The use of bakery wastes as a nutritional source for fish.

Martin Richard Jaffa.

A thesis submitted to the University of Aston in Birmingham for the award of Doctor of Philosophy.

July 1983.

SUMMARY.

The Use of Bakery Wastes as a Nutritional Source for Fish.

Martin Richard Jaffa B.Sc.

A thesis submitted to the University of Aston in Birmingham for the award of Doctor of Philosophy.

July 1983.

The growth responses and food utilisation of mirror carp fed complete diets containing varying levels of waste bread and meat pie production waste were investigated under controlled conditions. Diets containing high levels of these wastes produced good growth with no deleterious effects.
Diets containing high lipid levels with the fat supplied by the bakery waste were shown to spare protein. 17% fat in the diet allowed for a reduction in the protein level from 34% to 24% with similar growth responses.
Hard fat as used in bakery products were found to require supplements of oils to produce the best growth responses. This was due to an apparent deficiency of some essential fatty acids.
The growth responses of mirror carp grown in extensive ponds fed a supplemental diet of only bakery wastes were investigated. Protein sparing of natural protein from zooplankton in the pond was seen to occur at all levels of feeding. This was supported by a simulation of the pond trial held under controlled conditions in the laboratory.
Varying methods involved in the manufacture of a production pellet were investigated.
Air drying of bread at room temperature was found to be an effective method of reducing excess moisture with a reduction of 40% by simple air drying and 60% by blowing the air with a fan.
Preservation of bakery waste pellets was found to be practical with 5% salt or a mixture of 3% salt and 0·5% calcium propionate. Pellets could be held at room temperature for a month without any adverse microbial growth.
Binding of a bakery waste pellet was found to be effective without the addition of a binding agent.
It was concluded that bakery wastes are a good nutritional source for fish, especially carp, with the most promising use being as an energy feed in extensive pond culture.

KEY WORDS: Fish - Nutrition - Bakery wastes - Carp.

ACKNOWLEDGEMENTS.

I would like to thank my supervisor, Professor A.J. Matty and the head of the Aston fish culture unit, Dr N.R.Bromage for their help and advice.

I would also like to thank all those at Warburtons Ltd of Bolton, who provided the financial assistance for this research and I would especially like to mention Mr T. Warburton, Mr J. Mason and Mr K. Colwell.

I would also like to thank all those at Newhay Fisheries for their help during the pond trials with special thanks to Mr and Mrs V. Michaels.

I would finally like to thank Dr S. Smith for his help in the GLC analyses and all those in the Microbiological section of Warburtons for their help in the analysis of microbial stability of the pellets.

CONTENTS.

LIST OF TABLES.

LIST OF FIGURES.

List of fish species mentioned in the thesis.

Carp.	Cyprinus carpio L.

All mention of carp in the text refer to the common carp or one of its domesticated strains.
The fish used in these experiments are a mirror strain of common carp supplied by Warburtons fish hatchery. This strain originates from Dinkelsbühle in Germany but is now known as the Newhay carp.

Yellowtail.	Seriola quinqueradiata.
Chinook salmon.	Oncorhynchus tshawytscha (Walbaum).
Rainbow trout.	Salmo gairdneri Richardson.
Channel catfish.	Ictalurus punctatus (Rafinesque).
Brown trout.	Salmo trutta fario L.
Bluegill sunfish.	Lepomis macrochirus Rafinesque.
Brook trout.	Salvelinus fontinalis (Mitchill).
Tilapia.	Tilapia aurea.

(Tilapia have been renamed due to reclassification however the fish mentioned above are as quoted in published work).

PREFACE.

This project started as one of several ideas to improve the utilisation of waste products from Warburtons Ltd, a large Lancashire bakery group based in Bolton.

The original proposals of feeding scrap bread to rainbow trout were considered during 1974, however it soon became apparent that this idea was not feasible since trout have a much higher requirement for protein than could be supplied by the waste.

The intial research into fish feeds showed that a possible alternative to trout was the carp which being omnivorous could be fed on a variety of waste products. This is especially seen in traditional methods of carp farming which uses several waste products as supplementary feed.

Work was therefore started using this species of fish, however two problems soon became evident. The first was that carp being warmwater fish, require higher than typical British water temperatures in which to grow and feed. The second was the supply of carp fry was erratic and unpredictable. It was therefore decided that before any serious evaluation of the wastes could be undertaken, it would be necessary to overcome these two issues. The research team diverted its' attention to these problems leaving the feed question until the other difficulties had been solved.

By 1978, the fish farming section at Warburtons had shown enough progress in the development of a warmwater recirculation system for the culture of carp and in the production of carp larvae through induced spawning, that it was felt that a renewed effort should be directed into the consideration of the feed question.

It was thought that this problem was rather specialised and therefore could be better tackled by someone from outside the company and so an approach was made to the Fish Culture Unit of the University of Aston in Birmingham. The result of discussions held between the University and the Company was that the problem warranted the attention of a research student and therefore a post was advertised to this effect.

At the same time as talks were taking place with the University of Aston, Warburtons were also holding discussions with a commercial carp farm, Newhay Fisheries Ltd of Selby, Yorkshire. Newhay had started in a different way to the approach made by Warburtons, in fact derelict ponds were developed as outdoor growing facilities using the traditional carp farming methods.

Fingerlings were intially imported from Dinkelsbühle in Germany and were ongrown to table size. However in 1975, the British government introduced legislation which prohibited the import of all live fish other than fancy aquarium fish. The fingerlings were consequently grown

to breeding size in the hope of using them for spawning purposes and so the continuation of the farming operation.

Since 1980, during the second year of this project, Newhay and Warburtons have combined to work together, both gaining benefits from each others facilities and knowledge. Fish are now spawned by induced methods in the warmwater indoor system and then transfered for ongrowing to the outside growing ponds.

Thus the nature of this research changed with the emphasis being placed on the use of bakery wastes in extensive pond systems. This meant that the project became even more applied than before necessitating a complete change in attitude to the whole aspect of fish culture.

INTRODUCTION.

In 1973, Peru banned the export of products from the anchovy fishery which heralded a world shortage of fish meal and precipitated a crisis in the fish farming industry (Spinelli *et al.*, 1979). This was because fish meal is the basic ingredient in most fishfeeds designed for use in intensive fish farming. The shortage of fish meal together with a threefold increase in price spelled catastrophe for the fish farmer.

The situation eased after 1975 which resulted in an increase in aquaculture production to over 6 million tonnes or over 10% of total fish production (Glude, 1977).

In Japan, during 1973, production of yellowtail required 680,000 tonnes of food of which about 95% was of fish origin (Furukawa, 1975) and in the United States, trout requirement was about 35,000 (dry) tonnes (Spinelli *et al.*, 1979). Estimates for 1990 indicate that over 40,000 tonnes of fishmeal will be required for salmon alone.

Rumsey (1973) posed the question "Is aquaculture held to fishmeal supply?" The answer to this is to look for alternative cheaper protein sources especially as the goal in fish farming is neither to produce nor use the ideal diet but to gain the most advantageous growth and quality at the lowest possible cost (Rasmussen, 1969).

The use of expensive fishmeal protein is mainly restricted to highly intensive aquacultures where protein

is a limiting factor in the rate of production. This is not the case with extensive fish farms where protein is obtained from natural feeds within the ponds. This usually takes the form of zooplankton which are rich in protein and are readily taken by the fish.

In order to maximise the utilisation of this natural protein, it is necessary to introduce cheaper energy rich feed sources such as cereals which contain high levels of carbohydrates. Besides these obvious energy feed sources, there are also many unconventional feedstuffs available such as wastes from the food industry (Ben Gera & Kramer, 1969).

One such waste is that produced by the bakery industry. This makes a potentially ideal food source since it is made from carbohydrate rich cereals. These wastes can be used directly in an extensive system or can be incorporated into complete diets for use in more intensive pond farming where the methods used are still those of the extensive system but on a more intensive scale. Such systems are found today in Israel where yields of 7 tonnes hectare^{-1} are obtained (Brown, 1977).

