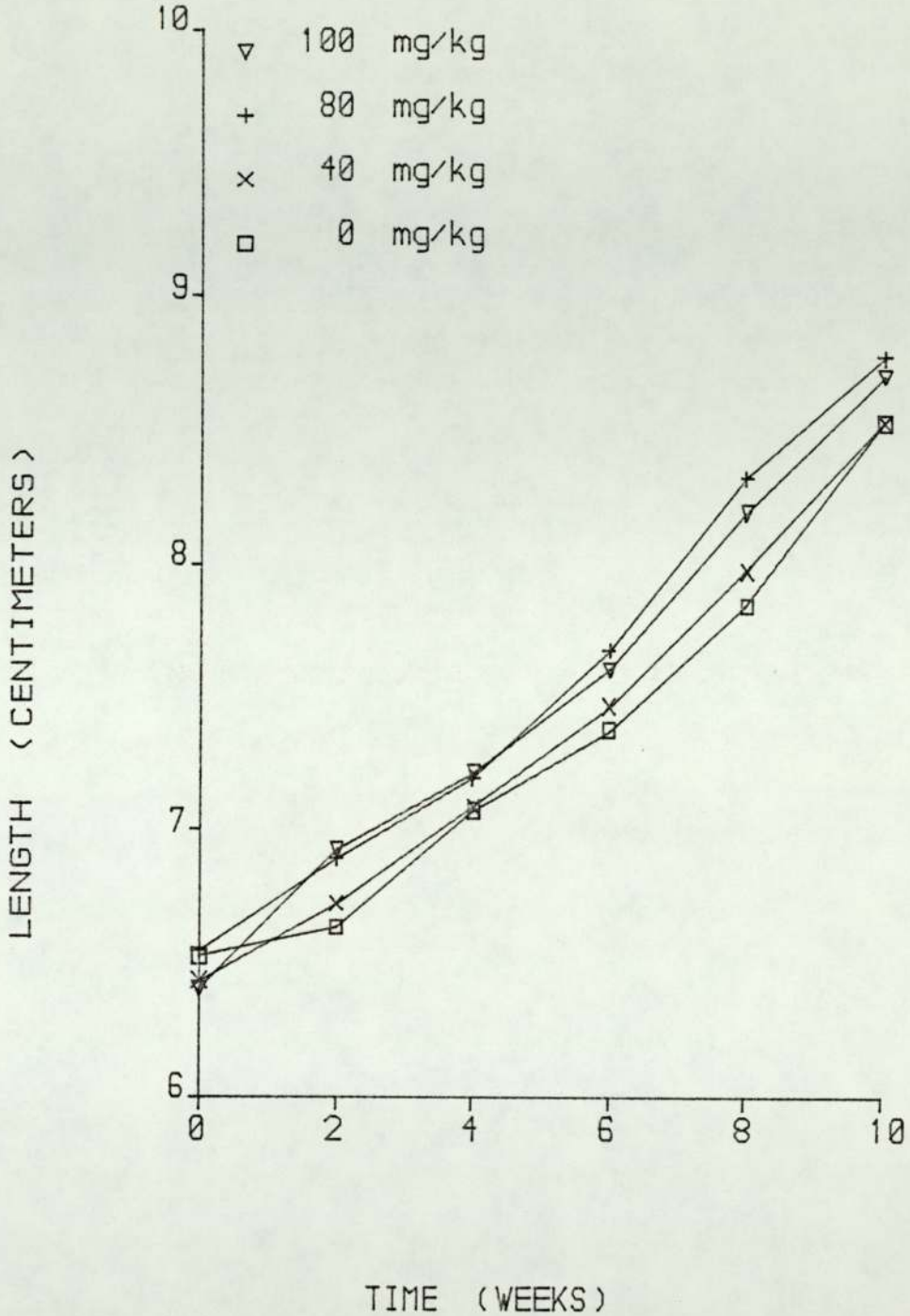


Figure 5.34 Effect of virginiamycin on the length of carp fed LP diet

during the last two weeks, the fish receiving 100 ppm virginiamycin gained less than the control. The overall DG in weight was significantly greater than the control, with the highest in the 80 ppm group. The DG in length was higher in all the treated fish than the control throughout the experiment. The DG in weight and length of the fish fed the LP diet showed an uneven pattern, as the increases varied during the experiment. However the overall increase was slightly higher in the treated groups and it showed that the higher the dosage the greater the increase. Results are shown in tables 5.46 and 5.47 and figures 5.35 and 5.36.

5.6.3 The Condition Factor :

The initial values for the condition factor (C) were higher in all the groups of fish than (except one) at the end of the experiment. This is probably due to the fact that the fish were feeding on high protein (50%) commercial trout diet during the acclimation period. The fish fed the HP diet showed higher C values than their control while the fish fed the LP diet had lower C than their control. Results are given in table 5.48.

5.6.4 Feed Utilization Efficiency (FUE) :

The virginiamycin-treated carp fed on the HP diet showed an increase in FUE over that of the control throughout the experiment (except the 100 mg group). Adding the drug to the LP diet also resulted in an increase in the FUE in the treated fish, except during the

Table 5.46 : Effect of feeding virginiamycin on the specific growth rate (SGR) of carp (weight).

Period in Weeks	Concentration of virginiamycin (mg/kg food)							
	High protein diet				Low protein diet			
	100	80	40	0	0	40	80	100
0-2	1.31	2.10	1.07	0.82	0.55	0.99	0.97	1.32
2-4	1.70	2.11	1.70	1.20	1.20	1.20	1.30	1.21
4-6	1.70	2.00	1.75	1.30	0.91	0.85	1.00	0.86
6-8	2.00	2.50	2.30	1.80	1.03	1.26	1.30	1.34
8-10	1.60	2.30	2.00	1.90	1.80	1.60	1.84	1.84
0-10	1.70	2.20	1.76	1.40	1.10	1.10	1.19	1.22

Table 5.47 : Effect of feeding virginiamycin on the specific growth rate (SGR) of carp (length).

Period in Weeks	Concentration of virginiamycin (mg/kg food)							
	High protein diet				Low protein diet			
	100	80	40	0	0	40	80	100
0-2	0.56	0.79	0.45	0.37	0.13	0.34	0.40	0.60
2-4	0.58	0.62	0.47	0.42	0.49	0.40	0.33	0.32
4-6	0.62	0.75	0.57	0.68	0.32	0.40	0.50	0.40
6-8	0.62	0.86	0.84	0.37	0.47	0.51	0.62	0.57
8-10	0.69	0.74	0.72	0.67	0.65	0.52	0.43	0.46
0-10	0.61	0.75	0.61	0.50	0.41	0.43	0.46	0.47

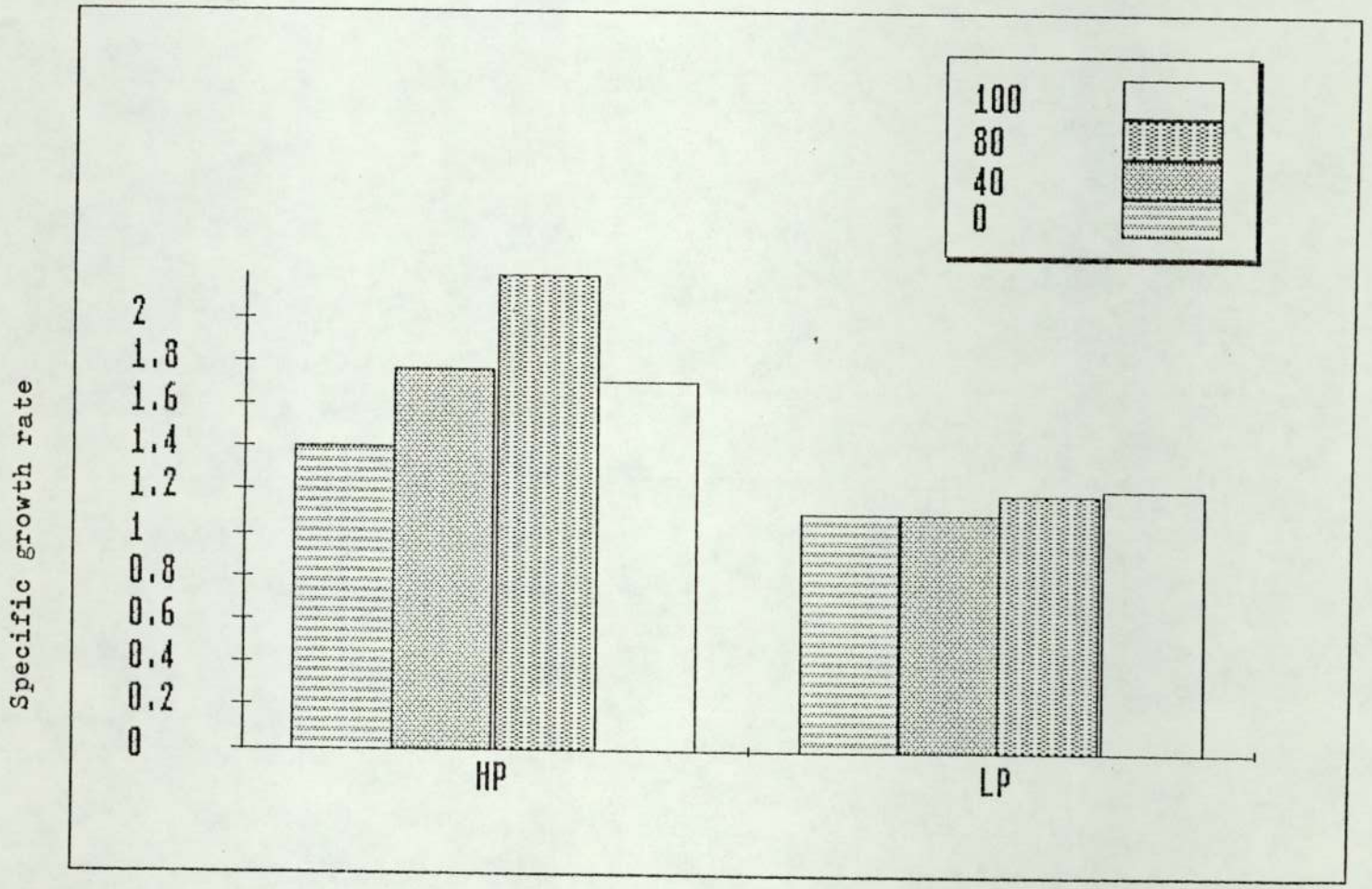


Figure 5.35 Effect of virginiamycin on the specific growth rate (weight) of carp

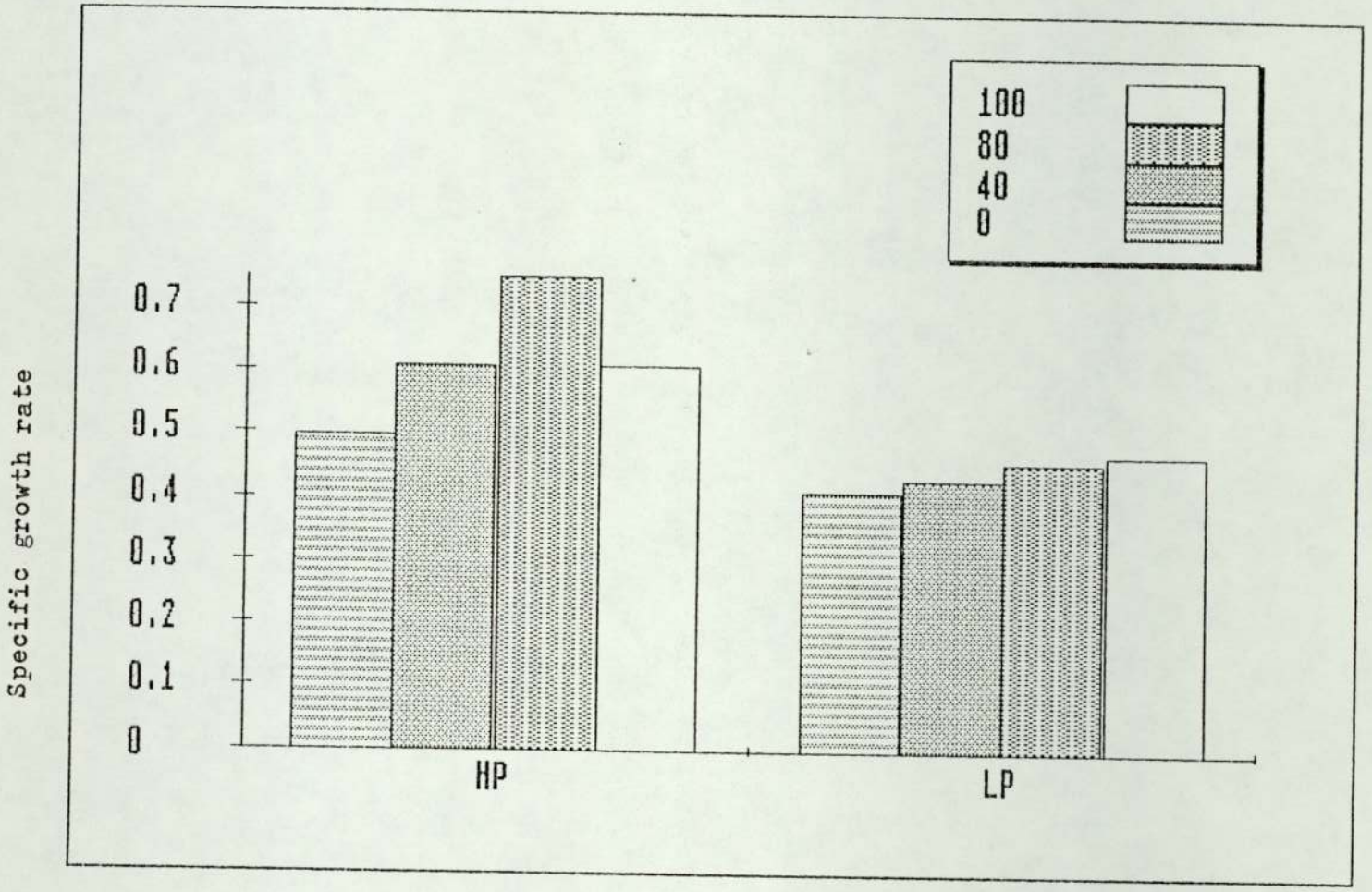


Figure 5.36 Effect of virginiamycin on the specific growth rate (length) of carp

Table 5.48 : Effect of feeding virginiamycin on the condition factor of carp. Values given are mean of 16 fish ±S.E.

(a) High Protein Diet

Duration in Weeks	Concentration of virginiamycin (mg/kg food)			
	100	80	40	control
0	1.7±0.03	1.6±0.03	1.5±0.02	1.6±0.02
2	1.5±0.02	1.5±0.02	1.4±0.02	1.5±0.02
4	1.5±0.02	1.5±0.02	1.5±0.02	1.5±0.02
6	1.5±0.02	1.5±0.02	1.5±0.02	1.5±0.03
8	1.5±0.02	1.5±0.04	1.5±0.03	1.5±0.02
10	1.5±0.02	1.5±0.02	1.5±0.02	1.44±0.02

Table 5.48 (cont'd.)

(b) Low Protein Diet

Duration in Weeks	Concentration of virginiamycin (mg/kg food)			
	100	80	40	control
0	1.5±0.02	1.6±0.03	1.5±0.03	1.6±0.02
2	1.4±0.03	1.5±0.03	1.5±0.03	1.6±0.02
4	1.5±0.02	1.5±0.02	1.5±0.02	1.5±0.03
6	1.5±0.01	1.5±0.02	1.6±0.04	1.5±0.04
8	1.5±0.02	1.5±0.02	1.5±0.03	1.5±0.04
10	1.4±0.02	1.4±0.02	1.4±0.02	1.5±0.02

the 3rd and 5th period when the control had higher FUE than the treated fish. However the overall effect was in favour of the treated fish. The FUE's of the fish fed the LP diet were similar. Results are shown in table 5.49.

5.6.5 Proximate Analysis Of Body Compostion :

The dietary protein did not have any effect on the biochemical content of the fish. Virginiamycin-treated fish seemed to have less water in their muscle than the control fish fed the HP diet; the fat content of the muscle of the HP fish was significantly higher than in the control. The protein content of the body was similar in all fish. No effect of the drug was seen on the biochemical content of the fish fed the LP diet. The PER and NPR of the treated fish were higher than in that of the controls. Results are given in table 5.50.

5.6.6 Body Somatic Indices :

The calculation of these indices was made on the group fed the 80 ppm virginiamycin-supplemented diet, the dose that gave the best response in growth rate. This group was compared with those receiving the same dosage of the drug in the LP diet. Neither virginiamycin nor the protein of the diet had any effect on the RSI or the VSI, whereas there was a significant effect of the drug in increasing the HSI of the fish fed the HP diet. Results are shown in table 5.51.

5.6.7 Bacterial Count :

Table 5.49 : Effect of feeding virginiamycin on feed utilization efficiency of carp over a period of ten weeks.

Period in weeks	Concentration of virginiamycin (mg/kg food)							
	High protein diet				Low protein diet			
	100	80	40	0	0	40	80	100
0-2	0.28	0.50	0.20	0.17	0.11	0.21	0.20	0.29
2-4	0.38	0.49	0.38	0.27	0.26	0.27	0.28	0.27
4-6	0.37	0.46	0.40	0.29	0.19	0.18	0.22	0.18
6-8	0.45	0.59	0.50	0.40	0.22	0.27	0.28	0.30
8-10	0.36	0.53	0.47	0.44	0.46	0.31	0.42	0.42
0-10	0.37	0.51	0.39	0.31	0.25	0.25	0.28	0.29

Table 5.50 : Proximate analysis of body composition of carp fed diet containing virginiamycin. Number of samples eight fish ±S.E.

Body composition[a]	Concentration of virginiamycin (mg/kg food)			
	High protein diet		Low protein diet	
	80	control	80	control
Water	76.0±1.00	78.5±0.37	78.0±0.38	78.5±0.34
Protein	72.8±0.94	72.2±1.50	73.0±0.77	73.5±0.60
Fat	14.7±0.80	9.6±0.30	9.9±0.10	10.2±0.92
Ash	7.3±0.10	7.8±0.44	7.9±0.35	8.5±0.70
PER[b]	1.38	0.88	0.77	0.70
NPR[c]	24.6	13.0	18.4	16.0

[a] : calculated on dry basis

[b] : protein efficiency ratio

[c] : net protein retention

Table 5.51 : Hepato-somatic, Reno-somatic and viscero-somatic indices of carp fed diet containing virginiamycin. Number of samples eight fish ±S.E.

Tissue indices	Concentration of virginiamycin (mg/kg food)			
	High protein diet		Low protein diet	
	80	control	80	control
Hepato-somatic indices (HSI)	1.79±0.25	1.44±0.05	1.65±0.26	1.58±0.34
Reno-somatic indices (RSI)	0.65±0.07	0.50±0.01	0.47±0.20	0.44±0.06
Viscero-somatic indices (VSI)	3.85±0.10	3.70±0.90	3.69±0.13	3.85±0.22

The number of bacteria found in one gram of gut content was measured in the fish receiving 80 ppm virginiamycin in the HP and LP diets and their controls. The fish fed the LP diet had a significantly higher bacterial count than the fish fed the HP diet. Virginiamycin supplementation increased the number of bacteria in both diets, however the increase was only significant when the drug was fed to the LP but not the HP diet. Results are given in table 5.52 and figure 5.37.

