The Physiological Effect of Angling Bait on Fish

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BSFS III. 1999.

A Dissertation submitted in partial fulfilment of the requirements of the B.Sc. in Aquaculture and Fishery Management.

ABSTRACT

There is growing concern within Fisheries Management with regard to recent management trends in recreational fisheries; an important concern is the increasing number of heavily (overstocked) stocked stillwater fisheries.

Bait can form a major part of the available food resource for fish in these often 'overstocked' fisheries that are exploited by anglers for recreational purposes. Little regard is shown for the suitability of angler's bait, for what is often the primary source of fish nutrition. The common carp *Cyprinus carpio* L. has become the most commonly stocked species in this type of fishery.

In situations where the high biomass of fish depletes the natural food resource, the suitability of angler's bait for nutritional purposes may be critical. Poor nutrition can have detrimental effects on fish health, whilst poor baiting regimes by the anglers can effect environmental conditions.

This study looks at the suitability of five anglers bait items with regard to their suitability as bait by investigating their physiological effects on the five study groups. Fish were fed iso-energetic meals, the meals consisted of a bait item and a two-percent of body weight supplement of trout pellet to ensure compliance with Home Office experimental procedures. Each of the five diets was fed in triplicate to groups of twelve fish maintained in glass aquaria at 21°C. The mean final weight of the fish under study was 9.08g

The results showed that there was a significant difference ($\leq$ P 0.05) between diet E (shelf-life boilies) and the other four diets. There were some other significant differences ($\leq$ P 0.05) between some diets, however these were performance differences and not differences that reflected any negative nutritional qualities as an anglers bait or as fish nutrition.

The study showed conclusively that shelf-life boilies made by Streamselect Limited are not suitable for fish nutrition for the size of fish under study, and so it is suggested that where bait is relied upon as a nutritional input, there use is highly questionable.

DISCLAIMER

This report is an original piece of work carried out at the students home in Hindhead, Surrey in a purpose built recirculation system and in the laboratory at Sparsholt College Hampshire and was supervised by Mr P.J. Haughton.

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ACKNOWLEDGMENTS

I would like to thank the staff of the Sparsholt College library and in particular Miss J Austin for her considerable help in obtaining texts and scientific papers for the literature review that formed part of this dissertation.

Thanks are due to Mr D. Hide for giving so freely of his time, particularly during the summer recess of 1998, to supervise laboratory work and also for his guidance in nutritional matters relating to the study. I must also thank Mrs A. Fox and her staff, for their assistance and flexibility in making 'bench space' for me in the laboratory.

I would also like to take the opportunity to thank Mr M. Lunn of Grayshott tackle for the supply of maggots; Mr K. Thomas for the loan of a pair of digital scales and Mr I. Couchman and Mr I. Wellby for proof reading the draft of this document.

My sincere thanks are due to Mr J. Branford and Mr S. Leonard in particularly Mr P. Haughton for their support throughout the course. However I am particularly grateful to Dr M. Burdass who amongst all the excellent lecturers at Sparsholt College has inspired and motivated me to extend my horizons of achievement.

My greatest thanks are reserved for my wife Frances, without her support none of this would have been possible.

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1.0 INTRODUCTION

This dissertation is submitted in partial fulfilment of the requirements of the B.Sc. (Hons.) in Aquaculture and Fishery Management Year III.

1.1 Terms of reference

Within Fisheries Management there is increasing concern with regard to recent management trends in recreational fisheries an important concern is the increasing number of heavily stocked stillwater fisheries that support fish at an artificially high biomass. The species most often selected for stocking into these waters is the common carp *Cyprinus carpio* L.; in one or more of its various forms (phenotype) i.e. common, mirror and ghost varieties. These waters tend to be smaller ponds with areas under 2.5 ha. Mr Ian Wellby a Fisheries Scientist at the Environment Agency's National Fisheries Laboratory reported to the 1998 annual conference of the Institute of Fisheries Management (Wellby 1998), that these ponds are often stocked at a biomass in excess of 2,000 kg/ha.

Natural food resources in these relatively small water bodies are often depleted by the high biomass of fish. This depletion of natural food can occur to such an extent that the fish often become reliant on the bait introduced by the angler. The majority of this bait enters the water, as loose feed or free offerings (not attached to angling tackle). The depletion of natural food is encouraged as a deliberate ploy by fishery mangers to make the catching of the fish easier on many of these 'overstocked' fisheries.

Such are the environmental conditions in many of the waters that they have become known as 'carp holes' 'pig pits' and other such colourful labels.

A major management concern and it must be stated potential threat to the well being of the fish maintained at these high-stocking levels is from a fish health aspect.

It is suggested that the health aspect of the fish in this type of fishery can be divided into two discrete and separate areas of study. The physiological effect on the fish of deteriorating environmental conditions and the physiological effect of angler's bait as a major source of nutrition for the fish.

The effect of environmental conditions on fish in recreational and sport fisheries is well-documented in fish health texts (Roberts 1989; Schlotfeldt and Alderman 1995). Pickering (1998) states "when a fish is exposed repeatedly to acute stresses or to chronically poor environmental conditions from which there is no escape, the stress response ceases to be adaptive and becomes, instead, maladaptive. Particularly damaging impacts can be seen on growth, disease resistance and reproductive capacity". The relevance of environmental factors and that many of the associated problems of fish health in these heavily stocked fisheries is inextricably linked to environment is acknowledged. However, many of the environmental conditions are often directly linked to stocking density, the reliance on artificial feed and nutritional factors.

This study considers the effect that five predetermined anglers bait items as a nutritional source may have on the physiology of the fish. The study is not considered as, nor is it intended to represent, a growth trial. Growth will be treated as a physiological effect of the feed items on the fish.

At the onset of this study it was thought likely that there may be significant nutritional differences between some of the bait items when considering the physiological effect on *C. carpio*.

1.2 Background

The creation of these overstocked carp fisheries is part of a changing dimension and attitudes in coarse angling. The increased influence and importance of the Commercial Fishery sector has largely brought about the change. The development of these fisheries is, it would appear, in response to a demand from match anglers, and pleasure anglers who wish to catch ever larger numbers of *C. carpio* between 0.5 kg and 3-4 kg.

These two areas of angling have been highlighted as they are the two branches of angling that have availed themselves of the facilities of these relatively new fisheries, to the greatest extent. In general their quarry is not specialised, they just wish to catch what is available (stocked into the water by man) to be caught. This approach is not thought by some to be compatible with the more traditional approach to angling, i.e. fish at any cost.

The fish tend to be relatively easy to catch, as they rely heavily on the angler's bait for food. Some angling clubs who have seen their membership numbers declining in recent years have attributed this decline to the competition from the commercial sector. In order to compete for their share of the angling market, clubs have chosen to adopt management regimes based around the anglers desire to be assured of catching more and perhaps with less effort. This has seen the trend towards these 'overstocked' carp fisheries spreading through angling club waters as an increasing number stock them to naturally unsustainable high levels.

The Institute of Fisheries Management (IFM) recommends that general coarse fisheries are stocked to a level of 300 kg/ha. The IFM having identified the 'overstocked' carp fishery trend quote a figure of 1000 kg/ha (IFM, 1993) for *C. carpio*. The reader is reminded that Wellby (1998) reports stocking densities in excess of 2000 kg/ha, whilst Ellis (1998) of British Waterways, and Steel, O'Hara and Aprahamian (1998) report stocking levels in excess of 3000 kg/ha.

C. carpio have been found to be the most suitable species to be stocked at these levels as they are hardy (Stoskopf 1993), and are comparatively resilient to angler damage as they do not loose scales easily. Mr M. Moore (*pers. com.*) of Moore and Moore Carp and formerly the manager of the National Rivers Authority (NRA) fish farm in the Thames region states that anglers and managers of fisheries have recognised this fact, and that his business receives order for, and supplies many heavily stocked fisheries with small *C. carpio*. From the anglers view point, they are very hard fighters when hooked (Giles 1994).

From a management standpoint in an aquaculture scenario feeding regimes and diet are, together with environmental parameters, perhaps the most important component of growth and maximising yield, and directly influence the success of an aquaculture operation.

There is no direct comparison in recreational or sport fishery management, this anomaly means that once fish are stocked they are left to fend for themselves regardless of food availability and suitability.

Mans interference in the form of angling often has an impact on the aquatic environment (Wolos, Teodorowicz and Grabowska 1992) and indeed the fish, yet very little consideration is given to the vast amounts of bait that are deposited into many waters on an almost daily basis. In an aquaculture system this is managed by discharge consents, which control levels such as nitrogen (in its various forms), phosphorus, and biochemical oxygen demand. The potential polluting effects of the bait items has not been studied in this work, the literature review of the subject of anglers bait revealed that little work has been carried out on the subject of 'polluting' effect. Cryer and Edwards (1987) carried out work, which showed significant differences between the control and cereal and maggot baits in a natural system, showing that there was a considerable polluting effect.

This bait, which can be thought of simply as feed is often in the form of hook baits, free offerings and groundbait.

The introduction of bait (baiting) is often carried out without any or very little consideration as to its suitability as a source of nutrition in the fish's diet, or for any direct or indirect effects it may have on the aquatic environment.

Kaushik (1995) states that in aquaculture practical diets are formulated with little concern for the biological and environmental consequences of poor digestive or metabolic utilisation of dietary ingredients. There is no reason why either natural or formulated baits should differ from some aquacultural feeds. It does appear that very little consideration as to the nutritional worth is given to the vast amounts of bait that are deposited into many waters on an almost daily and continual basis.

The fact that fishery managers who manage these overstocked carp fisheries may in many respects rely upon anglers bait to be the sole source of nutrition to their valuable stock might appear somewhat strange to others charged with the care and well being of animals. However this appears to be the case.

It has become more common for fishery managers to also feed the fish in these fisheries with supplementary rations, as some realisation has occurred that fish need a regular supply of nutrition. Mr P. Gray the manager of Catch 22 in Norfolk feeds twelve tonne of supplementary feed per year on his recreational fishery (Mr I. Couchman, *pers. com.*). This feeding may often occur at perhaps times when anglers are not fishing (such as mid-week etc.). The feed may often be in the form of trout pellets.

Supplementary feeding is carried out in many cases without any or very little consideration as to the suitability of diet for the species of fish concerned, or for any direct or indirect effects it may have on the aquatic environment. These may include the polluting effect of uneaten feed and also increased ammonia levels from protein sources used for energy. A carpet of uneaten feed can also cause anaerobic conditions on the specific area of benthic mud (Cryer and Edwards 1987).

There can be little doubt that in waters stocked at levels above 1,000 kg/ha, that natural food will be at a premium. *C. carpio* is a voracious feeder and may come to rely upon the bait (attached to the hook) and free offerings (loose feed introduced by the angler to entice fish to feed freely), and any supplementary feed that is fed. Given that natural food production is insufficient to maintain the stock density present.

There may be occasions when the number of baits or potential feed items is limited, such as wet periods during warm weather (few anglers), and that the bait may not fulfil the energy and/or nutrient requirements of the fish.

1.3 Nutrition

Clearly before one can understand the effects and efficiencies of anglers bait as a source of fish nutrition and its physiological effects, one must have an understanding of the nutritional requirements that *C. carpio* may have.

Fish, in common with other animals, do not have a true protein requirement but have a requirement for a well balanced diet of essential (EAA) and nonessential amino acids .

O' Grady and Spillet (1983) estimate that the crude protein requirement of cyprinid fish grown in extensive culture is 35%, whilst Takeuchi, Watanabe and Ogino (1979) report that around 31% dietary protein is optimum for health and growth in *C. carpio*.

Nutrients is a term used to describe the various groups or constituent ingredients that may all be found in feed stuffs in varying proportions. It is the balance of these proportions, which vary in requirement with species, age, physiological and environmental factors. Fish that are offered bait inhabit a natural system (relatively speaking), with no controls and very little research data available, as revealed by the literature review. It may be for this reason that such areas of fish nutrition are overlooked. There is little nutritional information available on these or other species in an angling environment, so one might assume that there is some difficulty to draw generalised conclusions on the dietary needs of and the utilisation by *C. carpio* stocked into recreational fisheries.

Available data shows that there is remarkable homogeneity in the nutrition of teleost fish (National Research Council, 1993), (NRC). The basic principles of cyprinid fish nutrition have been clearly outlined by Halver (1989). The nutrient requirements as currently understood are listed in *Table 1.3.1*

Table 1.3.1 Description of nutrient requirements of carp *C. carpio.*

Nutrient group	Source, requirement and description
Proteins	Fish meal, casein. 30-38% [1] optimal protein level
Lipids - fat	Fish oil, vegetable oil. 10-20% [2]. Essential fatty acids (EFA). 1% of both 18:3ω3 and 18:2ω6.[3] Phospholipids are not generally limiting in dietary lipids.
Carbohydrates	No dietary requirement has been established [4]. Warmwater fish are able to utilise higher levels than coldwater or marine fish. The amount of digestible carbohydrate that can be utilised by carp (*C. carpio*) may be 30-40% [5]
Vitamins	Occur in dietary components. Some are synthesised from other essential nutrients. Required in trace amounts [6]
Minerals	Occur in dietary components and absorbed from the external aquatic environment. Many are essential. Phosphate and sulphates [7]

1. & 3. Taken from Kaushik (1995). 2. Taken from De Silva and Anderson (1995).
4. Taken from Wilson (1994). 5. Taken from Satoh (1991).
6. Taken from Poston (1992). 7. Taken from Lall (1989).

1.3.1 Protein

The quality of protein can be equated to its content of the essential or indispensable amino acids of which in fish there are ten, these are shown in *Table 1.3.2*; the bioavailability of these amino acids is central to the quality of the protein.

Table 1.3.2 Essential amino acid requirement in carp *C. carpio.*

Amino acid	As a % of protein
Arginine	4.4
Histidine	1.5
Isoleucine	2.6
Leucine	4.8
Lysine	6.0
Methionine + Cys/2	2.7
Phenylalanine + Tyrosine	5.7
Threonine	3.8
Tryptophan	0.8
Valine	3.4

Taken from Shepherd and Bromage (1992).

The exact quantities of essential amino acids required by *C. carpio* varies depending on the commentator, other sources are National Research Council (1993); De Silva and Anderson (1995) and Cowey and Sargent (1979).

1.3.2 Lipid

The lipid requirement of *C. carpio* should consist of a proportion of either fish or vegetable oil containing the required essential fatty acids (EFA)'s, which are:- 18:3ω3 (linolenic acid) and 18:2ω6 (linoleic acid).

Once these essential lipids have been supplied the remaining lipid content can be utilised by the fish as digestible energy (DE), dietary lipids are the only source of these two highly unsaturated fatty acids (HUFA's) (Shepherd and Bromage, 1992). Lipids are also important for the transport of fat soluble minerals, phospholipids are the main lipid of biological membranes (Jobling 1995).

Appleford and Anderson (1996) state that as lipid content of a diet increases above 10% inclusion, the digestibility of the lipid decreases. In common with other species such as salmonid species the increase of lipid in the diet will have a protein sparing effect (Takeuchi, Watanabe and Ogino 1987). It is thought that inclusion levels as high as 20% can be added to the diet without inducing any ill effects in *C. carpio* (Cowey and Sargent 1979).

1.3.3 Carbohydrates

Carbohydrates act as an immediate energy source or as a rapidly available energy source, which is normally stored as glycogen in the liver and muscle, alternatively they can be converted to fat in the body. Carbohydrates are not essential (Wilson 1994), their inclusion in commercial diets for aquaculture is partly because they are the most cost efficient form of energy, and that they can have a binding effect when used in forms such as wheat or maize gluten (De Silva and Anderson 1995). The addition of carbohydrate in a balanced diet, particularly in cyprinid fish and especially when compared to species such as salmonids, can have a protein sparing effect (Wilson 1994). Furuichi and Yone (1981), report that starch is the most efficient form of carbohydrate energy in *C. carpio* when compared to Dextrin and Glucose. Kaushik, (1995), reports that increasing the feeding frequency from 2 to 6 feeds per day seemed to improve the utilisation of carbohydrate, incorporated at 30% when fed to juvenile *C. carpio*.

1.3.4 Vitamins

Vitamins can be essential and are classified in two important groups; those that are water soluble and those that are fat soluble. Eight of the water soluble vitamins are required in relatively small amounts and have coenzyme functions (De Silva and Anderson 1995), these are known as the vitamin B complex. Some such as B_{12} can be synthesised by the fish (Kashiwada, Teshima and Kanazawa 1970). Three of the water-soluble vitamins are required in larger quantities, these are:- choline, inositol, ascorbic acid (De Silva and Anderson 1995).

Fat soluble vitamins include A, D, E, and K, some such as K may have coenzyme functions (De Silva and Anderson 1995). A list of known cyprinid fish's vitamin requirements is given in *Table 1.3.3.*

Table 1.3.3 Vitamin requirements for carp *C. carpio.*

Vitamin	Requirement
Vitamin A	4,000-20,000 IU
Vitamin E	100 mg/kg dry diet
Thiamin	0.5 mg/kg dry diet
Riboflavin	6.2 mg / 7 mg/kg dry diet
Vitamin B_6	5-6 mg/kg dry diet
Pantothenic acid	30-50 mg/kg dry diet
Niacin	28 mg/kg dry diet
Biotin	1 mg/kg dry diet
Vitamin B_{12}	NR
Folate	NR
Choline	1,500 mg/kg dry diet
Myoinositol	440 mg/kg dry diet
Ascorbic acid	30-50 mg/kg dry diet

Adapted from NRC (1993).

1.3.5 Minerals

Mineral knowledge is one of the least advanced areas of fish nutrition (Shepherd and Bromage 1992). Minerals differ from other nutrients in that they are neither used nor produced, about twenty inorganic elements are required to meet the structural and metabolic functions (NRC 1993), those that are pertinent to *C. carpio* and not taken directly from the aquatic environment are shown in *Table 1.3.4.*

Of all the minerals phosphorus is one of the most important, this statement is of course somewhat of a contradiction as all essential nutrients are necessary to ensure a healthy life.

Phosphorus however is required in the highest levels of all inorganic ions at 0.6-0.7% (Ogino and Takeda, 1976), for maximum growth and bone mineralization, as well as in lipid and carbohydrate metabolism.

Other minerals such as sodium, potassium and chlorine are the most abundant electrolytes in the body; they are readily absorbed from the aquatic environment and will occur in natural feed items, if available.

Table 1.3.4 Mineral dietary requirement in carp *C. carpio.*

Mineral	Requirement
Phosphorus (P)	0.6-0.7%
Magnesium (Mg)	0.04-0.05%
Zinc (Zn)	15-30 ppm
Manganese (Mn)	13 ppm
Copper (Cu)	3 ppm
Cobalt (Co)	0.1 ppm
Iron (Fe)	150 ppm
Calcium (Ca)	>0.028%

Adapted from Shepherd and Bromage (1992).

In an aquaculture situation the farmer would apply himself to meet the needs of the fish as outlined in this section. However in the recreational fisheries sector this appears to be left very much to chance. In those waters which are stocked to high biomass levels (above 1000 kg/ha). Little thought appears to be given to supplying fish the required nutrition. Given this assumption this study was constructed to gain information about the suitability of such baits to sustain and promote healthy growth in *C. carpio.*

2.0 MATERIALS AND METHODS

2.1 Study considerations

When planning the study there was concern that the area of interest 'anglers bait' only occurs as a feed item in a 'natural' albeit highly stocked system. The required degree of control and limited time dictated that the study was carried out in a recirculation system, however, the behaviour of the fish in the 'wild' was taken into consideration when deciding strategies such as feeding regimes and designing the experiment.

2.2 Selection of diet

Five feed items that are used as angling bait for *C. carpio* were selected for study, these are shown in *Table 2.2.1*. The items were selected using the criteria that they were commonly used and sometimes banned for use by angling clubs or fishery owners.

2.3 Selection of fish

C. carpio was chosen to carry out the study, as not only is it the species that occurs most commonly in the recreational fisheries of interest (over stocked carp fisheries), it is also one of the species that lends itself well to nutritional and growth type studies. The carp is hardy, disease resistant and it is comparatively domesticated (Tave 1993).

The fish used in the study were juvenile (0+) common carp. The fish were purchased from a single source, in this way it was hoped to limit any genetic variability of growth (Purdom 1995), as different strains or races can display different growth potentials (Hulata, Wohlfarth, and Moav 1985). The heritability (h^2) of growth in *C. carpio* is considered to be significant at 0 - 0.34 (S.E ± 0.50), (Tave 1993). The reality is that genetic growth potential can differ between parents of the same strain, so selection for a low variance was unlikely, and probably impractical from the perspective of a potential supplier.

Table 2.2.1 Five selected feed items used as diets in the study of the physiological effect of bait on carp *C. carpio*.

Bait item	Selection criteria
Trout pellet	Very popular as a hook bait (made into a paste), introduced in the pellet form into waters in large amounts as loose feed as an attracter. Nowadays trout pellet is available from tackle shops. Trout pellets are often used as supplementary feed by fishery managers operating 'overstocked' carp fisheries.
Peanut	Enjoyed high popularity in the eighties as a hook bait and also as an attractant when used as loose feed. Banned in many waters because they are thought to be 'bad' for fish. Little evidence to support this. There is anecdotal evidence to suggest that many of the reported problems with peanuts may have been due to age induced nutrient inhibiting factors. They require soaking and boiling before use. Again peanuts have been linked by anecdotal evidence to fish deaths via the use of un-soaked peanuts.
Sweetcorn	Very popular bait, readily available. The tinned variety is the most popular. It is used as both hook and loose feed.
Maggot	The traditional anglers bait. Used as a hook bait and as loose feed. No longer a cheap alternative at £2.00 per pint. Maggots are available in a multitude of colours and sizes. It is thought by many anglers, that the fact that the bait is live when entering the water, gives it attracting properties.
Shelf-life boilie	These specially prepared baits are available in a variety of sizes. Anglers often use larger sizes for the hook, whilst using a smaller size for loose feed. The shelf-life label is a generic term for these boiled baits that can be used and stored over an extended period. The alternative is for the angler to purchase the ingredients and make his own. This gives a much shorter life.

Juvenile fish were chosen as they are more easily acclimatised to new surroundings than older fish and may feed more readily (Jobling 1983) in new surroundings. They also do not have the disadvantage of using energy/protein for gonad development, whilst showing more pronounced responses to depressed growth (Jobling 1983) or deficiency symptoms to unfavourable conditions. A final and very relevant consideration was that juvenile fish also require fewer resources.