In systems where protein is the limiting factor such as in salmonid culture, the cost of the protein-rich feeds will increase to the point where it will become uneconomic to continue farming such species. This will be increasingly possible if the recent trends observed in fishmeal supply and price continue.

It is therefore probable that the culture of carnivorous fish on which the West specialises will in the future enter a period of decline and its place will be filled by a return to more extensive farming with warmwater species. The most likely candidate will be the carp as it is one of the easiest fish to culture. Of all the fish species utilised by man, the carp has the longest history of culture in the world.

Mention of carp for food was first recorded by Fan Li in 460 BC when he wrote his treatise 'Fish culture classic' in China (Brown, 1977). Aristotle mentioned carp and it is likely that both the Greeks and Romans fattened carp in ponds. Further introductions into Europe may have taken place around 1150. In Austria, carp were being farmed by 1227 and in Britain, carp were grown in ponds by 1600 (Taverner, 1600). By 1860, carp were common all over Europe (Bardach et al., 1972) and even today carp retains its popularity in most European countries.

In Britain, carp went out of favour due to the increase in commercial marine fisheries at the turn of the century. With the return to fish farming in the last twenty years, carp were dismissed as a potential species in favour of 'up-market' salmonids. Whether this type of fish remain as the mainstay of the British fish farming industry or whether they disappear will depend mainly on the price of feeds and in particular the price of fishmeal, however there is no

reason why extensive fish farming cannot develop alongside the existing industry. To some extent, this is already happening with a number of small extensive units operating around the country. They are producing carp for the table market with ethnic groups as the primary consumer. Due to the potential size of this market, the possibilities for expansion are great.

The aim of this thesis is to investigate the potential of bakery wastes as a nutritional component in fish culture. Intial consideration of the problems involved have identified three main areas for possible research.

1. The first area to consider is the nutritional status of bakery wastes and their subsequent incorporation into a complete and balanced fish diet. As there is commercial interest in the results, various levels of waste should be used in order to ascertain the highest acceptable level which takes advantage of the low cost of the waste but will produce reasonable growth rates in the fish. Whilst dealing with complete diets, other nutritional aspects can be investigated including the use of carbohydrates and fats in fish diets, as bakery wastes are rich in both these dietary components.

2. The second area for consideration is the use of bakery wastes as supplemental feed in extensive fish culture. Due to the nature of the wastes they appear to fulfill the requirements of supplementary feeds however trials must be conducted to ascertain their acceptability.

3. The final area for research is that of pellet technology and the need to be able to produce an acceptable medium for presentation of the waste to the fish. This not only includes the manufacture of a pellet but also consideration of the storage potential of any food containing mixtures of bakery wastes.

Summarising these areas, the aim of this thesis is to produce from raw bakery waste, an acceptable final product which can be used in the commercial culture of carp.

GENERAL METHODS.

The methods described here apply to the majority of the experiments included in this thesis. In cases where different methods have been used, reference to the changes are included in the methods section of the individual experiment.

Experimental system.

The experiments were conducted in a water recycling system consisting of eight 90 l circular tanks supplied with biologically filtered water maintained at 22^{o}C. The total volume of the whole system was 1300 l. The water flow into each tank was kept at a constant 1·75 l min^{-1}. A supply of freshwater was added to the system at a rate of 0·36 l min^{-1} in order to replace evaporative and leakage losses and also to maintain nitrate levels below 20 ppm. Ammonia was effectively removed by the filter with unionised ammonia always lower than 0·01 ppm. pH was maintained in the range 6·8 - 7·2. Although the system contained a solids trap of 80 l complete with mesh baffles to aid deposition of solids, an additional small trap was placed on top of the filter unit. This took the form of a small circular tray of 12 l capacity which was filled with foam strips. Water passed from the larger trap into this secondary trap and any undeposited solids were retained on the foam pieces. These were cleaned daily.

Diet preparation.

All experimental diets were prepared by mixing the dry and semi-moist ingredients in a HOBART food mixer, model no A120, followed by the oils and water to produce a homogenous dough. This was extruded through the mincer attachment of the food mixer. The resulting pellets were frozen on an open tray, bagged and then stored in a freezer.

Weighing proceedures.

All fish were weighed weekly. They were first starved for 15 hours and then anaesthestised with ethyl-4-amino benzoate (Benzocaine, BDH Chemicals Ltd.) at a dosage of 1:15000 prior to weighing. Each fish was drained to remove excess moisture before they were weighed on an electronic top ban balance to the nearest 0·01g. After weighing, the fish were returned to freshwater to recover. The fish on each experimental diet were moved to the adjoining tank to eliminate any differences in growth due to tank position, water supply, etc.

Proximate analyses.

Moisture by oven drying at 105^{o}C to constant weight.

Protein by Kjeldahl method using the conversion factor N x 6·25.

Fat by Ether extraction.

Ash by incineration at 550^{o}C for 24 hours.

Nitrogen Free Extract by difference.

(AOAC, 1975).

All proximate analyses were carried out as shown above with the exception of the analyses of the fish carcasses in which the method was slightly modified. Nitrogen Free Extract (NFE) was assumed to be no more than 0·5% of the total analysis so that protein was determined by difference (Caulton & Bursell, 1977).

Analyses of experimental data.

Specific growth rate.

In the early stages of life, the growth of a fish under controlled conditions follows an exponential curve, the equation of which is:-

$$w_2 = w_1 e^{g(T-t)}.$$

where w_2 - Final weight at time T.

w_1 - Intial weight at time t.

e - base natural log.

g - constant for a particular curve known as the specific growth rate.

Rearrangement of the equation to obtain g and multiplication by 100 gives the rate of change of weight of the fish expressed as the percentage per day.

$(\log_e w_2 - \log_e w_1$ / No of days) x 100. (Brown, 1957).

Food conversion ratio.

Feed intake / Weight gain.

In calculation, dry weight of food for feed intake was used whereas wet weight was used for weight gain.

Protein efficiency ratio.

Weight gain / Protein fed. (Osborne et al., 1919).

Net protein utilisation.

Apparent NPU =(Nb - Na / Ni) x 100.

where Nb = body nitrogen at the end of the experiment.

Na = body nitrogen at the start of the experiment.

Ni = nitrogen injested during the experiment.

(Bender & Miller, 1953; Miller & Bender, 1955).

Statistical methods.

Statistical comparisions were made using the analyses of variance. Mean differences were assessed using Duncans Multiple Range Test. Standard errors (±SE) were calculated to identify the range of the means (Duncan, 1955).

SECTION 1.

The use of bakery wastes in complete diets.

The evaluation of bakery wastes in complete diets.

INTRODUCTION.

Bakery products are rich in carbohydrates whereas the natural food of many fish species is often deficient in this dietary component so before evaluating the potential role of wastes from the bakery industry, the role of carbohydrates in fish nutrition will be discussed.

Dietary carbohydrate serves largely as a source of energy but it is often omitted from fish diets since protein can also be utilised as an energy source to provide the necessary calories. Phillips & Brockway (1959) intially calculated that trout could obtain 3·9 calories from every gram of protein whereas from the same amount of carbohydrate, only 1·6 calories were available. It has now been shown that this figure is very low and that the energy provided by these components is very similar with 4·5 calories from each gram of protein and 4 calories from every gram of carbohydrate (Cowey & Sargent, 1972).

Many fish diets contain protein levels of over 50% which is far in excess of the requirement of most fish, so much is wasted in conversion to energy. The use of carbohydrates as an energy source is therefore highly desirable (Pieper & Pfeffer, 1979), with the incorporation in the diet of as much as the fish is able to utilise without incurring deleterious effects (Cowey & Sargent, 1972).