Summary of the performance of carp fed diet containing virginiamycin over the control is seen in table 5.53

5.6.8 Testing The Sensitivity Of *A. hydrophila* To the Drug (Virginiamycin) *in vitro* :

The results of this test showed that *Aeromonas hydrophila* was unaffected by the presence of virginiamycin, as the plates which were incorporated with the drug and the controls had similar number of this bacterium.

5.6.9 Microbiological Assay Of Antibiotic In Food And Residues In Carp Muscles, In Water And In Faeces :

This work was undertaken by commercial laboratories, because of the absence of residue detecting facilities at the University. Samples of diet containing three concentrations of drug and control from HP and LP diets were taken to Wickham Laboratories, Wickham, England. Samples for the microbiological assay of the residues in fish muscle, faeces and water were sent to Rixensart Laboratories in

Table 5.52 : Number of *A.hydrophila* (millions) per one gram of mid-gut content of carp fed diet containing virginiamycin for ten weeks. Number of samples are 8 ±S.E.

Concentration of virginiamycin (mg/kg food)			
High protein diet		Low protein diet	
80	control	80	control
3.49±0.94	1.55±0.41	9.13±3.50	5.55±0.94

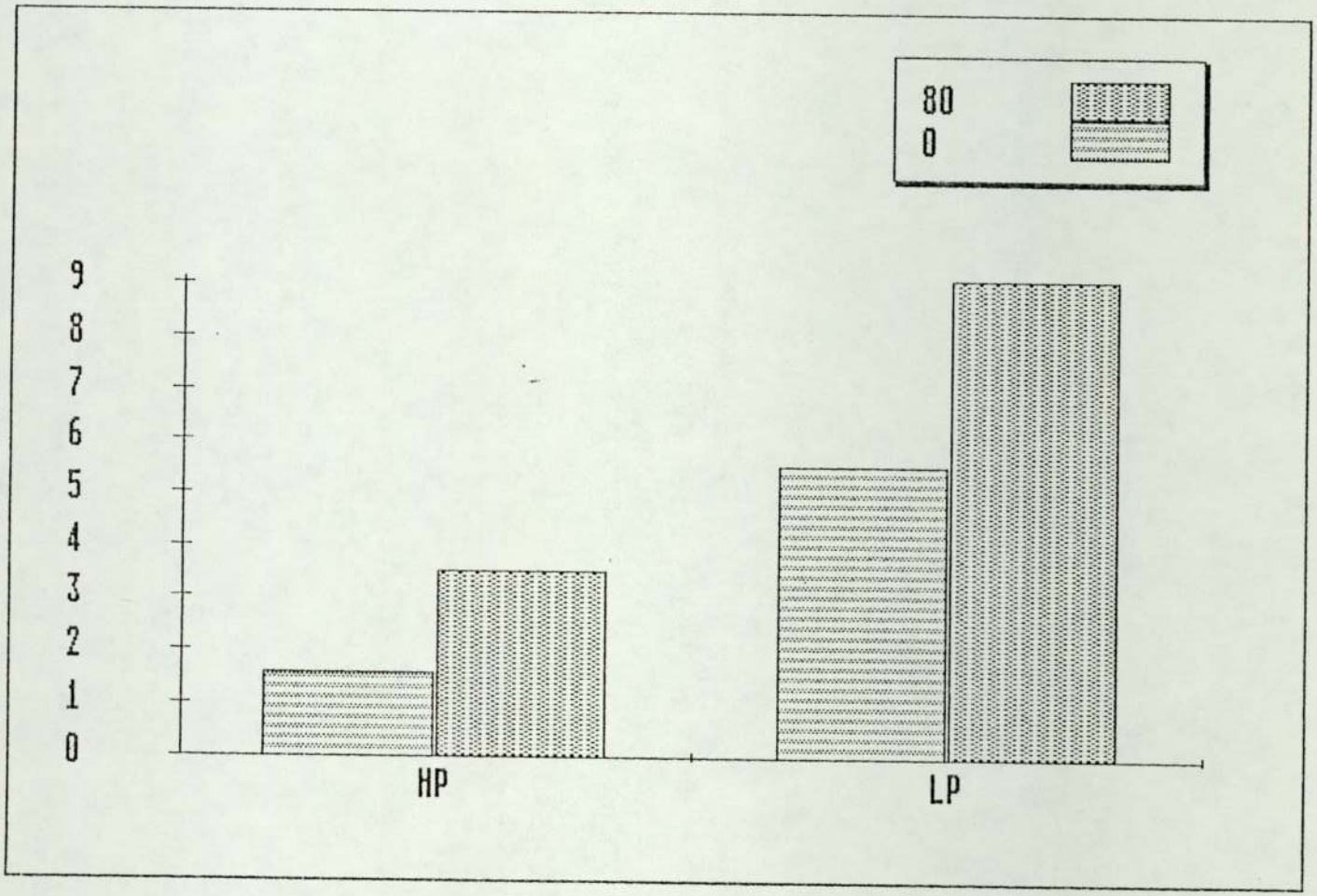


Figure 5.37 Effect of virginiamycin on the bacterial count of the mid-gut content of carp

Table 5.53 : Percentage increases in body weight and length, specific growth rate for weight and length and feed utilization efficiency of carp fed diet containing virginiamycin over the controls.

	Concentration of virginiamycin (mg/kg food)		
	100	80	40
a. HP diet :			
weight	31.0	111.6***	38.2*
length	25.6*	63.6***	27.2*
SGR (weight)	21.4*	57.3***	25.7*
SGR (length)	22.0*	50.0***	22.0*
FUE	19.4	64.5	25.8
b. LP diet :			
weight	11.4	14	-
length	15	10	5
SGR (weight)	10.9	8.2	-
SGR (length)	14.6	12.2	4.9
FUE	16	12	-

* = (P<0.05)

** = (P<0.01)

*** = (P<0.001)

Belgium. Samples of fish were taken at the end of the ten weeks experiment. These samples included fish taken one hour, 24 hours and 48 hours after feeding the HP and LP diets. Samples of water were taken from the filter. Faecal samples were collected from the bottom of the trap. All samples were immersed in liquid nitrogen and stored at -20°C until despatched to Rixensart Laboratories. The author took part in the work of measuring the residues at the Wickham Laboratories when the assay of the diet samples was made. The method is simply summarised as follows : The antibiotic activity is determined by measuring the diffusion of virginiamycin in an agar medium inoculated with *Micrococcus luteus (Sarcina lutea)*. Diffusion is indicated by the formation of zones of inhibition of growth of the microorganism. The diameter of these zones is compared with a standard curve. This method allows the measurment of virginiamycin residues equal to or greater than 50 ppb in fish tissues, faeces and water. The results are shown in tables 5.54, 5.55 and 5.56.

5.6.10 Digestibility Coefficient :

The digestibility coefficient of the HP diet was between 91.5-92% and between 89.2-90% for the LP diet. No effect of the drug on this parameter was seen with either diets.

5.6.11 Histological Examination :

The thickness of the intestine of samples from the

Table 5.54 : Expected and actual amount of virginiamycin in the diet fed to carp*

Type of diet	Virginiamycin (ppm)	
	Expected value	Actual value
Control high protein	0	trace
Control low protein	0	trace
40 ppm low protein	40	38.45
80 ppm high protein	80	77.9
80 ppm low protein	80	79.77
100 ppm high protein	100	98.95
100 ppm low protein	100	94.38

* Results supplied by Wickham laboratories, England.

Table 5.55 : Determination of virginiamycin in water and faeces from the carp trial[@].

Sample	Assay
Water	
1	*
2	*
3	*
4	*
Faeces[&]	
1	70 ng /g
2	60 ng /g

* : no detectable activity.

@ : Results supplied by Rixensart laboratories, Belgium.

& :faeces samples were pooled from all treatments including the control. The dry faeces content was estimated at 15% giving residue equivalent to 360-420 ng/g.

Table 5.56 : Virginiamycin residue in carp body tissue[@].

Virginiamycin in the diet[¬] (ppm)	Food	Hours after feeding	Assay
100	HP	1	*
=	LP	1	*
=	HP	24	*
=	LP	24	*
=	HP	48	*
=	LP	48	*
80	LP	1	*
40	LP	1	*
0	LP	1	*

[¬] : 3 fish for each treatment.

HP : high protein.

LP : low protein.

* : no detectable activity.

[@] : Results supplied by Rixensart laboratories, Belgium.

treated fish and the controls was similar.

5.7 Effect Of Feeding Virginiamycin To Rainbow Trout (Experiment 7) :

Virginiamycin was incorporated in a commercial trout diet (Edward Baker Ltd.) at concentrations of 40, 80 and 100 ppm and fed to juvenile rainbow trout *Salmo gairdneri* for ten weeks. The number of fish per treatment was 15.

5.7.1 Acceleration Of Growth :

The percentage gains in weight of the fish receiving 0, 40, 80 and 100 ppm were 119%, 147.5%, 162.1% and 183.4% respectively. In comparison to the control group the fish fed the drug supplement at 100 and 80 ppm were significantly heavier than the control ($P<0.01$ and $P<0.05$) respectively. The 40 ppm group weight gain was not significantly higher than the control ($P>0.05$). The percentage increases in length were 22.1%, 25.4%, 28% and 31.6% respectively. The increase in length was significant in the 100 and 80 ppm groups ($P<0.001$ and $P<0.01$) but not in the 40 ppm group ($P>0.05$). Results are shown in table 5.57 and 5.58 and figures 5.38 and 5.39.

5.7.2 Specific Growth Rate (Daily Gain) :

The $\hat{}^{overall}$ daily gain in weight of the fish fed the virginiamycin-supplemented diet at concentrations of 100 and 80 ppm was significantly greater than that of the control

Table 5.57 : Change in weight of rainbow trout *(Salmo gairdneri)* fed on diets containing virginiamycin for a period of ten weeks at 14±1°c. Numbers given are mean of 15 fish ±S.E.

Duration in Weeks	Concentration of virginiamycin (mg/kg food)			
	100	80	40	control
0	35.48±1.33	35.74±1.53	35.27±1.30	35.27±1.02
2	45.34±1.90 (27.8%)	42.49±2.30 (18.9%)	42.39±1.98 (20.2%)	39.87±1.92 (13.1%)
4	59.69±2.59 (68.2%)	54.15±3.40 (51.5%)	53.04±2.60 (50.4%)	50.36±3.20 (42.8%)
6	73.21±3.40 (106.3%)	66.06±3.90 (84.8%)	59.41±2.80 (68.4%)	60.74±3.70 (72.3%)
8	79.34±2.80 (123.6%)	76.20±5.30 (113.2%)	68.86±3.60 (95.2%)	68.58±4.30 (94.5%)
10	100.54±4.0 (183.4%)	93.67±6.0 (162.1%)	87.29±5.6 (147.5%)	77.27±5.2 (119.1%)

Table 5.58 : Change in length of rainbow trout (*Salmo gairdneri*) fed on diets containing virginiamycin for a period of ten weeks at 14±1°c. Numbers given are mean of 15 fish ±S.E.

Duration in Weeks	Concentration of virginiamycin (mg/kg food)			
	100	80	40	control
0	15.1±0.15	15.2±0.14	15.0±0.17	14.9±0.15
2	15.7±0.20 (4.4%)	15.3±0.22 (1.1%)	15.3±0.20 (2.0%)	15.2±0.16 (2.0%)
4	16.8±0.21 (11.9%)	16.3±0.24 (7.7%)	16.2±0.26 (8.0%)	16.0±0.25 (7.4%)
6	17.9±0.25 (18.9%)	17.3±0.27 (14.1%)	16.8±0.25 (12.0%)	16.9±0.30 (13.4%)
8	18.6±0.17 (23.6%)	18.1±0.30 (19.4%)	17.6±0.26 (17.3%)	17.5±0.37 (17.4%)
10	19.8±0.20 (31.6%)	19.4±0.34 (28.0%)	18.80±0.34 (25.4%)	18.2±0.38 (22.1%)

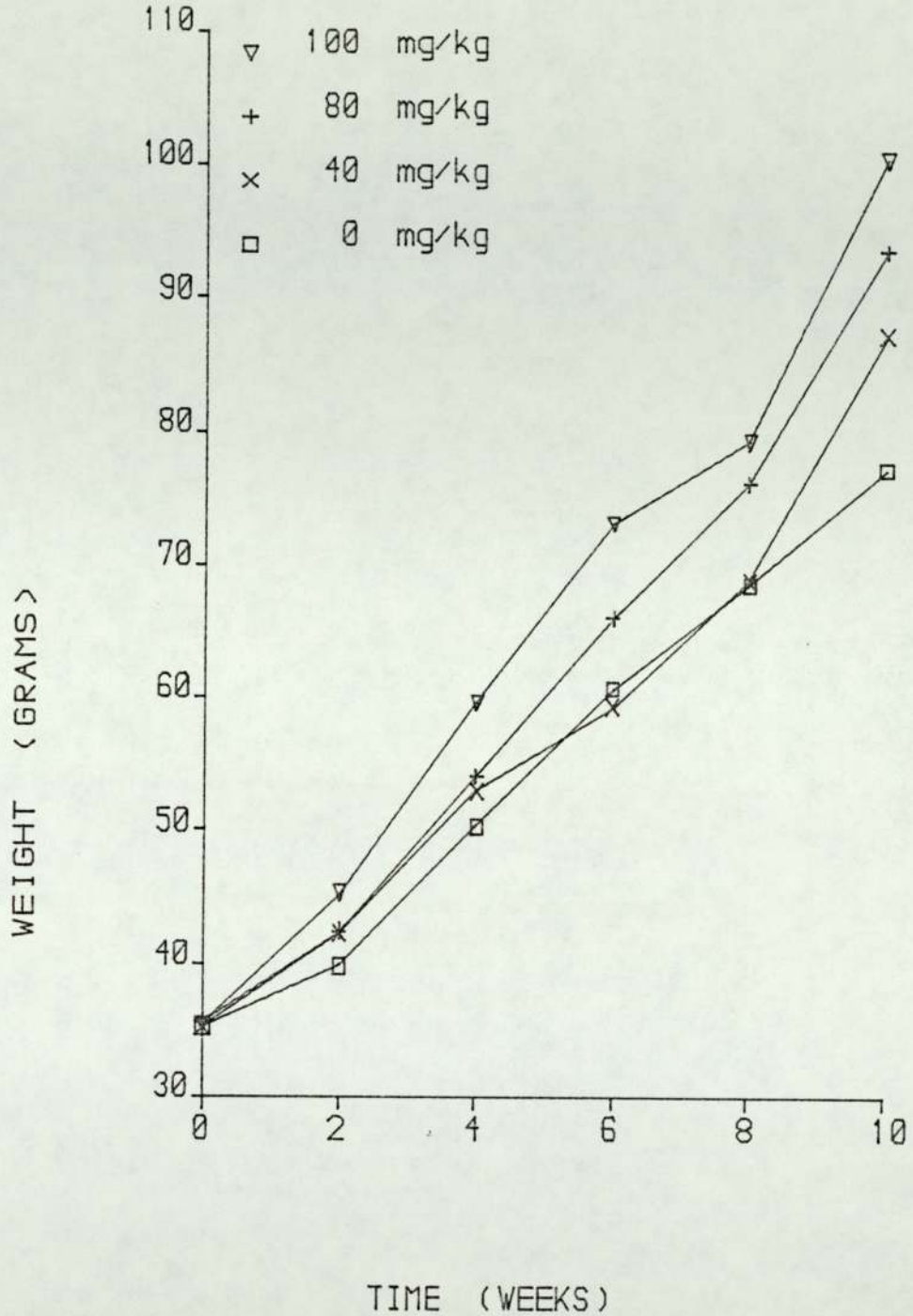


Figure 5.38 Effect of virginiamycin on the weight of rainbow trout

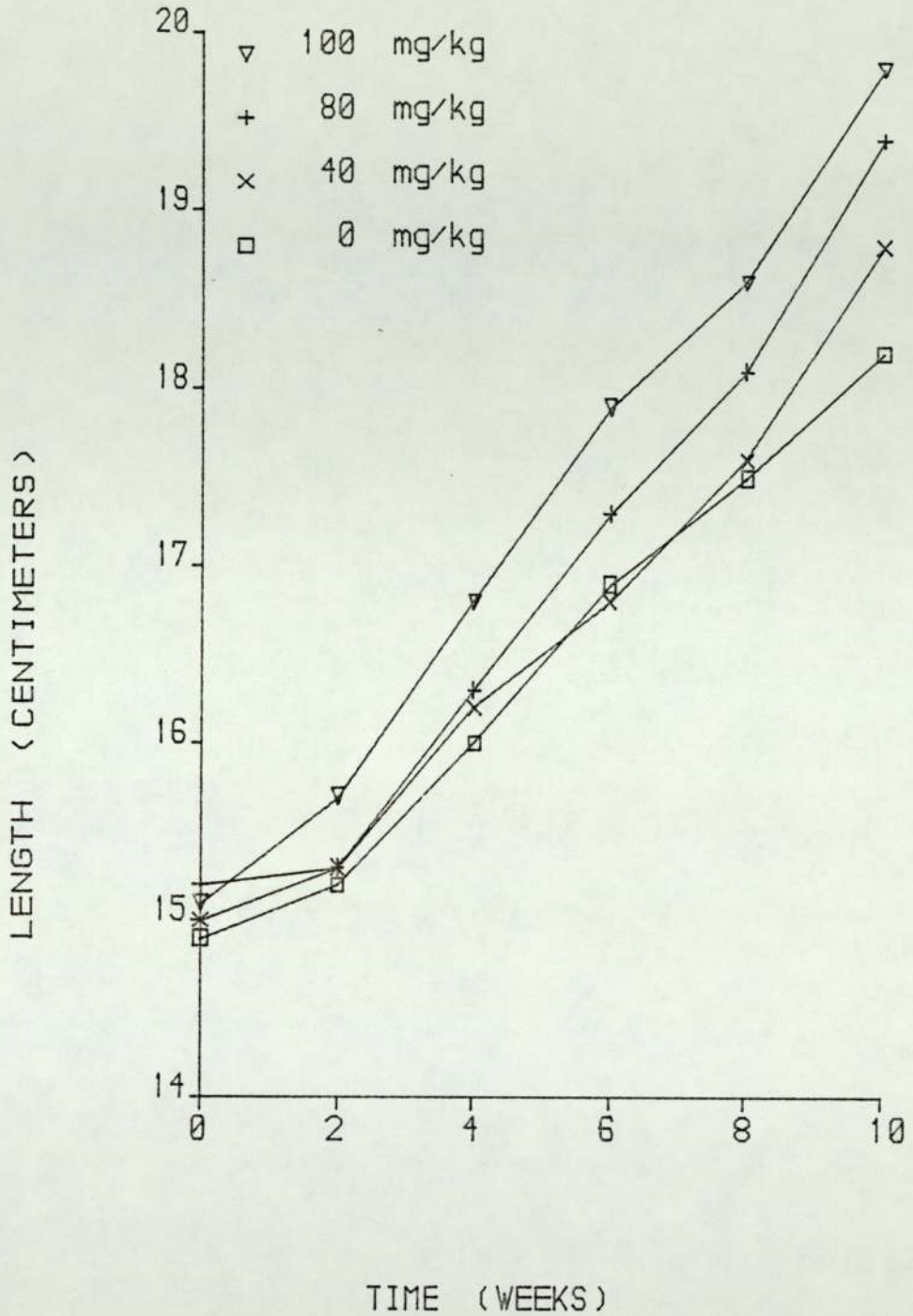


Figure 5.39 Effect of virginiamycin on the length of rainbow trout

(P<0.05). Although the 40 ppm group showed higher SGR the difference was not significant. The daily gain in length showed similar pattern to the daily gain in weight. Results are given in tables 5.59 and 5.60 and figures 5.40 and 5.41.