2.4 Diet presentation and feeding strategy

The feeding of a single bait/feed item to specific fish was initially considered to be the correct way to proceed with the study. However for this to be within the guidelines laid down by the Home Office for experimental work on live animals one would need to be sure that the food fed would supply the essential nutrient requirements of the fish, as discussed in section 1.3, page 6. The ability of the baits to supply the nutritional requirement of the fish was not certain with perhaps the exception of trout pellet.

After discussion with Mr D. Hide (*pers. com.),* a lecturer in fish nutrition at Sparsholt College, it was decided to feed all fish regardless of their experimental diet, at two-percent body weight with trout pellets. This would ensure that all fish would receive the essential nutrients that they required.

For the study to be relevant and applicable to the natural system, the assumption that *C. carpio* will feed on the most abundant food source in the fishery must be made. There have been many pieces of work carried out into feeding behaviour of fish that support this assumption including Chesson (1983); Gherking (1994); Ivlev (1961) and Strauss (1979).

The principal of diet switch and optimal foraging theory can be applied to *C. carpio* in overstocked fisheries where little natural food is available. Diet switch means that a group of individuals of the same species offered two food choices, will feed disproportionately on the more abundant of the two, this is known as optimal foraging theory (OFT) (Gherking 1994). There is more than one interpretation of OFT, another version is that when the reward rate reaches some low but unspecified threshold, the predator switches to the alternate food choice, which may have become the more abundant (Gherking 1994).

A further point to consider is that anglers now often add chemical attractants to their bait, which can be in many forms, often centred around crystaline amino acids and also trout pellets as part of a ground-bait mix. Food search and the practice of chemically enhancing baits is dealt with by Jones (1992). The subject of chemo attraction has developed significantly in recent year's as a trip to your local specialist angling (tackle) shop will reveal, with literally hundreds of chemical enhancing preparations available. The potential effects of these chemicals on the aquatic environment poses a separate question.

The study assumed that the presentation of the diet, which consisted of the selected bait item and a two-percent supplement of trout pellet, represented the food item that because of its abundance would represent a selected food (OFT) item in the natural system.

2.4.1 Size of feed items

The size of the feed items at the commencement of the study was to a greater extent dictated by the size of the fish, some consideration was however given to ensure that baits/feed items for each of the study diets were of a suitable and comparable size when fed. Hepher (1988) states that the milling of seeds and plant nutritional components can improve digestibility.

Busacker, Adelman and Goolish (1990) makes the point that the way in which a particular ration is presented can influence the response of the fish.

2.4.2 Feed frequency

Stomachless omnivorous fish such as *C. carpio* have evolved a different feeding strategy to that observed in fish with stomachs such as the carnivorous species (De Silva and Anderson 1995). This generally has developed around taking an increased number of smaller meals.

The number of feeds can have an effect on the efficiency or utilisation of the ration (Yamada, Tanaka and Katayama 1981). The digestibility of the diet will also affect the gut evacuation time (GET). De Silva and Anderson (1995) state that other factors such as parasitic infestation, food deprivation, stocking density and day length can also pre-long or shorten gut evacuation times.

The stomach plays an important role as a storage organ, whilst in those species without a true stomach such as cyprinid's, this capacity is partially replaced by the intestinal bulb, which is an extension of the foregut and is where many of the important digestion processes take place (Hofer 1991).

Yamada, Tanaka and Katayama (1981) reported that when feeding rate was increased for both crystaline 'free' amino acid and casein control diets the percentage growth of *C. carpio* fry increased.

However ultimately in experimental systems the number of feeds will be dictated by many other factors such as availability of manpower and convenience.

The study will adopt a regime of a feeding frequency of three times per day, the diet fed in excess of requirement will be close to the case in commercial fisheries.

2.5 Stress

Stress is an important factor in this study for at least two important reasons. Firstly stress is known to have an impact on studies of fish that are conducted in controlled systems, this is very similar to fish that are cultured for aquaculture (Wedemeyer, Bruce and Mcleay 1990). Stressors are present in many forms such as human disturbance and environmental conditions such as overcrowding, deteriorating water quality etc. This type of stress will often be present in more natural systems such as 'overstocked' fisheries.

For the purpose of this study, stress from these environmental sources was avoided if at all possible. As previously suggested, water quality may well differ from that encountered in overstocked fisheries and is likely to be a key factor, but it is beyond the scope of this study.

The second aspect of stress that it was thought should be taken into consideration was, stress, which derives from angling disturbance, and indeed the fish being caught and held in a keepnet until returned.

The most recent work carried out by Pottinger (1998) would suggest that there is no additional stress caused to fish in retaining them in keepnets than that caused initially by capture. Capture however showed a highly significant ($P<0.001$) elevation in plasma cortisol. It is clear that fish in a fishery similar to those of interest to this study would endure stressing factors and thus at least a degree of stress.

2.6 Materials

2.6.1 Fish

Three hundred juvenile (0+) mixed sex *C. carpio* fry of a mirror variety (mean weight 1.52g) were purchased from Hampshire Carp Hatcheries (Bowlake Fish Farm). The fish were held in a single aquaria (1 x .3 x .2 m) at a temperature of 18°C rising to 21°C (± .5°C) over a period of three days, the fish were then maintained at this temperature for a further 6 days. The fish were then divided randomly into their fifteen study groups of twelve and placed into the recirculation system and acclimatised for a further 7 days. The acclimatisation period was longer than recommended as a minimum (Hochachka and Somero 1984). This was to allow time for the maturation of the system biofilter and some engineering modifications to the system to be made. Whilst held in the acclimatisation tank, fish were fed sparingly with powdered trout pellet.

2.6.2 Recirculation system

A purpose build system was constructed at the writers home to carry out the study, the system consisted of fifteen glass aquaria (38 x 30.5 x 61 cm), which were fed with water that had passed through a 30 watt UV filter. The flow rate was set at .5 l/min from a pumped supply through a 6 mm inlet pipe. Return to the reservoir for recirculation was provided by an outlet pipe of 25 mm diameter, which was fitted with a mesh filter to avoid escapee fish. The return from each tank went from 25 mm tank outflow to a 50 mm main return pipe to the reservoir.

The return water then flowed through a brush filter system before entering the biofilter chamber. The biofilter design, although very similar in appearance to some commercial models suitable for pond filtration, was designed using criteria recommended by Lawson (1995). The water was further filtered by a three mat system (coarse, medium and fine) before being pumped back around the system for reuse. A plan of the biofilter is shown in *Appendix A*, page I.

The water was heated to 21°C ± 1° by a thermostatically controlled immersion heater. Aeration was supplied by a compressor running at .75 BAR, this supplied aeration to each tank individually. A diagrammatic representation of an aquaria is shown in *Appendix A*, page II., together with a list of ancillary equipment *Appendix A*, page III.

2.6.3 Feed – bait items

The five bait items to be used as feed were purchased in bulk as far as possible, although maggots were collected from Grayshott Tackle on a weekly basis. The need to present the feed items in as a uniform manner as was possible was given a great deal of consideration. It was decided that all the feed items should be macerated in a domestic blender to ensure that particle size was uniform both within and across diets.

2.7 Methods

2.7.1 Fish

The fish were randomly selected from the holding tank and placed in one of the system aquaria, each tank was stocked with twelve fish. The fish were then wet weighed as a group to establish a bulk weight for each tank, the weight was recorded to the nearest gram. The study was run in triplicate with there being three tanks for each of the diets:-

A Trout pellet

B. Peanut

C. Sweetcorn

D. Maggot

E. Shelf-life boilie

Each diet was then identifiable by a letter and a tank number. The diet identification and tank allocation is shown in *Table 2.7.1.*

Table 2.7.1 Diet description and tank allocation of the five study diets

Diet	Tanks	Feed item description
A	1 (A1), 2 (A2), 3 (A3).	Trout Pellet. Manufactured by Trouw Aquaculture Ltd. Amino Balance 30.
B	4 (B1), 5 (B2), 6 (B3).	Peanuts. Purchased from Health food outlet to ensure freshness.
C	7 (C1), 8 (C2), 9 (C3).	Sweetcorn. Purchased from grocery store/supermarket. Tinned variety used, manufactured under the brand name 'Jolly Green Giant'.
D	10 (D1), 11 (D2), 12 (D3).	Maggots. Obtained from a fishing tackle shop. Fresh on a weekly basis.
E	13 (E1), 14 (E2), 15 (E3).	Shelf-life boilies. Obtained in a bulk pack from fishing tackle shop. Manufactured by Richworth Baits Limited. Flavour was fishmeal special.

2.7.2 Operation and maintenance of the system

The chemical quality of the water was checked twice weekly to ensure adequate dissolved oxygen (DO) and that other important criteria for water quality such as ammonia and pH were kept well within normal tolerance limits of *C. carpio* (Boyd 1982). Twenty-five percent water changes were carried out on a weekly basis, the water change was part of the tank cleaning process rather than to maintain water quality, although this was also a net effect. The data recorded for water quality parameters is shown in *Appendix B*, page I

2.7.3 Feeding regime

The fish were fed three times a day at 07.30 a.m., 1.00 p.m. and again at 6.00 p.m. All tanks were cleaned each morning. (The daily routine protocol is shown in *Appendix B*, page IV. At weekly intervals the fish were weighed and the new rations calculated.

So as to induce similar stress to that encountered by the fish in a natural fishery situation, the fish were not starved prior to weekly weighing (equivalent to being caught by eating bait). All weighing with the exception of that at the conclusion of the study was conducted wet. Feed was calculated on a percentage body weight basis, which was then turned to an 'energy-supplied' basis. For clarity each feed ration was labelled for the correct tank, and was measured to 0.02 g accuracy.

The feeding of the fish in all tanks was carried out by hand at the rate detailed in Table 2.7.2, further details of the feed rate are shown in *Appendix B*, pageV.

The amino balance trout pellet manufactured by Trouw Aquaculture Limited was chosen as the control for the study as cyprinid fish are known to grow well on it (Scott 1987).

Information about the physiological effects of trout pellet on fish, as it is often used as supplementary feed and a bait in the fisheries of interest, would it was hoped be helpful for comparative purposes as part of the trial, representing a bait item in its own right. The literature review carried out as part of this study revealed that the subject of trout pellets and their suitability for carp nutrition has received very little attention and still requires researching.

Table 2.7.2 Feed rates and for the five study diets

Diet	kj/g	Feed rate	Detail
A	24.70	2% b.wt. in trout pellet, 6% b.wt. in trout pellet.	Total amount of kj/g fed is calculated and used as the control.
B	18.36	2% b.wt. in trout pellet. Equivalent to 6% b.wt (calorific value) in diet A1, fed as grams feed.	2% b.wt. is fed in amino balance the kj/g (45.4) is then subtracted from the total number of kj/g fed for diet A, tank 1. This amount is then divided by the kj/g for this diet (18.36) and fed as a weight in grams.
C	5.78	2% b.wt. in trout pellet. Equivalent to 6% b.wt (calorific value) in diet A1, fed as grams feed.	2% b.wt. is fed in amino balance the kj/g (45.4) is then subtracted from the total number of kj/g fed for diet A, tank 1. This amount is then divided by the kj/g for this diet (5.78) and fed as a weight in grams.
D	8.96	2% b.wt. in trout pellet. Equivalent to 6% b.wt (calorific value) in diet A1, fed as grams feed.	2% b.wt. is fed in amino balance the kj/g (45.4) is then subtracted from the total number of kj/g fed for diet A, tank 1. This amount is then divided by the kj/g for this diet (8.96) and fed as a weight in grams.
E	18.56	2% b.wt. in trout pellet. Equivalent to 6% b.wt. (calorific value) in diet A1, fed as grams feed.	2% b.wt. is fed in amino balance the kj/g (45.4) is then subtracted from the total number of kj/g fed for diet A, tank 1. This amount is then divided by the kj/g for this diet (18.56) and fed as a weight in grams.

The diets were fed as iso-energetic meals, this should not be confused with the popular method of testing diets, i.e. as iso-energetic diets. The diets used in the study were energetically very varied, but the amount of each diet in weight was varied by diet, to ensure that the same amount of energy was supplied to each tank.

The energy balance was achieved by testing each of the five feed items by bomb calorimetry according to accepted procedures (Gallenkamp 1988; Dowgiallo 1975). As this procedure was carried out as a pre study procedure the values are shown below in *Table 2.7.3.*, and have also been used in the table on the previous page (*Table 2.7.2.*).

Table 2.7.3 Calorific value of the five feed items used in the study.

Diet	Calorific value – kj/g
A – trout pellet	24.70
B – peanut	18.36
C – sweetcorn	5.78
D – maggot	8.96
E – boilie	18.56

Initially the amount of trout pellet at both 2% and 8% b.wt. was calculated for diet A, tank 1. This was then converted to wet weight kj/g. The amount of kj/g for the other four diets was then used to calculate the amount of energy to be fed to each of the study tanks. The kj/g was then converted back to grams of feed for each diet, and divided by three, the number of discrete meals per day.

A proximate analysis of the five diets was carried out, the methodology used was as follows. The Kjeldahl nitrogen determination method for protein content (Dowgiallo 1975). The soxhlet extraction method for oil percentage (Dowgiallo 1975).

Ash values were obtained by drying a sample of each diet at 500°C for 24 hr's (Dowgiallo 1975).

As this procedure was carried out as a pre study procedure the values are shown in graphic form in *Figure 2.7.4.*, and tabular form in *Table 2.7.5.* Full procedures for the analytical methods used in the laboratory can be found in literature published on standard methodologies by the American Association of Official Analytical Chemists (AAOAC).

Figure 2.7.4 Pie charts showing the proximate analysis of five feed items.

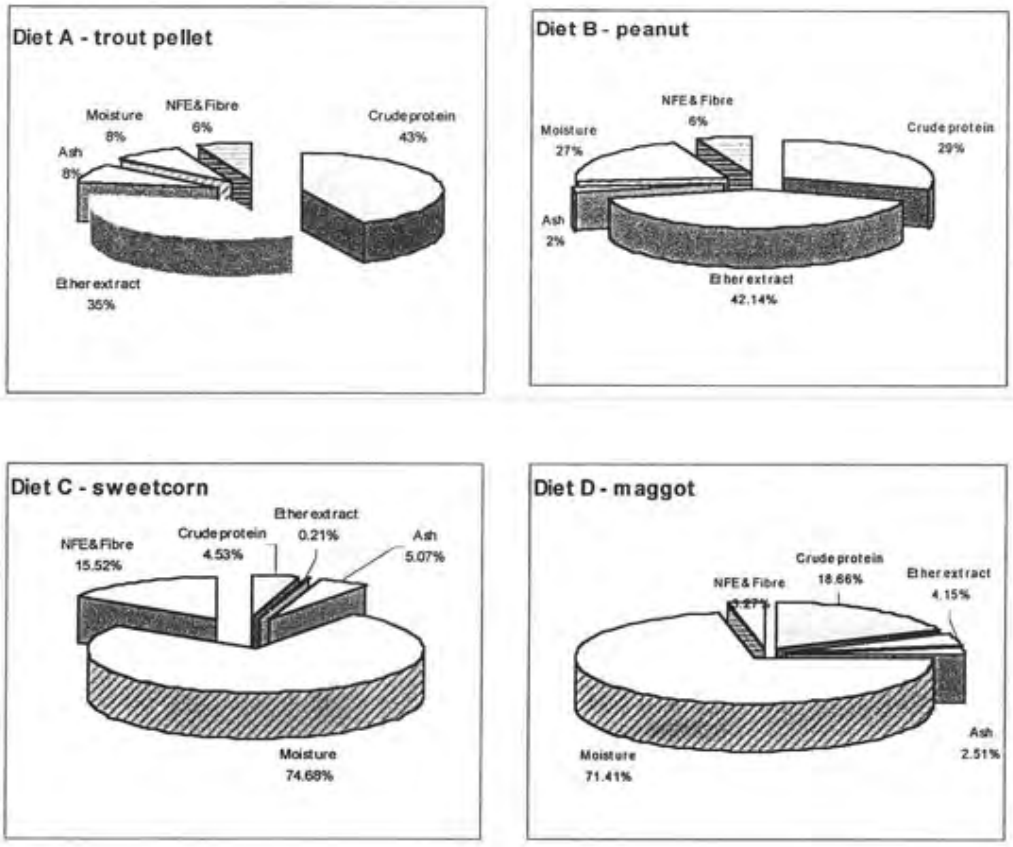


Figure 2.7.4 Pie charts showing the proximate analysis of five feed items.

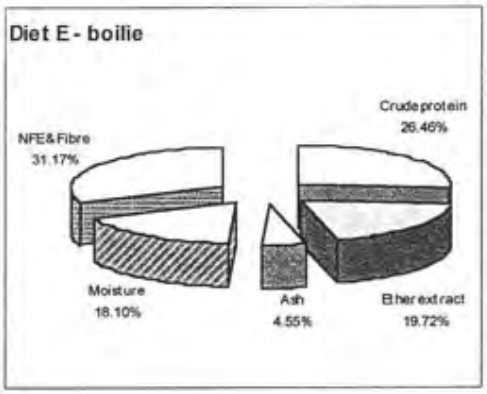


Table 2.7.5 Proximate analysis of the five diets

Diet	Moisture %	Protein %	Ether extract %	Ash %	NFE & Fibre
A	8.32	42.8	34.63	8.07	6.10
B	20.77	29.2	42.14	1.96	5.93
C	74.68	4.53	0.21	5.07	15.52
D	71.41	18.66	4.15	2.51	3.27
E	18.1	26.46	12.00	4.55	31.97

2.8 Parameters measured

1. Daily records were kept of fish feeding behaviour, waste, mortality (number but not weight), and food fed. These records were entered onto a spreadsheet and are now reproduced as *Appendix B*, page XI.
2. The bulk weight of fish in each tank was measured on a weekly basis. These records are shown in *Appendix B*, page V.
3. At the end of the study period a sample of the fish were weighed and measured and condition factor calculated. These are shown in *Appendix B*, page XII
4. From the data recorded the specific growth rate and percentage growth were calculated. These are shown in Appendix B, page XIV.

Other areas that were measured included:-

- Carcass moisture content Carcass lipid content
- Visceral-somatic index Liver-somatic index

2.9 Methodology for mortality analysis

Mortality percentage can be an indirect indicator of the nutritional suitability of a feed item when fed under comparative and controlled conditions. The mortality percentage per diet was compared for significant differences. As percentages or proportions are not normally distributed (Zar 1996) the data was transformed using arcsine transformation before undergoing parametric analysis.

2.10 Methodology for specific growth rate (S.G.R.) analysis

The assumption is made that the stage or life-cycle of the fish matches that of a stage where a change in size (growth) over time can be expected (Ricker 1979). SGR is the most widely used numerical expression of growth over time, it is calculated using the following formula:-

$$SGR = (LN\ Y_2 - LN\ Y_1) / (t_2 - t_1) \times 100$$

Where:- LN = Natural log Y_2 = Final weight t_1 = Start time
Y_1 = Start weight t_2 = Finish time

Ricker (1979) suggests that the instantaneous or SGR is a more appropriate measure of growth than expressions of absolute growth for study periods less than a year.

2.11 Percentage growth

Percentage growth is a relative expression of growth, which can be used to indicate the overall performance of a group of fish for comparative purposes. It is calculated using the formula:-

$$\% \text{ growth} = (Y_2 - Y_1) / Y_1 \times 100$$

Where: Y_1 = start weight Y_1 = Final weight

2.12 Methodology for carcass moisture content analysis

Moisture content in isolation is not a known indicator of either the physiological or nutritional condition of fish, however the relationship that is known to exist between moisture content and lipid content is important (Stoskopf 1993). This relationship, which is inversely proportional is very useful index of fish condition (Roberts 1989) and using the following procedure can be used to predict lipid levels for all the fish in all of the five groups. The level of moisture content in each of the five diets was obtained by drying three separate fish samples from each diet after each complete individual fish had been homogenised in a domestic blender. The paste like material was then spread on a glass dish and placed into an oven to dry for 24-h at 100°C. The amount of weight loss in drying gave the moisture content of the sample. The data is shown in *Appendix C*, page I

2.13 Methodology for carcass lipid content analysis

Lipid levels in a dry fish sample were measured by the Soxhlet extraction method (Dowgiallo 1975). Using nine samples from various diets, which had varying moisture contents, the oil content was plotted against their moisture content. The resulting regression equation was used to calculate the oil content of the remaining fish samples. The data is shown in *Appendix C*, page I.

2.14 Methodology for visceral somatic analysis

The visceral-somatic index (VSI) measures the viscera as a percentage of the total body weight (Adams and Mclean 1985). The viscera and associated cavity can be used as an area to store lipid, when fish are fed lipid rich diets (Adams and Mclean 1985; Kaushik 1995). The VSI is used as an expression if visceral lipid stores versus body weight (Delahunty and DeVlaming 1980). This indirect measure of growth rate is expressed as:-

VSI = (viscera weight/body weight) x 100

The VSI was calculated from a sample of 5 fish from each diet. The data is shown in *Appendix C*, page II.

2.15 Methodology for liver somatic index analysis

The liver is used by fish to accumulate energy during periods of high energy intake; much of this energy is stored in the form of glycogen (Busacker, Adelman and Goolish 1990). Therefore, the relative size of the liver should be able to give an indication as to the nutritional state of the fish. This indirect measure of growth rate is referred to as the liver-somatic index (LSI) (Adams and McLean 1985), and is expressed as:-

LSI = (liver weight/body weight) x 100

The LSI was calculated from a sample of nine fish from each diet. The data is shown in *Appendix C*, page III.

When carrying out research with fish that are mature or maturing, the LSI should not be confused with the hepatosomatic index (HSI). Whilst being very similar it is not exactly the same as the LSI, the gonads are subtracted from the fish's body weight when wishing to arrive at an HSI, before carrying out the equation as per the LSI. Technically of course the juvenile fish did not have gonads to remove.

2.16 Methodology for condition factor analysis

The condition factor (CF) can indicate both fluctuations and any anomalies in somatic growth, data is simply analysed by the use of the coefficient of condition (K), where:-

$K = \text{weight}/(\text{length})^3$.

The condition factor will reflect the nutritional state or "well-being" of an individual fish (Busacker, Adelman and Goolish 1990), although CF can show a wide variation in weight of fish of the same length within populations (Anderson and Neumann 1996). The condition factor was calculated for each individual fish in the study. The data is shown in *Appendix B*, page XII.