The use of carbohydrates or fats for energy to save protein for growth is known as the sparing action (Phillips & Brockway, 1956).

Since carbohydrates can be used to spare protein and are relatively cheap constituents to obtain, there is a temptation to feed them at high levels. This has been found to be acceptable to some extent for both herbivorous and omnivorous fish but is unacceptable for carnivorous species (Shimeno et al., 1979) because they have very little natural carbohydrate in the wild (Edwards et al., 1977). For this reason most of the work on carbohydrate nutrition has been carried out on these fish.

Some of the earliest work was carried out by McLaren et al. (1946; 1947). In the earlier, they found that the growth of trout fed diets containing 48% carbohydrate was equal to that produced by the standard hatchery diet but that the fish developed large yellow lobulated livers and eventually died. They concluded that to avoid this, the carbohydrate content of the diet should not exceed 20%. Phillips et al. (1948) also found that feeding carbohydrate to trout over long periods of time resulted in high glycogen livers and mortality. This was because trout have no mechanism for excreting excess carbohydrate. They suggested that trout diets should not contain more than 9% digestible carbohydrate or that the fish should not be fed more than 4•5 grams of digestible carbohydrate per kilogram of body

weight per day. Buhler & Halver (1961) fed a well characterised semi-synthetic diet based on that of Halver (1957) but containing varying amounts of carbohydrate. They found that fingerlings of chinook salmon fed an adequate diet could tolerate up to 48% carbohydrate for long periods of time. Good fish growth resulted with no increase in mortality or gross liver pathology. This is in agreement with the work of McLaren et al. (1947) who concluded that their earlier figure could be increased to 45% provided that the diet contained natural products such as vegetable meals instead of casein which they had previously used.

Edwards et al. (1977) working with different strains of trout found that the amount of carbohydrate utilised depended on the strain of fish although in most cases a level of 17 - 25% carbohydrate was acceptable. A 38% replacement of protein by carbohydrate in a diet produced a reduction in growth rate. The differences found by the different workers as to an acceptable level of dietary carbohydrate in salmonid fish may be due to variations in the test diets or in the type of carbohydrate used.

Buhler & Halver (1961) monitored the effects of different carbohydrates. They found that the fish were capable of utilising glucose, maltose, sucrose, dextrose and potato starch, although fish growth decreased with increasing molecular weight of the carbohydrate. Pieper

& Pfeffer (1979) replaced about 30% of a standard diet with either glucose, sucrose, lactose or gelatinised starch. They found that lactose was totally unacceptable whilst the others produced growth as good as the basic diet. Starch replaced to higher levels of about 40% did not appear to produce inferior growth to the protein replaced.

The utilisation of different carbohydrates depends on the digestibility of each. This is reflected in the amount actually fed to a fish. Phillips & Brockway (1959) calculated the number of dietary calories available to trout assuming a digestibility of 40%; this would result in 1·6 calories available for every gram of carbohydrate. This figure is now thought to be very low. In 1956, Phillips & Brockway calculated the absorption of different carbohydrates after 36 hours. Glucose produced the best absorption with 99%, maltose 93%, sucrose 73%, lactose 60%, cooked corn starch 47% and raw corn starch 38%. Singh & Nose (1967) worked out the digestibilities of these carbohydrates when fed at different levels from 20% to 60% of the diet. Digestibility was reduced with increasing molecular weight as well as increasing carbohydrate in the diet. The worst digestibility therefore resulted from potato starch fed at 60% of the total diet. The complex carbohydrates resulted in growth inferior to the other sugars especially at high feeding levels.

Inaba et al. (1963) measured digestibility using practical diets. The digestibility of protein and ∝ starch varied with their content in the diet. These were present in the form of white fishmeal and bread crumb respectively. Protein digestibilty was high when the protein level was high in the diet whereas the digestibility of ∝ starch was high when it was low in the diet. Starch digestibility decreased with increasing content. Raw starch was considerably lower in digestibility than when it was cooked. Kitamikado et al. (1964) found that the digestibility of protein decreased as the starch content increased. This is supported by Rychly & Spannhof (1979) who suggested that protein digestibility was reduced due to the low digestibility of the non-protein part.

Most of these studies have been carried out with regard to salmonid or carnivorous species whereas much less work has been aimed at the utilisation of carbohydrates by warmwater fish such as carp. This is because they have always been considered capable of dealing with large amounts of carbohydrate due to their natural omnivorous diet. Chiou & Ogino (1975); Shimeno et al. (1977) and Shimeno et al. (1979) all found that the digestibility of carbohydrate in carp remained constant at between 85% -90% regardless of the level of the carbohydrate in the diet provided that it did not amount to more than 50% of the total diet.

Growth trials investigating the effects of varying carbohydrate levels in carp diets are inconclusive. Ogino et al. (1976) replaced casein (60-0%) with dextrin (0-60%), thus the effects of varying carbohydrate was confounded by reduced protein levels and the conclusions of these workers that carbohydrate was effectively used by carp cannot be supported. Sen et al. (1978) also conducted a similar experiment in which dextrin (71-26%) was replaced by casein (0-45%). They suggested that the optimum growth of carp occured with a diet containing 45% protein and 26% carbohydrate. This was the diet with the lowest carbohydrate and highest protein level, which would be expected to produce the best growth.

Most work on carbohydrate nutrition in carp has been connected with biochemical studies which seem to indicate that carp are capable of dealing with carbohydrate in their diet due to high amylase activity (Cowey & Sargent, 1972). It would appear that diets with a high carbohydrate component are promising as feed for carp provided that the rest of the diet is nutritionally adequate. As carp are warmwater fish with a temperature optimum for growth of 28^{o}C, the metabolic energy requirements are far greater than those of salmonid species reared at 12-15^{o}C, consequently the sparing action of carbohydrate on dietary protein becomes more significant.

It can therefore be seen that carbohydrate can be very important as a dietary component in fish nutrition especially with regard to warmwater species. The use of bakery wastes as an ingredient in fish diets can provide this component very cheaply. One of the predominant wastes from the bakery industry is bread.

In Britain, it has been estimated that the public buy about 11 million loaves of bread every day (Agricapital Group, 1978) and about 0•1% of this production goes to waste in the form of unsold loaves (Roy, 1976). Much of this waste is bought by farmers for feeding to pigs, however bread has never been evaluated as a realistic food source for animals in Britain. This is not the case in the United States where there has been some work on the evaluation of bakery waste as an animal feed.

Kirk & Peacock (1969) have described Blended Dried Bakery Product (DBP) as a feed made from a combination of bakery products and surplus baked goods. The ingredients used to make DBP include bread, dough, cakes, flour, biscuits and crackers which are all blended together to a consistant analysis (Wallace, 1965). Arrington (1965) gave the proximate analysis of DBP as:- moisture - 6%. crude protein - 9•1%, Ether extract - 13%, ash - 3•4%, crude fibre - 1% and Nitrogen Free Extract - 67•5%. In 1965, over 1000 tons of DBP were produced from one production unit (Wallace, 1965). It is still widely available in the United States and in

January 1981 the price was about $160/ton. The analysis of this product is shown in table 1.1. (Enns, International Bakerage Inc, Atlanta Georgia, Personal communication). Since DBP is so widely available, it has been evaluated as a feed for several farm animals.

Wallace (1965) found that in pig rations, DBP could be substituted for yellow corn up to levels of 30% of the total diet. Growth of the pigs was found to be as good as those on the control diets. For younger pigs, he found that dried skimmed milk could be replaced by DBP up to levels of 20% in starter diets with no significant change in growth. Similar results were obtained by Meacham & Thomas (1965) whereas Peo (1965) found that the level of replacement could be increased to 30% of the diet. This was confirmed by Sewell (1966) but in this case, the DBP replaced corn and not skimmed milk. Kornegay (1974) showed that for older pigs, inclusion of DBP at between 12 and 24% of the diet improved daily weight gain and feed efficiency.