5.7.3 The Condition Factor :

At the start of the experiment the mean value of C was between 0.96-1.04. These values changed throughout the experiment to be between 1.23-1.29 at the end of the experiment. The fish treated with virginiamycin had slightly higher C than the control. Results are given in table 5.61.

5.7.4 Feed Utilization Efficiency (FUE) :

The trout fed the drug-treated diet generally utilized their food more efficiently than the control The overall improvement was greater the higher the dosage. Results are shown in table 5.62.

5.7.5 Proximate Analysis Of The Body Composition :

The biochemical composition of the dry muscle of the fish was analysed. The virginiamycin-treated fish appeared to have more water in their muscle than the control group, but the differences were not significant except for the 40 ppm group. The protein content in the muscle of the drug-treated fish was higher than the control, especially the 100 and 40 ppm groups (P<0.05). The trout fed the virginiamycin-supplemented diet, on the other hand, had less fat in their muscle than the fish fed the unsupplemented diet particularly

Table 5.59 : Effect of feeding virginiamycin on the specific growth rate (SGR) of rainbow trout (weight). The values are mean of 15 fish ±S.E.

Period in Weeks	Concentration of virginiamycin (mg/kg food)			
	100	80	40	control
0-2	1.9	1.3	1.4	0.95
2-4	2.1	1.9	1.7	1.8
4-6	1.6	1.5	0.90	1.4
6-8	0.62	1.1	1.1	0.90
8-10	1.8	1.6	1.8	0.90
0-10	1.49	1.36	1.27	1.1

Table 5.60 : Effect of feeding virginiamycin on the specific growth rate (SGR) of rainbow trout (length). The values are mean of 15 fish ±S.E.

Period in Weeks	Concentration of virginiamycin (mg/kg food)			
	100	80	40	control
0-2	0.33	0.10	0.15	0.15
2-4	0.53	0.49	0.44	0.39
4-6	0.47	0.44	0.28	0.42
6-8	0.30	0.34	0.36	0.27
8-10	0.48	0.53	0.51	0.30
0-10	0.39	0.35	0.32	0.29

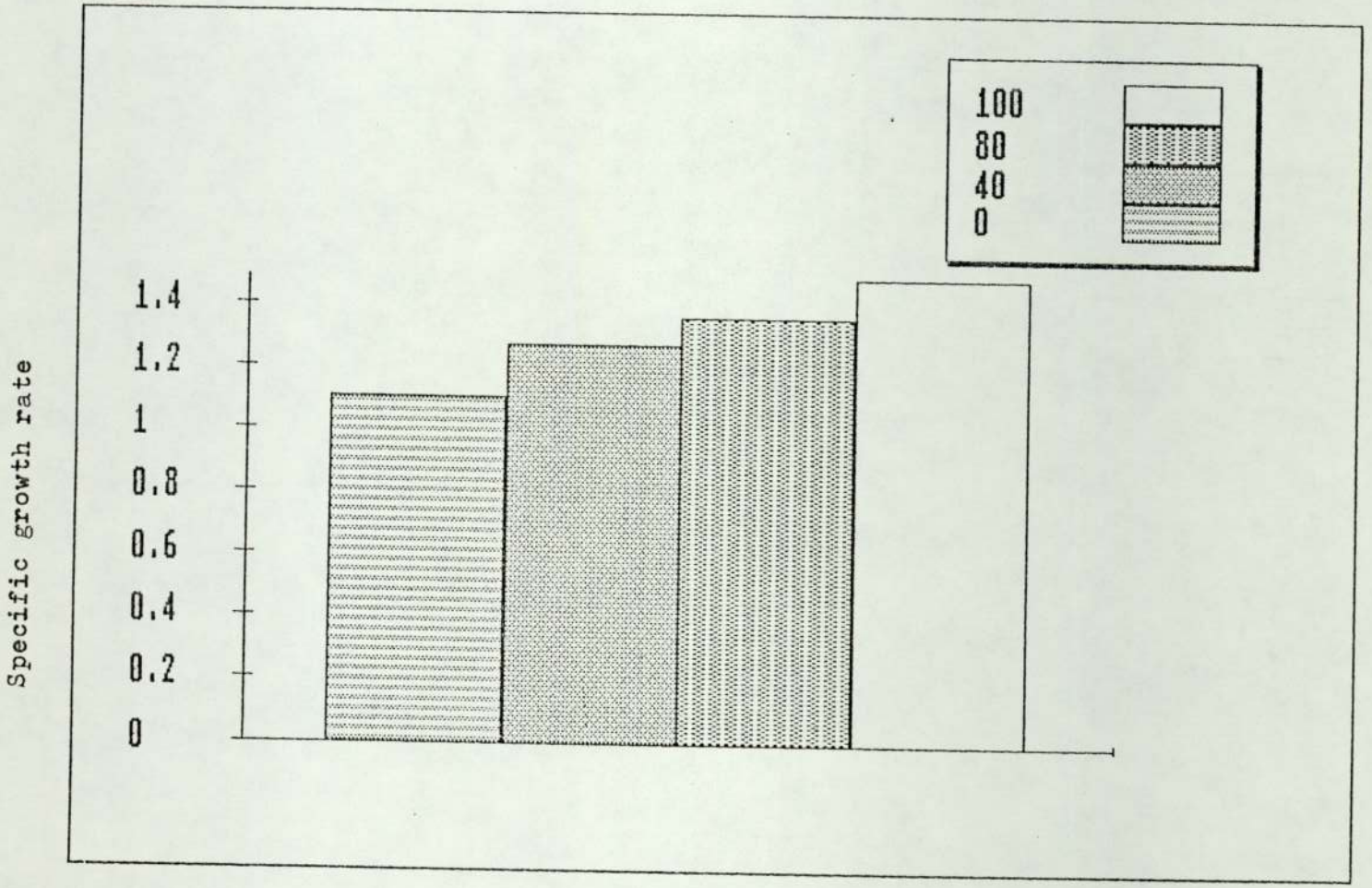


Figure 5.40 Effect of virginiamycin on the specific growth rate (weight) of rainbow trout

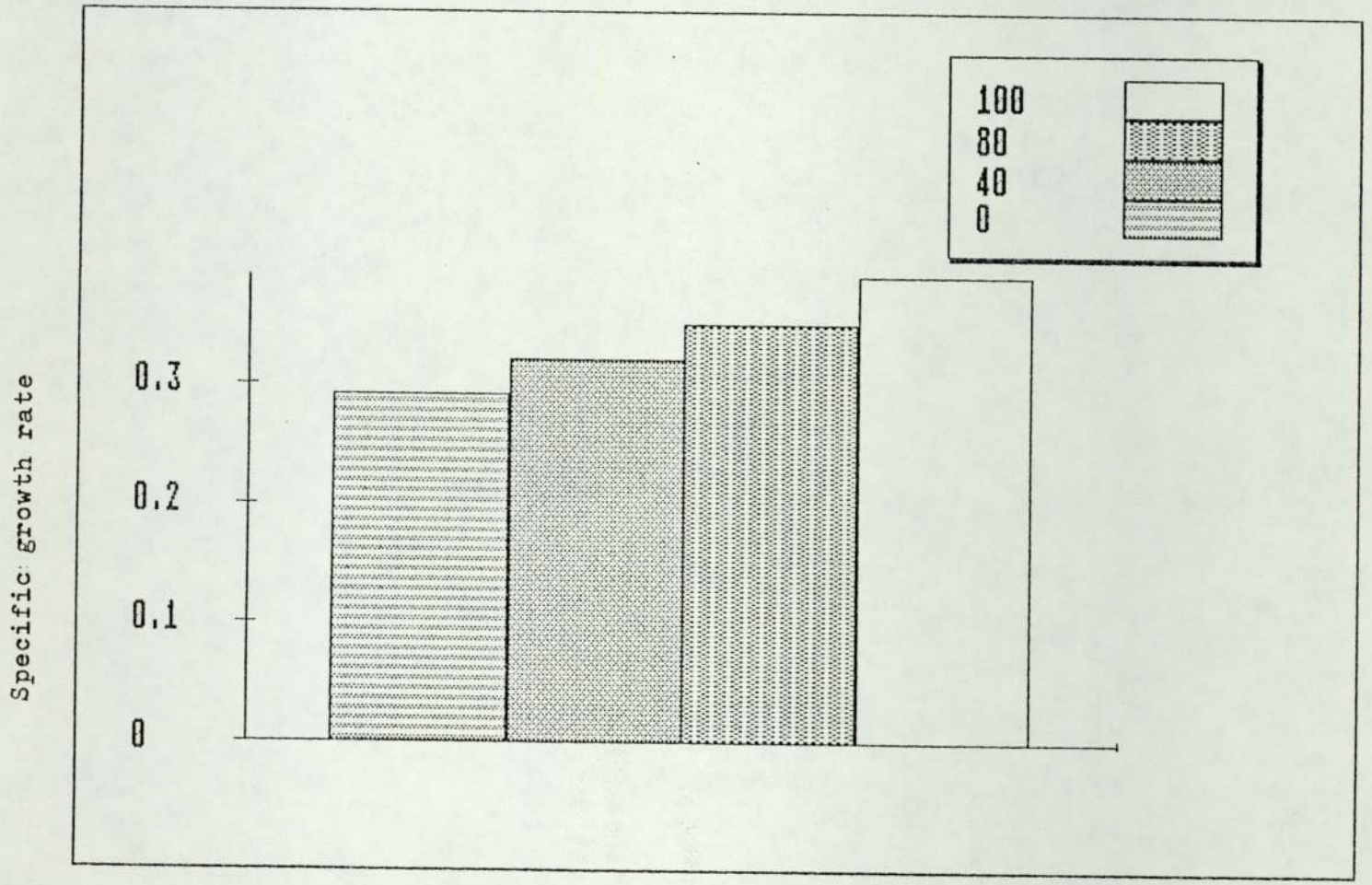


Figure 5.41 Effect of virginiamycin on the specific growth rate (length) of rainbow trout

Table 5.61 : Effect of feeding virginiamycin on the condition factor of rainbow trout. Values given are mean of 15 fish ±S.E.

Duration in Weeks	Concentration of virginiamycin (mg/kg food)			
	100	80	40	control
0	0.96±0.06	1.01±0.02	1.04±0.14	1.03±0.02
2	1.14±0.03	1.15±0.02	1.24±0.06	1.11±0.02
4	1.23±0.02	1.21±0.02	1.22±0.02	1.18±0.03
6	1.25±0.02	1.25±0.02	1.23±0.02	1.22±0.03
8	1.22±0.02	1.26±0.02	1.25±0.02	1.24±0.03
10	1.28±0.018	1.26±0.02	1.29±0.03	1.23±0.03

Table 5.62 : Effect of feeding virginiamycin on feed utilization efficiency of rainbow trout.

Period in Weeks	Concentration of virginiamycin (mg/kg food)			
	100	80	40	control
0-2	0.71	0.49	0.52	0.34
2-4	0.81	0.71	0.65	0.67
4-6	0.58	0.57	0.31	0.52
6-8	0.21	0.39	0.41	0.33
8-10	0.68	0.59	0.69	0.32
0-10	0.60	0.55	0.52	0.44

in the 100 ppm group. The ash content was similar in the treated and untreated fish. The PER and NPR were higher in the treated than in the controls. Results are shown in table 5.63.

5.7.6 Somatic Indices :

The ratio of the weight of the liver, kidney, stomach and intestine to the body weight of the trout were measured at the end of the experiment. There were no significant differences in these values between the fish fed the drug supplemented diets and the control. Results are in table 5.64.

5.7.7 Qualitative Bacteriology :

Samples were collected aseptically from the stomach, intestine and faeces and were examined qualitatively in the treated and in the controls. Table 5.65 shows the species of bacteria found in these samples. These bacteria were mainly Gram-negative rods. Gram-positive bacteria were found in a few samples of the treated and the control. In one sample of intestinal content of treated fish a yeast was found.

Further investigation was made by using scanning electron microscopy (SEM) according to the method described by Savage and Blushaw (1974) to study the bacterial settlement in the stomach and intestine coating. By using SEM it was only able to see a possible long rectilinear bacteria in the intestine samples (figure 5.42) but not in the stomach samples of the control and treated fish, as in figure 5.43.

Table 5.63 : Proximate analysis of body composition of rainbow trout fed diet containing virginiamycin. Number of samples eight fish ±S.E.

Body composition[a]	Concentration of virginiamycin (mg/kg food)			
	100	80	40	control
Water	73.5±0.11	74.2±0.10	77.0±0.61	73.0±0.16
Protein	72.3±0.13	71.6±0.30	72.6±0.38	69.12±0.30
Fat	13.5±0.07	14.0±0.19	14.75±0.26	15.5±0.08
Ash	7.6±0.06	7.05±0.09	6.9±0.06	7.4±0.03
PER[b]	7.1	6.07	5.72	4.76
NPR[c]	23.2	20.1	17.0	16.9

[a] : calculated on dry basis

[b] : protein efficiency ratio

[c] : net protein retention

Table 5.64 : Hepato-somatic, Reno-somatic and viscero-somatic indices of rainbow trout fed diet containing virginiamycin. Number of samples eight fish ±S.E.

Tissue indices	Concentration of virginiamycin (mg/kg food)			
	100	80	40	control
Hepato-somatic indices (HSI)	1.47±0.05	1.45±0.05	1.59±0.04	1.53±0.15
Reno-somatic indices (RSI)	0.77±0.07	0.98±0.06	0.76±0.06	0.89±0.05
Viscero-somatic indices (VSI)	1.02±0.10	0.89±0.03	1.02±0.13	1.30±0.10
Stomach	1.50±0.04	1.59±0.04	1.43±0.10	1.48±0.10

Table 5.65 : Bacterial species found in the content of the digestive tract of rainbow trout fed diet containing virginiamycin.

Bacterial species	Gram-stain & shape	Stomach	Intestine	Faeces
Pseudommonas maltophilia	g- rod	-	++	+
Serratia liquefacins	g- rod	+	++	-
Yersinia pseudotuberclosis	g- rod	+	++	+
Pseudomonas cepacia	g- rod	+	++	+
Pseudomonas paucimobilis	g- rod	+	++	+
Micrococcus	g+ cocci	+	+	+
Yeast		-	+	-

- = not found

+ = few

++ = abundant

Figure 5.42 Medial intestine of trout showing ^ long rod bacteria SEM X 3600

a possible

Figure 5.43 Stomach of trout. No bacterial presence SEM X 400

Summary of the performance of rainbow trout fed diet containing virginiamycin over the control is seen in table 5.66.

5.8 Effect Of Feeding Emtryl To Carp (Experiment 8) :

The protozocide emtryl was added to a high protein diet of juvenile carp at concentrations of 50, 100, and 150 mg per kg for ten weeks. The number of fish was 15.

5.8.1 Acceleration Of Growth :

The percentage increases in the weight of the fish fed emtryl at concentrations of 0, 50, 100 and 150 were 178.2%, 181.2%, 180.5% and 181.7% respectively, and the percentage increases in length were 38%, 40.2%, 39.2% and 41.4% respectively. The differences in the gain in weight and length between the treated fish and that of the group fed the unsupplemented diet were not significant ($P>0.05$). The results are shown in tables 5.67 and 5.68 and figures 5.44 and 5.45.

5.8.2 Specific Growth Rate (Daily Gain) :

There were no significant differences between the daily gain in weight and length of the fish treated with emtryl and the control throughout the experiment. The results are in tables 5.69 and figure 5.46.

5.8.3 Feed Utilization Efficiency (FUE) :

The ratio of weight gain to the food given of the fish

Table 5.66 : Percentage increases in body weight and length, specific growth rate for weight and length and feed utilization efficiency of rainbow trout fed diet containing virginiamycin over the controls.

	Concentration of virginiamycin (mg/kg food)		
	100	80	40
weight	54.9**	37.9*	23.9
length	42.4**	27.3*	15.5
SGR (weight)	35.5**	23.6*	10.1
SGR (length)	34.5**	20.7*	10.3
FUE	36.7	25.0	18.2

* = ($P<0.05$)

** = ($P<0.01$)

Table 5.67 : Change in weight of carp *(Cyprinus carpio)* fed on diets containing emtryl for a period of ten weeks at 25±1°c. Numbers given are mean of 15 fish ±S.E.

Duration in Weeks	Concentration of emtryl (mg/kg food)			
	150	100	50	control
0	16.4±0.8	16.4±1.1	16.5±1.1	16.5±0.7
2	20.2±0.19 (23.2%)	19.8±1.5 (20.7%)	22.2±1.6 (34.5%)	21.3±1.3 (29.1%)
4	26.3±1.9 (60.4%)	25.5±1.9 (55.5%)	29.0±2.2 (75.8%)	27.7±2.0 (67.9%)
6	34.0±2.2 (107.3%)	32.4±2.3 (96.6%)	36.0±3.2 (118.2%)	35.1±2.8 (112.7%)
8	39.4±3.5 (140.2%)	39.3±3.1 (139.6%)	40.3±4.2 (144.2%)	38.7±3.3 (134.5%)
10	46.2±4.0 (181.7%)	46.0±4.1 (180.5%)	46.5±5.3 (181.2%)	45.9±4.2 (178.2%)

Table 5.68 : Change in length of carp *(Cyprinus carpio)* fed on diets containing emtryl for a period of ten weeks at 25±1°c. Numbers given are mean of 15 fish ±S.E.