2.17 Data analysis

The data as detailed above and shown in the various appendix's was analysed by using one way analysis of variance (ANOVA) (Sokal and Rohlf 1995). Each set of data to be tested for ANOVA was first tested for homogeneity of variance by using the F_{max} test . Data that subsequently failed the F_{max} test was log transformed (Sokal and Rolfe 1995), before being resubmitted to further testing using ANOVA, data that subsequently failed, was submitted to further testing with the Tukey test to establish between which means there were differences (Zar 1996).

Mortality percentage was analysed by first carrying out arcsine transformation (Cohen and Fowler 1990).

The relationship between moisture and lipid content was analysed by using linear regression (Cohen and Fowler 1990).

.

The source of statistical tables are shown in *Table 2.17.1*

Table 2.17.1 Source of statistical tables

Table	Statistic	Source
F_{max}	F_{max} 0.05	Fowler and Cohen (1990).
ANOVA	F_{tab}	Murdoch and Barnes (1998)
Tukey	q 0.0.5	Murdoch and Barnes (1998)

All data analysis and statistical workings was carried out using Microsoft Excel 7 for Windows 98 on a Pentium II- 400 personal computer.

2.18 Anaesthetic and post mortem procedure.

A 24-h starvation period was allowed before fish were killed by overdosing with 98% ethyl 4-aminobenzoate (benzocaine). Approximately 5 grams was dissolved in 100 ml of acetone, which was then slowly added to 5 litres of water. The fish were introduced to the container and only removed when they were dead. Each fish was then weighed in grams (± 0.02) and its length recorded in centimetres (± 0.1). Each fish was then individually subjected to a post mortem procedure, which included an external examination. An internal examination followed, which included removing and weighing the liver in grams (± 0.00005), and also removing and weighing the viscera, also weighed in grams to the same accuracy. The post mortem procedure is detailed in *Appendix D*, page I.

3.0 RESULTS

The result section is divided into two sections. The first deals with results relating to the performance of the fish. Initial and final weights, specific growth rate (SGR), percentage growth, and mortality.

The second section deals with results relating to the carcass and indirect measurement of growth or physiological factors thought to relate to diet. Moisture and lipid levels, condition factor, and liver and visceral somatic indexes.

3.1 Performance

3.1.1 Initial weight

The initial weight for all five diets is represented graphically in *Figure 3.1.1.* There was a highly significant differences between initial weights ($P \leq 0.01$, F: 30.41, df : 4, 10) of the diets. The data was further analysed using a Tukey test to establish the means between which there were significant differences. The test showed that there were significant ($P \leq 0.05$, Q: 4.65, df: 4,10) differences between diet E and the other four diets The ANOVA and Tukey test is shown in *Appendix E*, page I.

Unfortunately due to the variability of the data between replicates of the same diet, displaying the data graphically was not straightforward.

Displaying the data in a meaningful format was only possible by showing the mean diet weight with error bars showing the smallest and largest mean weights of the other replicates of the same diet. However the 95% C.I. for the data is shown in *Table 3.1.2.*

Figure 3.1.1 **Mean initial weight and replicate (highest and lowest) range of *C. carpio* fed five diets.**

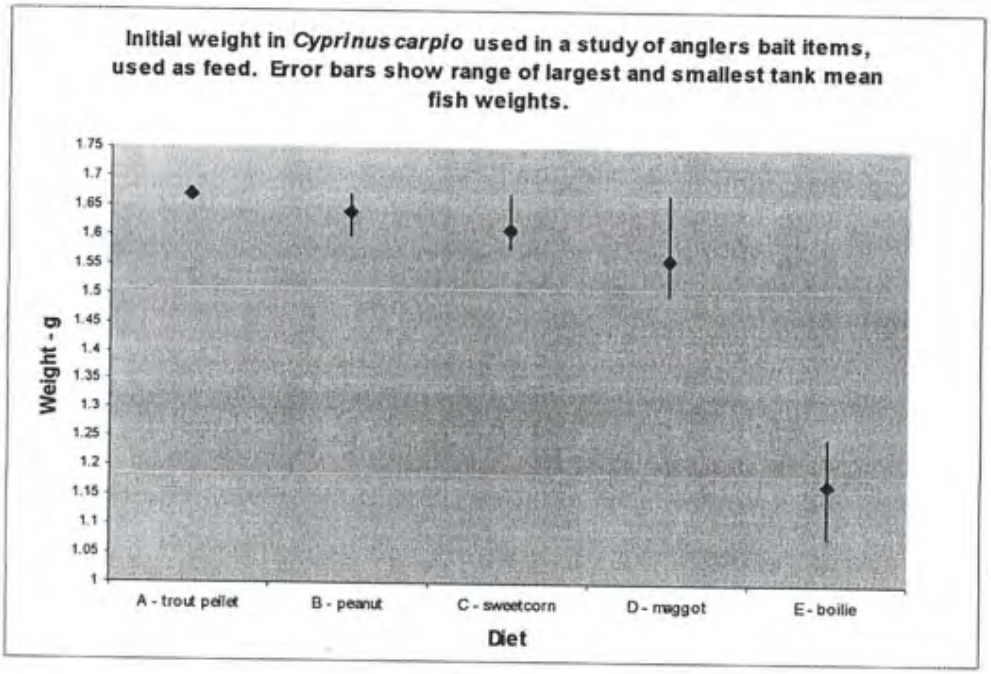


Table 3.1.2 **Mean initial weight and range (highest and lowest) of *C. carpio* fed five diets.**

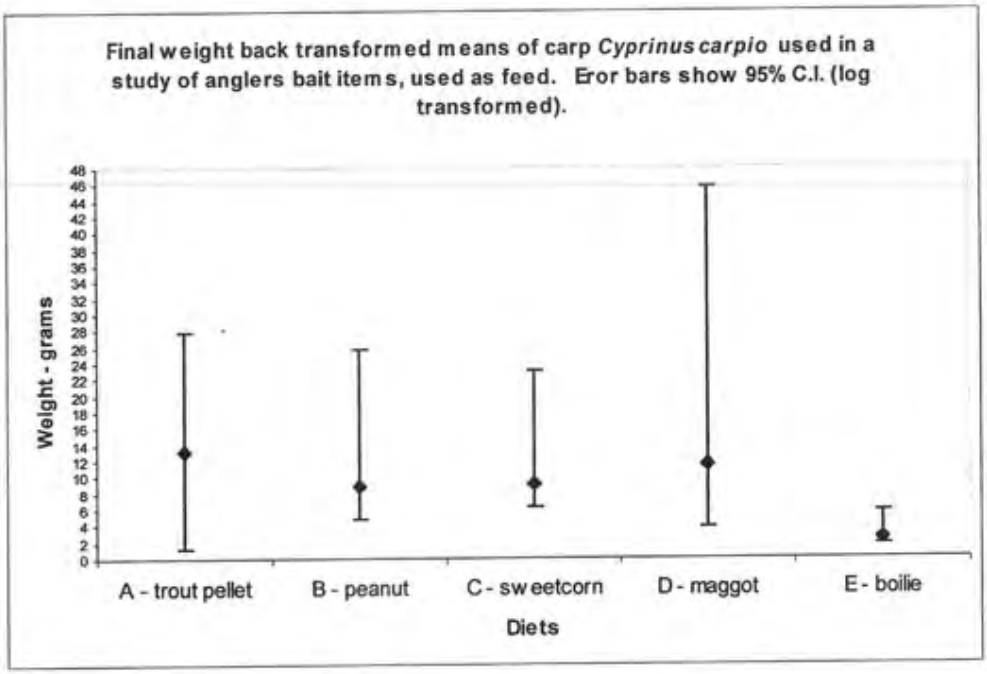
Diet	Smallest mean replicate fish weight (-)(g)	Mean of three replicates - fish weight (g) (SD)	Largest mean replicate fish weight (+)(g)	95% C.I. of mean of three replicates fish weight
A - trout pellet	0	1.67 (2.98E – 08)	0	0
B - peanut	0.04	1.64 (0.479)	0.03	0.120
C - sweetcorn	0.03	1.61 (0.479)	0.06	0.120
D - maggot	0.06	1.56 (0.096)	0.11	0.239
E - boilie	0.09	1.17 (0.085)	0.08	0.120

3.1.2 Final weight

The final weight for all five diets is represented graphically in *Figure 3.1.3.* It is shown as backtransformed data with 95% C.l . In a similar fashion to the initial weight data, the data could not be presented in an acceptable form without this adjustment. The mean final weights and 95% C.I. are also shown in *Table 3.1.4*

The data failed the F_{max} test for homogeneity of variance, and for this reason the data was log transformed and was reanalysed. There was a significant ($P \leq 0.05$, F: 22.45, df: 4, 10) differences between final weights of the diets. The data was further analysed using a Tukey test to establish the means between which there were significant differences.

Figure 3.1.3 Mean final weight and 95% C.I. of *C. carpio* fed five diets.



The Tukey test showed that there were significant differences between diet E and the other four diets (P≤ 0.05, Q: 4.65, df: 4,10). The ANOVA and Tukey test is shown in *Appendix E*, page I and II.

Table 3.1.4. Mean final weight and 95% C. I. for *C. carpio* fed five diets.

Diet	Mean of three replicates - fish weight (g) (SD)	95% C.I.
A - trout pellet	13.26 (0.536)	1.327
B - peanut	8.99 (2.666)	5.134
C - sweetcorn	9.22 (1.457)	3.621
D - maggot	11.51 (5.472)	13.594
E - boilie	2.41 (0.344)	0.855

3.1.3 Specific growth rate – SGR

The SGR for all five diets is represented graphically in *Figure 3.1.5.* There was a highly significant (P≤0.01, F: 12.01, df : 4,10) differences between SGR's of the diets. The data was further analysed using a Tukey test to establish the means between which there were significant differences. The test showed that there were significant differences between diet E and the other four diets (P≤ 0.05, Q:4.65, df: 4,10). The mean SGR's and 95% C.I., are also shown in *Table 3.1.6.* The ANOVA and Tukey test is shown in *Appendix E*, page II.

Figure 3.1.5 Mean cumulative S.G.R and 95% C.I. for each of five diets fed to *C. carpio.*

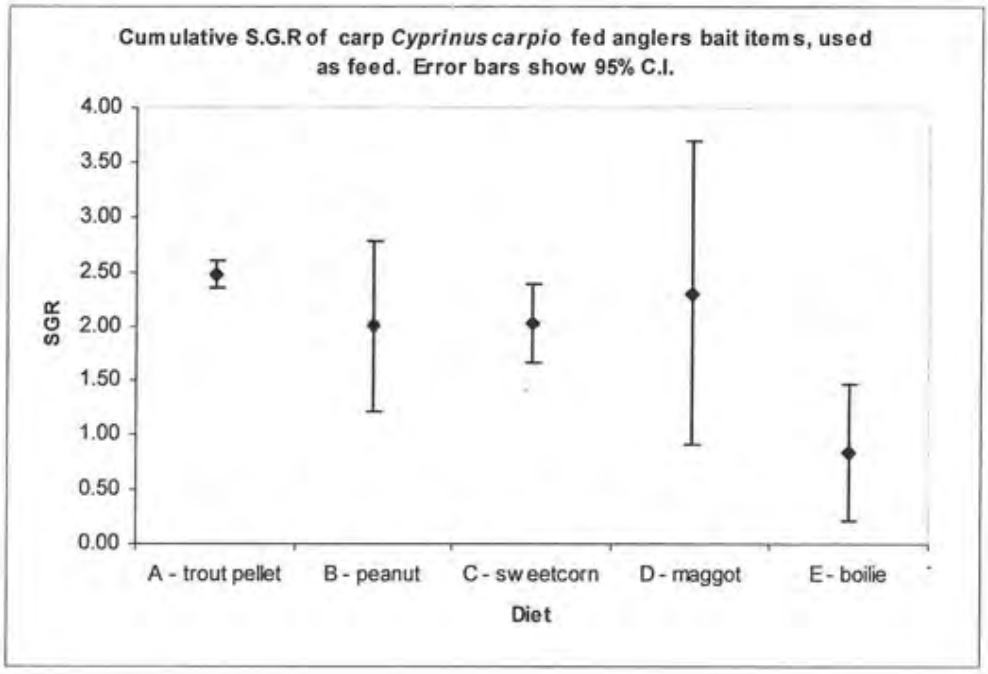


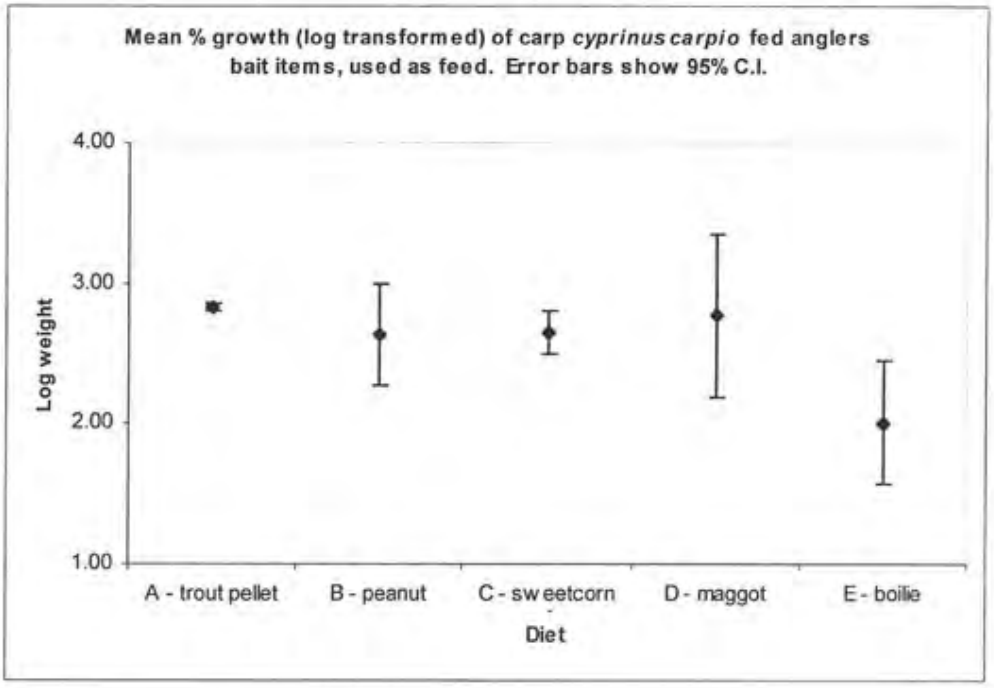
Table 3.1.6 Mean cumulative S.G.R and 95% C.I. for *C. carpio* fed five diets

Diet	SGR (SD)	95% C.I.
A – trout pellet	2.47 (0.050)	0.125
B – peanut	2.00 (0.316)	0.784
C – sweetcorn	2.02 (0.144)	0.357
D – maggot	2.30 (0.561)	1.395
E - boilie	0.85 (0.252)	0.627

3.1.4 Percentage growth

The percentage growth for all five diets is represented graphically in *Figure 3.1.7.* However due to the variability both within and across the groups it was not possible to represent the data in graphic form without first carrying out a log transformation. The data is represented in a biological sense in *Table 3.1.8.*

Figure 3.1.7 Mean total % growth and 95% C. l. of *C. carpio* fed five diets.



The data failed the F_{max} test for homogeneity of variance, and for this reason the data was log transformed and was reanalysed. There was a significant ($P \leq 0.05$, F: 14.54, df : 4,10) differences between % growth of the diets. The data was further analysed using a Tukey test to establish the means between which there were significant differences. The Tukey test showed that there were significant ($P \leq 0.05$, Q: 4.65, df: 4,10) differences between diet E and the other four diets The ANOVA and Tukey test is shown in *Appendix E*, page III

Table 3.1.8 95% C.I. for mean total % growth for *C. carpio* fed five diets.

Diet	% growth (SD)	95% C.I.
A - trout pellet	672.37 (15.436)	38.346
B - peanut	449.54 (135.551)	336.729
C - sweetcorn	450.36 (62.825)	156.066
D - maggot	647.44 (381.821)	948.496
E - boilie	107.30 (45.487)	112.996

3.1.5 Mortality

The percentage mortality for all five diets is represented graphically in *Figure 3.1.9.* The data for percentage mortality had arcsine transformation carried out on it. The data was then analysed. There was shown to be a highly significant (P≤0.01, F: 6.92, df: 4, 10) difference between percentage mortality of the diets. The data was further analysed using a Tukey test to establish the means between which there were significant differences. The test showed that there were significant (P≤ 0.05, Q: 4.65, df: 4, 10) differences between diet E and the other four diets, however due to the variability both within and across the groups it was not possible to represent the data in graphic form using 95% C.l. After discussions with Dr M. Burdass (*pers. com.)* a lecturer in data analysis at Sparsholt College, it was decided to show the data as a range of each diet. This is shown in *Table 3.1.10.* The ANOVA and Tukey test is shown in *Appendix E,* page III.

Figure 3.1.9 Mean mortality rate for *C. carpio* fed five diets

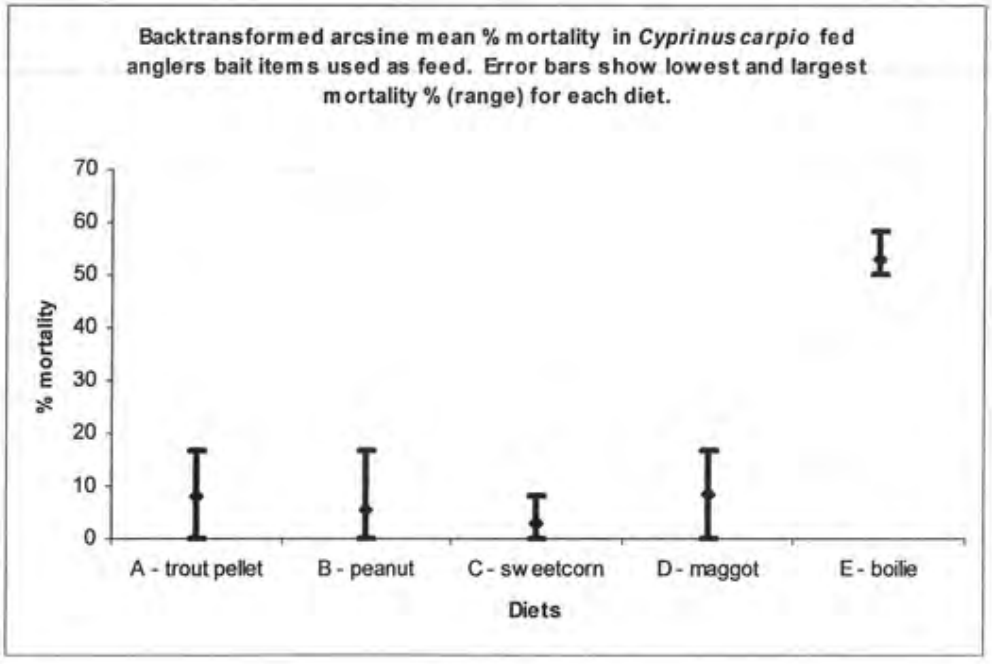


Table 3.1.10 Mean mortality rate and range (highest and lowest) for *C. carpio* fed five diets

Diet	Lowest mean replicate % mortality	Mean of three replicates % mortality (SD)	Highest mean replicate % mortality
A - trout pellet	0	8.33 (8.333)	16.67
B - peanut	0	5.56 (9.622)	16.67
C - sweetcorn	0	2.78 (4.811)	8.33
D - maggot	0	8.33 (8.333)	16.67
E - boilie	50	52.78 (4.811)	58.3

3.2 Indirect measurements and physiological factors

3.2.1 Moisture and lipids

The relationship between moisture and lipid levels was analysed using linear regression. This relationship is graphically shown in *Figure 3.2.1*. The data used to model the linear relationship is shown in *Table 3.2.2*. The coefficient of determination (r^2) shows a value of 0.6219. This means that 38.67 % of the variation in lipid % in fish composition is accounted for by variation in moisture content.

The analysis of the significance of the regression line showed that there was no significant ($P \geq 0.01$, t: 0.685, df: 6) linear relationship between y and x variables. Given the failure of the data to show a significant relationship the lipid percentage for each diet was analysed by ANOVA. The data is shown in graphical form in *Figure 3.2.3*. The data with 95% C.I. is shown in *Table 3.2.4*

Figure 3.2.1 **Linear regression analysis of the relationship between moisture and lipid for *C. carpio* fed five diets.**

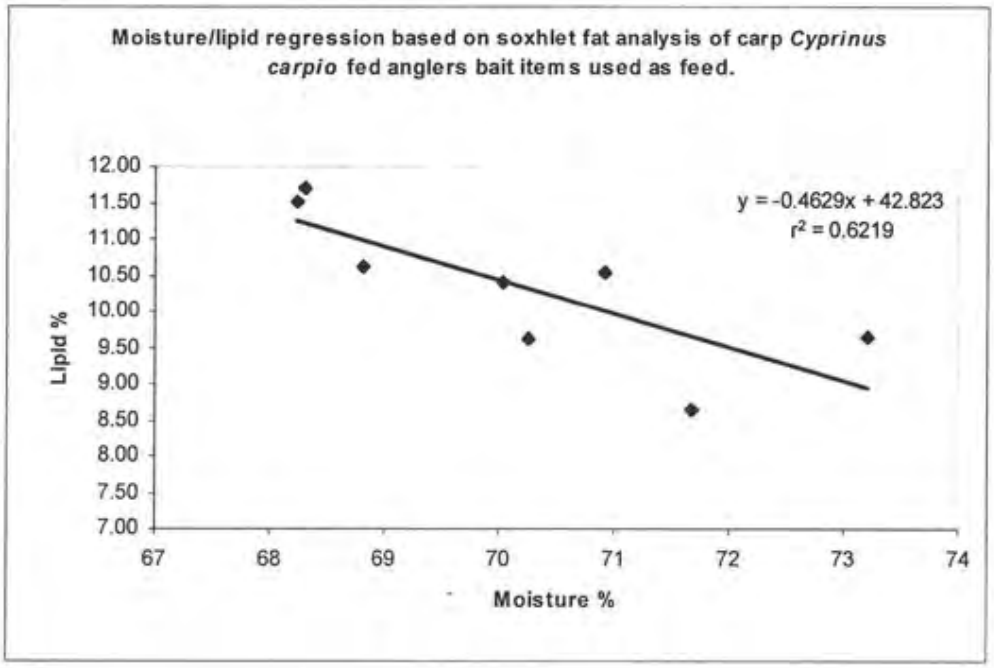


Table 3.2.2 **Data used to model linear relationship for *C. carpio* fed five diets.**

Diet	Oil - g	Moisture %	Oil %
A3-6	1.04	68.24	11.51
A1-10	1.15	70.04	10.40
D3-6	0.85	69.87	10.62
C1-5	0.74	68.82	10.62
C3-6	0.89	71.67	8.64
B1-5	1.16	68.31	11.71
D2-5	1.58	73.2	9.66
E3-1	0.66	70.92	10.54
B3-4	1.29	70.26	9.63

There was shown to be a highly significant ($P \leq 0.01$, F: 5.59, df: 4, 25) differences between lipid composition of the fish carcass of the different diets. The data was further analysed using a Tukey test to establish the means between which there were significant differences. The test showed that there were significant ($P \leq 0.05$, Q: 4.65, df: 4, 25). differences between diet E and diet A., and diet E and diet B.