Damron et al. (1965) fed DBP to chickens at a level of 10% and growth was unaffected. Turkeys fed DBP at the same level of inclusion, increased feed efficiency by 2·9% and decreased feed consumption by 2·7% (Potter et al., 1971).

Dairy cattle have been fed DBP as a substitute for corn and citrus pulp by Wing (1964; 1965) at a level of 30%. He suggested that the cooked ingredients may be beneficial for the rumen organisms. DBP has also been fed to steers

Table 1.1. Proximate analysis of Blended Dried Bakery Product, DBP, (as supplied by International Bakerage Inc, Atlanta, Ge).

Crude Protein.	8·50%
Ether Extract (fat).	11·50%
Crude Fibre.	2·00%
Ash.	5·40%
Moisture.	7·50%
Sugars.	9·20%
Metabolizable energy.	3969·00 Cal/Kg.
Productive energy.	2976·75 Cal/Kg.

Available amino acids expressed as a percentage of the total ingredients.

Arginine.	0·32%
Methionine.	0·16%
Cystine.	0·18%
Meth. + Cystine.	0·34%
Lysine.	0·045%
Histidine.	0·10%
Isoleucine.	0·55%
Leucine.	0·80%
Phenylalinine.	0·40%
Phenyl. + Tyrosine.	0·90%
Threonine.	0·60%
Valine.	0·45%
Glycine.	0·90%

continued.

Table 1.1. continued.

Minerals and Vitamins.	
Calcium.	0•10%
Available Phosphorus.	0•19%
Salt. (maximum)	3•50%
Sodium.	1•38%
Manganese.	100•11 mg/Kg.
Iron.	29•11 mg/Kg.
Copper.	5•07 mg/Kg.
Cobalt.	1•01 mg/Kg.
Potassium.	0•80%
Magnesium.	0•32%
True vitamin A.	6747•30 IU/Kg.
Carotene.	4•63 mg/Kg.
Vitamin E.	59•97 IU/Kg.
Thiamin.	1•49 mg/Kg.
Niacin.	25•91 mcs/Kg.
Riboflavin.	2•20 mg/Kg.
Pantothenic acid.	4•63 mgs/Kg.
Vitamin B12.	0•0
Choline.	1102•50 mgs/Kg.
Pyridoxine.	5•51 mgs/Kg.
Xanthophyll.	1•76 mgs/Kg.
Folacin.	trace.
Ethoxyquin.	trace.

as a fattening ration at levels of 9·4% (Baker, 1964) and 10% (Kirk & Peacock, 1969). At over 10%, the weight of the steers increased by 11% over the control rations and at a level of 20%, the weight increased by 10·3%.

All this work has confirmed that bakery wastes are an excellent filler in all types of animal ration. The level at which it can be incorporated is dependent on the desired composition of the final diet. Because these workers look on bakery waste as an energy feed, it has not been included at very high levels. At the levels at which it has been used, the provision of energy from a cooked product appears to have increased the efficiency of the whole diet. This can especially be seen in the experiments where the DBP has replaced a raw carbohydrate source such as corn (Sewell, 1966).

Bakery wastes are mainly thought of as being either bread or dough type wastes which are relatively low in protein, high in carbohydrate and devoid of fat such as in bread waste, however there are wastes that originate from associated baking industries which have a completely different nutritional profile. One such waste is that from the producers of meat pies and although a large proportion of this type of waste consists of pastry, the remainder is made up from protein and fat. Because of the higher levels of protein and fat than bread wastes, pie waste therefore has the potential for increasing the nutritional value of other low grade

bakery wastes. There are however, one or two drawbacks to its use in fish diets. The first is that the waste is made up of two separate ingredients: the filling and the pastry and their presence is variable as a result of the time during the manufacturing process at which the waste is generated. It is possible that the waste could consist of only low protein pastry or conversely it could contain only the protein and fat rich filling. This makes the formulation of diets difficult since the analysis of the waste may not reflect the actual nutritional profile. This could lead to nutritional deficiencies which in turn could lead to unexpected changes in growth rates or in certain cases even death. There is really no satisfactory answer to this problem except to constantly carry out proximate analyses or to hope that over a period of time the analyses of the waste will even out to an average value. The second associated problem is that the waste does not only contain nutritional components but also includes some inedible material, the bulk of which is aluminium foil in which the pies are manufactured. The removal of this foil will be dealt with in a later section.

The nutritional value of the different components in the waste is very important since the incorporation of as much as possible in a diet is very desirable because of its relatively low cost. The use of this waste would reduce the need for additional expensive ingredients

especially of those which are protein sources. This is because many diets are formulated to least cost formulations (Cowey & Sargent, 1972) where the cheapest possible ingredients are used to produce the required results. The cost of diets can be further reduced through a reduction in the protein level which can be achieved through sparing the protein with either fats or carbohydrates. Protein sparing by fat is more efficient than by carbohydrates because of the higher energy level of fats. This is 9 Kcal g^{-1} as compared with 4 Kcal g^{-1} for carbohydrates. Although the pie waste contains higher levels of protein than other bakery wastes it is still below that required by fish so the presence of the fat should aid in its utilisation to the fullest through its sparing potential.

There appears to be no work published on the inclusion of any type of bakery waste in complete fish rations although their use as a supplementary feed was mentioned by Schaperclaus (1933). That fish like certain bakery products, there is no doubt for Hunt (1971) observed that wild populations of barbel were able to survive on bread that had been supplied by anglers who used it as ground-bait. Analyses of stomach contents from these fish showed that 47% consisted of anglers baits.

The evidence from the work on DBP fed to farm animals together with that from anglers reports indicate that in nutritional terms, bakery waste constitutes a good dietary

source. Although it contains a limited amount of protein, its real value is for the provision of energy and the component that supplies the major part of this is the carbohydrate.

The aim of the following experiments is to evaluate two different types of bakery waste when incorporated into complete fish diets. These experimental diets contain all the components required for good fish growth. This is essential as the experiments all take place in controlled conditions where the only nutrient source is from the diets. Although these experiments take place in recycling systems which require a total nutritional input and in economic terms are expensive to run, there are a few examples of carp culture taking place in intensive sysytems which require the same nutritional input in the form of a complete diet. Evaluation of bakery waste in complete diets can therefore have some relevance to a commercial system.

The bakery wastes to be considered in this section are bread wastes which take the form of whole loaves of bread and pie production wastes which consist of a mixture of pastry and filling in various quantities. The experiments carried out in this section, evaluate bread alone and then a mixture of the two. A third experiment compares the best diet from each group.

The evaluation of waste bread for use in complete diets.

MATERIALS & METHODS.

Diets.

Bread was obtained from Warburtons Ltd of Bolton as a mixture of brown and white, sliced and unsliced loaves. Each loaf was crumbed using the liquidiser attachment of a KENWOOD chef food mixer (model A707A). The crumb was seived through a 2 mm sieve, larger pieces were recrumbed and seived again. A mixture of 50% brown and 50% white crumb was used in the diets. Proximate analyses were carried out on both white and brown samples (Table 1.2.).

A control diet was formulated using herring meal and wheat middlings as the main ingredients. Six experimental diets were derived from the control diet by the substitution of bread for the wheat middlings (Table 1.3.). The level of the ingredients were adjusted so as to balance the protein level at 33%, which was considered to be sufficient to meet the protein requirement of carp, and the fat levels at 9% (Table 1.4.). A commercial trout diet (Edward Baker Ltd, Omega no 4) was repelleted as a moist diet and then frozen. The moisture content of all eight diets was measured and a proximate analysis was carried out on each (Table 1.4.).

Table 1.2. Proximate analyses of white and brown bread.

	White bread. (%)	Brown bread. (%)
Moisture.	39•70	39•50
Protein.	7•83	8•36
Fat.	0•17	0•42
Crude fibre.	0•15	4•36
Ash.	1.93	2•36
Nitrogen Free Extract.	50•16	45•09

Experimental system.