Duration in Weeks	Concentration of emtryl (mg/kg food)			
	150	100	50	control
0	9.9±0.04	9.7±0.07	9.7±0.07	10.0±0.07
2	10.6±0.2 (7.0%)	10.5±0.3 (8.0%)	10.7±0.2 (10.3%)	10.8±0.2 (8.0%)
4	11.3±0.3 (14.0%)	11.2±0.3 (15.5%)	11.7±0.3 (20.6%)	11.6±0.3 (16.0%)
6	12.5±0.4 (26.0%)	12.2±0.3 (25.8%)	12.7±0.3 (30.9%)	12.3±0.3 (23.0%)
8	13.4±0.4 (35.0%)	12.8±0.4 (32.0%)	13.1±0.4 (35.0%)	13.3±0.4 (33.0%)
10	14.0±0.4 (41.4%)	13.5±0.4 (39.2%)	13.6±0.4 (40.2%)	13.8±0.4 (38.0%)

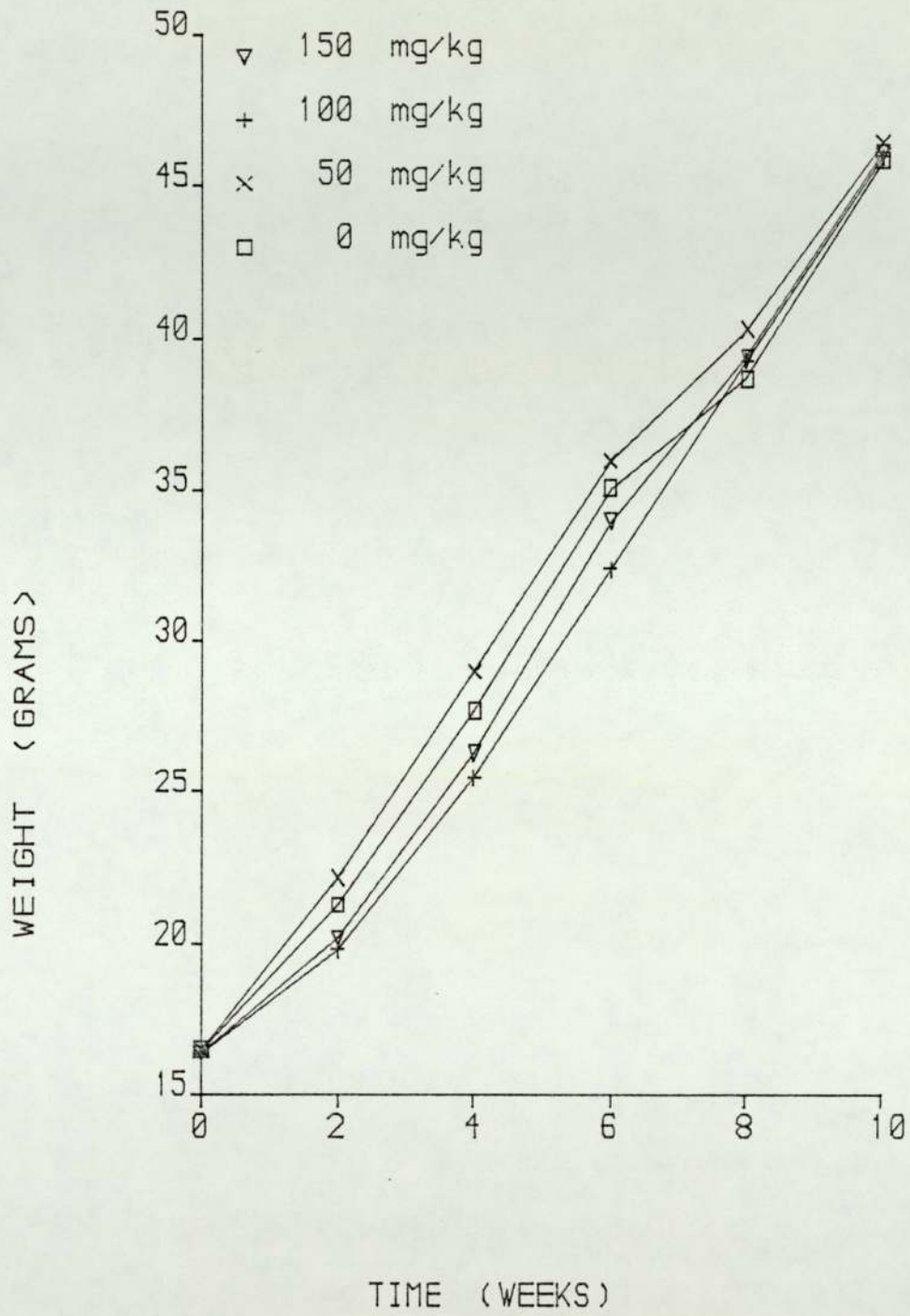


Figure 5.44 Effect of emtryl on the weight of carp

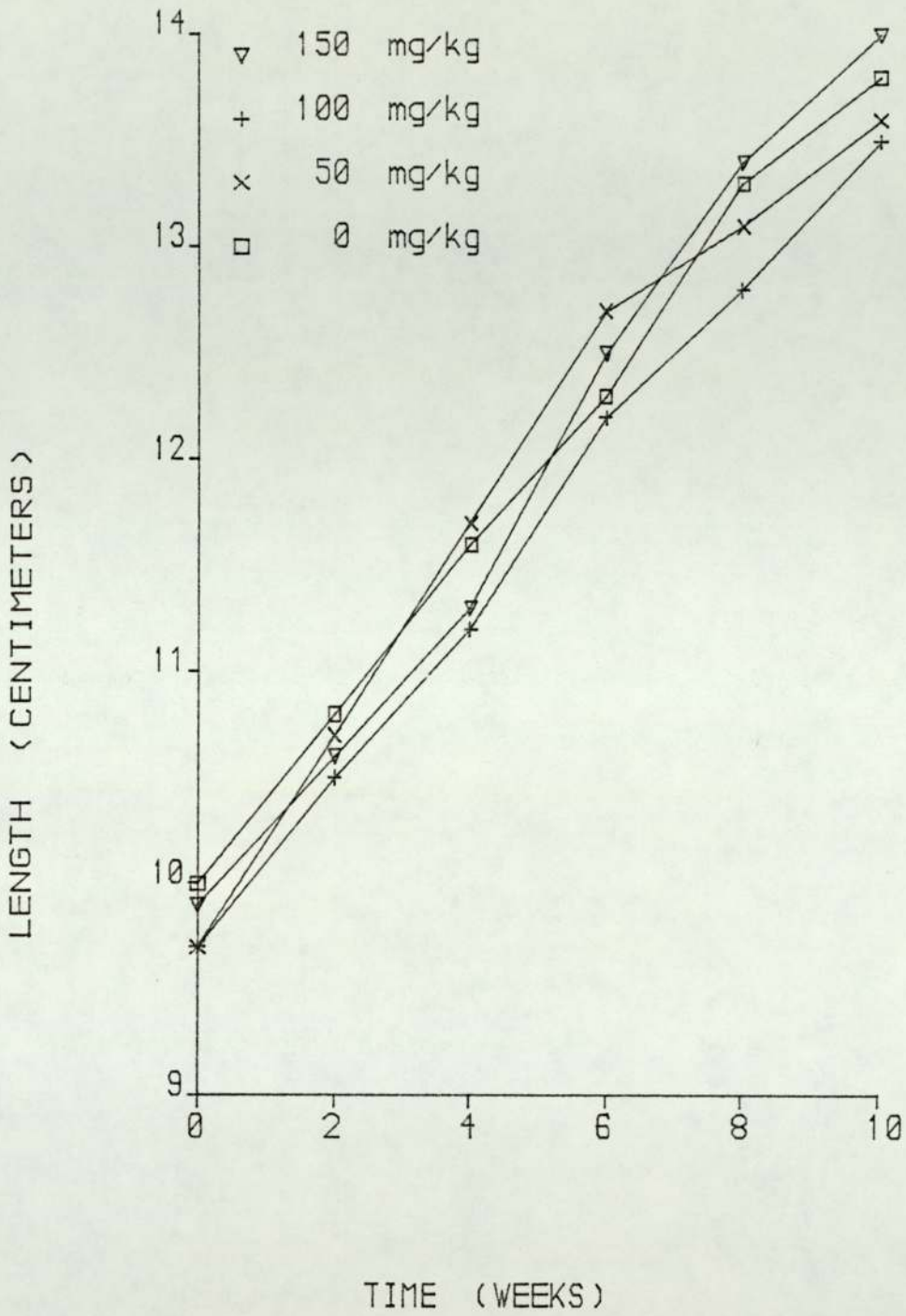


Figure 5.45 Effect of emtryl on the length of carp

Table 5.69 : Effect of feeding emtryl on the specific growth rate (SGR) of carp (weight). The values are mean of 15 fish ±S.E.

Period in Weeks	Concentration of emtryl (mg/kg food)			
	150	100	50	control
0-2	1.6	1.4	2.3	1.96
2-4	2.0	1.95	2.1	2.0
4-6	2.0	1.8	1.7	1.8
6-8	1.1	1.5	0.87	0.75
8-10	1.2	1.2	1.1	1.3
0-10	1.58	1.57	1.60	1.56

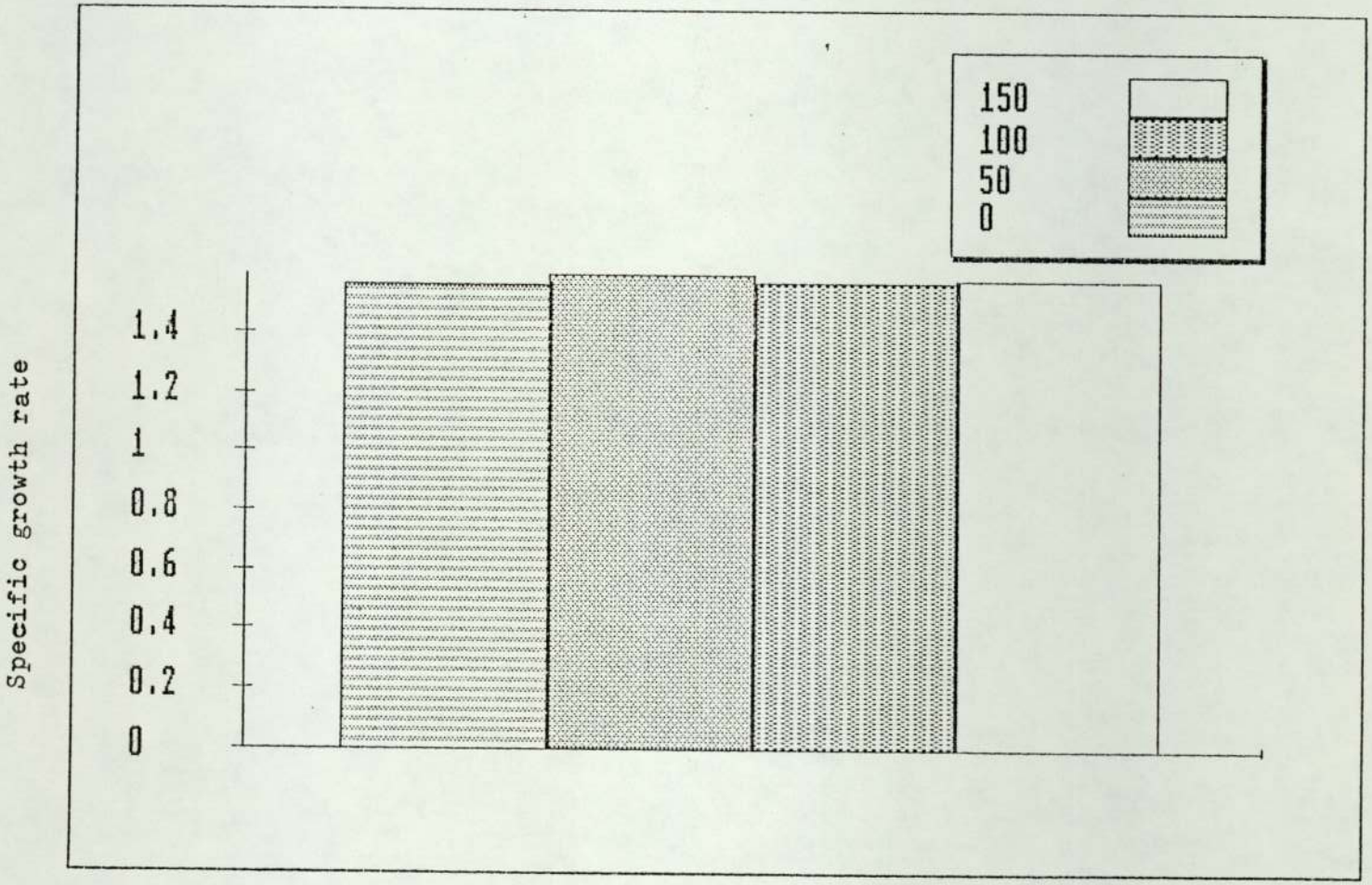


Figure 5.46 Effect of emtryl on the specific growth rate (weight) of carp

fed the supplemented diet was similar to that of the fish fed the unsupplemented diet. Results are shown in table 5.70.

5.8.4 Proximate Analysis Of The Body Composition :

The muscle of all groups of fish were analysed for their water, protein, fat and ash content. No differences were found between groups in any of these parameters. The PER and NPR were similar in the treated and untreated fish. Results are given in table 5.71.

5.8.5 Somatic Indices :

The ratios of the weight of the liver, kidney and intestine to the body weight of the fish treated with emtryl were similar to those of the control as shown in table 5.72.

5.8.6 Bacterial Count :

Although emtryl is a protozocide rather than antibacterial agent, the bacterial count of one gram of the mid-gut content was also measured, to compare the results of this experiment with the experiments where antibacterial agents were used. No significant differences in the bacterial count of the fish receiving the drug-supplemented diet and the control were found. Results are shown in table 5.73 and figure 5.47.

5.8.7 Histological Examination :

The comparison between the intestinal sections of the fish consuming the drug-treated diet and the control revealed

Table 5.70 : Effect of feeding emtryl on feed utilization efficiency of carp.

Period in Weeks	Concentration of emtryl (mg/kg food)			
	150	100	50	control
0-2	0.30	0.32	0.53	0.45
2-4	0.46	0.44	0.47	0.46
4-6	0.56	0.52	0.46	0.51
6-8	0.31	0.41	0.36	0.20
8-10	0.33	0.30	0.30	0.36
0-10	0.39	0.40	0.41	0.38

Table 5.71 : Proximate analysis of body composition of carp fed diet containing emtryl. Number of samples eight fish ±S.E.

Body composition[a]	Concentration of emtryl (mg/kg food)			
	150	100	50	control
Water	77.0±0.75	77.3±0.20	78.2±0.20	77.7±0.31
Protein	73.3±0.86	75.6±0.83	73.5±0.90	73.9±0.80
Fat	9.2±1.0	10.2±0.90	8.9±0.70	10.0±0.80
Ash	7.0±0.12	6.9±0.21	7.2±0.10	7.0±0.13
PER[b]	1.0	1.0	0.98	1.0
NPR[c]	17.0	17.2	16.2	16.5

[a] : calculated on dry basis

[b] : protein efficiency ratio

[c] : net protein retention

Table 5.72 : Hepato-somatic, Reno-somatic and viscero-somatic indices of carp fed diet containing emtryl. Number of samples eight fish ±S.E.

Tissue indices	Concentration of emtryl (mg/kg food)			
	150	100	50	control
Hepato-somatic indices (HSI)	1.7±0.14	1.8±0.14	1.9±0.10	1.8±0.12
Reno-somatic indices (RSI)	0.58±0.03	0.64±0.05	0.57±0.05	0.50±0.02
Viscero-somatic indices (VSI)	3.7±0.20	4.1±0.10	3.4±0.12	3.9±0.20

Table 5.73 : Number of *A.hydrophila* (millions) per one gram of mid-gut content of carp fed diet containing emtryl for ten weeks. Number of samples are 8 ±S.E.

Concentration of emtryl (mg/kg food)			
150	100	50	control
1.69±0.30	1.60±0.61	1.31±0.36	1.72±0.85

Figure 5.47 Effect of emtryl on the bacterial count of mid-gut content of carp

that these sections were similar.

5.9 Effect Of Injection Of Zeranol Into Carp (Experiment 9) :

In this experiment the weak hormone-like compound zeranol was injected subcutaneously. The zeranol-containing tablets (12mg) were each dissolved in 3 ml distilled water, and 0.2 ml of the solution was injected into each fish; the actual dosage per fish per week was 0.8 mg, for eight weeks. The control fish were injected with 0.2 ml distilled water only. The fish were offered commercial trout diet during the experiment.

5.9.1 Acceleration Of Growth :

The percentage increase in the weight of the zeranol-injected fish was 73.3% and 64.4% for the control; the percentage increase in length was 20.3% and 15.6% for the control group. The increments in the weight and length of the injected fish were not significantly different from the control ($P>0.05$). Results are shown in tables 5.74 and 5.75 and figures 5.48 and 5.49.

5.9.2 Specific Growth Rate (Daily Gain) :

The mean daily weight and length gain of carp injected with zeranol were greater than the control in all but one case the experiment. The overall improvement in the SGR of weight and length of the treated fish were significantly greater ($P<0.01$) and ($P<0.001$) respectively. Results are given in

Table 5.74 : Change in weight of carp *(Cyprinus carpio)* injected with 0.8 mg zeranol per fish per week for eight weeks at 25±1°c. Numbers given are mean of 12 fish ±S.E.

Duration in Weeks	Concentration of zeranol (mg/fish)	
	0.8	control
0	76.71±4.7	82.50±4.3
2	84.3±5.2 (9.8%)	90.00±4.8 (9.1%)
4	102.51±6.9 (33.6%)	106.69±5.6 (29.3%)
6	116.6±7.7 (52.0%)	118.70±6.9 (43.9%)
8	133.1±7.9 (73.5%)	135.6±7.2 (64.4%)

Table 5.75 : Change in length of carp *(Cyprinus carpio)* injected with 0.8 mg zeranol per fish per week for eight weeks period at 25±1°c. Numbers given are mean of 12 fish ±S.E.

Duration in Weeks	Concentration of zeranol (mg/fish)	
	0.8	control
0	16.3±0.34	16.7±0.30
2	16.8±0.32 (3.0%)	17.0±0.32 (1.8%)
4	17.4±0.30 (6.7%)	17.4±0.32 (4.3%)
6	18.9±0.50 (16.0%)	18.8±0.40 (12.6%)
8	19.6±0.5 (20.3%)	19.3±0.6 (15.6%)