Figure 3.2.3 Mean % lipid and 95% C.I. of *C. carpio* fed five diets.

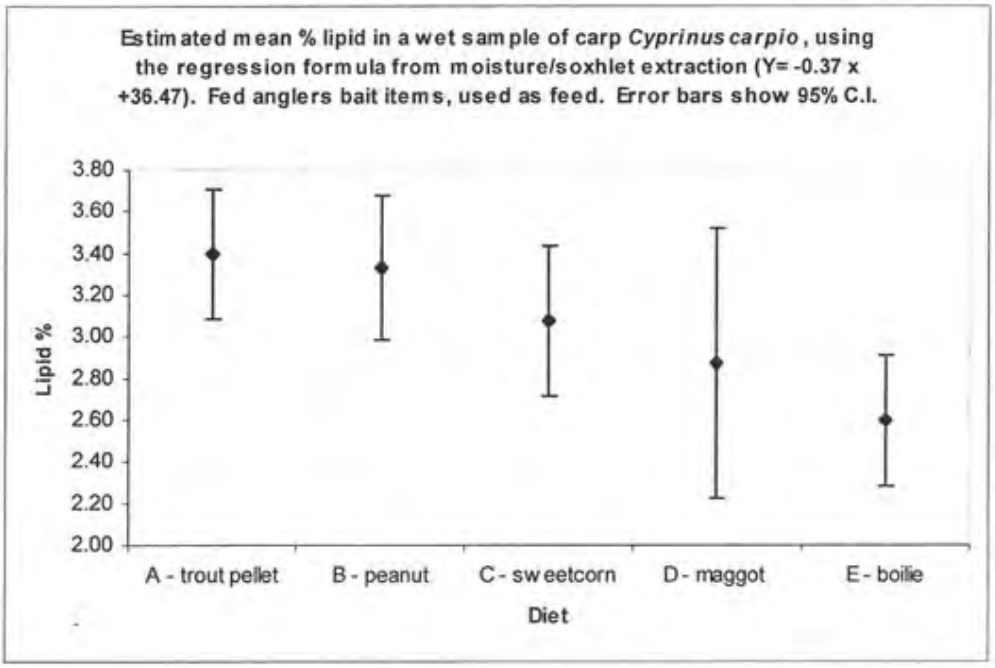


Table 3.2.4 Mean % lipid in body composition with 95% C.I. for *C. carpio* fed on five diets.

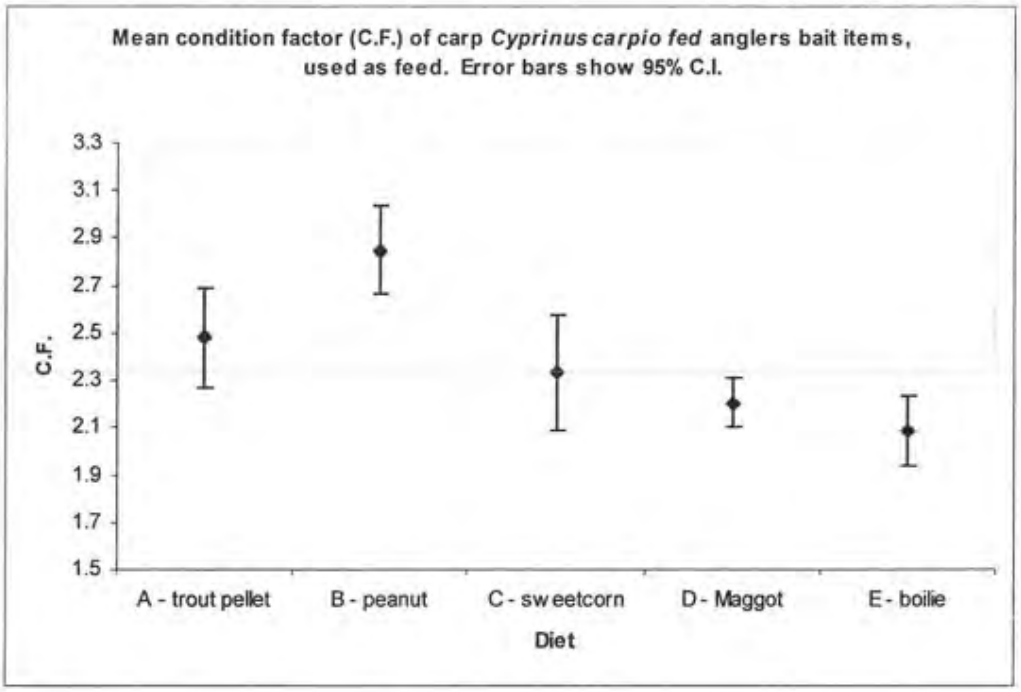
Diet	Mean % (SD)	95% C.I.
A - trout pellet	3.42 (.296)	0.337
B - peanut	3.33 (.331)	0.348
C - sweetcorn	3.08 (.343)	0.360
D - maggot	2.87 (.314)	0.647
E - boilie	2.60 (.297)	0.313

The ANOVA and Tukey test and significance of the regression line (S.E.) test is shown in *Appendix E,* page IV.

3.2.2 Condition factor

The condition factor (CF) for all five diets is represented graphically in *Figure 3.2.5.* The data was then analysed using ANOVA. There was shown to be a highly significant (P≤0.01, F: 13.58, df: 4, 40) difference between the CF of the diets. The data with 95% C.I. is shown in *Table 3.2.6.* The data was further analysed using a Tukey test to establish the means between which there were significant differences. The test showed that there were significant (P≤ 0.05, Q: 4.04, df: 4, 40) differences between diets:- A and D, A and E, B and the other four diets, and C and E.

Figure 3.2.5 Mean condition factor for *C. carpio* with 95% C.I. fed five diets



The ANOVA and Tukey test is shown in *Appendix E,* page V.

Table 3.2.6 Mean condition factor with 95% C.I. for *C. carpio* fed five diets.

Diet	Mean C.F. (SD)	95% C.I.
A - trout pellet	2.48 (0.275)	0.211
B - peanut	2.85 (0.243)	0.187
C - sweetcorn	2.33 (0.315)	0.242
D - Maggot	2.20 (0.135)	0.104
E - boilie	2.09 (0.193)	0.148

3.2.3 Liver somatic index - LSI

The LSI for all five diets is represented graphically in *Figure 3.2.6*. The data failed the F_{max} test for homogeneity of variance, and for this reason the data was log transformed and was reanalysed. The backtransfomed means and 95% C.I. is shown in *Table 3.2.7*

Figure 3.2.6 Liver somatic index with 95% C.I. for *C. carpio* fed five diets

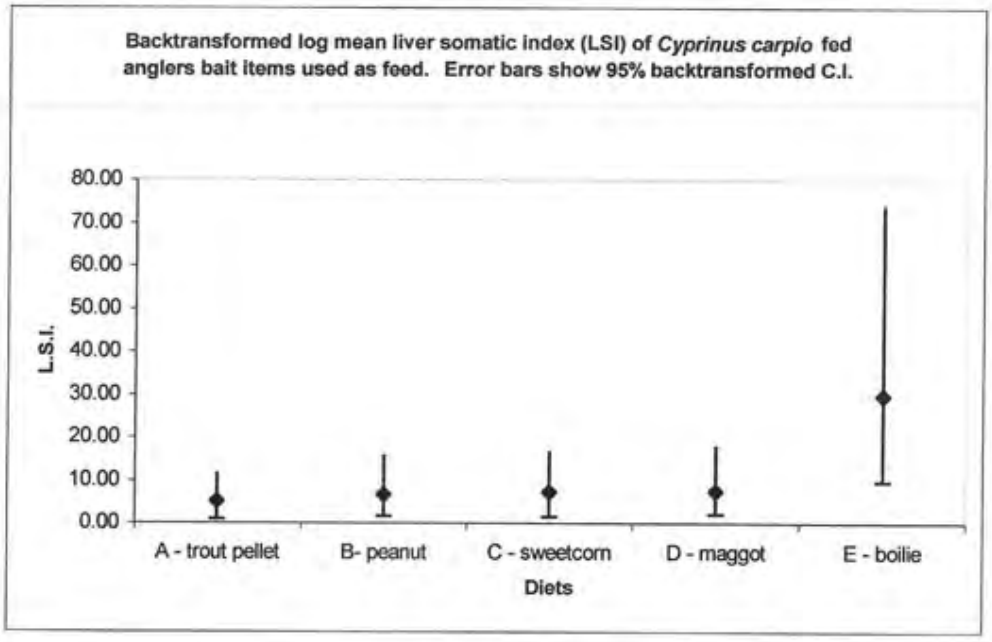


Table 3.2.7 Log backtransformed means for LSI in *C. carpio* fed five diets.

Diet	Mean L.S.I. (SD)	95% C.I	upper 95% Cl.	lower 95% C.l.
A - trout pellet	5.12 (1.542)	1.216	6.224	4.208
B- peanut	6.74 (2.490)	1.317	8.875	5.119
C - sweetcorn	7.36 (2.070	1.235	9.094	5.960
D - maggot	7.49 (3.018)	1.376	10.313	5.445
E - boilie	29.80 (13.09)	1.477	44.013	20.183

There was a highly significant ($P \leq 0.01$, F: 28.56, df: 4, 40) difference between LSI of the diets. The data was further analysed using a Tukey test to establish the means between which there were significant ($P \leq 0.05$, Q: 4.04, df: 4, 40) differences. The diets between which there were significant differences were diet A and the other four diets and diet E and the other four diets.

The ANOVA and Tukey test is shown in *Appendix E*, page VI.

3.2.4 Visceral somatic index - VSI

The VSI for all five diets is represented graphically in *Figure 3.2.8.* The data failed the F_{max} test for homogeneity of variance, and for this reason the data was log transformed and was reanalysed. The backtransformed means and 95% C.l. are shown in *Table 3.2.9.*

There was a highly significant ($P \leq 0.01$, F, 103.66, df : 4, 20) difference between VSI of the diets. The data was further analysed using a Tukey test to establish the means between which there were significant differences.

The diets between which there were significant (P≤ 0.05, Q: 4.23, df: 4, 20) differences were diet A with diets C, D, and E.; diet B with diets C, D, and E.; diet C with diet E.; and diet D with diet E.

The ANOVA and Tukey test are shown in *Appendix E,* page VI.

Figure 3.2.8 Visceral somatic index with 95% C.l. for *C. carpio* fed five diets

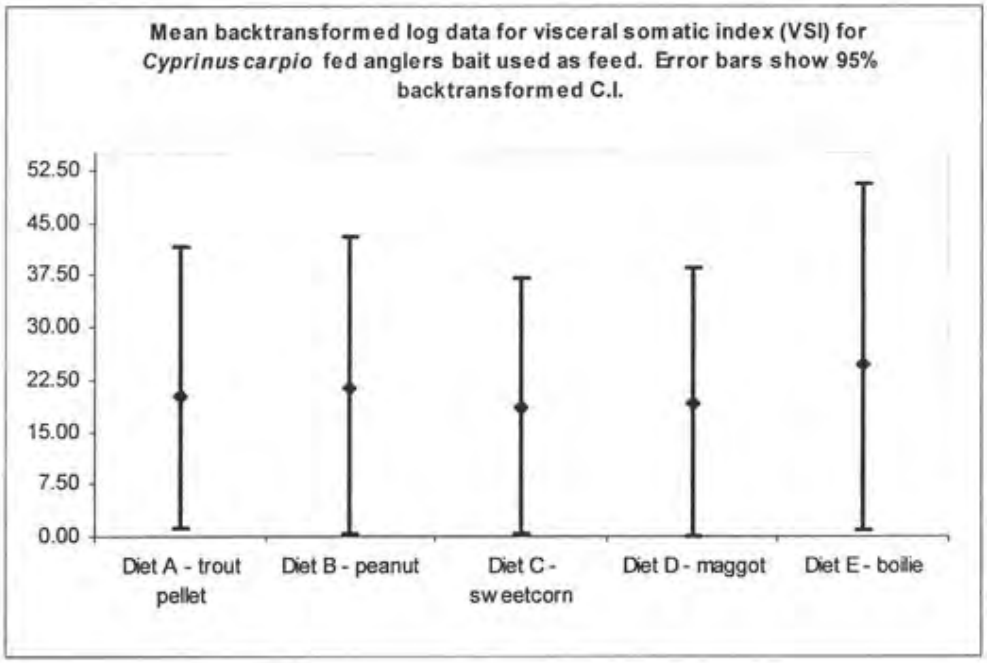


Table 3.2.9 Log backtransformed means for VSI with 95% CI and C.l. for *C. carpio* fed five diets.

Diet	Mean V.S.I. (SD)	95% CI.	upper 95% C.l.	lower 95% C.l.
A - trout pellet	20.17 (0.992)	1.061	21.396	19.019
B - peanut	21.34 (0.163)	1.009	21.546	21.144
C - sweetcorn	18.42 (0.261)	1.018	18.743	18.095
D - maggot	19.20 (0.101)	1.007	19.330	19.079
E - boilie	24.83 (0.646)	1.033	25.642	24.040

4.0 DISCUSSION

This discussion section is divided into three parts, the first deals with parameters measured that relate directly to fish growth and performance, whilst the second part of the section will deal with the results relating to the carcass and indirect measurement of growth and physiological factors. The third part of the section will draw together and consider the significance of all the results holistically.

4.1 Growth and performance analysis

There was found to be a significant ($P \leq 0.05$) difference between final weight, specific growth rate (SGR), percentage growth and percentage mortality between diet E and the other four diets. The mean initial weight of the replicates of all five diets also showed a significant ($P \leq 0.05$) difference between diet E and the other four diets.

The analysis of data shows that anglers bait can have a physiological effect on fish.

4.1.1 Initial weight

The analysis of initial weight by ANOVA showed that there was a highly significant ($P \leq 0.01$, F: 30.41, df: 4, 10) difference between diets. Further analysis of the data by Tukey test showed that there was a significant ($P \leq 0.05$, Q: 4.65, df: 4, 10) difference between the mean of diet E and the other four diets.

Whilst the difference that was recorded between diet E and the other four diets was perhaps less than ideal, the study was not a growth trial and the objective was not to directly compare diets. Due to the fact that juvenile fish are known to be in a comparatively rapid growth phase of their life-cycle (Lovell 1989), valid comparisons could still be made.

4.1.2 Final weight

The analysis of final weight by ANOVA showed that there was a significant ($P \leq 0.05$, F: 22.45, df: 4, 10) difference between diets. Further analysis by Tukey test showed that there was a significant ($P \leq 0.05$, Q: 4.65, df: 4, 10) difference between the mean of diet E (shelf-life boilies) and the other four diets.

Whilst the results in terms of significant differences between diets would indicate no difference between final weight and the initial weight, in as much as the only diet showing a significant difference was diet E (shelf-life boilies). It can be seen from the graph (Figure 3.1.3, page 37) that diets A, B, C, and D all performed relatively well in comparison to each other; whilst diet E (shelf-life boilies) had not performed at all well, even in isolation. Diet E (shelf-life boilies) performed very badly in comparison with the other diets. Given the assumption that the two-percent supplement of trout pellet in each diet is providing the essential vitamins and minerals (section 1, pages 11 and 12), the growth of the fish can be seen as an indication of the nutritional value of the diet.

4.1.3 Specific growth rate (SGR)

The SGR is presented as a cumulative figure across the period of the entire study, this avoids the small fluctuations that are thought to relate to environmental and natural growth effects when considering weekly fluctuations (Ricker 1979).

The analysis of SGR by ANOVA showed that there was a highly significant ($P \leq 0.01$, F: 12.01, df: 4, 10) difference between diets. Further analysis of the data by Tukey test showed that there was a significant ($P \leq 0.05$, Q: 4.65, df: 4, 10) difference between the mean of diet E (shelf-life boilies) and the other four diets.

Diet A (trout pellet) showed the highest SGR at 2.47, this is not surprising as *C. carpio* are known to grow well on trout pellet (Scott 1997). Given that all study diets received a two-percent of body weight (fed as kj/g) supplement, all diets can have been expected to return at least a modest SGR. The figure for diet E (shelf-life boilies) of 0.85 when compared to the next lowest of 2.30 for diet D (maggot) is very low. The difference between the SGR for diets may simply have been due to bioavailability of nutritional components of the diet, or given the availability of the separate nutrients, the digestibility of them. Whilst the proximate analysis (Table 2.7.5, page 28) of the diets shows diet E has a relatively low moisture level, at 18.1%, compared for example with diet D (maggot) at 71.41%. An important factor is that the diets were all fed as iso-energetic meals, each tank in the study received the same amount of energy. This would suggest that bioavailability or digestibility of the diets varies greatly.

However this convenient theory does not account for the very low SGR for diet E (shelf-life boilie). The two-percent of body weight supplement in the diet would be sufficient to give better growth than this. Mr M. Moore (*pers. com.*) states that on a two-percent supplement of trout pellets in a stock pond he would expect substantial growth from *C. carpio*. It therefore appears that the shelf-life boilie component of diet E is having a negative or anti-nutritional effect on the two-percent trout pellet component.

All SGR figures returned in the study (other than diet E) showed only a moderate performance. Workers conducting feed trials with carp at similar temperatures (Takeuchi, Watanabe and Ogino 1979; Nandeesha *et al* 1998), have often reported SGR figures in excess of 2.75 – 3.00. These studies were conducted using balanced diets, where the ratio of the major nutrients was in balance. Nutrient ratio is not thought to be an issue as the trout pellet proportion of some of the diets (other than diet E) has been available for growth, for example in the low protein and lipid diet C (sweetcorn).

Genetic variability can account for differences between growth rates, Hepher (1988) reports that environment can suppress growth in some strains, and that inbreeding will cause decreased growth, whilst cross breeding often results in heterosis and improved growth. It would therefore appear that there is little to be gained by comparing SGR in unrelated studies, on unrelated populations, in varying environmental conditions.

4.1.4. Percentage growth

Generally at least a doubling of weight is considered to be appropriate when conducting feed trials (Mr D. Hide *pers com.*), whilst Lovell (1989) suggests experiments should be run until statistical differences can be shown between study diets. The percentage growth data (Table 3.1.8, page 40) meets both criteria, with Diet E (shelf-life boilies) returning the smallest increase at 107.30%.

The analysis of percentage growth by ANOVA showed that there was a significant ($P \leq 0.05$, F: 14.54, df: 4, 10) difference between diets. Further analysis of the data by Tukey test showed that there was a significant ($P \leq 0.05$, Q: 4.65, df: 4, 10) difference between the mean of diet E (shelf-life boilies) and the other four diets.

Diet A (trout pellet) showed the greatest increase at 672.37%, this mean figure between replicates showed a relatively small variation (Table 3.1.8, page 40). Whilst diet D (maggot) also showed a high percentage growth of 647.44%, there was however a very large variation across replicates. Examination of C.F. data (Appendix B, page XIII) for the study end, shows that tank 2 of diet D had some comparatively large fish. Tank two of diet D it would appear had a disproportionate number of 'shooters'. Some consideration was given to winsorising the data (Sokal and Rohlf 1995), however removing the largest fish had only a limited effect on variability across the sample, this being the case it was decided to leave the data intact.

Diet B (peanut) and diet C (sweetcorn) showed considerable similarity considering how different the nutritional make up of the two diets was (Table 2.7.5, page 28), returning growth figures of 449.54% and 450.36% respectively. This is thought to reflect the omnivorous *C. carpio's* flexibility in utilising the available nutrition from varying sources when fed as iso-energetic meals (Kaushik 1995). As previously stated diet E only grew to 107.30%, this shows that the nutrition available as a comparable meal as kj/g of energy was not efficiently utilised for growth. Percentage growth analysis is thought also to lead to the conclusion that because even the very low protein and energy diet C (sweetcorn), when fed with a two-percent supplement of trout pellet sustained a reasonable growth, that the shelf-life boilie component of diet E is having a anti-nutritional or toxic effect. If this were not the case then the source of energy and bioavailability of the essential amino acids (EAA's) may well be considered as potential problem areas.

4.1.5. Mortality percentage

The analysis of percentage mortality by ANOVA showed that there was a highly significant ($P \leq 0.01$, F: 6.92, df: 4, 10) difference between diets. Further analysis of the data by Tukey test showed that there was a significant ($P \leq 0.05$, Q: 4.65, df: 4, 10) difference between the mean of diet E (shelf-life boilies) and the other four diets.

The mortality percentage across the diets A, B, C, and D showed no significant differences; the range of the data, discounting diet E (shelf-life boilies) was from 2.78% (diet C – sweetcorn) to 8.33% (diet A – trout pellet).

These mortality rates compare favourably with results obtained with *C. carpio* under study conditions (Nandeesha *et al* 1998). This assumption means that there is no link between mortality and diet, in diets A, B, C, and D. However the mortality percentage for diet E (shelf-life boilies) at 58.3% is very significant (Table 3.1.10, page 42), as each replicate had a mortality percentage of over 50%.

The well being of the fish fed on the other four diets would lead to the conclusion that environmental factors played no part in the high mortality of diet E (shelf-life boilies).

The fact that all replicates of diet E (shelf-life boilies) displayed similar and high mortality rates would very strongly suggest that the mortalities were linked to diet. This is somewhat unexpected as all diets were fed a two-percent of body weight (fed as kj/g) of trout pellet as a supplement to the main feed item, as a maintenance diet.

The high mortality rate for diet E (shelf-life boilies) reinforces the theory that an anti-nutritional or toxic component must have a role to play in the poor performance of diet E.

4.2 Indirect measurements and physiological factors

There were found to be a significant ($P \leq 0.05$) difference between lipid body composition percentage, condition factor (CF), liver somatic index (LSI) and visceral somatic index (VSI) between various diets.