A single water recirculation system of eight tanks was maintained at $22^{o}C$. Each tank was stocked with 12 fish of average weight of 49g.

All fish used were mirror strain of common carp, Cyprinus carpio L. and were obtained from Warburtons fish hatchery. This strain of carp (now called the Newhay carp) originated from German stock called Dinkelsbühle named after an area of Germany near Nurnberg. This German stock can be traced back to the original Franconian race of domesticated carp.

Feeding.

Diets were fed at a rate of 2% of body weight day^{-1} of dry feed. This was calculated on a weekly basis. The amount of diet fed was adjusted to allow for differences in the moisture content. Feeding was by hand and took place thrice a day. The experiment ran for 35 days.

Carcass analyses.

At the end of the experiment, six fish were removed from each treatment for carcass analyses. This involved carrying out a proximate analysis on each fish.

Table 1.3. Constituents of the test diets (% by weight).

Diet. Ingredient.	A.	B.	C.	D.	E.	F.	G.	H.
Trout pellet.[1]	66	-	-	-	-	-	-	-
Herring meal.[2]	-	22	25	28	29	30	32	35
Wheat middlings.	-	39	25	17	13	8	4	-
Skimmed milk.	-	2	-	-	-	-	-	-
Bread.	-	-	14	22	26	30	35	41
Oils.[3]	-	3	4	4	4	4	4	4
IVY.[4]	-	2	2	2	2	2	2	2
Binder.[5]	-	1	1	1	1	1	1	1
Water.[6]	34	32	31	27	26	25	22	17

1. Edward Baker Ltd. Omega no 4 trout food.
2. Edward Baker Ltd. Norseamink.
3. Mixture 50% corn oil, 50% cod liver oil.
4. Edward Baker Ltd. IVY mineral & vitamin mix.
5. Alginate Industries, London. Alginate binder.
6. Additional water required to make the diet semi-moist.

Table 1.4. Proximate analyses of the test diets. (% by dry weight).

Diet. Component.	A.	B.	C.	D.	E.	F.	G.	H.
Protein.	45•59	33•38	33•05	33•86	34•34	33•75	34•09	34•87
Fat.	6•19	7•26	9•04	9•41	8•66	10•01	9•82	10•27
Ash.	12•97	10•81	10•96	11•64	12•53	12•47	12•34	12•69
N.F.E.[1]	35•25	48•55	46•95	45•09	44•47	43•77	43•75	42•17
Overall moisture.[2]	42•00	40•36	45•41	44•34	44•80	44•26	43•72	42•17

1. Nitrogen free extract.

2. Figures given in the proximate analyses refer to the dry weight of each component and these in total make 100%. The figures given for overall moisture refer to the finished semi-moist product. The dry components account for the remaining 55-60%.

RESULTS.

In all the treatments, the fish soon became accustomed to the experimental diets and consumed all the food that was given. All the fish grew rapidly and the average weight gains are shown in table 1.5. Statistical analysis showed that the growth of the fish fed the three diets containing the highest levels of bread were not significantly different ($p>0.05$) from the control diet. The largest increase occurred with the diet containing bread at a level of 30%. This is also shown by the specific growth rates (Figure 1.) and the food conversion ratios (Table 1.5.). For the 30% bread diet, non of these indices were significantly different ($p>0.05$) from the control. Average growth rates increased with increasing levels of bread up to 30% inclusion and then they were seen to show a slight decline.

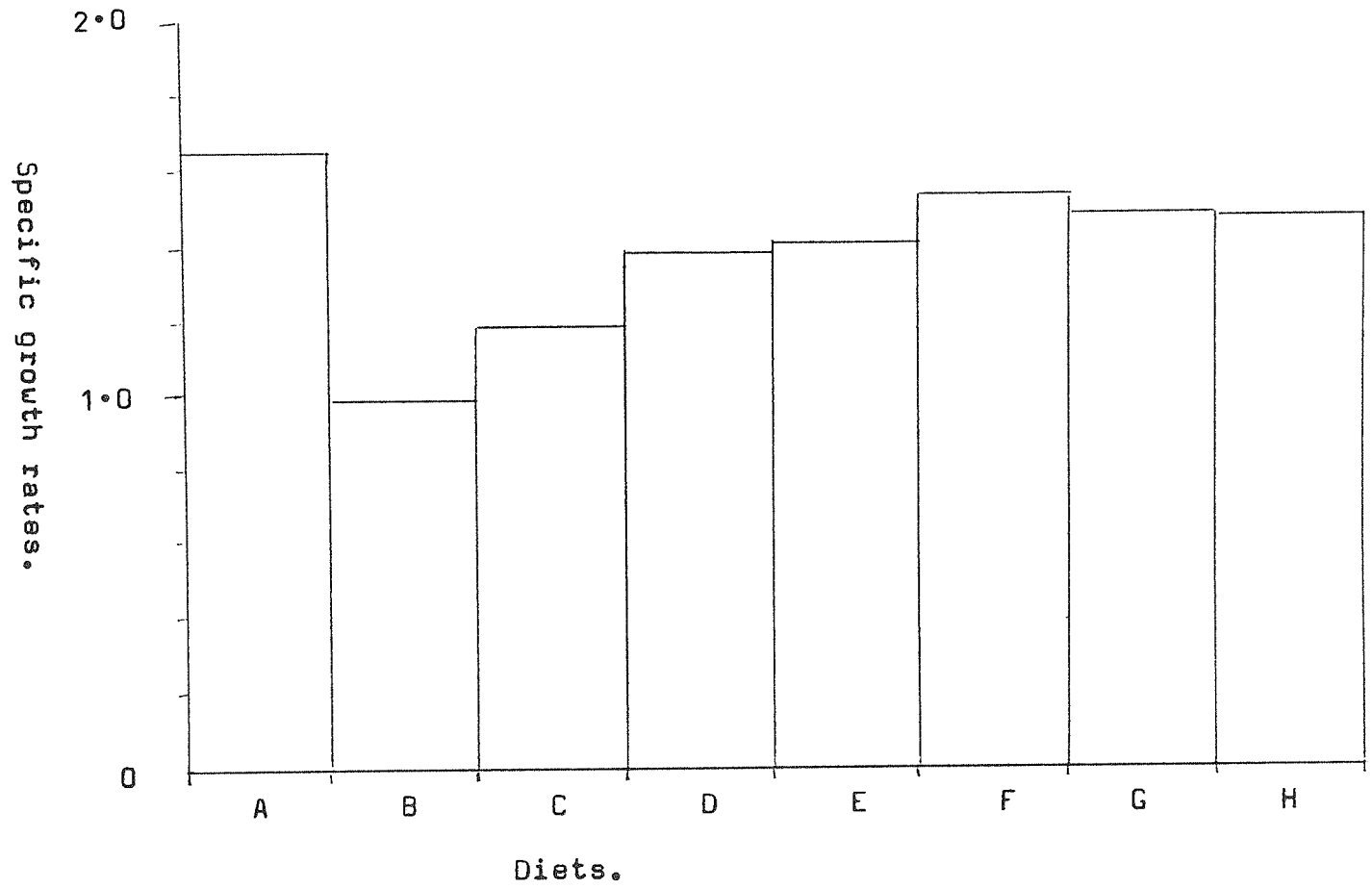
The results of the proximate analyses of the fish carcasses are shown in table 1.6. There is very little difference between the different components for each treatment, although the differences are in some cases, significant ($p<0.05$). With increasing levels of bread in the diet there is a slight reduction in the moisture content of the whole body. There is a definite trend which follows the pattern of the specific growth rates. Feeding diet F (30% bread) resulted in the lowest carcass moisture level whereas the commercial diet produced the highest. Protein, fat and ash levels of the fish follow

Table 1.5. Performance of carp fed on the test diets for 5 weeks at 22°C. (n=12 for each treatment).