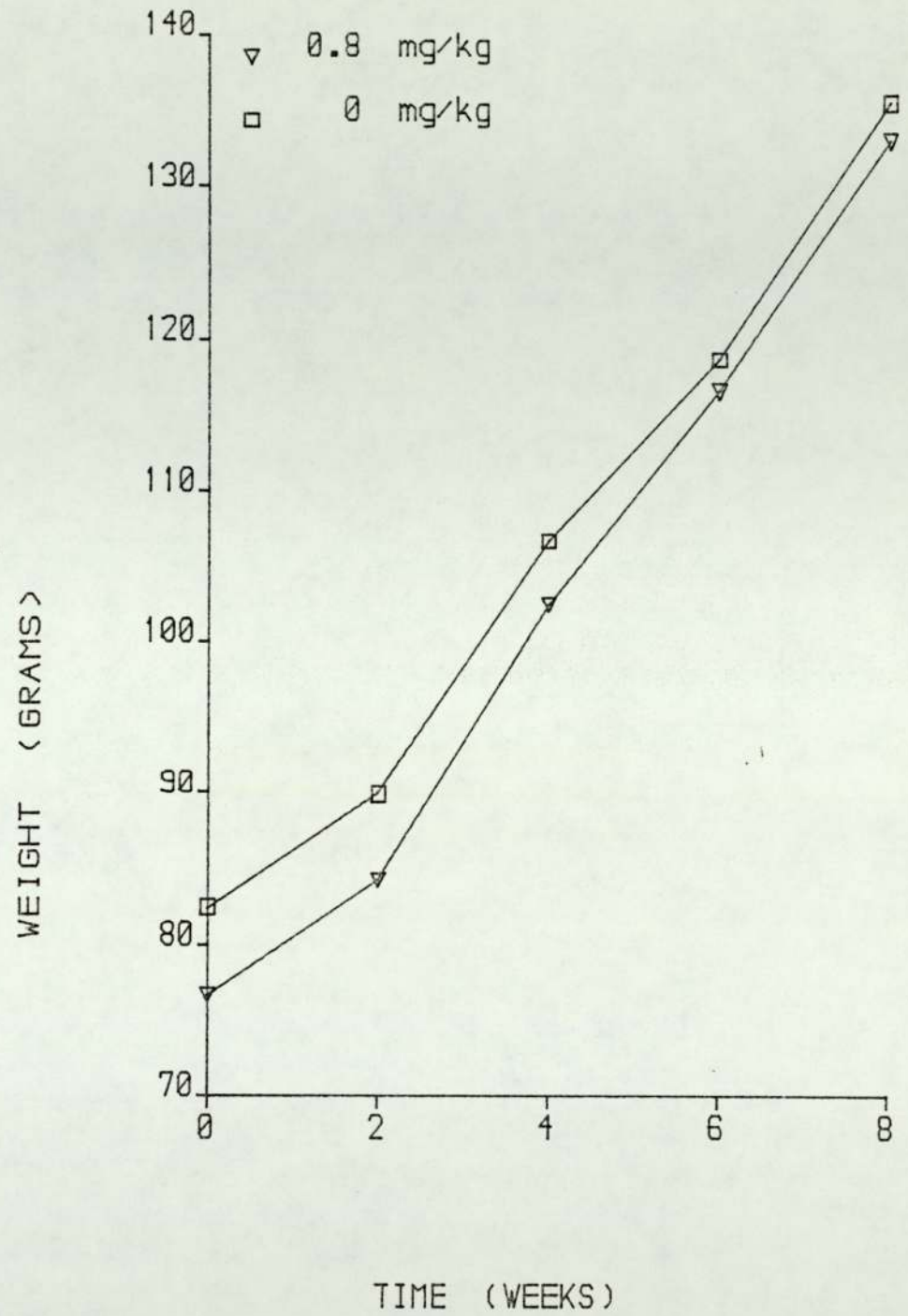


Figure 5.48 Effect of injecting zeranol on the weight of carp

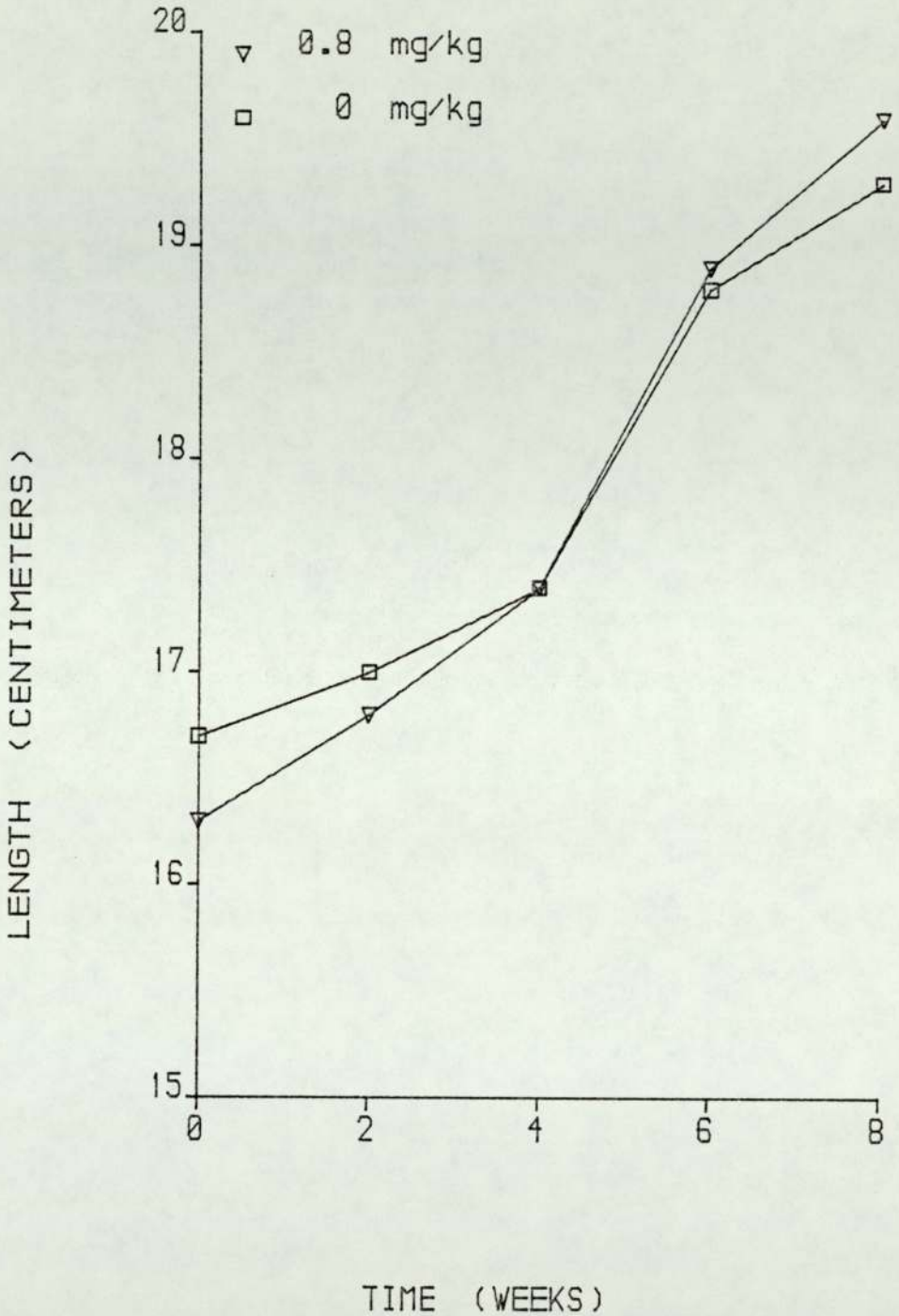


Figure 5.49 Effect of injecting zeranol on the length of carp

tables 5.76 and 5.77 and figures 5.50 and 5.51

5.9.3 Feed Utilization Efficiency (FUE) :

The fish injected with zeranol had higher FUE values than the control group throughout the experiment except during the last two weeks when both groups had the same FUE values. However the overall values were higher in the treated than in the control. Results are shown in table 5.78.

5.9.4 Proximate Analysis Of The Body Composition :

The biochemical composition of the dried muscle of the fish treated with zeranol was compared with that of the control. The protein and water content of the treated fish were significantly higher than those of the control. The fat content on the other hand was significantly lower in the treated fish than in the control. The PER and NPR were higher in the injected fish than in the control. Results are given in table 5.79.

5.9.5 Somatic Indices :

The ratios of the weight of the body organ (liver, kidney and intestine) to the body weight were measured for both groups of fish. The results showed that zeranol did not have a significant effect on these parameters as shown in table 5.80.

5.10 Effect Of Injecting Terramycin Or Virginiamycin Into Carp (Experiment 10) :

Pure terramycin was dissolved in 0.9% saline and 0.2 ml

Table 5.76 : Effect of injecting 0.8 mg zeranol per fish, weekly on the specific growth rate (SGR) of carp (weight). The values are mean of 12 fish ±S.E.

Period in Weeks	Concentration of zeranol (mg/fish)	
	0.8	control
0-2	0.73	0.67
2-4	1.50	1.31
4-6	0.99	0.82
6-8	1.02	1.02
0-8	1.00	0.90

Table 5.77 : Effect of injecting 0.8 mg zeranol per fish, weekly on the specific growth rate (SGR) of carp (length). The values are mean of 12 fish ±S.E.

Period in Weeks	Concentration of zeranol (mg/fish)	
	0.8	control
0-2	0.23	0.14
2-4	0.27	0.18
4-6	0.64	0.59
6-8	0.28	0.20
0-8	0.33	0.26

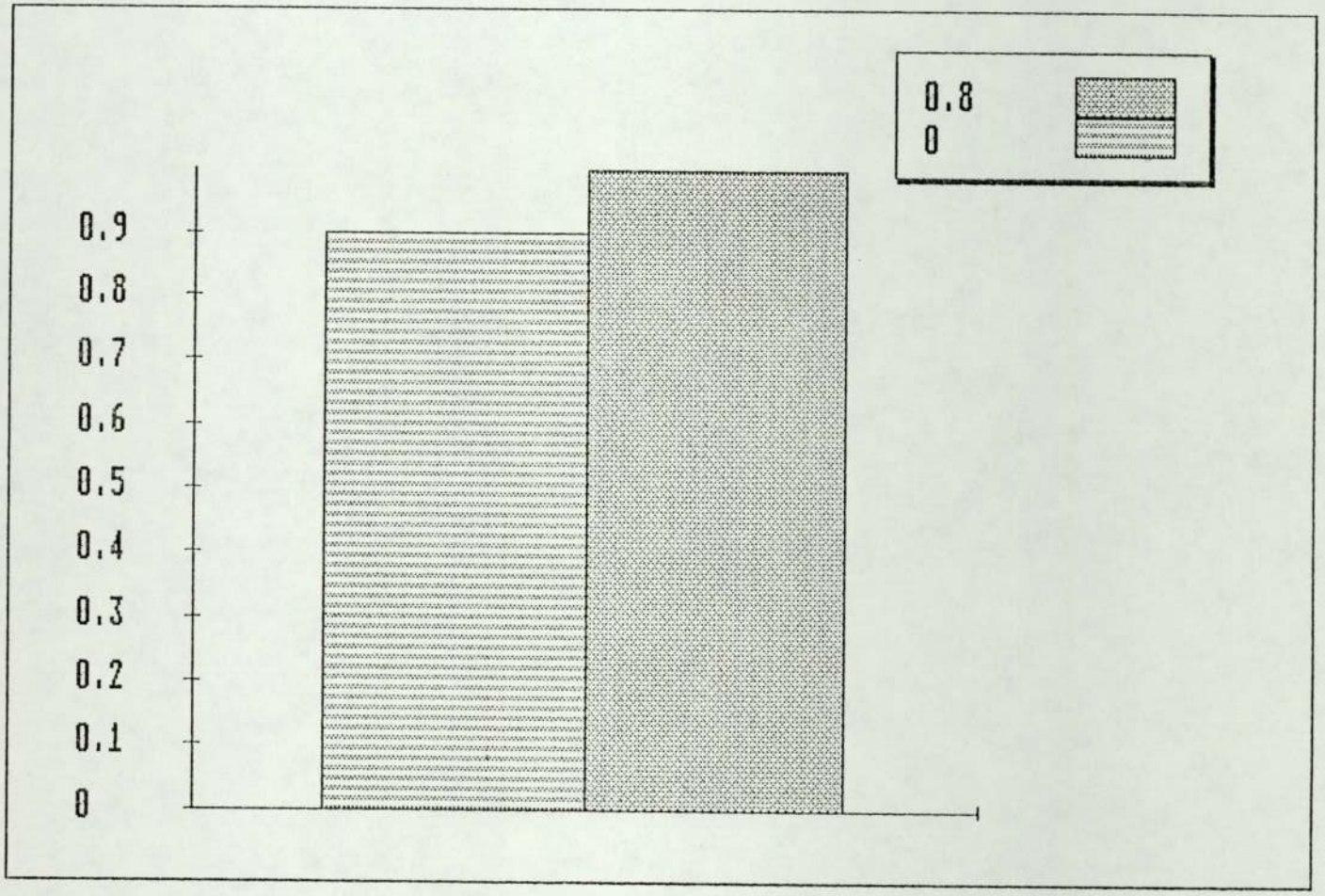


Figure 5.50 Effect of injecting zeranol on the specific growth rate (weight) of carp

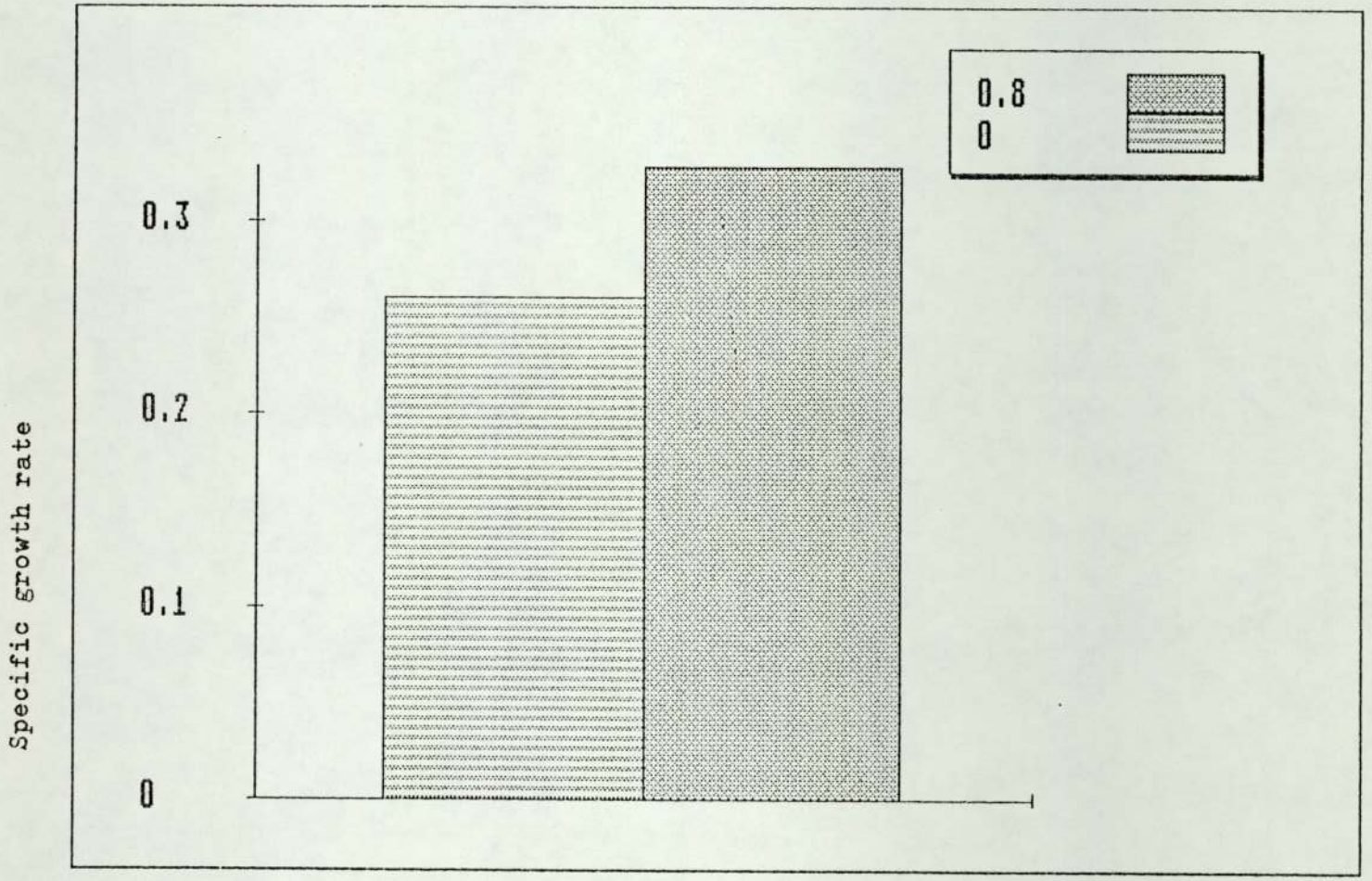


Figure 5.51 Effect of injecting zeranol on the specific growth rate (length) of carp

Table 5.78 : Effect of injecting 0.8 mg zeranol on feed utilization efficiency of carp.

Period in Weeks	Concentration of zeranol (mg/kg food)	
	0.8	control
0-2	0.38	0.35
2-4	0.83	0.70
4-6	0.59	0.45
6-8	0.60	0.60
0-8	0.60	0.53

Table 5.79 : Proximate analysis of body composition of carp injected with zeranol. Number of samples four fish ±S.E.

Body composition[a]	Concentration of zeranol (mg/fish)	
	0.8	control
Water	78.0±0.7	77.15±0.86
Protein	74.9±1.7	71.5±1.2
Fat	10.0±0.7	10.85±0.7
PER[b]	1.28	1.06
NPR[c]	22.0	18.0

[a] : calculated on dry basis

[b] : protein efficiency ratio

[c] : net protein retention

Table 5.80: Hepato-somatic, Reno-somatic and virscero-somatic indices of carp injected zeranol. Number of samples four fish ±S.E.

Tissue indices	Concentration of zeranol (mg/fish)	
	0.8	control
Hepato-somatic indices (HSI)	3.29±0.29	2.94±0.30
Reno-somatic indices (RSI)	0.56±0.09	0.54±0.06
Viscero-somatic indices (VSI)	5.3±1.0	5.41±1.1

of the solution, to give a dosage of 2 mg per fish, was injected each week. Another group of fish was injected with virginiamycin by dissolving a powder containing 10% active drug in 0.9% saline, and 0.2 ml (containing 13 mg drug) was injected into the fish each week. The control fish were injected with 0.2 ml saline only. The number of fish in each treatment was 12 and the drugs were injected for four weeks. Drug treatment was stopped during the final week of the experiment.

5.10.1 Acceleration Of Growth :

The percentage increases in weight of the fish injected with 2 mg terramycin per fish and the control were 18.3% and 19.8%, and the percentage increases in body length were 6.0% and 6.2% respectively. The percentage increase in the weight and length of fish injected with 13 mg virginiamycin per fish was 19% and 5.9% respectively. Using the "t" test it was shown that the differences in the growth of the fish injected with both drugs and the control were not significant ($P>0.05$). Results are given in table 5.81 and 5.82 and figures 5.52 and 5.53.

5.10.2 Specific Growth Rate(Daily Gain) :

The daily increase in weight and length of the treated fish over that of the control was not significant. Results are shown in table 5.83 and figure 5.54.

Table 5.81 : Change in weight of carp *(Cyprinus carpio)* injected with drugs for four weeks at 25±1°c. Numbers given are mean of 12 fish ±S.E.

Duration in Weeks	Concentration of the drug injected per fish		
	terramycin (2.0 mg)	virginiamycin (13.0 mg)	saline (control) (0.2 ml)
0	56.4±3.7	56.7±3.7	57.0±4.7
1	59.2±3.9 (5.0%)	58.5±3.6 (3.2%)	58.6±4.9 (2.8%)
2	60.5±3.9 (7.3%)	64.7±3.9 (14.1%)	61.6±5.0 (8.1%)
3	62.4±4.0 (10.6%)	64.5±3.8 (14.0%)	63.9±5.0 (12.0%)
4	66.7±4.3 (18.3%)	67.5±3.9 (19.0%)	68.3±5.0 (19.8%)
5*	74.0±4.7 (31.2%)	71.6±4.0 (26.3%)	73.8±5.5 (29.5%)

* The drug was withdrawn after four weeks.

Table 5.82 : Change in length of carp *(Cyprinus carpio)* injected with drugs for four weeks at 25±1°c. Numbers given are mean of 12 fish ±S.E.

Duration in Weeks	Concentration of the drug injected per fish		
	terramycin (2.0 mg)	virginiamycin (13.0 mg)	saline (control) (0.2 ml)
0	15.0±0.34	15.2±0.33	15.2±0.43
1	15.4±0.35 (2.7%)	15.5±0.32 (2.0%)	15.6±0.42 (2.6%)
2	15.5±0.34 (3.3%)	15.7±0.30 (3.3%)	15.7±0.39 (3.3%)
3	15.7±0.35 (4.7%)	15.8±0.32 (3.9%)	16.0±0.40 (5.3%)
4	15.9±0.36 (6.0%)	16.1±0.39 (5.3%)	16.2±0.39 (6.2%)
5*	16.4±0.30 (8.7%)	16.3±0.30 (6.6%)	16.5±0.40 (8.5%)

* The drug was withdrawn after four weeks.