The differences between diets A, B, C and D are thought to represent the difference in the base composition of each diet, as all were fed a two-percent trout pellet supplement. The difference between diet E (shelf-life boilies) and the other diets is thought to represent a difference in the availability of the nutrients to the fish. This could be because of anti-nutritional or toxic factors, which may be coupled with nutrient deficiencies, which may have caused the mortality rate for diet E (shelf-life boilies).

4.2.1 Moisture and lipid relationship

The coefficient of determination (r^2) for the data was .6219, therefore 38.67% of the variation can be accounted for by variation in moisture content. The r^2 value is low compared to other studies (*Nandeesha et al 1998*). Analysis of the regression line showed there to be no significant linear relationship ($P \geq 0.01$, t: 0.685, df: 6) between moisture and lipid body composition across the diets. The analysis of lipid composition by ANOVA showed that there was a highly significant ($P \leq 0.01$, F: 5.59, df: 4, 25) difference between diets. Further analysis of the data by Tukey test showed that there was a significant ($P \leq 0.05$, Q: 4.65, df: 4, 25) difference between the mean of diet E (shelf-life boilies) and diet A (trout pellet) and diet B (peanut).

The mean percentage of lipid in body composition of 3.18% and moisture of 70.15% across the study diets is low (Table 3.2.2, page 43), when compared to values obtained by workers conducting similar studies with carp. Takeuchi, Watanabe and Ogino (1979) found mean lipid levels in body composition of 7.18% and moisture levels of 76.25%.

Nandeesha *et al* (1998) report values of 3.76% (lipid) and 76.3% (moisture). Variability in moisture lipid levels is to be expected (Mr D. Hide *pers. com.).*

The results follow the trend that the higher the lipid inclusion in the diet the higher the proportion found in body composition (Martyshev 1983). The exception to this is diet E (shelf-life boilies), which had a higher dietary lipid inclussion than both diet C. (sweetcorn) and diet D (maggot), but had the lowest % lipid in the carcass analysis.

It would appear that the fish fed diet E (shelf-life boilie) and a two-percent of body weight supplement of trout pellet did not digest the same proportion of lipid from their diet, which we know was fed on an equivalent energy basis to all the other diets. Lipid can be metabolised for storage in the liver, however this is not thought to be the case as lipid values were obtained from macerated whole fish, which therefore included the liver.

Soya anti-nutritional factors have been shown to affect lipid metabolism, such as trypsin inhibitors, which have been reported to cause a decrease in intestinal lipase activity in catfish (NEC 1993).

The reason why there is a low r^2 value between moisture and lipid for fish in this study is not clearly understood, however the results may indicate that the specific diet of a fish will affect the lipid /moisture relationship in the carcass of the fish.

It seems reasonable to accept that such factors as lipid digestibility, the ability of fish to utilise the lipid, and indeed that what the fish does with the lipid may affect the carcass composition and therefore the inversely proportional relationship with moisture

Experimental design and error should not be ruled out however. The regression formula used to predict lipid levels in the study fish was arrived at with the use of only one fish from diet E. The reason for this was because of the size of fish from the replicates of diet E were comparatively small, compared to that required by the procedure and the other sample diets. It was impossible to obtain a sample of the right weight for soxhlet analysis after drying in the oven, only one fish was large enough. This in itself may have biased the results away from the mean values of diet E. In the opinion of Mrs A. Fox *(pers. com.) who runs the* Sparsholt College laboratory the decreased size of the sample used for analysis should not have unduly affected the accuracy.

.4.2.2 Condition factor (CF)

The analysis of condition factor by ANOVA showed that there was a highly significant ($P \leq 0.01$, F: 13.58, df: 4, 40) difference between diets. Further analysis of the data by Tukey test showed that there was a significant ($P \leq 0.05$, Q: 4.04, df: 4, 40) difference between the means of diets:- A and D, A and E, B and the other four diets, and C and diet E.

The results of the CF analysis are a little unusual as there are shown to be significant differences in CF between diets, where there were no significant differences in growth.

This would suggest that not all weight gain is being used or laid down in the same way by the fish feeding on the different diets. The CF of fish is often very variable (section 2.16, page 32).

It can be theorised that the largest proportion of the combination of protein and lipid percentage for diet B (peanut) and the two-percent trout pellet supplement had been laid down in these fish as somatic growth. This growth is shown as the superior CF between the diets. However the exact nature of that growth cannot be ascertained for diet B or any other diet for that matter. All though the diets were fed as iso-energetic meals, there are bound to be variabilities in the digestibility of the nutritional components between diets. This variability is likely to have led to the differences between the diets showing significant differences, and indeed for the smaller differences between diets, showing no significant ($\geq$ P 0.05) differences.

It is thought that this equality and relatively good performance of the diets showing no difference in performance between them, is thought to reflect *C. carpio*'s ability to use both carbohydrate and lipid for protein sparing (Wilson 1994). The efficiency of the protein sparing is reliant on the presence of EAA's in the right quantities and balance.

Using the arguments employed above, diet E (shelf-life boilies) should potentially have displayed a CF superior to B, C, and D instead of the most inferior (CF 2.09). The most probable reason is that which has already been discussed. From the growth analysis carried out (SGR and % growth) we know that fish fed on diet E (shelf-life boilies) had been unable to utilise the protein in the diet or that available from the two-percent supplement of trout pellet. It has already been stated that *C. carpio* are known to grow well on trout pellet.

The low and significantly different CF of diet E (shelf-life boilies) reflects the inability of the fish fed on this diet to utilise the nutrients in the diet, which is the probable cause of the poor performance. The inability to feed successfully is thought to be due to an anti-nutritional or toxic factor.

4.2.3 Liver somatic index (LSI)

The analysis of liver somatic index by ANOVA showed that there was a highly significant ($P \leq 0.01$, F: 28.56, df: 4, 40) difference between diets. Further analysis of the data by Tukey test showed that there was a significant ($P \leq 0.05$, Q: 4.04, df: 4, 40) difference between the means of diets:- A and the other four diets and diet E and the other four diets.

The difference between the mean of diet A (trout pellet) and the other diets is not thought to be substantial, the failure of diet A by the Tukey test was marginal compared to the failure of diet E (shelf-life boilies).

The reason for the significant difference between diet A (trout pellets) and the other four diets is thought to reflect the variability due to hierarchical influence (see section 4.3.1.3, page 62), more than too a nutritional difference between diets.

The comparatively very high mean LSI for diet E (shelf-life boilies) of 29.40, it was initially thought may be due to either the high carbohydrate levels present in the diet at 31.97% (glycogen) or high lipid deposition in the liver. The relatively low levels of lipid found in the carcass/lipid analysis (section 4.2.1, page 55) for diet E (shelf-life boilies) at 2.6%, may indicate that the enlarged liver is due to lipid storage. However given the high carbohydrate content of diet E (shelf-life boilies) the liver being enlarged due to glycogen was also a possibility. The troubling factor with both these theories is that it would appear unlikely that carbohydrate and or lipid were being metabolised for energy storage, yet protein or energy were not being utilised for anything but the smallest amount of growth. The anti-nutritional factor would have to be blocking the metabolic pathway of the protein (EAA's). Protease inhibitors are substances that have the ability to inhibit the proteolytic activity of certain enzymes (De Silva and Anderson 1995), in principal these heat labile substances should be broken down when diet E (shelf-life boilies) are manufactured.

With regard to the high carbohydrate levels it should be noted that Satoh (1991) reports carbohydrate levels of between 30 – 40% as acceptable in *C. carpio.* Satoh's paper relates to the *C. carpio* in an aquaculture situation where the carbohydrate proportion was part of a balanced diet.

The balance of the diet with regard to availability of the carbohydrate to the fish was unknown and unfortunately beyond the scope of this study.

4.2.4 Visceral somatic index (VSI)

The analysis of visceral somatic index by ANOVA showed that there was a highly significant ($P \leq 0.01$, F: 103.33, df: 4, 20) difference between diets Further analysis by Tukey test showed that there was a significant ($P \leq 0.05$, Q: 4.234, df: 4, 20) difference between the means of diets:- Diet A with diets C, D, and E. Diet B with diets C, D, and E. Diet C with diet E. Diet D with diet E.

Based on the results of this study it would appear that VSI is not a useful indicator of physiological change in this type of study. Two diet combinations, diets A and B and diets C and D showed no significant difference between them. Although a significant difference exists between the other diets its is difficult to offer an explanation as to the cause, other than to state that variability between replicates complicates and makes the analysis less clear

No unusual changes to internal organs were noted when examined at post mortem. The fish were however very small, particularly in the case of diet E (shelf-life boilies).

It can be said that diet E (shelf-life boilies) shows the highest VSI at 24.83. This is significantly ($P \leq 0.05$, Q: 4.23, df: 4, 20) higher than the second ranked diet B (peanut) at 21.34 and is probably due to the enlarged livers present in the fish fed on diet E (shelf-life boilies).

4.3 Summary of results

4.3.1 Variability of growth

The growth relationship, both between diets and indeed between replicates of diets was made more complicated for the purpose of statistical analysis by the degree of variability in the samples. However this is to be expected when carrying out studies involving growth with fish (Jobling 1983).

Both genetic variability for growth and dominance play a part in ensuring that it is very difficult to select a sample of fish for a study that will all have the same potential for growth, given the availability of a diet of equal nutritional and energetic values (bioavailability). The reasons for this variability are discussed in the next section.

4.3.1.2 Genetic variability

C. carpio despite many hundreds of years of domestication as a farmed fish remains reasonably true to its wild ancestors, except for the development of scale phenotypes and possibly body form, as seen when comparing a fish of Asian origin with a European form (Purdom 1993).

Growth rate is not thought to be responsive to selection in *C. carpio.* Moav and Wohlfarth (1976) carried out a major study of the inheritance of growth rate in carp; the study was centred on a mass selection experiment at various farms in Israel. The results showed that *C. carpio* over a five generation breeding programme displayed no response to selection for the growth trait. Interestingly to this study the authors found that within the different farms used in the study, growth rate varied by as much as 300% within any group of fish. Purdom (1993) suggests that this was due to the growth traits reliance on environmental conditions.

From the point of view of this study, the above information might lead one to discount the growth analysis when considering the suitability of the diets to maintain *C. carpio* in natural/semi-natural fisheries. However significant ($P \leq 0.05$) variation occurred between diet E and the other four diets, so whilst genetic variation can explain the high variability in growth between the diets it cannot explain the poor performance of diet E (shelf-life boilies).

The variation seen between replicates of diets was it is thought also due to dominance hierarchy, which is discussed in the next section

4.3.1.3 Dominance hierarchy

Jobling (1994) describes the subordination of smaller fish to larger fish, which feed on a disproportionate high share of the available food resource as 'size related dominance hierarchy'.

Simply stated this effect means that even when food is in abundance the social and behavioural interaction of the group will ensure that not all members of the group receive an equal proportion of the available food.

Observations made during feeding would indicate that this interaction and competition for food did occur within the study system and within all diets, although this interaction and competition appeared to stop in the fish fed on diet E (shelf-life boilies) after two or three weeks. The larger fish consumed more of the ration, with the smaller fish receiving proportionally much less and therefore consequently growing less. Once again this is inconvenient with regard to analysis of data, but it is what actually occurs in the semi-natural fishery where anglers bait may provide the nutritional mainstay for the fish population. Koebele (1985) states "dominant-subordinate differences in activity are responsible for more than a negligible growth depensation" in sub-optimal foraging habitat in the natural system.

4.3.2 Physiological effect of the baits

The effect of angler's bait on the physiology of fish is of course an effect that would normally only occur in a fishery (a body of water stocked with fish that are exploited by anglers for pleasure). The results achieved in this study, it is thought, can be applied to a semi-natural/natural fishery situation.

The inclusion of trout pellet as both a bait item (study diet) and as the control, as a two-percent supplement for maintenance is considered to be fully justified and applicable to the fishery situation. Viola, Arieli and Lahav (1990) state that 1% of body weight of cereal grain is sufficient to sustain *C. carpio*. The trout pellet as a commercially available diet for trout also provided vitamins and minerals considered to be essential to fish (Table 1.3.3, page 12).

The analysis of the data produced showed that the combination of trout pellet with peanut, sweetcorn and maggot has no detrimental effect to the fish in the study. The inference of the data was supported by the external and internal examination of the fish from diets A, B, C and D.

The internal and external examination of the fish also showed the fish to be free form both ecto and endo parasites. Therefore it can be assumed that parasitic pathology had no detrimental effect on the fish under study.

The analysis of the data produced for diet E (shelf-life boilies) showed that the combination of trout pellet and shelf-life boilies failed to support even reasonable growth, but also caused a significantly higher mortality rate than the other diets studied. The external and internal examination of the fish from diet E showed that the liver was visually disproportionately large compared to fish from the other four diets as shown by the LSI analysis (section 3.2.3, page 46).

As previously stated the maintenance diet for *C. carpio* can be thought of in terms of the nutrition supplied by 1% body weight of cereal grain, therefore there should be no doubt that trout pellets fed at two-percent body weight would provide nutrition for growth. It can also be stated that trout pellet was fed in combination with the other feed items and in diets A, B, C and D growth occurred at varying but acceptable rates. This was not the case for diet E and the two-percent supplement of trout pellets. The assumption must be made that shelf-life boilies actually have a detrimental if not negative effect on growth. The reality is that the fish that were still extant at the end of the trial were very lethargic and appeared to be near to death.

That trout pellet was not able to provide the nutrition required to sustain the fish in diet E is surprising. There would appear to be only two reasonable explanations for this poor performance, either the shelf-life boilies had an anti-nutritional property or they had a toxic or pathological effect on the fish.

4.3.3 Suitability of bait items as fish nutrition

The suitability of a bait item used in this study is dependent upon it being used in a semi-natural system that has an available source of any nutritional components lacking in that specific bait item. In other words it should be acknowledged that no bait item under study is thought to be a nutritionally complete and balanced meal for *C. carpio*, when fed in isolation .

Whilst no physical evidence has been shown by this study to suggest that diet A (trout pellet) would have a detrimental effect on *C. carpio,* it is widely acknowledged that commercial trout feed contains levels of both protein and lipid that are considered to be too high for cyprinid fish (Spillet and O'Grady 1983; Takeuchi, Watanabe and Ogino 1979; Kaushik 1995).

Whilst trout pellet is not considered nutritionally suitable as a mono feed for *C. carpio*, it has been shown by the results in this study that in various combinations with other baits, the nutritional components have worked to provide protein, lipid, carbohydrate and minerals and vitamins in a balance that will sustain *C. carpio*. For example it appears that protein sparing may have been provided by carbohydrate in the case of sweetcorn and by lipid in the case of peanut. It can be said these combinations do not appear to have had a detrimental effect on the fish, whilst this is true in the short-term, the long-term effect is unknown.

4.3.4 Suitability of shelf-life boilies as anglers bait

The results of the study indicate conclusively that as nutrition for fish that diet E (shelf-life boilies) is not acceptable as a bait item (for fish of the size under study). Where they are likely to become dependant upon it as part of their diet for anything other than an occasional meal. The mortality percentage would indicate that not only do the shelf-life boilies in question have a poor nutritional profile they may have a serious pathological consequence for fish that feed on them.

During the trial, observation of feeding gave the impression that the fish were not growing because they found the shelf-life boilies to be unpalatable. They made no attempt to eat the feed of the surface or as it was falling through the water body, as occurred with the other diets. At least 50% of the diet was removed from the tank during cleaning.

The diet therefore may contain a feeding deterrent, which becomes effective with quantity and/or time fed, this is the conclusion that must be drawn as diet E is the only one to be fed shelf-life boilies. As the trial progressed it was noticed that the fish fed shelf-life boilies appeared to avoid the areas where the feed settled out on the bottom of the tank.

There is no doubt that either a component or an awareness by the fish that the feed was not good for them led to a reduced intake of the feed item. It should perhaps be remembered that these shelf-life boilies are primarily designed and made to be attractive to the fish.

Without further composition and chemical analysis it was impossible to arrive at a definitive reason for the reluctance of the fish to eat the shelf-life boilies. By half way through the trial the fish had even stopped making the attempt to eat the trout pellet proportion of the diet. The reluctance of the fish to eat diet E (shelf-life boilies) may explain the enlarged liver present in fish fed on diet E. this is discussed further in section 4.3.5, page 71.

The importance of feeding stimulants as part of the behavioural component of feeding is documented by Hara (1993). He suggests that some species of fish that rely heavily on chemoreception are also sensitive to antinutritional and toxic components. Anecdotal evidence from specimen (large) carp anglers would suggest that *C. carpio* will reject baits that contain rancid oils.

The presentation of feed items was the same for each diet and replicates of the diet fed, as far as possible all feed was presented at a uniform and equal size across all diets. Feed was administered from the container to the water with a stainless steel spoon, so as to avoid human taints. Experimental or design error is ruled out as being responsible for the poor performance of diet E (shelf life boilies).

A poor or imbalance of protein/energy ratio of a diet may encourage the development of fatty livers, excess visceral fat reduced feeding activity and failure to grow (Stoskopf 1993). The effect that diet E (shelf-life boilies) item had on the liver of the fish is a tangible change in the physiology of the fish.

On internal examination it was visually very obvious that the fish fed on diet E (shelf-life boilies) had a grossly enlarged liver, the subsequently calculated L.S.I confirmed this, being some 3.98 times larger than the next largest LSI.

A review of health texts indicates that an enlarged liver as found in the fish fed on diet E (shelf-life boilies) is entirely concurrent with symptoms found in fish fed a high carbohydrate diet, the liver being a store for glycogen. These fish are often lethargic and show a lack of appetite (Stoskopf 1993). It is of course possible that once the fish stopped eating an amount that was maintaining them, then dietary deficiencies may have occurred. Glycogen is only one possible reason for the enlarged liver, the subject is discussed below.

4.3.5 Factors effecting liver size

Lipoid liver disease is most often associated with rancidity of the dietary lipid content (Roberts 1989). The most common cause of rancidity is poor storage for prolonged periods at high temperatures. The inclussion of antioxidants such as Vitamin E (Roberts 1989) will significantly reduce the rate and degree of deterioration.

Rancid lipids are toxic and can react with protein to lower its biological value and have a deleterious effect on vitamins (Roberts 1989), which are not themselves antioxidants. Although an enlarged liver is symptomatic of lipoid liver disease, diagnosis is carried out by histological methods, so no conclusion could be made.

Other symptoms of rancid lipid problems are exophthalmia, streatitis, darkening, splenic haemosiderosis and skeletal myopathy (Roberts 1989).

A further pathological syndrome involving dietary lipids is the infiltration of fat into the liver, which may accompany the use of high lipid diets. The liver will appear yellow to ochre and often mottled (Stoskopf 1993). Diagnosis is carried out by staining techniques and examination with a high powered microscope.

Fish can also suffer disease related to carbohydrate overload. Liver glycogen is normally 0.5 to 5% of the liver mass, but may increase to as high as 17% of the liver mass when fish are fed rations high in digestible carbohydrate. Symptoms of high glycogen levels include the fish becoming lethargic, dark, refusal to take food and swimming near the surface of the water (Stoskopf 1993). Diagnosis of the condition is by blood glucose and liver glycogen analysis (Stoskopf 1993).

Enlarged liver can be symptomatic of starvation (Stoskopf 1993), unfortunately inappetence is one of the more common clinical descriptions or symptoms of a nutritional deficiency, so this neatly takes the investigation full circle.

Given the specialised methods of diagnosis and the complexity of the subject, it was not possible to advance the diagnosis of the cause of the poor performance of diet E (shelf-life boilies) any further.

5.0 CONCLUSION

In an attempt to obtain further insight into the syndrome related to diet E, the manufacturers of the shelf-life boilies were contacted in the hope of obtaining a further insight into the composition of the boilies. The boilies were made by Streamselect Limited who market the self-life boilies under the style of 'Richworth Baits'. M. Lunn (*pers. com.)* of Grayshott tackle states that the company are a market leader in this type of angling bait.

Mr R.G. Baker (*pers. com.*) the managing director of Streamselect Limited was very guarded in his comments with regard to the shelf-life boilies, he would not give anything more than a very sketchy description of the composition stating that the bait contained high quality fish meal protein and fish oils. When questioned further about composition Mr Baker agreed that the baits main ingredient was carbohydrate, he would not say of what type, but added that the entire composition was highly palatable and digestible.

Given the apparent undesirable traits of the 'seafood special' flavoured shelf-life boilie, it was decided to run a short post-study trial of three other flavoured shelf-life boilies also manufactured by Streamselect Limited under the same trading style. The short trial (four weeks), based on observation was carried out to examine the feeding responce of *C. carpio*, which had previously been fed a trout pellet/maize diet when fed on shelf-life boilies. All fish initially fed on the three different boilies. The boilies were fed using the same regime as the main study, also in triplicate. After five days it was noticeable that the amount of feed being left had increased.

After twenty-eight days the fish feeding on all diets had all but stopped feeding. At this stage the trial was stopped to safeguard the welfare of the fish.

The results of this study would not entirely agree with the description of shelf-life boilies given by Mr Baker on behalf of the manufacturers. It would appear that they are not highly digestible and that the palatability is questionable, to the size of fish under study. It can also be said that shelf-life boilies appear also to have a detrimental effect on the fish when fed in combination with at least one other bait (trout pellet). Trout pellet, peanut, sweetcorn and maggot would all appear to be individually suitable for use as anglers bait in a semi-natural system where there are other sources of nutrition available. Trout pellet will undoubtedly sustain fish as a complete diet, there is however a question of its suitability for cyprinid fish over an extended period.

An angler's short-term requirement from his bait is not the same as his quarries requirement. An angler requires the bait to attract his chosen quarry and to appear palatable so as the fish will take the bait into its mouth, so the angler may hook it. There is no requirement on the part of the angler for the bait item to be either nutritional or digestible, there is a need for these traits in a long-term bait.

It may well be that the responsibility for ensuring that only nutritional items to be used as bait lies with those responsible for managing waters rather than anglers, who's prime concern is to catch fish.

The importance of the nutritional value of bait items in heavily stocked waters, where the fish may become dependent on this introduced food source should be stressed.

The health aspect is often little considered in this type of fishery scenario, however poor nutrition can have a significant effect on fish health (Roberts 1989; Stoskopf 1993 and Schlofeldt and Alderman 1995). Glittino (1988) links spring viraemia in carp (SVC) to nutrition. Starvation will lead to stress (Stoskopf 1993), which will in turn lead to a shut down or suppression of the fish's immune system.

The results obtained in this study would not lend evidence or any weight to the many bait bans that are reported regularly in the angling press, for items studied other than diet E (shelf-life boilies) when fed to fish of a similar size to those studied.