Diet.	A.	B.	C.	D.	E.	F.	G.	H.	±SE[1]
Intial Wt (g).	$49{\cdot}28^{a}$	$49{\cdot}47^{a}$	$49{\cdot}58^{a}$	$49{\cdot}52^{a}$	$49{\cdot}03^{a}$	$49{\cdot}63^{a}$	$50{\cdot}73^{a}$	$50{\cdot}89^{a}$	4·36
Final Wt (g).	$87{\cdot}53^{a}$	$69{\cdot}69^{a}$	$74{\cdot}58^{a}$	$80{\cdot}04^{a}$	$80{\cdot}16^{a}$	$84{\cdot}70^{a}$	$84{\cdot}87^{a}$	$84{\cdot}82^{a}$	7·40
Average weekly weight gain (g).	$7{\cdot}65^{c}$	$4{\cdot}04^{a}$	$5{\cdot}00^{ab}$	$6{\cdot}10^{bc}$	$6{\cdot}23^{bc}$	$7{\cdot}01^{c}$	$6{\cdot}83^{c}$	$6{\cdot}79^{c}$	1·79
Specific growth rate.[2]	$1{\cdot}64^{c}$	$0{\cdot}98^{a}$	$1{\cdot}17^{a}$	$1{\cdot}37^{ab}$	$1{\cdot}40^{b}$	$1{\cdot}52^{bc}$	$1{\cdot}47^{bc}$	$1{\cdot}46^{bc}$	0·15
Food conversion ratio.[2]	$1{\cdot}16^{a}$	$1{\cdot}99^{c}$	$1{\cdot}68^{b}$	$1{\cdot}39^{a}$	$1{\cdot}39^{a}$	$1{\cdot}24^{a}$	$1{\cdot}30^{a}$	$1{\cdot}33^{a}$	0·04

1. SE = Standard error, calculated from residual mean square in the analysis of variance. abc = Mean values for components with the same superscripts are not significantly different (Duncans multiple range test. $p>0{\cdot}05$).
2. S.G.R. and F.C.R. calculated as mean weekly values.

Figure 1. Specific growth rates produced by the experimental diets when fed to carp at 2% body weight day^{-1} dry diet. (n = 12 for each treatment).



no obvious pattern and although there are significant differences ($p<0.05$) in some of these components, they appear to be random. Diets with the higher measured fat levels resulted in higher body fat levels.

Table 1.6. Proximate analyses of the fish carcasses (n=6 for each treatment).

Diet. Component.	Pre.[1]	A.	B.	C.	D.
Moisture.	$74 \cdot 11 \pm 0 \cdot 34^{ab}$	$74 \cdot 85 \pm 0 \cdot 90^{a}$	$74 \cdot 70 \pm 1 \cdot 33^{a}$	$73 \cdot 56 \pm 1 \cdot 48^{abc}$	$72 \cdot 65 \pm 1 \cdot 17^{bc}$
Protein.	$14 \cdot 27 \pm 1 \cdot 67^{ab}$	$14 \cdot 22 \pm 1 \cdot 59^{ab}$	$16 \cdot 33 \pm 1 \cdot 55^{a}$	$14 \cdot 05 \pm 1 \cdot 53^{b}$	$15 \cdot 34 \pm 1 \cdot 39^{ab}$
Fat.	$7 \cdot 95 \pm 0 \cdot 90^{cd}$	$8 \cdot 46 \pm 1 \cdot 07^{bc}$	$6 \cdot 52 \pm 1 \cdot 47^{d}$	$10 \cdot 17 \pm 1 \cdot 52^{a}$	$9 \cdot 63 \pm 0 \cdot 65^{ab}$
Ash.	$3 \cdot 17 \pm 1 \cdot 69^{a}$	$2 \cdot 10 \pm 1 \cdot 57^{a}$	$1 \cdot 95 \pm 1 \cdot 12^{a}$	$1 \cdot 71 \pm 0 \cdot 92^{a}$	$1 \cdot 85 \pm 0 \cdot 84^{a}$

Diet.	E.	F.	G.	H.	$\pm SE^{2}$
Moisture.	$72 \cdot 72 \pm 1 \cdot 04^{bc}$	$72 \cdot 10 \pm 0 \cdot 95^{c}$	$72 \cdot 65 \pm 0 \cdot 67^{bc}$	$73 \cdot 11 \pm 1 \cdot 38^{bc}$	0·4740
Protein.	$14 \cdot 97 \pm 1 \cdot 99^{ab}$	$14 \cdot 81 \pm 1 \cdot 71^{ab}$	$15 \cdot 14 \pm 1 \cdot 20^{ab}$	$14 \cdot 87 \pm 2 \cdot 23^{ab}$	0·6783
Fat.	$9 \cdot 38 \pm 1 \cdot 28^{abc}$	$10 \cdot 34 \pm 1 \cdot 07^{a}$	$9 \cdot 71 \pm 1 \cdot 05^{ab}$	$9 \cdot 95 \pm 1 \cdot 47^{ab}$	0·5271
Ash.	$2 \cdot 43 \pm 1 \cdot 60^{a}$	$2 \cdot 25 \pm 1 \cdot 24^{a}$	$1 \cdot 99 \pm 0 \cdot 97^{a}$	$1 \cdot 56 \pm 0 \cdot 73^{a}$	0·4942

1. Fish taken for analyses before the start of the experiment. 2. SE = Standard error. abcd = Mean values for each component with the same superscript are not significantly different (Duncans multiple range test $p > 0 \cdot 05$).

The evaluation of a mixture of waste bread and pie production waste for use in complete diets.

MATERIALS & METHODS.

Diets.

Production waste from pie manufacture was obtained from Warburtons Ltd of Bolton as a mixture of uncooked pastry, meat filling and aluminium foil trays. The trays were removed manually and the remaining mixture was minced through the mincer attachment of a HOBART food mixer. Proximate analysis was carried out on the resultant mixture (Table 1.7.).

Bread was treated as in the previous experiment.

Seven experimental diets were formulated, based on a constant amount of fishmeal. Varying amounts of bread and pie waste were included so that as one increased, the other decreased (Table 1.8.). The level of each ingredient was adjusted so as to balance the protein level at 24% and the fat at 17% (Table 1.9.). Very little extra water was required due to the moist nature of the pie waste. Proximate analyses were carried out on all the diets (Table 1.9.). A commercial trout diet was repelleted as a moist diet and used as a control.

Experimental systems.

A single water recirculation unit of eight tanks was maintained at 22^{o}C. Each tank was stocked with 10 fish of average weight, 106g.

Feeding.

Diets were fed at a rate of 1% body weight day^{-1} of dry feed. This was calculated on a weekly basis. The amount of diet fed was adjusted to allow for differences in the moisture content. Feeding was by hand and took place twice a day. The experiment ran for 35 days.

Carcass analysis.

At the end of the experiment, six fish were removed from each treatment for carcass analysis. This involved carrying out a proximate analysis on each fish.

Table 1.7. Proximate analysis of the pie waste.

a) complete waste.

Component.	%.
Moisture.	35·9
Protein.	6·7
Fat.	18·5
Ash.	5·7
Nitrogen free extract.	33·0

b) separate portions.

Component.	Filling (%).	Pastry (%).
Moisture.	74·65	28·79
Protein.	9·75	7·07
Fat.	9·30	15·06
Ash.	1·37	1·16
Nitrogen free extract.	4·93	47·92

Table 1.8. Constituents of the test diets (% by weight).