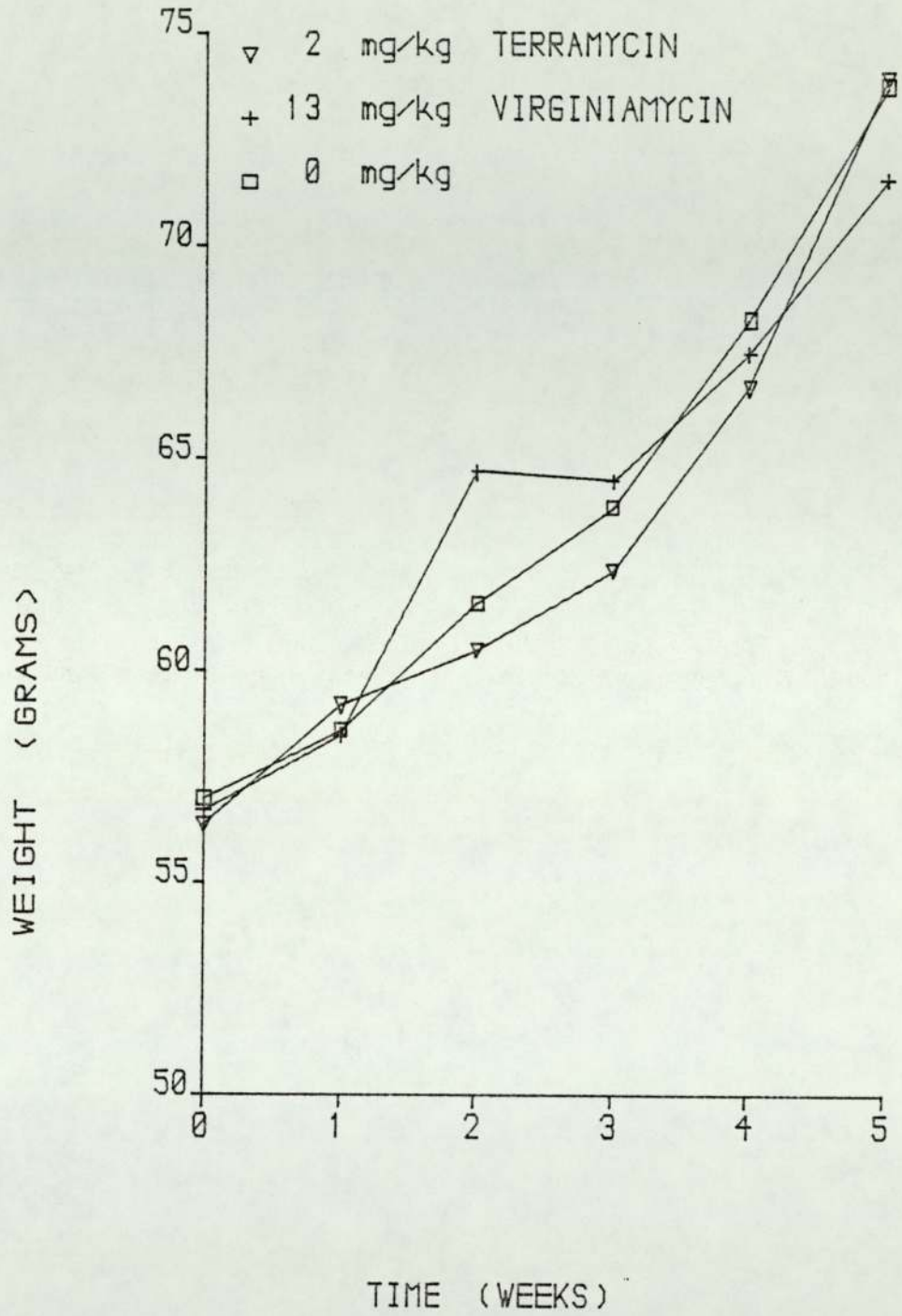


Figure 5.52 Effect of injecting terramycin or virginiamycin on the weight of carp

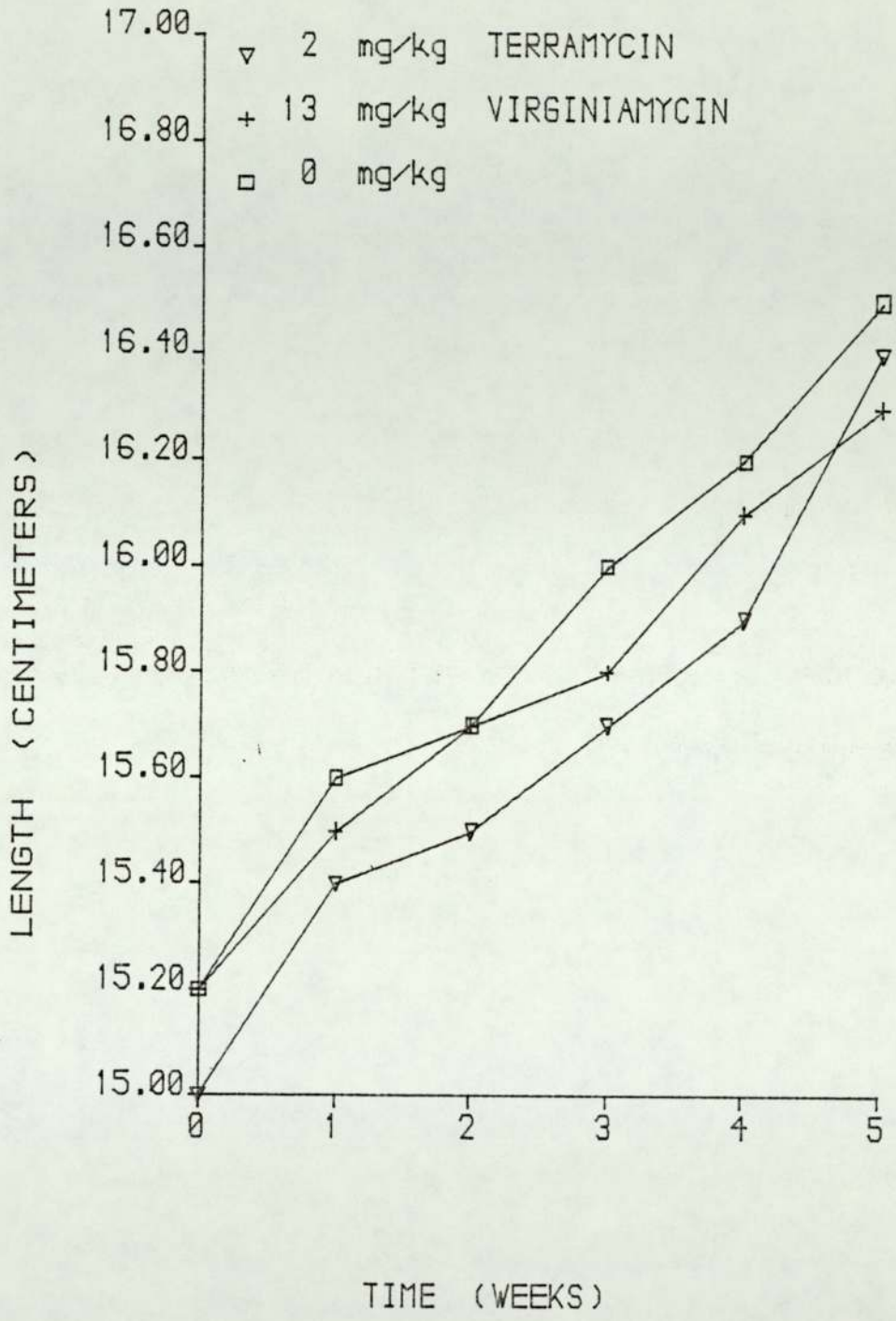


Figure 5.53 Effect of injecting terramycin or virginiamycin on the length of carp

Table 5.83 : Specific growth rate in weight and length of carp injected with terramycin and virginiamycin for four weeks at 25±1°c. Numbers given are mean of 12 fish ±S.E.

Duration in Weeks	Dosage of drug injected per fish per week		
	terramycin (2.0 mg)	virginiamycin (13.0 mg)	control
a) weight			
0-4	0.70	0.72	0.75
4-5*	1.79	1.02	1.28
b) length			
0-4	0.19	0.20	0.20
4-5*	0.54	0.23	0.38

* The drug was withdrawn after four weeks.

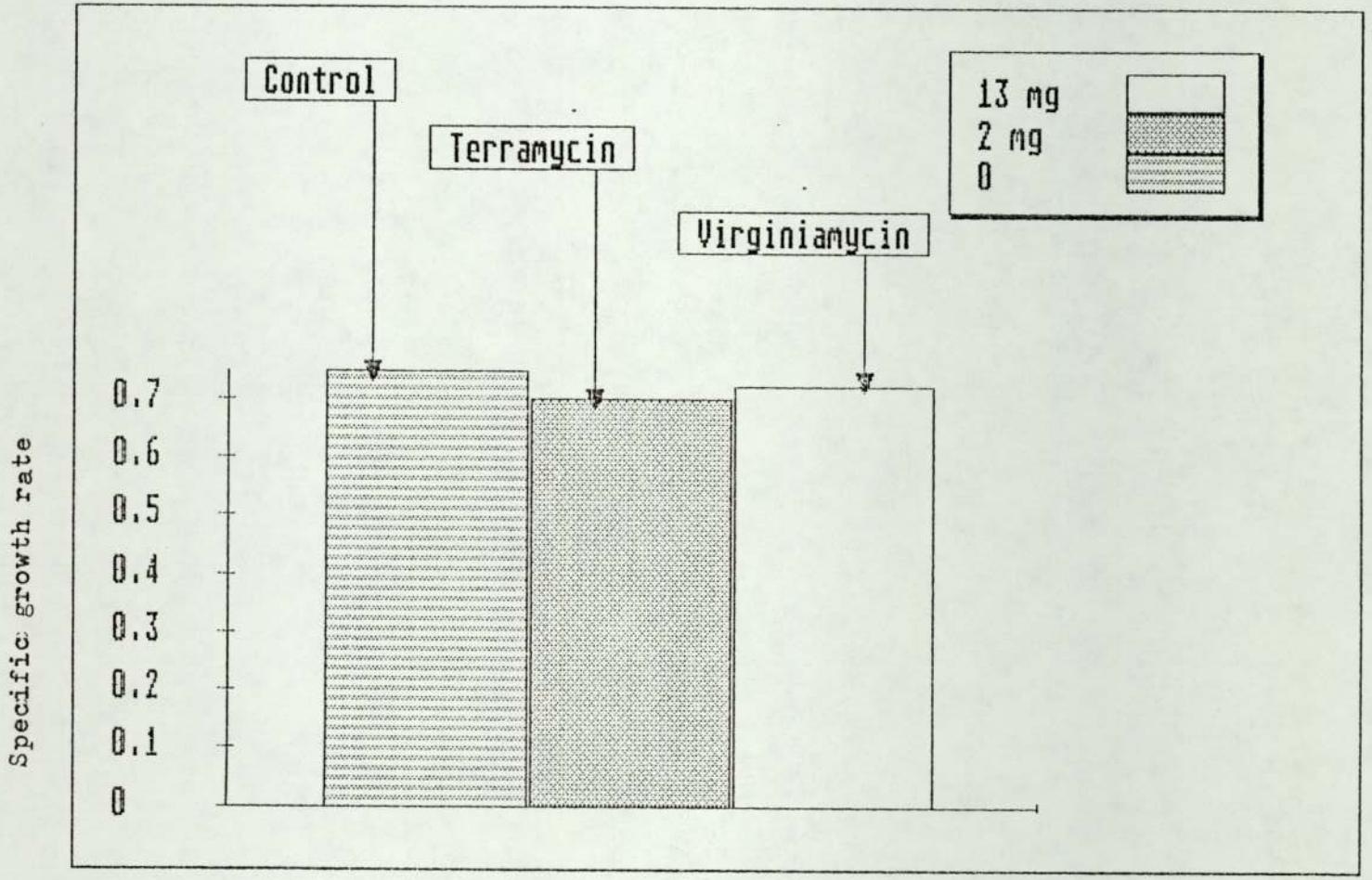


Figure 5.54 Effect of injecting terramycin or virginiamycin on the specific growth rate (weight) of carp

5.10.3 After Drug Treatment :

When terramycin injection was stopped for one week, the percentage increase in weight of the treated group was 10.9% and 8% for the control group, and the percentage increases in length were 3% and 2.5% respectively. The differences between the growth of those groups were not significant. However the increase in daily gain in weight and length of the treated fish were significantly higher than those of the control ($P<0.01$) and ($P<0.05$) respectively.

When the injection of virginiamycin was stopped, the percentage increase in weight and length of the treated group were 6% and 1.9%. It appears that the treated fish were not significantly lighter than the control, however the decrease in the daily gain in weight below the control was significant ($P>0.05$). Table 5.83 and figure 5.53.

CHAPTER SIX

6. DISCUSSION AND CONCLUSION

6.1 Tylosin :

The apparent increase in SGR during the first two weeks and in feed utilization efficiency (FUE) during the first month of the control above the values of the treated fish, irrespective of the drug dosages in both diets, suggests that the fish needed time to adapt themselves to the drug. It also could be due to the stress caused by the marking method (freeze branding) which might have stressed the fish in general and the treated fish perhaps could not cope with the two factors. Freeze branding was not really successful because the marks only lasted for a month, after that they were too difficult to read and the fish had to be remarked. Anglesea (1982) found that this kind of mark lasted only ten weeks at the most with trout depending on the growth speed of the fish. Tylosin has not been used in fish before and its most common use in livestock is on pigs and steers. The former have always shown improvement in growth rate, SGR and FUE (Jordan et al. 1960; Shively et al. 1981 and Jones and Tarrant 1982), while in steers tylosin either depressed the growth or did not have any effect on growth or FUE (Horton and Nicholson 1980 and Horton et al. 1981). Therefore the tylosin effect might be considered as being species dependent. In the present study there was some evidence of growth promotion. However, the effect of tylosin was more pronounced in improving the SGR and FUE. The proximate

analysis of the body composition showed that the fat content of the treated fish muscle was higher than in the control, but this happened only when tylosin was added to the HP diet. As there were no significant differences in protein and water content of the muscle this means that tylosin caused the fish to accumulate lipid in their bodies which is, according to Love (1970), also a sign of growth. This effect has not been reported for tylosin before. Although tylosin in the present study showed only slight improvement in growth rate, the combination of individual animal variation may have prevented consistent detection. The other reason may be the special conditions in this experiment that affected the growth especially at the beginning of the experiment (the stress caused by the freez branding). Other work should be undertaken by using smaller fish or other environmental conditions, lower temperature for instance.

Tylosin is thought to be more active when the animals were reared in old pens with relatively bad conditions and in smaller animals. Jones and Tarrant (1982), however, showed that this is only true when the improvements are expressed as a proportion of the control value and the animals were newly weaned and subjected to stresses, such as change of diet, and exposure to unfamiliar strains of infective organisms. Cracknell (1983) claimed that since its development, tylosin has not posed any threat to man nor has there been any spread of multiple infectious drug resistance in animals. Burrows et

al. (1983) stated that tylosin is rapidly eliminated from the body of the calves exposed to tylosin. The present study also showed that tylosin did not change the weight of liver and kidney. This shows that tylosin is free from hypertrophic effect. A combination of this and and the above-mentioned points, with the knowledge that tylosin is active mainly on Gram-positive bacteria, suggests that if this drug is going to be used in fish it will be safe.

6.2 Payzone :

Parovà et al. (1982) in their experiment on carp (species not specified) claimed that payzone at a dosage of 25 mg per kg food for 112 days resulted in an increase in weight gain of 11.8% and that the consumption of feed mixture per one kilogram of weight gain decreased by 10%. This effect was accompanied, so they claimed, by a decrease in water, protein and ash and increase in the fat content. In their abstract they did not refer to whether the results had been analysed statistically. In a field trial Chua and Teng (1980) rearing estuary grouper *Epinephelus salmoides* in floating net cages, payzone was incorporated as growth promoter in a 38% protein diet for 5 months at a concentration of 0.25-1 gm per kg. The 1 gm per kg dose gave 62.8% increase in weight over the control. In the present study the percentage increase in weight was similar to that of Parovà etal. However, this increase was achieved in a shorter time (70

days) and may suggest that there is no need to expose the fish for longer times to improve growth. The present study showed that payzone increased the length of the fish more efficiently with the LP diet, this perhaps being due to most of the protein in this diet being used for maintaining the body composition; the fish fed the medicated HP diet had a higher fat content in their muscles, and it looks as though the excess protein in this diet was used by the fish to increase the weight gain and fat deposition. Reinitz (1983) reported that the fat content of the muscle of fish was only increased in fish fed high fat diet. Since the HP diet of the present study which was fed to the treated and the control was the same except for the drug, payzone apparently was responsible for increasing fat in the body. This is in agreement with the conclusion of Parovà et al. The increase in fat content of the body coincided with the decrease in liver weight.

Primary examination of the bacterial count of the carp fed the treated and control diets showed a similarity between the two groups. A histochemical procedure was undertaken to determine the effect of flora changes on the intestinal enzymes acid and alkaline phosphatase. No effect could be noticed. The difference in response to payzone feeding between the carp and estuary grouper may either be species dependent or due to the fact that in the second case payzone was used in a field trial. Interestingly Hays and Muir (1976)

reported that in field trials on pigs, the observed improvements in average daily gain and feed conversion results from use of antibacterial agents are nearly double those observed in experimental station trials. They suggested this was due to the researcher for paying too much attention to providing optimal conditions. Ultimately it seems from this study and others that a dosage of 25 mg per kg is the optimum in carp feeding, since Parovà and his colleagues did not find positive results with a dosage of 15 mg, and the higher dosage of the present study depressed growth. The use of payzone did not cause any mortalities and the fish health was satisfactory. As was described earlier in this study payzone (nitrovin) is used successfully and safely in other animals and several points should be considered for the future with fish:

i) A field trial may be worthwhile.

ii) A diet of intermediate protein content could be used by replacing fish meal by other cheap and readily available materials such as wheat bran or soyabean meal since the LP diet in the present study gave a reasonable increase in growth rate and FUE.

iii) Other species of fish could be tried with a payzone-supplemented diet.

6.3 Avoparcin :

From the result of this experiment it is clear that avoparcin is more effective in improving FUE than in

increasing the weight and length gain. Although avoparcin has not been used in fish before, the results with other animals quite agree with this finding. (Johanson etal.(1979) in cattle; Dyer etal.(1980) in cattle; [illegible] and Leeson et al. (1980) in broiler chickens). Avoparcin use in chickens showed improvement in weight gain and FUE in older but not younger chickens. In the present study on fish, avoparcin has no significant effect on the body composition (water, protein and fat) of carp fed the supplemented diet. All the above mentioned work also showed no significant effect of avoparcin on these parameters in the animals studied.

From the above it can be concluded that 40 ppm avoparcin is a dosage that produces interesting results but perhaps using a higher dosage in the future might produce better result. Since avoparcin has been proved to be active only on Gram-positive bacteria (Redin and Dornbush 1968), its use as a growth promoter may be considered safe.

6.4 Terramycin :

The experiments carried out on fish in the past showed that terramycin either diminished growth (Wagner 1954; Herman 1969 Snieszko 1957) or positively increased it (Mitra and Ghosh 1967; Sukhoverkhov 1967). There was no statistical analysis of these experiments.