Indeed the angling club that the author of the dissertation belongs to, has a ban on peanuts, which has been in existence for many years, he has been unable to find out why, other than an explanation given by the Secretary Mr Huskisson *(pers. com.)* "peanuts are bad for fish". There is anecdotal evidence to suggest that *C. carpio* have died from eating poorly cooked peanuts that have expended in the gut after they have been eaten by the fish. This is poor angling rather than a poor nutritional bait. There is a need for further study of the effect of bait in general on fish from a nutritional point of view and also from the aspect of its effect on the environment.

The difficulty is that conducting experiments in laboratory conditions is far removed from the semi-natural fisheries of interest.

This study has shown that shelf-life boilies of one specific type are not suitable nutrition for *C. carpio* of the size under study. A specific requirement for further research related to this study should be to isolate the cause of the poor performance of the shelf-life boilies, and to establish if all shelf-boilies have this effect or whether it is isolated to those manufactured by Streamselect Limited. It is also important to establish the range of fish that they can potentially effect. A further study with larger fish should be carried out to ensure that the poor performance of diet E (shelf-life boilies) was not related to the inability of the juvenile *C. carpio* to utilise the diet. This inability could be due to the gut morphology of the juvenile fish (De Silva and Anderson 1995) or poor development of associated bacterial flora and enzymes (Hepher 1988). This is actually not thought to be the case as the fish used in the post-study experiment to observe feeding response all at an initial weight of over 10g.

Further research is required to investigate the effects of feeding fish with diet E (shelf-life boilies with regard to the high mortality rate and the enlarged liver. A trial could be run using fish fed only on the shelf-life boilies.

Attention to studying the nature and pattern of feeding is recommended, to obtain further information about the apparent anti-feeding component of the diet. Fish that die during the trial should have a full post-mortem examination, which should include a chemical analysis of the liver and histology of the vital organs. Extant fish at the end of the trial should also be thoroughly examined to the same degree.

The exact composition of the shelf-life boilies should be obtained by further analysis, to establish for example the type and composition of carbohydrate.

If the study were to be repeated it is recommended that larger fish were used, and that they were selected for lack of variability (no obvious slow or fast growing fish) from a larger group. Greater attention should be paid to the initial bulk tank weight. It would also be very useful to carry out histology on the livers, and proximate analysis for protein, lipid, carbohydrate, ash and moisture of the fish carcass for each diet under study.

Perhaps a final point should be that those that manage these fisheries (overstocked and/or little natural food); may need to look at the ethical practices of managing these waters at naturally unsustainable levels from the nutritional, health and environmental aspects of best practice in fishery management.

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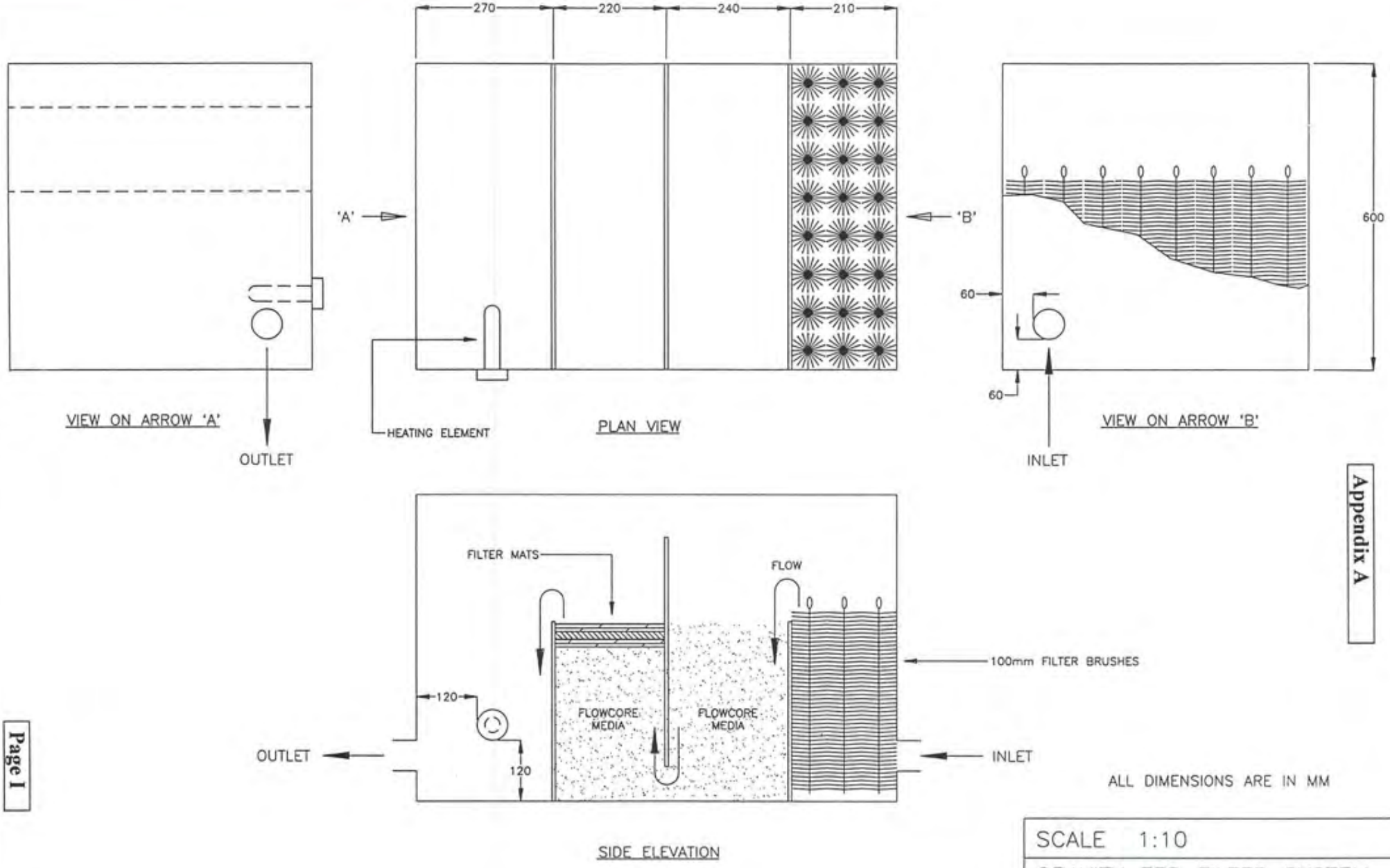


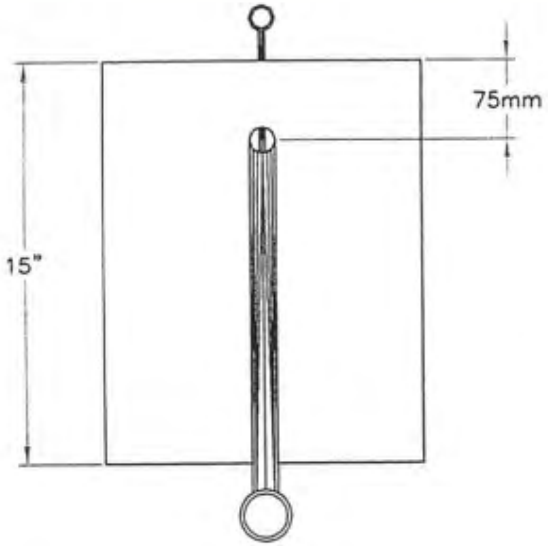
ALL DIMENSIONS ARE IN MM

SCALE 1:10

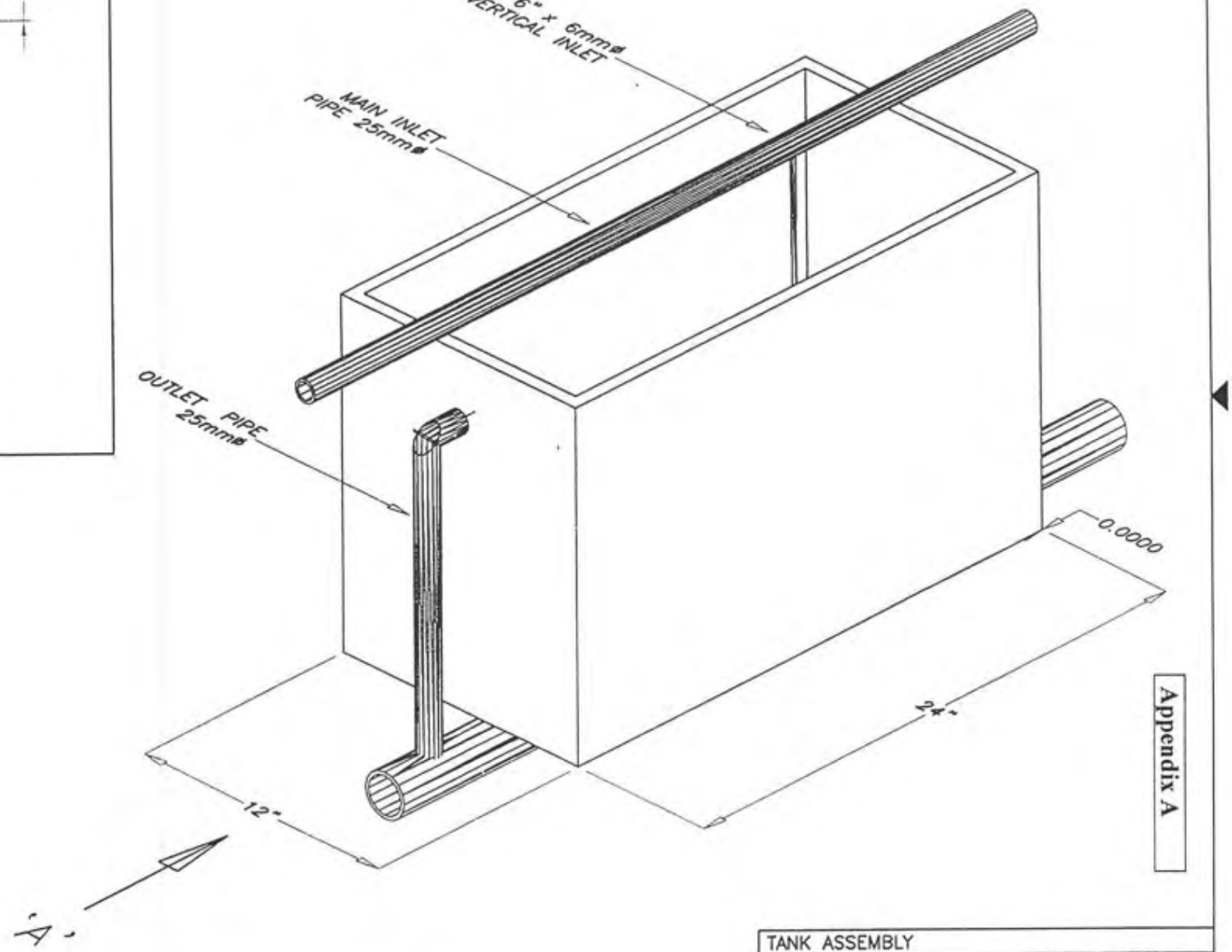
GRAVITY FED FILTER SYSTEM
30/01/99

Appendix A

Page I



VIEW ON ARROW 'A'



TANK ASSEMBLY	
SCALE : N.T.S.	24/02/99

Appendix A

Table. Ancillary equipment used in the recirculation system and the study generally.

Component	Description
Compressor – Compton D245S	240V single phase compressor
Immersion heater	Domestic water tank heater, fitted into plastic water tank with a special collar (2-1/4 Essex Flange).
Pump – 'Granfoss'	Domestic central heating pump
Ultra violet filter -	30 watt – 'Yamitsu Algae Master'
Bio-filter media – 'Flowcore'	Cylindrical light plastic media available under trade name 'flow core'.
Filter brushes	Wire brushes with plastic filaments.
Filter mat set	Comprising of a coarse, medium and fine mat.
Supply pipe – 25 mm 'Hep-flow' pipe	Grey push fit pipe with a full range of joints available.
Aquaria tank return (overflow pipe)	25 mm 'poly' pipe (waste pipe)
Return pipe – 50 mm 'OSMA'	Push fit domestic water pipe.
'Tanita' digital scales	3 kg ± 1g
'AE' digital scales	240 g ± 0.02g
Water quality testing kits.	'Tetra' kits - pH, HN_4^{+}.
Dissolved oxygen (DO) monitoring	'pHox' electronic DO meter.

Appendix B

Water quality dated recorded from the recirculation system during the period of the study is shown in *Table B1*.

Dissolved oxygen (DO) was taken with a pHox meter, model 62T, that read percentage saturation (% DO).

Temperature was also recorded with the temperature facility on the pHox oxygen meter.

Ammonia levels were taken with a Tetra test kit. pH levels were also recorded with a Tetra pH test kit.

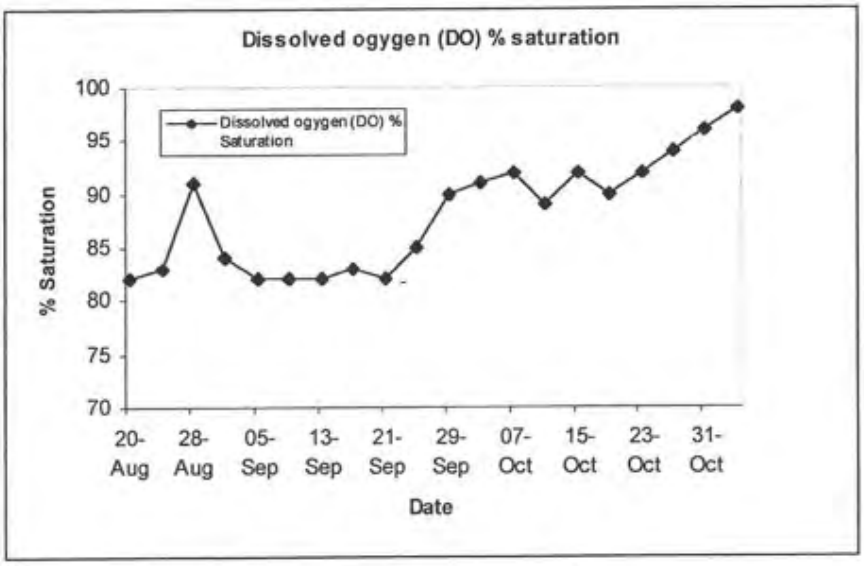
Measurements were taken before the evening feed.

Table B1. Water quality criteria for the recirculation system used in this study.

Date	% DO	Ammonia	pH	Temp
20-Aug	82	0	7.5	22.0
24-Aug	83	0	7.5	22.0
28-Aug	91	0	7.5	22.0
01-Sep	84	0	7.5	21.0
05-Sep	82	0	7.5	21.5
09-Sep	82	0	7.5	21.0
13-Sep	82	0	7.5	21.5
17-Sep	83	0	7.5	22.0
21-Sep	82	0	7.5	21.0
25-Sep	85	0	7.5	20.0
29-Sep	90	0	7.5	23.0
03-Oct	91	0	7.5	22.2
07-Oct	92	0	7.5	21.0
11-Oct	89	0	7.5	21.0
15-Oct	92	0	7.5	21.5
19-Oct	90	0	7.5	20.5
23-Oct	92	0	7.5	20.5
27-Oct	94	0	7.5	20.0
31-Oct	96	0	7.5	21.5
04-Nov	98	0	7.5	20.5

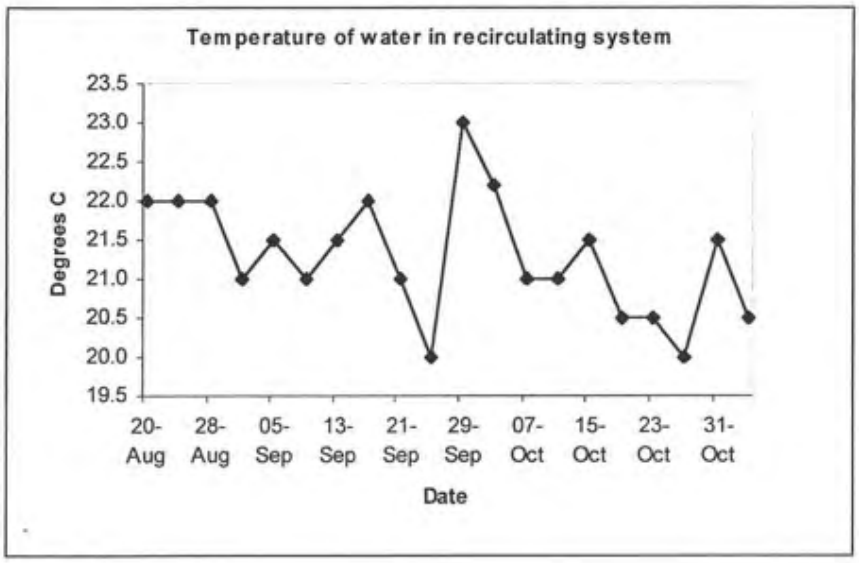
It can be seen that DO levels increased during the period of the study this is due to changes in air stones, as the original ones fitted to the system appeared not to be efficient, this is supported by the data illustrated in the graph (*Figure B2*).

Figure B.2. Oxygen saturation of the recirculation system.



The temperature profile of the system (*Figure B3.)* shows a slight fluctuation of temperature around the target temperature of 21° C. Fluctuations were in the main due to the inefficiency of the thermostat, which was of the type commonly employed in domestic immersion heaters.

Figure B3. Water temperature of the recirculation system over the period of the study.



Ammonia never tested positively, which would signify that the biofilter was effective at controlling the nitrogen cycle within the system.

B4. Daily routine protocol

Any unusual behaviour of fish was observed and recorded. The flow into the tanks was checked and regulated to approximately 0.5 l/min.

After the inspection, all faeces and waste were siphoned out of each tank. After the cleaning, the fish were fed. Each tank's rations was fed fed approx. 33% a.m. 33% midday, and 33% p.m.

The feeding to was conducted slowly over one minute in time to ensure minimum wastage.

The biofilter/reservoir level was checked

Tank outflow screens were checked and cleaned.

07.30 – 08.00	inspection, cleaning and feeding
13.00 - 13.30	feeding
18.00 - 18.30	feeding

A 25% water change was carried out weekly to replace water lost during the siphoning of tanks and cleaning and to ensure water quality. At the same time the biofilter brushes and mat filters were cleaned.

B5. Feed rate calculations

Readings taken from bomb calorimetry.

Diet	Galvo rise	kj per galvo unit	Wt. of sample	Gross energy	Ave. Kj/g
A1	5.675	1.839	0.3996	26.117	26.20
A2	5.875	1.839	0.4112	26.275	
B1	5.675	1.839	0.3276	31.857	32.52
B2	5.575	1.839	0.309	33.179	
C1	6.575	1.839	0.6319	19.135	19.38
C2	5.475	1.839	0.513	19.627	
D1	6.675	1.839	0.3959	31.006	31.40
D2	6.575	1.839	0.3804	31.786	
E1	4.875	1.839	0.4019	22.307	22.43
E2	5.125	1.839	0.4179	22.553	

Energy values for the individual feed items fed during the study.

Average gross energy	Dry Kj/g	Ave. Moisture Content %	Wet wt. =Dry wt Kj/g	Wet 1 g = Kj/g	Dry matter %	Wet Kj/g
Amino Balance - (A)	26.20	5.72	1.057	24.78	94.28	24.70
Peanut - (B)	32.52	43.53	1.435	22.66	56.47	18.36
Sweetcorn - (C)	19.38	70.20	1.702	11.39	29.80	5.78
Maggots - (D)	31.40	71.46	1.715	18.31	28.54	8.96
Bollies - (E)	22.43	17.27	1.173	19.13	82.73	18.56

Week 0 - Feed calculations, gross weight, feed fed, per tank.

Tank	Number of fish per tank	Total bulk wt. g	Wet feed energy per g - kj/g	2% B. wt. amino g. D. wt.	Maint. diet 2% B. Wt as kj	6% B. wt. diet g Dry wt.	8% B. wt. total diet g – Dry wt.	Moist. % of diet	Total energy fed	6% Energy required	Other feed required to supply energy	Total feed fed per day g
A1	12	20	24.70	0.40	9.88	1.2	1.6	5.72	39.52	29.64	1.20	1.60
A2	12	20	24.70	0.40	9.88	1.2	1.6	5.72	39.52	29.64	1.20	1.60
A3	12	20	24.70	0.40	9.88	1.20	1.6	5.72	39.52	29.64	1.20	1.60
B1	12	19	18.36	0.38	9.39			43.53	37.55	28.16	1.53	1.91
B2	12	20	18.36	0.40	9.88			43.53	39.52	29.64	1.61	2.01
B3	12	20	18.36	0.40	9.88			43.53	39.52	29.64	1.61	2.01
C1	12	19	5.78	0.38	9.39			70.2	37.55	28.16	4.88	5.26
C2	12	19	5.78	0.38	9.39			70.2	37.55	28.16	4.88	5.26
C3	12	20	5.78	0.40	9.88			70.2	39.52	29.64	5.13	5.53
D1	12	20	8.96	0.40	9.88			71.46	39.52	29.64	3.31	3.71
D2	12	18	8.96	0.36	8.89			71.46	35.57	26.68	2.98	3.34
D3	12	18	8.96	0.36	8.89			71.46	35.57	26.68	2.98	3.34
E1	12	14	18.56	0.28	6.92			17.27	27.67	20.75	1.12	1.40
E2	12	13	18.56	0.26	6.42			17.27	25.69	19.27	1.04	1.30
E3	12	15	18.56	0.30	7.41			17.27	29.64	22.23	1.20	1.50

Week 1 - Feed calculations, gross weight, feed fed per tank.