Diet. Ingredient.	A.	I.	J.	K.	L.	M.	N.	O.
Trout pellet.[1]	66	-	-	-	-	-	-	-
Herring meal.[2]	-	19	19	19	19	19	19	19
Bread.	-	20	25	30	35	40	45	50
Pie waste.	-	56	49	43	37	29•5	23•5	17
Oils.[3]	-	-	1	2	3	4•5	5•5	7
IVY.[4]	-	2	2	2	2	2	2	2
Binder.[5]	-	1	1	1	1	1	1	1
Water.[6]	34	2	3	3	3	4	4	4

1. Edward Baker Ltd. Omega no 4 trout food.
2. Edward Baker Ltd. Norseamink.
3. Mixture 50% corn oil/ 50% codliver oil.
4. Edward Baker Ltd. IVY mineral & vitamin mix.
5. Alginate Industries, London. Alginate binder.
6. Additional water required to make the diet semi-moist.

Table 1.9. Proximate analyses of the test diets (% by weight).

Diet. / Component.	A.	I.	J.	K.	L.	M.	N.	O.
Protein.	45•59	24•62	23•75	24•18	25•78	24•67	26•13	26•97
Fat.	6•19	17•29	17•16	17•24	16•39	16•16	15•92	15•37
Ash.	12•97	9•60	9•80	10•16	9•77	9•33	9•31	9•56
N.F.E.[1]	35•25	48•49	49•29	48•42	48•06	49•84	48•64	48•10
Moisture.[2]	32•43	32•27	32•65	32•65	32•64	32•95	32•52	32•50

1. Nitrogen free extract.

2. Overall moisture of the finished pellet.

Table 1.10. Percentage make up of the fat content of the test diets. (% of total fat).

Diet.	I.	J.	K.	L.	M.	N.	O.
Fat.							
Hard fat.	100·0	90·2	79·8	69·6	54·8	44·2	31·0
Corn oil.	-	4·9	10·1	15·2	22·6	27·9	34·5
Cod liver oil.	-	4·9	10·1	15·2	22·6	27·9	34·5

RESULTS.

In all the treatments, the fish soon became accustomed to the experimental diets and consumed all that was given. All the fish grew rapidly and the average weekly weight gains are shown in table 1.11. The best growth was shown by diet K (30% bread/43% pie waste). Statistical analysis showed that this diet was not significantly different ($p>0.05$) from the control. Diets J (25% bread/49% pie waste) and L (35% bread/37% pie waste) also gave good growth. These results are reflected in the specific growth rates (Figure 2) which was not significantly different ($p>0.05$) from the control. Diets with high bread levels showed the worst growth rates (Table 1.11.).

Table 1.12. shows the proximate analyses of the whole fish carcasses. The moisture contents of the fish fed the experimental diets were significantly lower ($p<0.05$) than the fish fed the control. The analyses for the protein content showed a significant ($p<0.05$) decrease with decreasing levels of pie waste in the diet. The actual level decreased from 16% for diet I to 12% for diet N. Diet O showed a slight increase up to 14%. The fat levels demonstrated a reverse in the trend to that of the protein so that a decrease in the pie waste resulted in increased body fat. The changes were significant ($p<0.05$) over the whole range with an increase from 6% for diet I to 13% for diet N. A slight decrease was shown by diet O.

Table 1.11. Performance of carp fed on the test diets for 5 weeks at 22°C. (n=10 for each treatment).

Diets.	A.	I.	J.	K.	L.	M.	N.	O.	±SE[1]
Intial Wt (g).	109·05	107·13	107·30	110·50	107·64	104·72	104·36	104·99	
Final Wt (g).	143·15	129·15	133·35	141·20	133·13	121·21	120·41	123·69	
Average weekly weight gain (g).	6·82^{a}	4·40^{bc}	5·21^{abc}	6·14^{ab}	5·15^{abc}	3·29^{c}	3·21^{c}	3·74^{c}	0·64
Specific growth rate.	0·78^{a}	0·53^{bc}	0·62^{abc}	0·70^{ab}	0·61^{abc}	0·41^{c}	0·41^{c}	0·47^{c}	0·07
Food conversion ratio.	1·34^{a}	1·92^{ab}	1·59^{a}	1·47^{a}	1·85^{ab}	2·55^{b}	2·59^{b}	2·22^{ab}	0·29

1. SE= Standard error, calculated from residual mean square in the analysis of variance.

abc = Mean values for components with the same superscripts are not significantly different (Duncans multiple range test p>0·05).

Figure 2. Specific growth rates produced by experimental diets when fed to carp at 1% body weight day^{-1} dry diet. (n = 10 for each treatment).

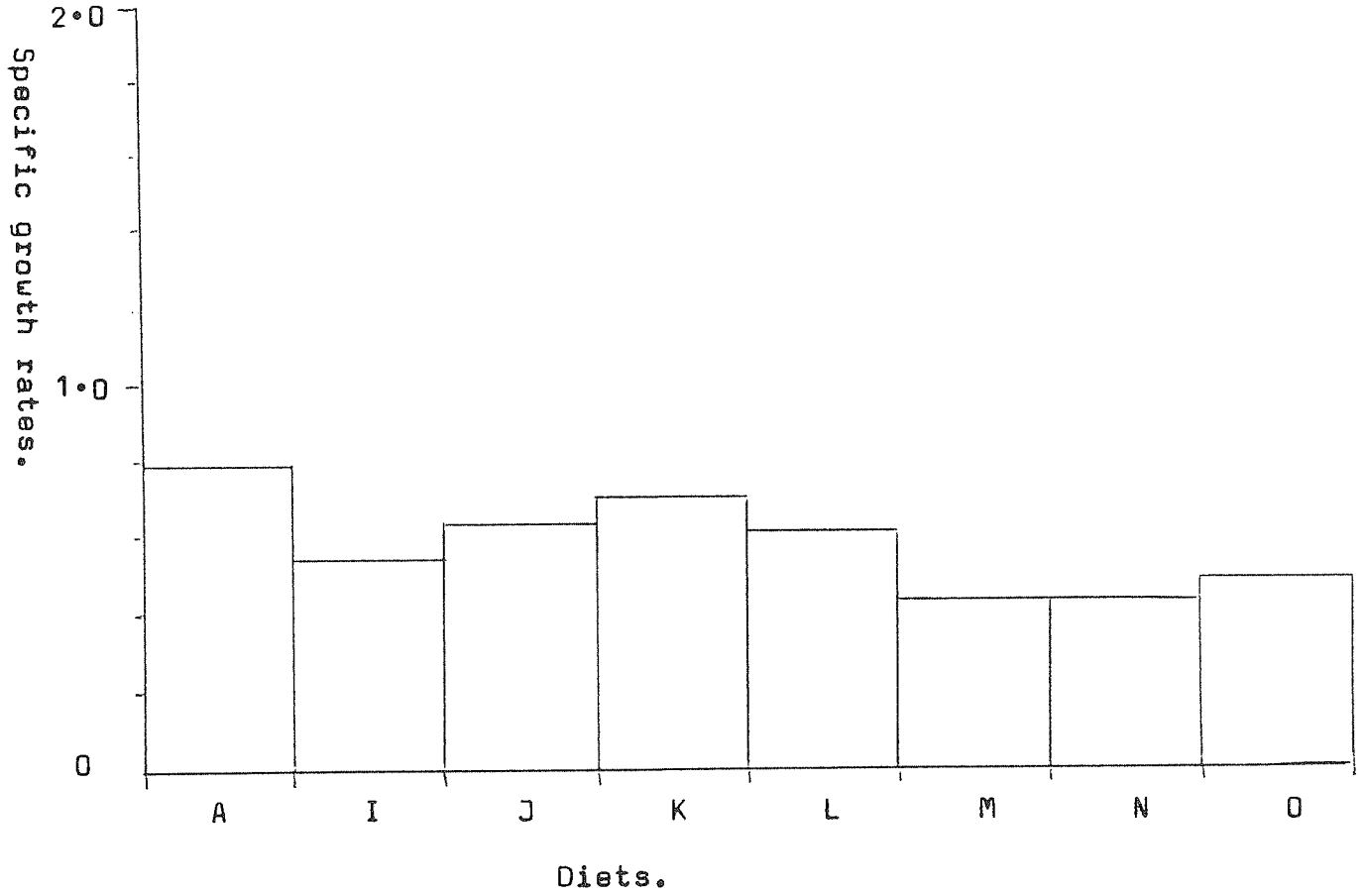


Table 1.12. Proximate analyses of the fish carcasses (n=6 for each treatment).