The mechanism by which terramycin acts is not very

clear; however, the increase in the bacterial count as a result of terramycin treatment may have a beneficial effect. Several authors have pointed out the role of intestinal microflora in nutrient availability for terrestrial animals and fish, e.g. Combe et al. (1976); the microflora are essential in cellulose and starch degradation, which are incomplete when they are absent. Halver (1976) reported that some fish harbour gut bacteria that hydrolyze and make available some monosaccharides from cellulose. Cowey and Sargent (1979) concluded from an experiment on catfish that cellulase activity was associated with microflora in the alimentary tract. In their experiment starved catfish exposed to 200mg streptomycin lost their cellulase activity while untreated starved controls did not. This activity has been already reported in carp *(Cyprinus carpio)* by Ogino and Satio (1970) and Cowey and Sargent (1972). On the other hand, Bergot (1981) reported that carp and rainbow trout cannot degrade purified cellulose. This finding suggests that the microflora are not always capable of cellulose degradation and the addition of antibiotics may increase their number and in turn increase their metabolic activity. The diet of the present experiment contained highly cellulosic material (wheat middlings) and the antibiotic may have increased growth of *A. hydrophila* , the sole bacterium found in the carp gut. This increase in bacterial count may have resulted in the production of metabolites which were beneficial to the

fish and which were absorbed by them.

The increase in fat content of the body was significant in fish receiving the HP drug-supplemented diet. Increment in body fat means an increase in energy content (Zeitler et al. 1984). The effect of terramycin in sparing energy was cited by Francois (1962) when terramycin was fed to piglets. This was dependent on the protein and fat content of the diet. It appears that terramycin in the present study may have the same effect considering the fact that the diet was relatively high in protein content for this species of fish. Carp, unlike other fish such as yellowtail, catfish and rainbow trout have a lower energy requirement (Takeuchi et al. 1979; and Murai et al. 1984). Many species of fish use the protein of the diet as an energy source (Phillips, 1972 and Cowey and Sargent, 1979).

Including terramycin in diets for the treatment of fish diseases faced a lot of argument in regard to its withdrawal period prior to marketing. Salte (1982) reported that this period depends on the temperature used during the treatment and that at 7.7°C the withdrawal time should be 75 days prior to marketing when trout were treated with 75 mg terramycin per kg diet. In another experiment Salte and Liestol (1983) recommended that the withdrawal period should be 60 days at water temperatures above 10°C and 100 days at temperatures between 7-10°C. Herman et al. (1969) examined the excretion rate of terramycin, and was unable to detect

residues in trout muscle after 10 days at high temperature and after 35 days at the low temperature after oral adminsitration of the drug. McCracken et al. (1976) reported an investigation into residues of terramycin in rainbow trout. No residues could be detected after 28 days at 5°C and after 10 days at 10°C following intraperitoneal injection. Terramycin residues fall to a low concentration within 10 days after oral adminstration; it was concluded that the water temperature affects fish metabolism and that this leads to an increase in the rate of elimination of antibiotic residues. This means that the withdrawal period is inversely related to the water temperature. The carp in the present experiment were reared at 25 ±1°C which means shorter withdrawal periods and the residues being eliminated even faster. McCracken et al. cited Fribourgh et al. (1969) found no detectable residues after 2 days of oral adminstration of terramycin to channel catfish presumably because of the high temperature used to rear this warmwater species.

One can conclude now that the use of terramycin as a growth promotant in fish may be profitable especially with LP diets. However, the withdrawal period should be studied before recommending it to be used practically. Incorporation of terramycin in diets of locally available cheap ingredients should also be investigated.

6.5 Virginiamycin (1) :

The microbiological study showed that the bacterial count in one gram of the gut content was increased in the fish receiving the virginiamycin-supplemented diet. However, the improvement in the growth rate was inversely related to the bacterial count of the gut content i.e., the group that showed the greatest improvement in weight and length showed a slight increase in the number of bacteria. The ability of virginiamycin to increase bacteria was reported by Aviotti et al. (1980) who found an increase in coliform count in pigs fed 27.5 ppm virginiamycin supplemented diet. It appears that the fish benefit from the mild increase in the number of *A . hydrophila* as was described in Experiment 4, but with no further improvement in growth beyond a certain bacterial number.

The intestine of the treated fish was lighter than the intestine of the control fish. Similarly Madge (1971) found that when virginiamycin was fed to mice the weight of their intestine was lighter, but in contrast to the present study the mice showed a decrease in body weight. The histological examination did not show any difference between the groups examined. It is premature then to suggest that the decrease in the weight of the intestine led to the improvement in the growth rate and to FUE.

The virginiamycin-treated fish deposited more fat in their body than the control. Love (1970) claimed that muscle

lipid increases during the growth of fish. Kennedy et al. (1981) noted an increase in the fat content of pigs treated with virginiamycin.

From the result of this experiment virginiamycin can be regarded as growth promoter. To confirm these positive results it was decided to carry out another experiment with larger number of fish and with a low protein diet.

6.6 Virginiamycin (2) :

It appears that virginiamycin was more effective when mixed with the HP diet. The insignificant improvement in the growth rate and FUE of carp fed the drug-supplemented LP diet may be attributed to the significant increase ($P < 0.01$) in the number of A. hydrophilain the gut content of the treated fish. Although the fish did not show any infectious symptoms, the bacteria may have competed with the host fish for the nutrients available. On the other hand the moderate, though not significant, increase in the number of bacteria with the HP diet may have spared nutrient for the host fish. This agrees with the results obtained from the previous experiment using virginiamycin in the feed of carp where the 80 ppm dosage gave the best result in improving the growth rate and FUE but brought about an insignificant increase in the number of bacteria. On the other hand *in vitro* studies showed that virginiamycin had no effect on increasing the number of this bacterium.

The body composition of the fish receiving virginiamycin

was different from their control. The treated fish showed significant decrease in the water content and a nonsignificant decrease in the protein content. The fat content was higher in the muscle of the treated fish, than in the control. This finding is also similar to the previous experiment which showed increase in the muscle fat of the treated fish and again it seems that fat deposition in the muscle is a response to virginiamycin feeding. This is confirmed by the finding that the fish on the LP diet did not have any increase in fat content and showed no improvement in growth rate either. Although it seemed that the protein content was inversely related to the fat content the correlation coefficient was not significant ($r = 0.059$).

The effect of virginiamycin on the HSI is not known, however the slight decrease in the protein content of the body may have an effect. The VSI seemed higher in the control fish than in the virginiamycin-treated fish, however the differences were not significant. It was mentioned before that the improvement in growth was not conditioned by the decrease in the weight of the intestine.

Microbiological assay showed that there were no detectable tissue residues in the carp fed virginiamycin ten weeks. Samples were taken 1, 24 and 48 hours after ceasing to feed the drug. Madge (1971) claimed that virginiamycin remains unabsorbed from the digestive tract. Residues have not been detected in the body tissue. This characteristic and

the fact that virginiamycin is highly effective against certain common Gram-positive pathogens but ineffective against most aerobic Gram-negative commensals, fungi and viruses makes safe the use of virginiamycin as a feed additive in fish.

From this experiment and the previous one it seems that 80 ppm dosage is optimum for feeding to carp for growth promotion. Using this drug in farms will reduce the time that the fish take to reach market size and reduce the cost by improving the FUE.

6.7 Virginiamycin (rainbow trout) :

SmithKline (1980, personal communication, unpublished data) incorporated virginiamycin into the diet of farmed rainbow trout for three months in two trials; no calculation of the results was made for the first trial and no statistical analysis for either. In the first trial a dosage of 40 ppm seemed to increase the growth rate and FUE. The other two dosages used were 20 ppm and 10 ppm and they showed no differences from the control. In the second trial using a higher dosage of 80 ppm they reported an improvement of 8.9% in weight gain and -9.2% in the food conversion index (calculated as feed intake/ weight gain). In summary virginiamycin positively improved the weight gain and FUE. A comparison between results of the present experiment and the result of these two trials is not possible. However, it seems

from their results that the 40 ppm dose gave the best result, while in this study the improvement in growth rate was greater the higher the dosage. This contradiction is probably due to the way of interpreting the data or more likely that their experiment was carried out on a farm. A measurement of residues of virginiamycin was also made in this investigation by SmithKline according to the me thod described by The Medicines Regulations No. 273, (1985). The results showed no detectable residues in water, faeces, blood and bile, muscles, liver and skin. This is in agreement with the residu e results found in the carp trial of the present study.

Microbiological analysis showed that the bacterial population of the stomach and intestine of rainbow trout was similar whether virginiamycin was added to the diet or not. The absence of *A. hydrophila* was noticeable although it has been reported to be present in the digestive tract of rainbow trout (Trust et al., 1979 ; Niemi and Taipalinen, 1982). The species of bacteria found in the stomach and intestine of rainbow trout in the present study seem identical in number in the fish receiving treated and the control diets.

Scanning electron microscopy showed that the bacteria attached to the coating of the digestive system were also similar in both the control and the treated fish. The number of bacteria in the stomach and intestinal content was greater than the number attached to the digestive system.

Similar findings were reported by Lesel and Pointel (1979). It is clear now that virginiamycin has no effect on the quantitative and qualitative existence of bacteria in the digestive system of trout. Moreover it seems that the mode of action of virginiamycin, certainly for trout, is not necessarily associated with the change of bacterial count in general and *A. hydrophila* in particular.

The protein efficiency ratio (PER) was higher for the treated groups [23.2% (100 ppm), 20.1% (80 ppm) and 17% (40 ppm)] than for the control (16.9%). Reinitz (1983) found similar values of 22.9-23.6% in trout fed high-protein diet (52.8%). It appears that virginiamycin improves the PER, as was also demonstrated by the significant increase in the muscle protein of the treated fish. Apparently the protein of the diet was efficiently used for anabolic purposes instead of energy purposes. It seemed that the fat content of the muscle tended to decrease when the protein content increased. However the correlation coefficient was not significant ($r=0.515$). This means the increase in protein was not a result of a decrease in the fat.

Virginiamycin had no effect on somatic indices, which makes the use of this drug in fish feeding favourable. The conclusion is that virginiamycin acts positively in improving the growth rate and FUE without any side effect on the health of the fish, body organs or leaving any residues in the fish flesh or in the environment.

The three experiments involving the use of virginiamycin in the diet of carp (Experiment 5 and 6) and of rainbow trout (Experiment 7) showed that in these two species, virginiamycin acted similarly in some aspects and differently in others. In both species of fish, virginiamycin improved the growth rate and FUE, however the optimum dosage for carp was 80 ppm while in the trout the higher the dosage the greater the improvement. The reason for the differences could be due to :(i) species differences,(ii) diet and (iii) water temperature in which the fish were kept, 25±1°C for carp and 14±1°C for rainbow trout. Virginiamycin increased the bacterial count of the gut content of carp while no change was observed in the trout. The changes in body composition of fish were also different. In carp the drug increased the fat content and decreased the moisture and protein while in trout the protein was increased and the fat was decreased. This may be due to the fact that trout readily use protein as a caloric source and a high degree of digestion and absorption is expected from most protein used in trout diet (Phillips 1972).

From the above one can conclude that virginiamycin can be used successfully and safely in carp and trout. Other species, which have economic value, such as salmon might be fed a diet containing virginiamycin. Further work could include studies of the effect of virginiamycin on metabolism of the bacterial flora and fish, since

quantitative and qualitative studies showed no obvious effect.

6.8 Emtryl :

The aim of including the protozocide emtryl in the feed of carp was to compare it with the antibacterial feed additives used in this study and to elaborate knowledge about the way(s) in which antibacterial compounds might act. The bacteria count in the mid gut content was studied in the same way as with antibacterial agents. The result of this experiment showed that the growth rate, the SGR and FUE were similar between the fish treated with emtryl and the control. These results coincide with the bacterial count measurement which showed no differences in the number of bacteria between the treated fish and the control. This result was expected with a compound which has no antibacterial activity. However, a compound with fungicidal or antihelminthic activity like copper sulphate has been found to improve daily liveweight gain and FUE when supplied to pigs feed at concentration of 0.1%; perhaps a protozocide would act in the same manner. Madge (1973) stated that the bacterial action of copper supplementation in the alimentary canal is inconclusive. He attributed the improvement in growth rate and FUE to an increase in nitrogen retention and improved nitrogen digestibility. Emtryl in this study did not show any effect on protein digestibility.

From the above, one can conclude that emtryl is not

suitable as a growth promoter at the dosages used. However, higher dosages might prove to have a better effect especially if the drug acts in its characteristic manner of not changing the body somatic indices.

6.9 Zeranol :

A slight but not significant improvement in growth of carp receiving 0.8 mg zeranol per week was shown. Zeranol-treated fish grew more quickly in length (26.9%) than in weight (6.2%) compared with controls. Ashby (1957) reported similar results with brown trout when treated with progesterone. However, zeranol significantly increased the daily increase in weight ($P<0.05$)and length and ($p<0.01$) of the injected carp. Individual animal variation might have prevented consistent detection of improvement in total weight and length gain. Zeranol in the present study also improved FUE in the injected carp by 13.2% over that of the control. Improvement in FUE probably was a product of increased digestion. Similarly Hirose and Hibiya (1968) found improvement in FUE as a result of treatment with sex hormones.

Fat content of the wet muscle of the injected fish was significantly lower than the control. This is contrary to the result obtained from steroid hormone administration reported by Fagerland and McBride (1975,1977), Simpson (1976), and Lone and Matty (1980) who found an increase in

lipid content of steroid-treated fish. The fat in this study might have been deposited in the liver as the weight of this organ in treated fish was slightly higher than that in the control. The protein and moisture of the treated fish were significantly higher than the control. This finding is in agreement with the results obtained by Sharp and Dyer (1971) in heifers treated with zeranol. Apparently zeranol treatment resulted in a better utilization of the relatively high protein (47%) diet so the fish accumulated the excess protein in the muscle. An inverse relationship between fat and moisture content of the body is reported (Love 1970, Murai et al. 1985). This relationship seemed true in this experiment, however the correlation coefficient between the two parameters was not significant (r= -0.919). Zeranol did not have any effect on altering the HSI, RSI and VSI. It appears that the action of zeranol in carp is free from the hepatotrophic or renotrophic side effect found with some anabolic steroids. This side effect was reported in fish like rainbow trout, goldfish and brown trout (Ashby 1957; Hirose and Hibiya 1968; Cowey and Sargent 1972). Since zeranol, a non-steroid oestrogen-like hormone, has few of the disadvantages of androgenic hormones it can be considered as a safe growth promotant. Bennett et al. (1974) reported that when zeranol and diethylstilboestrol (DES) were administered at the same dosage, zeranol had only 1/2500th the estrogenic activity of DES in the mouse and 1/2000th in the rat.

In summary the improvement in DG of zeranol-injected fish was due either to improvement in FUE or to the deposition of protein in the muscle, and one can conclude from the results of this experiment that zeranol is a potential growth promoter in carp without fear of side effects. However, a withdrawal period should be determined before it is used in the fish farm. This experiment opens up wide aspects and topics for the future such as using other routes of adminstration, in different species of fish, and different dosages.

6.10 Terramycin And Virginiamycin Injection :

From the results of this experiment it is seen that the injection of terramycin into carp does not improve the gain in weight and length. This is in agreement with the results obtained by Rijkers et al. (1980) who found that the injection of terramycin into carp *(Cyprinus carpio)* at concentrations higher than the one used in this experiment (60 and 180 mg per fish every three days) did not accelerate their growth. From this one can conclude that neither high dosage nor low dosage of injected terramycin produce positive results. However different results were obtained when another member of the tetracycline group, aureomycin was injected into calves at 400 mg per kg diet. The injected animals showed higher gain in weight and better condition than the control (Rusoff et al 1953). The results of the present study are also in contrast to the results obtained from feeding

terramycin to carp in an earlier experiment, which showed significant increase in the growth rate of the fish consuming the medicated diet. Rijkers et al. (1980) also found an unexpected apparent increase in weight of fish fed a terramycin-supplemented diet (no statistics were performed). After ceasing drug injection, the specific growth rate of weight and length was improved in the treated fish over that of the control. This may suggest that the injection of terramycin actually suppressed growth and this was corrected after stopping the injection for one week.

Injection of fish with virginiamycin did not decrease the growth significantly. In fact, during the first two weeks of the experiment the increase in weight seemed to be greater in the virginiamycin-injected fish than the control. However, when injection of virginiamycin was stopped the specific growth rate of weight and length were significantly lower than the control. This may lead to the conclusion that injection with virginiamycin actually disturbed the normal growth of the treated fish. But this is not the case when virginiamycin was mixed with the food in earlier experiments in this study. The virginiamycin-fed fish were significantly larger than the control. Positive results were also found by Smith Kline (1980) in a trout experiment. Hence one can recommend that virginiamycin is better used as a feed additive rather than injected.

Further work should include trials of other injected

dosages (higher and lower) to confirm and extend the present results.

CHAPTER SEVEN

7. GENERAL DISCUSSION AND CONCLUSION

7.1 Protein :

The protein requirement of common carp *(Cyprinus carpio)* has been extensively investigated, as the worldwide production of this fish contributes more to the freshwater fishery yield than any other species. These requirements are influenced by biotic and abiotic determinants. Murai et al. (1985) found that maximum growth rate was achieved by fish fed a diet containing 33% protein. Ogino (1980) reported that optimum crude protein content in the diet could be decreased from 50% to 35% with increase in daily feeding rate from 2.5% to 3.5% of the live weight. Goolish and Adelman (1984) found that maximum growth of juvenile carp (8g) was obtained at a temperature between 24-30°C and food converison efficiency reached maximum at 24°C with 4% ration size. The optimum dietary protein for maximum protein accumulation was 38% when casein was the sole protein source in the diet (Ogino and Satio 1970). The effect of dietary energy (DE) was shown by Takeuchi et al. (1979) who reported that growth rate and feed conversion on the diet containing 23% protein were quite low regardless of DE content in the diet. These values improved as the dietary protein was increased reaching maximum in the 31-32% protein diets with DE contents of 310-360 kcal/100g, which they concluded was optimum for carp. The effect of temperature was also studied for this species Suzuki et al. (1977) found that the growth

rate of Japanese and mirror carp increased over temperature range 15-25°C and then showed no change at 30°C. Halver (1976) concluded that *Cyprinus carpio* , eel and channel catfish required high protein for very young fish, the requirement decreasing with age. Recently Watson (1985) found that 37-38% protein was optimum for carp fed piggery waste. AL-Asgah and Bedawi(1984) found that by using locally available protein sources, the best growth was obtained by the diet containing 43%; however the growth rate obtained was poor and they attributed these results to the absence of the natural food organisms, low digestibility or unbalanced nutritive element in the feed. Kim (1974) reported that the true digestibility of white fish meal protein showed a constant value regardless of the protein content of the diet.

The high protein (HP) diet in the present study can be considered optimum as other factors that influence the protein requirement of carp were met. These factors are more or less the same in the the HP and LP diets. The temperature was 25±1°C, gross energy was 4136 kcal/kg for the HP diet and 4178 kcal/kg for the LP diet, and the protein digestibility coefficients were 91.5% for the HP and 89.1% for the LP diet. So it would appear that any effect on body composition will be due to the interaction between the drug and the dietary protein. The protein sparing effect will be due to the drug used.