Tank	Number of fish per tank	Total bulk wt. g	Wet feed energy per g - kj/g	2% B. wt. amino g. D. wt.	Maint. diet 2% B. Wt as kj	6% B. wt. diet g Dry wt.	8% B. wt. total diet g – Dry wt.	Moist. % of diet	Total energy fed	6% Energy required	Other feed required to supply energy	Total feed fed per day g
A1	12	25	24.70	0.50	12.35	1.5	2	5.72	49.40	37.05	1.50	2.00
A2	12	26	24.70	0.52	12.84	1.56	2.08	5.72	51.38	38.53	1.56	2.08
A3	12	29	24.70	0.58	14.33	1.74	2.32	5.72	57.31	42.98	1.74	2.32
B1	12	28	18.36	0.56	13.83			43.53	55.33	41.50	2.26	2.82
B2	12	29	18.36	0.58	14.33			43.53	57.31	42.98	2.34	2.92
B3	12	25	18.36	0.50	12.35			43.53	49.40	37.05	2.02	2.52
C1	12	24	5.78	0.48	11.86			70.2	47.43	35.57	6.16	6.64
C2	12	23	5.78	0.46	11.36			70.2	45.45	34.09	5.90	6.36
C3	12	26	5.78	0.52	12.84			70.2	51.38	38.53	6.67	7.19
D1	12	25	8.96	0.50	12.35			71.46	49.40	37.05	4.13	4.63
D2	12	24	8.96	0.48	11.86			71.46	47.43	35.57	3.97	4.45
D3	12	25	8.96	0.50	12.35			71.46	49.40	37.05	4.13	4.63
E1	12	17	18.56	0.34	8.40			17.27	33.59	25.20	1.36	1.70
E2	12	16	18.56	0.32	7.90			17.27	31.62	23.71	1.28	1.60
E3	12	16	18.56	0.32	7.90			17.27	31.62	23.71	1.28	1.60

Week 2 - Feed calculations, gross weight, feed fed, per tank.

Tank	Number of fish per tank	Total bulk wt. g	Wet feed energy per g - kj/g	2% B. wt. amino g. D. wt.	Maint. diet 2% B. Wt as kj	6% B. wt. diet g Dry wt.	8% B. wt. total diet g – Dry wt.	Moist. % of diet	Total energy fed	6% Energy required	Other feed required to supply energy	Total feed fed per day g
A1	12	26	24.70	0.52	12.84	1.56	2.08	5.72	51.38	38.53	1.56	2.08
A2	12	26	24.70	0.52	12.84	1.56	2.08	5.72	51.38	38.53	1.56	2.08
A3	12	30	24.70	0.60	14.82	1.80	2.4	5.72	59.28	44.46	1.80	2.40
B1	12	34	18.36	0.68	16.80			43.53	67.19	50.39	2.74	3.42
B2	12	31	18.36	0.62	15.31			43.53	61.26	45.94	2.50	3.12
B3	12	33	18.36	0.66	16.30			43.53	65.21	48.91	2.66	3.32
C1	12	30	5.78	0.60	14.82			70.2	59.28	44.46	7.70	8.30
C2	12	28	5.78	0.56	13.83			70.2	55.33	41.50	7.19	7.75
C3	12	31	5.78	0.62	15.31			70.2	61.26	45.94	7.96	8.58
D1	12	27	8.96	0.54	13.34			71.46	53.35	40.02	4.47	5.01
D2	12	27	8.96	0.54	13.34			71.46	53.35	40.02	4.47	5.01
D3	12	27	8.96	0.54	13.34			71.46	53.35	40.02	4.47	5.01
E1	12	20	18.56	0.40	9.88			17.27	39.52	29.64	1.60	2.00
E2	12	19	18.56	0.38	9.39			17.27	37.55	28.16	1.52	1.90
E3	12	19	18.56	0.38	9.39			17.27	37.55	28.16	1.52	1.90

Week 3 - Feed calculations, gross weight, feed fed, per tank.

Tank	Number of fish per tank	Total bulk wt. g	Wet feed energy per g - kj/g	2% B. wt. amino g. D. wt.	Maint. diet 2% B. Wt as kj	6% B. wt. diet g Dry wt.	8% B. wt. total diet g – Dry wt.	Moist. % of diet	Total energy fed	6% Energy required	Other feed required to supply energy	Total feed fed per day g
A1	12	30	24.70	0.60	14.82	1.8	2.4	5.72	59.28	44.46	1.80	2.40
A2	12	39	24.70	0.78	19.27	2.34	3.12	5.72	77.07	57.80	2.34	3.12
A3	12	34	24.70	0.68	16.80	2.04	2.72	5.72	67.19	50.39	2.04	2.72
B1	12	38	18.36	0.76	18.77			43.53	75.09	56.32	3.07	3.83
B2	12	34	18.36	0.68	16.80			43.53	67.19	50.39	2.74	3.42
B3	12	35	18.36	0.70	17.29			43.53	69.16	51.87	2.82	3.52
C1	12	33	5.78	0.66	16.30			70.2	65.21	48.91	8.47	9.13
C2	12	32	5.78	0.64	15.81			70.2	63.24	47.43	8.21	8.85
C3	12	34	5.78	0.68	16.80			70.2	67.19	50.39	8.73	9.41
D1	11	22	8.96	0.44	10.87			71.46	43.47	32.61	3.64	4.08
D2	12	30	8.96	0.60	14.82			71.46	59.28	44.46	4.96	5.56
D3	12	27	8.96	0.54	13.34			71.46	53.35	40.02	4.47	5.01
E1	12	15	18.56	0.30	7.41			17.27	29.64	22.23	1.20	1.50
E2	11	18	18.56	0.36	8.89			17.27	35.57	26.68	1.44	1.80
E3	11	16	18.56	0.32	7.90			17.27	31.62	23.71	1.28	1.60

Week 4 - Feed calculations, gross weight, feed fed per tank.

Tank	Number of fish per tank	Total bulk wt. g	Wet feed energy per g - kj/g	2% B. wt. amino g. D. wt.	Maint. diet 2% B. Wt as kj	6% B. wt. diet g Dry wt.	8% B. wt. total diet g – Dry wt.	Moist. % of diet	Total energy fed	6% Energy required	Other feed required to supply energy	Total feed fed per day g
A1	11	34	24.70	0.68	16.80	2.04	2.72	5.72	67.19	50.39	2.04	2.72
A2	11	39	24.70	0.78	19.27	2.34	3.12	5.72	77.07	57.80	2.34	3.12
A3	12	40	24.70	0.80	19.76	2.40	3.2	5.72	79.04	59.28	2.40	3.20
B1	12	44	18.36	0.88	21.74			43.53	86.95	65.21	3.55	4.43
B2	11	29	18.36	0.58	14.33			43.53	57.31	42.98	2.34	2.92
B3	12	42	18.36	0.84	20.75			43.53	83.00	62.25	3.39	4.23
C1	11	35	5.78	0.70	17.29			70.2	69.16	51.87	8.98	9.68
C2	12	36	5.78	0.72	17.78			70.2	71.14	53.35	9.24	9.96
C3	12	41	5.78	0.82	20.26			70.2	81.02	60.77	10.52	11.34
D1	10	26	8.96	0.52	12.84			71.46	51.38	38.53	4.30	4.82
D2	11	34	8.96	0.68	16.80			71.46	67.19	50.39	5.62	6.30
D3	12	32	8.96	0.64	15.81			71.46	63.24	47.43	5.29	5.93
E1	12	18	18.56	0.36	8.89			17.27	35.57	26.68	1.44	1.80
E2	9	15	18.56	0.30	7.41			17.27	29.64	22.23	1.20	1.50
E3	9	15	18.56	0.30	7.41			17.27	29.64	22.23	1.20	1.50

Week 5 - Feed calculations, gross weight, feed fed, per tank.

Tank	Number of fish per tank	Total bulk wt. g	Wet feed energy per g - kj/g	2% B. wt. amino g. D. wt.	Maint. diet 2% B. Wt as kj	6% B. wt. diet g Dry wt.	8% B. wt. total diet g – Dry wt.	Moist. % of diet	Total energy fed	6% Energy required	Other feed required to supply energy	Total feed fed per day g
A1	11	38	24.70	0.76	18.77	2.28	3.04	5.72	75.09	56.32	2.28	3.04
A2	11	48	24.70	0.96	23.71	2.88	3.84	5.72	94.85	71.14	2.88	3.84
A3	12	51	24.70	1.02	25.20	3.06	4.08	5.72	100.78	75.59	3.06	4.08
B1	12	53	18.36	1.06	26.18			43.53	104.73	78.55	4.28	5.34
B2	11	30	18.36	0.60	14.82			43.53	59.28	44.46	2.42	3.02
B3	12	48	18.36	0.96	23.71			43.53	94.85	71.14	3.87	4.83
C1	11	45	5.78	0.90	22.23			70.2	88.92	66.69	11.55	12.45
C2	12	42	5.78	0.84	20.75			70.2	83.00	62.25	10.78	11.62
C3	12	51	5.78	1.02	25.20			70.2	100.78	75.59	13.09	14.11
D1	10	36	8.96	0.72	17.78			71.46	71.14	53.35	5.95	6.67
D2	11	48	8.96	0.96	23.71			71.46	94.85	71.14	7.94	8.90
D3	12	40	8.96	0.80	19.76			71.46	79.04	59.28	6.62	7.42
E1	9	13	18.56	0.26	6.42			17.27	25.69	19.27	1.04	1.30
E2	8	15	18.56	0.30	7.41			17.27	29.64	22.23	1.20	1.50
E3	7	11	18.56	0.22	5.43			17.27	21.74	16.30	0.88	1.10

Week 6 - Feed calculations, gross weight, feed fed per tank.

Tank	Number of fish per tank	Total bulk wt. g	Wet feed energy per g - kj/g	2% B. wt. amino g. D. wt.	Maint. diet 2% B. Wt as kj	6% B. wt. diet g Dry wt.	8% B. wt. total diet g – Dry wt.	Moist. % of diet	Total energy fed	6% Energy required	Other feed required to supply energy	Total feed fed per day g
A1	11	54	24.70	1.08	26.68	3.24	4.32	5.72	106.71	80.03	3.24	4.32
A2	11	67	24.70	1.34	33.10	4.02	5.36	5.72	132.40	99.30	4.02	5.36
A3	12	64	24.70	1.28	31.62	3.84	5.12	5.72	126.47	94.85	3.84	5.12
B1	12	63	18.36	1.26	31.12			43.53	124.49	93.37	5.08	6.34
B2	11	39	18.36	0.78	19.27			43.53	77.07	57.80	3.15	3.93
B3	12	58	18.36	1.16	28.65			43.53	114.61	85.96	4.68	5.84
C1	11	51	5.78	1.02	25.20			70.2	100.78	75.59	13.09	14.11
C2	12	48	5.78	0.96	23.71			70.2	94.85	71.14	12.32	13.28
C3	12	58	5.78	1.16	28.65			70.2	114.61	85.96	14.88	16.04
D1	10	44	8.96	0.88	21.74			71.46	86.95	65.21	7.28	8.16
D2	11	64	8.96	1.28	31.62			71.46	126.47	94.85	10.58	11.86
D3	12	49	8.96	0.98	24.21			71.46	96.83	72.62	8.10	9.08
E1	5	9	18.56	0.18	4.45			17.27	17.78	13.34	0.72	0.90
E2	6	14	18.56	0.28	6.92			17.27	27.67	20.75	1.12	1.40
E3	7	15	18.56	0.30	7.41			17.27	29.64	22.23	1.20	1.50

Week 7 - Feed calculations, gross weight, feed fed per tank.

Tank	Number of fish per tank	Total bulk wt. g	Wet feed energy per g - kj/g	2% B. wt. amino g. D. wt.	Maint. diet 2% B. Wt as kj	6% B. wt. diet g Dry wt.	8% B. wt. total diet g – Dry wt.	Moist. % of diet	Total energy fed	6% Energy required	Other feed required to supply energy	Total feed fed per day g
A1	11	71	24.70	1.42	35.08	4.26	5.68	5.72	140.30	105.23	4.26	5.68
A2	11	81	24.70	1.62	40.02	4.86	6.48	5.72	160.06	120.05	4.86	6.48
A3	12	79	24.70	1.58	39.03	4.74	6.32	5.72	156.11	117.08	4.74	6.32
B1	12	74	18.36	1.48	36.56			43.53	146.23	109.67	5.97	7.45
B2	11	46	18.36	0.92	22.73			43.53	90.90	68.18	3.71	4.63
B3	12	65	18.36	1.30	32.11			43.53	128.45	96.34	5.25	6.55
C1	11	61	5.78	1.22	30.14			70.2	120.54	90.41	15.65	16.87
C2	12	53	5.78	1.06	26.18			70.2	104.73	78.55	13.60	14.66
C3	12	72	5.78	1.44	35.57			70.2	142.28	106.71	18.48	19.92
D1	10	53	8.96	1.06	26.18			71.46	104.73	78.55	8.77	9.83
D2	11	81	8.96	1.62	40.02			71.46	160.06	120.05	13.40	15.02
D3	12	57	8.96	1.14	28.16			71.46	112.64	84.48	9.43	10.57
E1	5	13	18.56	0.26	6.42			17.27	25.69	19.27	1.04	1.30
E2	6	18	18.56	0.36	8.89			17.27	35.57	26.68	1.44	1.80
E3	6	19	18.56	0.38	9.39			17.27	37.55	28.16	1.52	1.90

Week 8 - Feed calculations, gross weight, mortality per tank.

Tank	Number of fish per tank	Total bulk wt. g	Wet feed energy per g - kj/g	2% B. wt. amino g. D. wt.	Maint. diet 2% B. Wt as kj	6% B. wt. diet g Dry wt.	8% B. wt. total diet g – Dry wt.	Moist. % of diet	Total energy fed	6% Energy required	Other feed required to supply energy	Total feed fed per day g
A1	11	89	24.70	1.78	43.97	5.34	7.12	5.72	175.87	131.91	5.34	7.12
A2	11	98	24.70	1.96	48.41	5.88	7.84	5.72	193.66	145.24	5.88	7.84
A3	12	89	24.70	1.78	43.97	5.34	7.12	5.72	175.87	131.91	5.34	7.12
B1	12	88	18.36	1.76	43.47			43.53	173.90	130.42	7.10	8.86
B2	11	51	18.36	1.02	25.20			43.53	100.78	75.59	4.12	5.14
B3	12	85	18.36	1.70	41.99			43.53	167.97	125.98	6.86	8.56
C1	11	73	5.78	1.46	36.06			70.2	144.26	108.19	18.73	20.19
C2	12	64	5.78	1.28	31.62			70.2	126.47	94.85	16.42	17.70
C3	12	85	5.78	1.70	41.99			70.2	167.97	125.98	21.81	23.51
D1	10	63	8.96	1.26	31.12			71.46	124.49	93.37	10.42	11.68
D2	11	105	8.96	2.10	51.87			71.46	207.49	155.62	17.37	19.47
D3	12	66	8.96	1.32	32.61			71.46	130.42	97.82	10.92	12.24
E1	5	9	18.56	0.18	4.45			17.27	17.78	13.34	0.72	0.90
E2	6	18	18.56	0.36	8.89			17.27	35.57	26.68	1.44	1.80
E3	6	18	18.56	0.36	8.89			17.27	35.57	26.68	1.44	1.80

Week 9 - Feed calculations, gross weight, feed fed per tank.

Tank	Number of fish per tank	Total bulk wt. g	Wet feed energy per g - kj/g	2% B. wt. amino g. D. wt.	Maint. diet 2% B. Wt as kj	6% B. wt. diet g Dry wt.	8% B. wt. total diet g – Dry wt.	Moist. % of diet	Total energy fed	6% Energy required	Other feed required to supply energy	Total feed fed per day g
A1	11	108	24.70	2.16	53.35	6.48	8.64	5.72	213.42	160.06	6.48	8.64
A2	11	108	24.70	2.16	53.35	6.48	8.64	5.72	213.42	160.06	6.48	8.64
A3	12	110	24.70	2.20	54.34	6.60	8.8	5.72	217.37	163.03	6.60	8.80
B1	12	104	18.36	2.08	51.38			43.53	205.52	154.14	8.39	10.47
B2	11	56	18.36	1.12	27.67			43.53	110.66	83.00	4.52	5.64
B3	12	97	18.36	1.94	47.92			43.53	191.68	143.76	7.83	9.77
C1	11	92	5.78	1.84	45.45			70.2	181.80	136.35	23.61	25.45
C2	12	82	5.78	1.64	40.51			70.2	162.04	121.53	21.04	22.68
C3	12	94	5.78	1.88	46.44			70.2	185.75	139.32	24.12	26.00
D1	10	68	8.96	1.36	33.59			71.46	134.38	100.78	11.25	12.61
D2	11	128	8.96	2.56	63.24			71.46	252.94	189.71	21.17	23.73
D3	12	76	8.96	1.52	37.55			71.46	150.18	112.64	12.57	14.09
E1	5	8	18.56	0.16	3.95			17.27	15.81	11.86	0.64	0.80
E2	6	15	18.56	0.30	7.41			17.27	29.64	22.23	1.20	1.50
E3	6	14	18.56	0.28	6.92			17.27	27.67	20.75	1.12	1.40

Week 10 - Feed calculations, gross weight, feed fed per tank.

Tank	Number of fish per tank	Total bulk wt. g	Wet feed energy per g - kj/g	2% B. wt. amino g. D. wt.	Maint. diet 2% B. Wt as kj	6% B. wt. diet g Dry wt.	8% B. wt. total diet g – Dry wt.	Moist. % of diet	Total energy fed	6% Energy required	Other feed required to supply energy	Total feed fed per day g
A1	11	129	24.70	2.58	63.73	7.74	10.32	5.72	254.92	191.19	7.74	10.32
A2	10	127	24.70	2.54	62.74	7.62	10.16	5.72	250.97	188.22	7.62	10.16
A3	12	122	24.70	2.44	60.27	7.32	9.76	5.72	241.09	180.81	7.32	9.76
B1	12	114	18.36	2.28	56.32			43.53	225.28	168.96	9.20	11.48
B2	10	62	18.36	1.24	30.63			43.53	122.52	91.89	5.00	6.24
B3	12	111	18.36	2.22	54.84			43.53	219.35	164.51	8.96	11.18
C1	11	105	5.78	2.10	51.87			70.2	207.49	155.62	26.95	29.05
C2	12	83	5.78	1.66	41.00			70.2	164.02	123.01	21.30	22.96
C3	12	102	5.78	2.04	50.39			70.2	201.56	151.17	26.18	28.22
D1	10	78	8.96	1.56	38.53			71.46	154.14	115.60	12.90	14.46
D2	11	161	8.96	3.22	79.54			71.46	318.15	238.62	26.63	29.85
D3	12	90	8.96	1.80	44.46			71.46	177.85	133.39	14.88	16.68
E1	5	8	18.56	0.16	3.95			17.27	15.81	11.86	0.64	0.80
E2	6	15	18.56	0.30	7.41			17.27	29.64	22.23	1.20	1.50
E3	6	14	18.56	0.28	6.92			17.27	27.67	20.75	1.12	1.40

Week 11 - Feed calculations, gross weight, feed fed per tank

Tank	Number of fish per tank	Total bulk wt. g	Wet feed energy per g - kj/g	2% B. wt. amino g. D. wt.	Maint. diet 2% B. Wt as kj	6% B. wt. diet g Dry wt.	8% B. wt. total diet g – Dry wt.	Moist. % of diet	Total energy fed	6% Energy required	Other feed required to supply energy	Total feed fed per day g
A1	11	145	24.70	1.45	35.82	4.35	5.8	5.72	107.45	71.63	2.90	4.35
A2	10	127	24.70	1.27	31.37	3.81	5.08	5.72	94.11	62.74	2.54	3.81
A3	12	135	24.70	1.35	33.35	4.05	5.4	5.72	100.04	66.69	2.70	4.05
B1	12	124	18.36	1.24	22.77			43.53	91.89	69.12	3.76	5.00
B2	10	66	18.36	0.66	12.12			43.53	48.91	36.79	2.00	2.66
B3	12	120	18.36	1.20	22.04			43.53	88.92	66.89	3.64	4.84
C1	11	104	5.78	1.04	6.01			70.2	77.07	71.06	12.30	13.34
C2	12	88	5.78	0.88	5.08			70.2	65.21	60.13	10.41	11.29
C3	12	115	5.78	1.15	6.64			70.2	85.22	78.58	13.61	14.76
D1	10	85	8.96	0.85	7.62			71.46	62.99	55.37	6.18	7.03
D2	11	196	8.96	1.96	17.56			71.46	145.24	127.68	14.25	16.21
D3	12	98	8.96	0.98	8.78			71.46	72.62	63.84	7.12	8.10
E1	5	6	18.56	0.06	1.11			17.27	4.45	3.33	0.18	0.24
E2	6	14	18.56	0.14	2.60			17.27	10.37	7.78	0.42	0.56
E3	6	12	18.56	0.12	2.23			17.27	8.89	6.67	0.36	0.48

B6. Mortality

Tank	Start No:	End No:	% mortality
A1	12	11	8.33
A2	12	10	16.67
A3	12	12	0.00
B1	12	12	0.00
B2	12	10	16.67
B3	12	12	0.00
C1	12	11	8.33
C2	12	12	0.00
C3	12	12	0.00
D1	12	10	16.67
D2	12	11	8.33
D3	12	12	0.00
E1	12	5	58.33
E2	12	6	50.00
E3	12	6	50.00

B7. Condition factor (CF)

Diet A – trout pellet C.F.

Tank 1				Tank 2			Tank 3		
Fish No:	Wt. - g	Length - cm	C.F.	Wt. - g	Length - cm	C.F.	Wt. - g	Length - cm	C.F.
1	24.82	9.8	2.64	29.70	11.50	1.95	11.72	7.60	2.67
2	11.54	7.9	2.34	20.58	9.10	2.73	9.78	7.70	2.14
3	28.12	10.1	2.73	16.80	8.60	2.64	5.52	6.00	2.55
4	18.06	9.2	2.32	14.70	8.10	2.76	12.2	7.90	2.47
5	13.30	8.3	2.33	9.41	7.20	2.52	8.36	7.10	2.33
6	11.80	7.5	2.80	4.08	5.40	2.59	12.09	8.00	2.36
7	10.10	7.2	2.71	12.68	7.70	2.77	15.07	8.70	2.28
8	8.84	7.1	2.47	12.21	7.90	2.47	16.94	8.70	2.57
9	5.58	6.3	2.23	8.49	7.00	2.47	11.74	7.90	2.38
10	5.92	6.2	2.48	9.46	7.70	2.07	13.16	7.80	2.77
11	7.32	6.5	2.67	0.00			25.82	10.00	2.58
12							10.52	7.70	2.30
Tank wt.	145.40			138.11			152.92		
Average	12.12	7.83	2.52	13.81	8.02	2.50	12.74	7.93	2.45

Diet B – peanut C.F.

Tank 1				Tank 2			Tank 3		
Fish No:	Wt. - g	Length - cm	C.F.	Wt. - g	Length - cm	C.F.	Wt. - g	Length - cm	C.F.
1	16.04	7.9	3.25	13.26	7.7	2.90	15.52	8.10	2.92
2	12.05	7.4	2.97	6.04	6.1	2.66	15.24	7.90	3.09
3	3.55	5.1	2.68	5.76	6	2.67	8.54	7.00	2.49
4	8.74	6.7	2.91	3.4	5.2	2.42	5.17	5.90	2.51
5	9.83	7.1	2.75	1.06	3.7	2.09	8.36	6.60	2.90
6	5.39	6	2.50	2.44	5	1.95	6.22	6.00	2.88
7	13.06	8.7	1.98	1.92	4.7	1.85	6.39	6.50	2.32
8	2.4	5	1.92	6.88	6.5	2.51	7.04	6.20	2.95
9	10.64	7.1	2.97	8.04	6.6	2.80	10.5	7.00	3.06
10	31.24	11.2	2.22	17.26	8.7	2.62	7.06	6.40	2.69
11	5.58	5.1	4.21				8.57	6.70	2.84
12	5.4	5.7	2.92				21.72	8.90	3.08
Tank wt.	123.92			66.06			120.33		
Average	10.326	6.92	2.77	6.606	6.02	2.45	10.03	6.93	2.81

Diet C – sweetcorn C.F.

Tank 1				Tank 2				Tank 3	
Fish No:	Wt. - g	Length - cm	C.F.	Wt. - g	Length - cm	C.F.	Wt. - g	Length - cm	C.F.
1	9.94	7.8	2.09	8.92	6.60	3.10	11.61	8.1	2.18
2	12.32	8.1	2.32	7.03	6.80	2.24	8.12	6.9	2.47
3	10.96	7.9	2.22	5.72	6.30	2.29	6.46	6.8	2.05
4	6.08	6.7	2.02	5.78	6.40	2.20	5.28	6.3	2.11
5	11.25	7.9	2.28	5.18	6.00	2.40	10.64	7.9	2.16
6	9.12	7.1	2.55	2.56	4.80	2.31	12.74	8.5	2.07
7	8.54	7.4	2.11	7.16	6.30	2.86	11.55	8	2.26
8	8.12	6.9	2.47	9.08	7.00	2.65	10.68	8	2.09
9	15.68	8.9	2.22	9.02	6.90	2.75	6.05	6.5	2.20
10	12.3	8.2	2.23	10.54	7.80	2.22	12.06	8.1	2.27
11				7.53	6.60	2.62	7.47	6.9	2.27
12				12.72	8.50	2.07	12.76	8.6	2.01
Tank wt.	104.31			91.24			115.42		
Average	10.43	7.69	2.25	7.60	6.67	2.48	9.62	7.55	2.18

Diet D – maggot C.F.

Tank 1				Tank 2			Tank 3		
Fish No:	Wt. - g	Length - cm	C.F.	Wt. - g	Length - cm	C.F.	Wt. - g	Length - cm	C.F.
1	7.4	6.8	2.35	23.36	10.2	2.20	4.68	5.9	2.28
2	9.6	7.5	2.28	23.16	10.6	1.94	7.4	6.8	2.35
3	6.8	6.9	2.07	15.56	9	2.13	7.26	6.9	2.21
4	10.54	7.9	2.14	13.34	8.7	2.03	7.82	7.1	2.18
5	7.52	7.3	1.93	11.98	8.4	2.02	9.6	7.5	2.28
6	8.1	7	2.36	7.72	7.4	1.91	8.66	7	2.52
7	7.83	7.1	2.19	17.56	9.5	2.05	5.58	6.4	2.13
8	7.26	6.8	2.31	10.66	8	2.08	6.14	6.5	2.24
9	10.62	8	2.07	18.72	9.6	2.12	6.76	6.9	2.06
10	9.5	7.4	2.34	26.8	10.9	2.07	13.88	8.5	2.26
11				27.26	11	2.05	7.46	6.9	2.27
12							13.06	8.2	2.37
Tank wt.	85.17			196.12			98.3		
Average	8.52	7.27	2.20	17.83	9.39	2.05	8.19	7.05	2.26

Diet E – boilie C.F.

Tank 1				Tank 2			Tank 3		
Fish No:	Wt. - g	Length - cm	C.F.	Wt. - g	Length - cm	C.F.	Wt. - g	Length - cm	C.F.
1	1.62	4.1	2.35	4.30	5.90	2.09	5.74	6.4	2.19
2	1.54	4.3	1.94	2.78	5.10	2.10	2.05	4.6	2.11
3	1.22	3.8	2.22	2.49	4.90	2.12	1.52	4.5	1.67
4	1.78	4.4	2.09	1.72	4.40	2.02	1.34	4.4	1.57
5	4.2	5.4	2.67	2.50	4.90	2.12	1.36	4	2.13
Tank wt.	10.36			13.79			12.01		
Average	2.072	4.4	2.25	2.76	5.04	2.09	2.402	4.78	1.93

B8. Specific growth rate for five study diets

SGR	Diet	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Tank 1	A1		3.19	0.56	2.04	3.03	1.59	5.02
Tank 2	A2		3.75	0.00	5.79	1.24	2.97	4.76
Tank 3	A3		5.31	0.48	1.79	2.32	3.47	3.24
Tank 4	B1		5.54	2.77	1.59	2.09	2.66	2.47
Tank 5	B2		5.31	0.95	1.32	-1.03	0.48	3.75
Tank 6	B3		3.19	3.97	0.84	2.60	1.91	2.70
Tank 7	C1		3.34	3.19	1.36	2.08	3.59	1.79
Tank 8	C2		2.73	2.81	1.91	1.68	2.20	1.91
Tank 9	C3		3.75	2.51	1.32	2.67	3.12	1.84
Tank 10	D1		3.19	1.10	-1.68	3.75	4.65	2.87
Tank 11	D2		4.11	1.68	1.51	3.03	4.93	4.11
Tank 12	D3		4.69	1.10	0.00	2.43	3.19	2.90
Tank 13	E1		2.77	2.32	-4.11	2.60	-0.54	3.14
Tank 14	E2		2.97	2.46	0.47	0.26	1.68	3.12
Tank 15	E3		0.92	2.46	-1.21	1.94	-0.84	4.43
SGR	Diet		Week 7	Week 8	Week 9	Week 10	Week 11	CUM. SGR
Tank 1	A1		3.91	3.23	2.76	2.54	1.67	2.46
Tank 2	A2		2.71	2.72	1.39	3.68	1.19	2.52
Tank 3	A3		3.01	2.63	2.09	2.93	1.79	2.42
Tank 4	B1		2.30	2.48	2.39	1.31	1.20	2.23
Tank 5	B2		2.36	1.47	1.34	1.45	2.25	1.64
Tank 6	B3		1.63	3.83	1.89	1.93	1.11	2.13
Tank 7	C1		2.56	2.57	3.30	1.89	-0.14	2.13
Tank 8	C2		1.42	2.69	3.54	0.17	1.31	1.86
Tank 9	C3		3.09	2.37	1.44	1.17	1.71	2.08
Tank 10	D1		2.66	2.47	1.09	1.96	1.23	1.94
Tank 11	D2		3.37	3.71	2.83	3.28	2.81	2.95
Tank 12	D3		2.16	2.09	2.02	2.42	1.22	2.02
Tank 13	E1		5.25	-5.25	-1.68	1.68	1.51	0.64
Tank 14	E2		3.59	0.00	-2.60	0.00	1.62	1.13
Tank 15	E3		5.58	-0.77	-3.59	0.00	0.40	0.78

Appendix C

C1. % lipid using regression formula to predict value from moisture content.

Diet & fish	Moisture %	Lipid % D. matter	D. matter	Lipid % in wet fish
A1-6	67.57	11.47	32.43	3.93
A1-10	70.04	10.55	29.96	3.18
A2-5	67.57	11.47	32.43	3.22
A2-6	67.58	11.46	32.42	3.01
A3-4	67.26	11.59	32.74	3.22
A3-6	68.24	11.22	31.76	3.35
B1-4	69.56	10.73	30.44	3.27
B1-5	68.31	11.19	31.69	3.55
B1-6	66.53	11.85	33.47	3.97
B2-4	69.54	10.74	30.46	3.27
B3-5	69.34	10.81	30.66	3.32
B3-4	70.26	10.47	29.74	3.11
C1-4	69.37	10.80	30.63	3.31
C1-5	68.82	11.01	31.18	3.43
C1-6	70.23	10.49	29.77	3.12
C2-6	68.17	11.25	31.83	3.58
C3-4	71.69	9.95	28.31	2.82
C3-6	71.67	9.95	28.33	2.82
D1-6	70.82	10.27	29.18	3.00
D2-4	69.12	10.89	30.88	3.36
D2-5	73.20	9.38	26.80	2.51
D3-4	70.79	10.28	29.21	3.00
D3-5	71.47	10.03	28.53	2.86
D3-6	69.87	10.62	30.13	3.20
E1-3	73.70	9.20	26.30	2.42
E2-1	71.72	9.94	28.28	2.81
E2-2	74.07	9.07	25.93	2.35
E3-1	70.92	10.23	29.08	2.97
E3-2	70.87	10.25	29.13	2.99
E3-4	71.45	10.03	28.55	2.86

C2. Data used to calculate the V.S.I of sample fish from each diet

Fish	Whole	gutted	Viscera wt.	VSI
A1-6	11.8	9.44	2.36	20.00
A2-6	4.08	3.22	0.86	21.00
A3-4	12.2	9.78	2.42	19.80
A1-10	5.92	4.75	1.17	19.82
A3-6	12.09	9.69	2.40	19.87
A2-5	9.41	7.33	2.08	22.11
B3-5	8.36	6.58	1.78	21.34
B3-4	5.17	4.08	1.09	21.02
B1-4	8.74	6.88	1.86	21.26
B1-5	9.83	7.70	2.13	21.62
B1-6	5.39	4.25	1.14	21.20
B2-4	3.4	2.68	0.72	21.10
B3-6	6.22	4.90	1.32	21.30
C1-4	6.08	4.96	1.12	18.50
C2-6	2.56	2.10	0.46	18.10
C3-4	5.28	4.33	0.95	17.90
C1-5	11.25	9.17	2.08	18.52
C3-6	12.74	10.42	2.32	18.21
C1-6	9.12	7.41	1.71	18.75
D2-4	13.34	10.79	2.55	19.10
D3-4	7.82	6.31	1.51	19.30
D2-5	11.98	9.67	2.31	19.32
D3-6	8.66	7.00	1.66	19.12
D3-5	9.60	7.76	1.84	19.18
E1-3	1.22	0.93	0.29	24.00
E2-3	2.49	1.87	0.62	24.90
E3-4	1.34	1.00	0.34	25.10
E3-2	2.05	1.54	0.51	25.10
E2-1	4.30	3.23	1.07	24.96
E3-1	5.74	4.30	1.44	25.10
E1-2	1.54	1.14	0.40	25.76
E2-2	2.78	2.10	0.68	24.52

C3. Data showing L.S.I. for five diets

Fish	A	B	C	D	E
1	4.023	3.832	6.203	8.449	37.358
2	4.543	5.079	5.019	6.573	39.052
3	5.795	11.451	5.576	9.149	50.475
4	4.074	4.556	6.989	4.011	14.321
5	4.301	8.336	8.819	4.096	21.842
6	4.070	9.094	10.932	4.618	24.189
7	5.398	5.575	5.430	13.318	11.078
8	4.983	5.449	7.591	8.693	29.429
9	8.870	7.288	9.700	8.534	40.500
Mean	5.117	6.740	7.362	7.493	29.805
95% C.I.	1.185	1.914	1.591	2.320	10.058

Appendix D

D1. Post mortem

All fish examined were killed by overdosing on anaesthetic, individuals were weighed (± 0.02g) and lengthed (0.1cm), and the data recorded before being submitted to a post mortem examination.

Fish were placed in groups of three on a dissection board, each fish was placed according to its numeric identification number across the board so it could be identified as an individual from a specific tank fed on a specific diet. A skin scrape was taken and examined for ectoparasites under a high powered compound microscope.

A vertical incision was made behind the operculum to facilitate the removal of the heart. (The heart was not examined internally but was examined externally under a 30x dissection microscope for fattiness. An operculum was removed to facilitate the removal of a sample piece of gill tissue for examination under the 30 x dissection microscope, a section of primary lamellae was taken and a squash prepared, this was examined under a high powered compound microscope.

A flap or window was opened in the fish by making a horizontal incision from the top of the original vertical incision along the body of the fish dropping down to the vent.

The general condition of the viscera including its fattiness was noted. The liver was dissected out of the fish and removed for weighing.

The viscera of the fish was then combined with the liver and weighed. All organs and tissue was kept in a petri dish containing phosphate buffered saline (PBS) between stages of the procedure. The intestinal tract of three fish from each tank were inspected for endoparasites by removing the gut and opening it along its length to examine for parasites. The gut as with other organs had to be kept with the fish to allow for accurate weighing of the viscera.

Appendix E

E1. DATA ANALYSIS

ANOVA – Initial weight (P=0.01)

Anova: Single Factor		P = 0.01	Initial weight			
Groups	*Count*	*Sum*	*Average*	*Variance*		
Diet A	3	4.998	1.67	8.88E-16		
Diet B	3	4.915	1.64	2.30E-03		
Diet C	3	4.832	1.61	2.30E-03		
Diet D	3	4.666	1.56	9.19E-03		
Diet E	3	3.496	1.17	7.23E-03		
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.5110	4	0.1277	**30.410**	1.422E-05	**5.994**
Within Groups	0.0420	10	0.0042			
Total	0.5530	14	**Fmax**	**4.00E+00**	**Fmax tab**	**202**

Tukey – Initial weight (P=0.05)

Tukey test	**P = 0.05**	**Initial weight for each diet**			
Mean	1.667	1.639	1.611	1.556	1.167
Sample	**A**	**B**	**C**	**D**	**E**
A		0.028	0.056	0.111	**0.5**
B			0.028	0.083	**0.472**
C				0.055	**0.444**
D					**0.389**
E					
T =	**0.169794**	**Q =**	4.65	Within variation	0.004
Number	3				

ANOVA – Final weight (P=0.05)

Anova: Single Factor		P= 0.05	Final weight (log transformed)			
Groups	*Count*	*Sum*	*Average*	*Variance*		
Diet A	3	3.367	1.122	0.0003		
Diet B	3	2.836	0.945	0.0118		
Diet C	3	2.882	0.961	0.0051		
Diet D	3	3.095	1.032	0.0362		
Diet E	3	1.138	0.379	0.0039		
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.028	4	0.2571	**22.453**	5.551E-05	**3.48**
Within Groups	0.115	10	0.0115			
Total	1.143	14	**Fmax**	**38.258**	**Fmax crit**	**202**

Tukey – Final weight (P=0.05)

Tukey	P=0.05		Final weight (log transformed)		
Mean	**1.122**	**0.945**	**0.961**	**1.032**	**0.379**
Sample	A	B	C	D	E
A		0.18	0.16	0.09	**0.74**
B			-0.02	-0.09	**0.57**
C				-0.07	**0.58**
D					**0.65**
E					
T =	0.287298	Q =	4.65	Within sample var	0.011
Number	3				

ANOVA – SGR (P=0.01)

Anova: Single Factor		P= 0.01	SGR			
Groups	*Count*	*Sum*	*Average*	*Variance*		
Diet A	3	7.4	2.4667	0.0025		
Diet B	3	6	2.0000	0.0997		
Diet C	3	6.07	2.0233	0.0206		
Diet D	3	6.91	2.3033	0.3152		
Diet E	3	2.55	0.8500	0.0637		
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	4.822	4	1.2055	**12.01**	0.0007796	**5.99**
Within Groups	1.004	10	0.1004			
Total	5.826	14	Fmax	**124.43**	**Fmax tab**	**202**

Tukey – SGR (P=0.05)

Tukey	P=0.05	SGR			
Mean	**2.470**	**2.000**	**2.020**	**2.300**	**0.850**
Sample	A	B	C	D	E
A		0.47	0.45	0.17	**1.62**
B			-0.02	-0.30	**1.15**
C				-0.28	**1.17**
D					**1.45**
E					
T =	0.850666	Q =	4.65	Within sample var	0.1004
Number	3				

ANOVA – % growth (P=0.05)

Anova: Single Factor		P = 0.05	% growth (log transformed data)			
Groups	*Count*	*Sum*	*Average*	*Variance*		
Diet A	3	8.48	2.828	9.8E-05		
Diet B	3	7.91	2.638	2.1E-02		
Diet C	3	7.95	2.651	4.0E-03		
Diet D	3	8.30	2.766	5.5E-02		
Diet E	3	6.02	2.006	3.1E-02		
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.301	4	0.325	**14.54**	0.0004	**3.48**
Within Groups	0.224	10	0.022			
Total	1.525	14	**Fmax**	**0.02**	**Fmaxcrit**	**202**

Tukey - % growth (P=0.05)

Tukey	**P=0.05**	**% gwth.**			
Mean	**2.828**	**2.638**	**2.651**	**2.766**	**2.006**
Sample	A	B	C	D	E
A		0.19	0.18	0.06	**0.82**
B			-0.01	-0.13	**0.63**
C				-0.12	**0.64**
D					**0.76**
E					
T =	0.4016	Q =	4.65	Within sample var	0.0224
Number	3				

ANOVA – % mortality (P=0.01)

Anova: Single Factor		P = 0.05	% Mortality - arcsine transformed			
Groups	*Count*	*Sum*	*Average*	*Variance*		
A	3	40.87	13.62	152.602		
B	3	24.09	8.03	193.520		
C	3	16.78	5.59	93.841		
D	3	40.87	13.62	152.602		
E	3	139.80	46.60	7.671		
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3324.663	4	831.166	**6.92**	0.01	**5.99**
Within Groups	1200.472	10	120.047			
Total	4525.135	14	**Fmax**	**25.23**	**Fmax tab**	**202**

Tukey - % mortality (P=0.05)

Tukey	P=0.05			% mortality - arcsine transformed data	
Mean	13.62	8.03	5.59	13.62	46.60
Sample	A	B	C	D	E
A		5.59	8.03	0.00	-32.98
B			2.44	-5.59	-38.57
C				-8.03	-41.01
D					-32.98
E					
T =	29.415	Q =	4.65	Within sample var	120.047
Number	3				

S.E – Significance of the regression line (p=0.01)

SE Significance of regression line	
Moisture/lipid	
Slope	0.4629
Standard Error	0.6755988
t	0.68517
t tab(0.01)	2.447

ANOVA – Lipid % (P=0.01)

Anova: Single Factor		P=0.01	Mosture / lipid			
Groups	*Count*	*Sum*	*Average*	*Variance*		
A	6	20.41	3.402	0.0874		
B	6	19.98	3.331	0.1097		
C	6	18.47	3.078	0.1179		
D	6	17.23	2.872	0.0986		
E	6	15.59	2.598	0.0892		
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2.632	4	0.6580	**6.54**	0.000956	**4.18**
Within Groups	2.515	25	0.1006			
Total	5.147	29	**Fmax**	**1.35**	**Fmax tab**	**16.3**

Tukey – lipid % (P=0.05)

Tukey	Lipid %	P=0.05			
Mean	3.317	3.331	3.078	2.872	2.598
Sample	A	B	C	D	E
A		-0.014	0.239	0.445	**0.719**
B			0.253	0.459	**0.733**
C				0.206	0.48
D					0.274
E					
T =	0.53866	Q =	4.16	Within sample var	0.1006
number	6				

ANOVA – C.F (P=0.01)

Anova: Single Factor		P=0.01	C.F.			
Groups	*Count*	*Sum*	*Average*	*Variance*		
A	9	22.32	2.48	0.0757		
B	9	25.64	2.85	0.0592		
C	9	20.97	2.33	0.0990		
D	9	19.82	2.20	0.0182		
E	9	18.78	2.09	0.0372		
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3.142	4	0.7855	**13.58**	4.442E-07	**3.83**
Within Groups	2.314	40	0.0579			
Total	5.456	44	Fmax	5.44	Fmaxtab	8.12

Tukey – C.F. (P=0.05)

Tukey	C.F.	P=0.05			
Mean	2.480	2.849	2.330	2.202	2.087
Sample	A	B	C	D	E
A		**-0.3684**	0.14985	**0.277772**	**0.393427**
B			**0.51821**	**0.646129**	**0.761784**
C				0.127924	**0.243579**
D					0.115655
E					
T =	0.153641	Q =	4.04	Within sample var	0.057851
Number	40				

ANOVA – L.S.I (P=0.01)

Anova: Single Factor		P=0.01	LSI			
Groups	*Count*	*Sum*	*Average*	*Variance*		
Diet A	9	6.26	0.70	0.0122		
Diet B	9	7.23	0.80	0.0242		
Diet C	9	7.67	0.85	0.0142		
Diet D	9	7.58	0.84	0.0326		
Diet E	9	12.87	1.43	0.0485		
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3.010	4	0.7524	**28.56**	2.99E-11	**3.83**
Within Groups	1.054	40	0.0263			
Total	4.063	44	**Fmax**	**3.97**	**Fmax tab**	**8.12**

Tukey – L.S.I. (P=0.05)

Tukey	LSI	P=0.05	L.S.I (log transformed)		
Mean	**0.695**	**0.804**	**0.852**	**0.842**	**1.430**
Sample	A	B	C	D	E
A		**-0.1084**	**-0.1571**	**-0.14722076**	**-0.73454**
B			-0.0487	-0.03880682	**-0.62613**
C				0.00990124	**-0.57742**
D					**-0.58732**
E					
T =	0.103677	Q =	4.04	Within sample var	0.026343
Number	40				

ANOVA – V.S.I. (P= 0.01)

Anova: Single Factor		P=0.01	VSI (log transformed)			
Groups	*Count*	*Sum*	*Average*	*Variance*		
Diet A	5	6.56	1.31	0.00042		
Diet B	5	6.65	1.33	0.00001		
Diet C	5	6.33	1.27	0.00004		
Diet D	5	6.42	1.28	0.00001		
Diet E	5	6.97	1.39	0.00013		
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.0502	4	0.0125542	**103.66**	4.487E-13	**4.43**
Within Groups	0.0024	20	0.0001211			
Total	0.0526	24	**Fmax**	**1.10**	**Fmax tab**	**25.42**

Tukey – V.S.I. (P=0.05)

Tukey	VSI	P=0.05	V.S.I (log transformed)		
Mean	**1.313**	**1.329**	**1.265**	**1.283**	**1.395**
Sample	A	B	C	D	E
A		-0.017	**0.04747**	**0.029241**	**-0.0822**
B			**0.06411**	**0.045879**	**-0.06556**
C				-0.018226	**-0.12966**
D					**-0.11144**
E					
T =	0.020809	Q =	4.23	Within sample var	0.000121
Number	5				