7.2 Fat :

The fish fed the HP diet and treated with the antibacterial agents (ABA) in the present study and which had significant increase in body weight tended to accumulate more fat in their muscles than the fish fed the LP treated diet and than their control. Experimentally the increase in the carcass fat could be due to different factors. Zeitler et al. (1984) found that an increase in protein content from 41.3 to 51.4% in the feed did not influence the carcass composition of the carp, *Cyprinus carpio* , whereas, an increase in the energy content in the feed from 18.3 to 20.1 MJ/kg diet increased the fat content and with it the energy content of the carcass increases. Watanabe (1977) and Reinitz et al. (1978) reported that increasing the amount of dietary lipid has a sparing action on dietary protein in trout rations. Reinitz (1983) reported that the fat content of rainbow trout was increased only in the fish fed high fat diet. Recently Murai et al. (1985) observed that the carcass lipid of carp became higher with increasing of dietary lipid and decrease of protein to less than 33%. In contrast to rainbow trout Takeuchi et al. (1979) found that the energy requirement of carp was low and that carp cannot efficiently utilize dietary lipid to spare protein being used as energy when supplemented at more than 5% in the diet. Atack et al. (1979) found a higher fat content in the body of carp fingerlings with a feed low in crude protein and

enriched in fat. Eckhardt et al. (1982) similarly found that fat supplies of 0 to 18% in feed also reduced the protein content in the carp and raised the fat content. In an intensive culture in ponds, oil-coated pellets increase the fat content of carp by 2% (Viola et al. 1981). Since the fat content of the HP and LP diets in the present study were similar (9.5% and 9.3%) and so was the gross energy of the diet, the drugs then should be responsible for the increase in body fat by utilising the excess protein of the diet, as it is known now that carp have a low energy need. This seems true, as the rainbow trout fed a diet containing adequate protein and treated with virginiamycin showed an increase in protein content in the muscle rather than fat. The tendency of the animal treated with ABA to deposit more fat has been reported in pigs, Vestal (1951) and Bowland et al. (1951) observed that the fat content of pig tissues increased when they were fed a diet containing aureomycin. Interestingly Groves (1970) stated that for sockeye salmon, fat tends to increase with body size and this may suggest that the drugs increased the weight of the fish and hence increased the fat content of the muscle.

The high percentage of fat in the body of the fish did not seem to affect the health of the carp although it is known historically that an increase in body fat is regarded as detrimental to the health of rainbow trout (Phillips and Podoliak 1957). However in more recent reports, no such

harmful effects have been detected (Lee and Wales 1973, Reinitz and Hitzel 1980). Reinitz (1983) claimed that the association between the percent of fat and moisture in fish carcass and individual weight indicated that additional energy stored as fat by fish simply replaced the body water and did not adversely affect the deposition of protein. Carp are known to contain more fat in their body than trout, as confirmed by Ogino et al.(1976) who obtained a slight weight gain even in the carp fed protein-free diet; they suggested that this might be due to the deposition of lipid in the body. In the present study, the increase in the fat content seems to have had no effect on the condition of carp. The condition factor (C) did not dramatically change in all experiments where the drugs improved the growth gain. The carp fed the terramycin-treated diet had even higher C values than their controls. It appears, however, that the mechanism by which zeranol affected the body composition of carp is different, as the fish injected with this drug showed higher protein and moisture in their muscle with a decrease in the fat content. It can be suggested that zeranol enhanced the conversion of the protein of the HP diet to fish protein.

7.3 Mode Of Action :

7.3.1 The Disease Theory :

Most of the literature surveyed for the present study was concerned with the effect of the drugs on growth rate,

rather than their mode of action. However, there is some consistency concerning the way in which they act, in that the response to ABA has been found to be higher in animals reared in old or frequently-used environments, or in animals described as having sub-clinical diseases. For example Wachholz and Heidenreich (1970) found a greater improvement in pigs in well-used earth-floored units with tylosin than in new ones. The concept was first outlined by Braude et al. (1953) and Taylor (1957) called it the disease theory, but stated that the action is shown by certain antibiotics and not all.

In the present experiments, the theory seemed to be true when virginiamycin was first incorporated in the diet of carp that came from a population whose fish were dying without any apparent infection. These fish showed significant increase in growth over that of the control. However in a second trial, using the same species of fish that were healthy and where there had been no mortalities when the fish were selected for the trial, a significant increase in growth rate was also shown. Moreover, prior to each experiment the whole of the tank system in which the fish were reared had been thoroughly cleaned and disinfected. Hence this concept is not well-supported for fish, in line with work of Coates and Harrison (1969) in chicken; Bloom and Knodt (1952) in calves and Jones (1978) and Jones and Tarrant (1982) in pigs. Other experiments in the future might be carried out by using both

clean and well-used environmental conditions on fish of the same size, age and coming from the same stock.

7.3.2 Size Of Animal :

The literature also indicated that feed additives might be more effective in slower-growing animals (Henson 1970; Wilson et al. 1972) and in younger animals (Jackson et al. 1974; Powley et al. 1981). However, some investigators found no difference such as Wiggins et al. (1979) who used zeranol in sheep, and Hulan and Proudfoot (1981) using avoparcin in turkeys. The results of the present study agree quite well with the last two authors as the fish which were small at the begining of the experiment had a small final weight. This was also clear from the wide variation between the growth of fish which in fact resulted in some apparently excellent growth-promoting activity of some of the drugs used being not statistically significant. This may suggest that feed additives do not specifically have the ability to improve the growth of animals with low genetic growth capability.

7.3.3 Nutrient-sparing And Digestibility :

The nutrient-sparing effect is established, but results are often contradictory and do not permit one to conclude that growth-promoting antibiotics always have a sparing effect. Including two concentrations of protein in the diet of the fish in the current experiments was meant to show this effect, the most desirable for economic rearing of animals in

farms, but in nearly all experiments, the effects were not sufficiently clear or direct enough to justify cutting down the dietary protein. Kennedy et al. (1980) found that a low-protein diet depressed the growth of pigs, and virginiamycin did not have a significant effect in increasing their growth. Fish fed the HP diet were heavier than the fish fed the LP diet, irrespective of the drug concentration except when terramycin was added at 100 mg/kg. This group showed similar gain in weight to that of the control of the HP diet. This result substantiates the findings of Biely et al. (1952); Wallace et al. (1954) and Powley et al. (1981), although some reports show on the contrary that additives usually have a protein-sparing effect (McGinnis 1951; Machlin et al. 1952). Some authors thought that an improvement in absorption or prevention of the degradation of amino acids were caused by ABA supplementation. In other words the effect of ABA is a sparing effect on particular amino acids rather than on protein as a whole (Vijayaraghavan et al.(1951), Carroll et al. (1953); Hensley et al.(1953); Francois (1962) and Froetschel et al.(1983)). On the other hand the difference between the two concentrations of protein used in this study is high and this may have masked the diet-sparing effect. By means of protein retention it should be possible directly to show up a sparing effect of feed additives for the protein supplied. Nearly all fish treated with ABA gave higher net protein retention (NPR) and protein efficiency ratio (PER)

than their controls on both concentration of protein. These results are similar to those obtained by Forbes (1954) and Hogue et al. (1956). Vervaeke et al.(1978) reported that virginiamycin had carbohydrate and some nitrogen-sparing effect. Other experiments however, showed no improvement in protein retention (Hoefer et al. 1952 and Braude and Johanson 1953). In addition to the improvement in NPR and PER zeranol-injected fish showed higher protein content in their muscles than the control.

Digestibility studies are often used for determining nutrient-sparing activity. Francois (1962), reviewing works on pigs, rats and calves by several authors and himself, stated that the digestibility of dry matter, organic matter and especially protein has led to conclusions which are relatively in agreement with one another. Antibiotics have little or no effect on these parameters. Schneider et al. (1970)found that payzone (nitrovin) supplements improved nitrogen digestibility significantly, however the improvement in growth rate was not significant. The present study revealed that in all experiments the total protein digestibility was not affected by the addition of the feed additives.

7.3.4 Bacterial Numbers And Metabolism :

An interesting sparing effect was suggested by Waible et al.(1952) and Wallace (1970) who suggested that ABA either

increase the number of bacteria synthesising nutrients for the host or suppress the organisms that compete with the host for the nutrient. Wallace also suggested that the effect may be a result of improving nutrient absorption through a thinner intestine. As far as the present study is concerned these hypotheses were not viable as is explained below.

Looking at the results of the present study, it seemed that the increase in the bacterial count of the gut content was responsible for the positive increase in growth rate and feed utilization efficiency (FUE). However, a closer look at the results by using statistical analysis showed that the significant increase in growth was not consistently related to the increase in the bacterial number. For instance, in the first virginiamycin experiment on carp, the increase in the number of bacteria was highly significant, but at the same time the group showing the highest increase in growth had a non- significant increase in bacterial number. This situation was repeated in the second experiment, in which the fish fed the same dosage that gave the highest increase in weight again had a non-significant but higher number of bacteria. When the fish on the LP diet were fed the corresponding dosage, they harboured significantly more bacteria in their gut than their control and the treated fish on the HP diet. However, increase in body weight was not significant. In the trout experiment, no difference in bacterial count could be traced, and even the organism found in the gut of carp which

was thought to be responsible for the growth was completely absent from the digestive system of the rainbow trout studied. Similar results were noticed in the terramycin experiment when it was used in the HP diet, however the treated fish on the LP diet showed that the significant increase in growth rate coincided with a significant increase in bacterial count. The payzone experiment also showed no apparent difference in bacterial count between the control and the treated fish. Gerlach (1970) reported that payzone increased the number of coliform of pigs significantly, in addition an increase in lactobacilli was also seen. After cessation of feeding the drug, the coliform count dropped to a figure similar to that of the control. However *in vitro* payzone showed no effect on bacterial count. This is similar to the finding of this study when virginiamycin did not bring about an increase in the bacterial count *in vitro* . Schönherr (1956) found that oxytetracycline (OTC) significantly increased the total number of aerobes in pigs, and Scaletti et al. (1955) found that it increases E. coli in rats; these changes were not always correlated with increase in growth. Although Decuypere et al. (1973) had observed that the number of coliforms and enterococci were increased by the addition of 50 mg virginiamycin per kg of feed. Virginiamycin was found by Vervaeke et al. (1978) to decrease ammonia concentration in the digestive tract of pigs. They also observed a general decrease in the number of bacteria along

the small intestine when the pigs were slaughtered. This led them to conclude that changes in bacterial metabolism have to be more nutritionally important for the animal than a simple change in numbers. Moreover they found that virginiamycin increased nitrogen and carbohydrate metabolism.

It can be concluded now that although ABA seemed to increase the bacterial count however, most increases were not statistically significant. For this reason it seems more logical to agree with Taylor (1957) and Visek (1978) who concluded that statistically significant differences in growth should be correlated with changes in the bacterial metabolism rather than changes in the number of bacteria. Visek (1980) again reported that ammonia released by bacteria, principally through urea hydrolysis can be responsible for the growth-promoting response. However further investigations need to be carried out in order to confirm this suggestion.

7.3.5 Intestine Thickness :

THe other possible mode of action of ABA investigated in the present work arose from the examination of the thickness of the intestine. Carp is a species lacking a stomach and the so called medial intestine may be regarded as corresponding to the small intestine in other species by supposing that it is in this part of the gut that most nutrient absorption takes place. However, Bucke (1976) found that lipids are absorbed by the columnar epithelial cells of

the anterior intestine whereas those of the medial to posterior intestine absorb protein. The thicknesses of these three parts were examined. No differences were found between the intestine of the carp treated with tylosin, payzone, avoparcin, terramycin and virginiamycin and their controls. Although the fish treated with virginiamycin in the first experiment had intestines which weighed less than their controls histological examination failed to show any differences. The ratio of the length of the whole intestine of the fish treated with terramycin to the total body length was also measured, and no difference from the control was noticed. This observation confirms that there is no relationship between the weight and morphology of the intestine and the increase in weight brought about by antibacterial feeding. This is in line with findings of Klaus and Fewson (1955), Taylor and Harrington (1955) and with Pepper et al.(1953) who reported that another dietary addition such as manganese may give rise to decreases in gut weight without increasing body weight.

From the above, one can generally conclude that although there is agreement about possible beneficial effects of the ABA there is no general consensus on how this positive result is obtained. For discussion purposes a separate consideration of the mode of action is a convenient approach. In reality they may overlap, interrelate and be confounded by one an other. This can be due to the fact that the experimental

conditions in different investigations are not comparable so that no conclusion can be reached or that the parameter which is measured is not exclusively influenced. Indeed it can be assumed that animal performance is influenced by three factors, nutritional, genetic or environmental.

7.4 Resistance to ABA :

With the successful use of ABA as growth promoters, concern has been expressed from time to time since 1957 by official bodies, such as the Swann Committee in the UK and the American Food and Drug Administration (FDA) about the long-term continuous use of these feed additives. Their concern relates to the possible hazard of proliferation of resistant bacteria in animals fed antibiotics. The significance of antibiotic-resistant bacteria was realised after the discovery by Japanese investigators in 1959 that the resistance to antibiotics was transferable (Watanabe 1963). Scientists began to fear that the resistant bacteria could be transmitted to humans making treatment of certain infections difficult. Regulations were laid down specifying that antibiotics to be used in animal feed for more than 2 weeks must meet certain safety criteria. Every antibiotic should be (i) effective and of economic value; (ii) have little or no use as a therapeutic agent in man and (iii) there must be complete elimination of drug residues and metabolites from edible tissues that are likely to be

consumed by humans, and a withdrawal period must be specified on any label (Wallace 1970, Sliver and Mercer 1976 , Corliss 1979 and FDA report 1984). During the 35 years since feed use of antibiotics was introduced, the average enhancement in rate of growth has remained relatively constant. Coates and Davis (1959) have indicated that over 5 years they had not observed any change in the effectiveness of penicillin, nor in the absolute weight of chicken treated with penicillin. Visek (1978) stated that despite reports that the number of bacterial cultures showing antibiotic resistance has risen significantly since antibiotics were introduced into feed use, the constancy of the growth response over time argued that growth promotion is independent of the mechanism responsible for acquired resistance or its transfer between strains or species of microorganisms. A similar conclusion was reached by Jukes (1971). Hays and Muir (1979) from their work in this field for 20 years claimed that the possibility that resistant organisms may be transferred to man is a theortical consideration and that data are not available to accurately estimate the relative magnitude of such a potential risk. The present author agrees that the continuous use of ABA such as tylosin, avoparcin, virginiamycin, monensin and bacteracin as growth promoters up till now makes it difficult to visualise a problem of increase in resistant pathogens in the cattle, pigs, chicken and fish. All the ABA used in the current study except terramycin

are seldom used in human medical treatment and they are mainly active againt Gram-positive bacteria. Therefore, there is no risk of transferable drug resistance, a phenomenon limited in practice to the Gram-negative bacteria. Evidence to support this is provided by Jordan et al. (1960) and Cracknell (1983) who stated that tylosin is inactive against Gram negative bacteria and causes no increase in *Salmonella* or coliforms resistant to drugs used in human medicine. Partial cross-resistance has been demonstrated between tylosin and some antibiotics such as spiramycin, lincomycin and virginiamycin but it has not been shown to cause any significant problem (McGuire et al. 1961).

In vivo studies conducted for Cyanamid in the University of Liverpool in 1972 with pigs and broilers it was shown that the use of avoparcin as a growth promoter did not favour the selection of bacteria resistant to it nor to any therapeutic antibiotic in the animal or in the contact human. An *in vivo* study conducted by Walton (1974) demonstrated that the continuous feeding of payzone to growing pigs at either 10 ppm or 100 ppm did not increase the resistant strains of *E. coli* in faeces. Furthermore, it did not cause the apperance of cross-resistance to any antibiotics in faecal *E. coli*. Smith Kline (personal communication) have claimed that *in vitro* and *in vivo* studies indicate that resistance to virginiamycin develops very slowly. Two studies with *Staphylococcus aureus* showed either a very small percentage

or none to be resistant to virginiamycin, and in addition it did not show cross-resistance to some other antibiotics. The conclusion was that resistance to virginiamycin in animals is so low that there was only a theoretical risk and that its toxicity to man prevents it being used in medical treatment (Madge 1971).

For terramycin there has often been failure to prove that its use as a growth promoter can cause a risk of bacterial resistance, and the argument between the official bodies and the drug sponsors led the FDA in 1984 to lift the ban on the use of this drug until further information was available and the picture became clear. Very recently Schnick et al (1986) reported permission to use terramycin in fish food. Aviotti et al.(1980) found that the increases in the bacteria resistant to chlortetracycline were similar in pigs fed diets containing subtherapeutic and therapeu tic dosages. This finding supports the claim of Hays and Muir (1979) that the occasional therapeutic use would maintain resistant bacteria.

7.5 Tissues Residues :

The importance of the rapid elimination of the feed additives from the tissues or animal product that is likely to be consum ed has been also carefully studied. All the feed additives used in the present study except terramycin and zeranol are believed not to be absorbed through the intestinal tract and most of the digested drugs are found in

faeces, as was explained in Chapter 3. This was proved practically in the present study when virginiamycin residues were only found in faeces of the carp fed the supplemented diet for 10 weeks. This is in line with the similar results for residues found in the gut content of trout fed virginiamycin in a farm trial by Smith Kline (1984) carried out at the same time as this experiment. Jukes (1971) claimed that OTC was used for delaying spoilage of poultry meat and fresh fish and was destroyed by cooking. Tarr (1956 quoted from Jukes) found that in salmon and lingcod flesh containing 5-10 ppm of chlortetracycline (CTC), cooking was followed by destruction of at least 80-90% of the antibiotic. Similarly Tomiyama et al.(1957) reported that this antibiotic disappeared from bonito fillets heated for 60 minutes at 93°C. Jukes and William (1953) had earlier stated that cooking destroyed aureomycin in all tissues tested. Meredith et al. (1965) reported that normal methods of roasting, frying and autoclaving destroyed all residues of OTC and CTC in tissues of poultry fed 2000 ppm OTC and 1000 ppm CTC. Broquist and Kohler (1953) noticed the disappearance of CTC from the meat of chicken roasted at 230°C. Goldberg et al. (1953) found that 2 ppm CTC added to ground beef disappeared after 96 hr. storage at 10°C. The studies with tetracyclines are cited here, firstly because terramycin is one of this group and secondly because it gave rise to information on how much remains in meat and on the rate of disappearance of such

residues. After 65 days withdrawal period of the injected zeranol, tissues were found to be free from the drug (Bennett et al. 1974). In this case if zeranol is fed to fish, it may not be absorbed through the intestinal tract.

The general conclusion of the present study is that despite the old hypothesis that ABA would not improve the growth of fish because their guts were thought to be sterile, ABA can be used as growth promotants. However, further investigations are needed, including the ultimate metabolic fate of the parent compound and the significance of metabolites and their role on the mode of action of ABA, before these growth promotants could be used in fish production. Residues that might remain in the flesh of fish treated with ABA under ordinary practical conditions need to be determined, and a withdrawal period prior to marketing must be set, during which complete elimination of any residues may be ensured; this period would be very short with compounds that are not readily absorbed through the intestine. The practice which is being used in animal production could also be used in the feeding of fish, that is the combination of two ABA compounds such as monensin (an antibiotic) plus avoparcin (Johanson et al. 1979, Dyer et al. 1980 and Froetschel et al. 1983;) monensin plus tylosin (Heinemann et al. 1978 and Horton et al. 1981) or compounds like copper sulphate plus virginiamycin (Aviotti et al. 1980 and Lima et al. 1981) and even monensin plus zeranol (Utley et al.

1976 and Stidham et al. 1981).

Appendix

Abbreviations Used

ABA	Antibacterial agents
API	Analytab Products Inc
C	Condition factor
CTC	Chlorotetracycline
DG	Daily gain
FCR	Feed conversion ratio
FUE	Feed utilization efficiency
HP	High protein diet
HSI	Hepato-somatic index
LP	Low protein diet
NPR	Net protein retention
OTC	Oxytetracycline
PER	Protein efficiency ratio
RSI	Reno-somatic index
SGR	Specific growth rate
VSI	Viscero-somatic index

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