Diet.	Pre.[1]	A.	I.	J.	K.
Moisture.	$74 \cdot 11 \pm 0 \cdot 34^{ab}$	$75 \cdot 05 \pm 0 \cdot 84^{a}$	$72 \cdot 47 \pm 1 \cdot 51^{b}$	$72 \cdot 79 \pm 0 \cdot 90^{b}$	$72 \cdot 25 \pm 1 \cdot 34^{b}$
Protein.	$14 \cdot 27 \pm 1 \cdot 67^{bc}$	$16 \cdot 12 \pm 1 \cdot 58^{ab}$	$16 \cdot 92 \pm 1 \cdot 06^{a}$	$15 \cdot 34 \pm 0 \cdot 64^{abc}$	$14 \cdot 70 \pm 1 \cdot 56^{bc}$
Fat.	$7 \cdot 95 \pm 0 \cdot 70^{c}$	$6 \cdot 47 \pm 0 \cdot 71^{c}$	$8 \cdot 33 \pm 1 \cdot 56^{c}$	$10 \cdot 72 \pm 1 \cdot 22^{b}$	$10 \cdot 60 \pm 1 \cdot 41^{b}$
Ash.	$3 \cdot 17 \pm 1 \cdot 69^{a}$	$1 \cdot 85 \pm 1 \cdot 46^{ab}$	$1 \cdot 77 \pm 1 \cdot 67^{ab}$	$0 \cdot 64 \pm 0 \cdot 66^{b}$	$1 \cdot 89 \pm 1 \cdot 18^{ab}$

Diet.	L.	M.	N.	O.	±SE.[2]
Moisture.	$73 \cdot 31 \pm 2 \cdot 67^{ab}$	$73 \cdot 95 \pm 1 \cdot 63^{ab}$	$72 \cdot 83 \pm 1 \cdot 15^{b}$	$72 \cdot 31 \pm 1 \cdot 26^{b}$	0·6403
Protein.	$13 \cdot 92 \pm 2 \cdot 88^{cd}$	$13 \cdot 76 \pm 1 \cdot 05^{cd}$	$12 \cdot 22 \pm 0 \cdot 86^{d}$	$14 \cdot 11 \pm 1 \cdot 43^{cd}$	0·6231
Fat.	$10 \cdot 72 \pm 2 \cdot 18^{b}$	$10 \cdot 50 \pm 2 \cdot 34^{b}$	$12 \cdot 91 \pm 1 \cdot 16^{a}$	$11 \cdot 31 \pm 1 \cdot 10^{ab}$	0·6611
Ash.	$1 \cdot 55 \pm 1 \cdot 62^{ab}$	$1 \cdot 28 \pm 1 \cdot 02^{b}$	$1 \cdot 53 \pm 0 \cdot 80^{ab}$	$1 \cdot 76 \pm 1 \cdot 47^{ab}$	0·5368

1. Fish taken for analyses before the start of the experiment. 2. SE= Standard error.

abcd = Mean values for each component with the same superscripts are not significantly different (Duncans multiple range test $p > 0 \cdot 05$).

Comparison of different bakery waste diets.

MATERIALS & METHODS.

Diets.

Diets were formulated as in the two previous experiments, diet F containing 30% bread and diet K, 30% bread and 43% pie waste and with 30% and 19% fishmeal respectively. Diet Q was also formulated consisting totally of bakery waste (table 1.13.). A commercial trout diet (Edward Baker Ltd) was repelleted as a semi-moist diet and used as a control - diet A. The moisture content of all the diets was measured and a proximate analysis carried out on each (table 1.14).

Experimental system.

A single water recirculation unit of eight tanks was maintained at 22^{o}C. Due to the large variation in the size of the available fish, they were split into two groups and each divided amongst four tanks with each tank containing 12 fish. One group had an initial mean weight of 8·75g and the other, 13·20g.

Feeding.

Diets were fed at a rate of 2% of body weight per day of dry feed. This was calculated on a weekly basis. The amount of diet fed was adjusted to allow for differences in moisture content. Each diet was fed in replicate, one to each group of fish. Feeding took place thrice daily. The experiment ran for 35 days.

Carcass analyses.

At the end of the experiment, six fish were removed from each diet, three from each size range, for carcass analyses. The results for each diet were combined to give mean values.

Table 1.13. Constituents of the test diets (% by weight).

Diet. / Ingredient.	A.	F.	K.	Q.
Trout pellet.[1]	66•0	-	-	-
Herring meal.[2]	-	30•0	19•0	-
Bread.	-	30•0	30•0	48•5
Pie waste.	-	-	43•0	48•5
Wheat middlings.	-	8•0	-	-
Oils.[3]	-	4•0	2•0	-
IVY.[4]	-	2•0	2•0	2•0
Binder.[5]	-	1•0	1•0	1•0
Water.[6]	34•0	25•0	3•0	-

1. Edward Baker Ltd. Omega no 4 trout food.
2. Edward Baker Ltd. Norseamink.
3. Mixture 50% corn oil/ 50% codliver oil.
4. Edward Baker Ltd. IVY mineral & vitamin mix.
5. Alginate Industries London. Alginate binder.
6. Additional water required to make diet semi-moist.

Table 1.14. Proximate analyses of the test diets.
(% by dry weight).

Diet. / Component.	A.	F.	K.	Q.
Protein.	45•59	34•38	24•18	15•49
Fat.	6•19	9•63	16•86	14•45
Ash.	12•97	9•95	9•21	7•20
N.F.E.[1]	35•25	43•77	48•42	62•86
Overall moisture.[2]	42•00	44•26	32•64	36•52
Total energy.[3]	4•02	4•17	4•54	4•51
PE/DE.[4]	51	37	24	16

1. Nitrogen free extract.
2. Overall moisture of the finished pellet.
3. Kcal/100g.
4. Protein energy:Digestible energy ratio.
   Calculated from the following values:-
   Protein - 4•5kcal/g.
   Fat - 9•0kcal/g.
   Carbohydrate - 4•0kcal/g.
   (Cowey & Sargent, 1979).

RESULTS.

In all the tanks the fish quickly became accustomed to the experimental diets and consumed all the food that was given. In both series of four diets, the fish grew fastest on the higher protein diets with the specific growth rates decreasing with decreasing protein content of the diet. Although the trends in both series are similar, differences between the two are apparent and it is therefore necessary to consider both series separately.

With fish of initial mean weight of 8·75g (table 1.15), the specific growth rate (figure 3) was significantly different ($p<0·05$) for all the treatments and the same applies to the food conversion ratios. Average weight gains are also shown in table 1.15. and these can be seen to decrease with decreasing dietary protein. The fish of larger initial mean weight showed the same trends as the other series with the exception of specific growth rate (figure 4). Diets F and K were not significantly different ($p>0·05$) from each other (table 1.16.).

Differences between the two series for each of the experimental diets have not been found to be significantly different ($p>0·05$) for specific growth rates and food conversion ratios. With the exception of diet F, the fish of smaller initial mean weight have grown at a slower rate than the larger fish. In the case of diet F, both specific growth rates and food conversion ratio are better for the smaller fish.

Table 1.15. Performance of the fish of initial mean weight of 8·75g fed the experimental diets for 5 weeks at 22°C. (n = 12 for each treatment).

Diets.	A.	F.	K.	Q.	±SE.[1]
Intial Wt. (g)	8·41[a]	9·02[a]	8·75[a]	8·84[a]	0·485
Final Wt. (g)	16·63[a]	14·16[b]	12·74[bc]	11·08[c]	0·737
Av. Wt. Inc.[2] (g)	1·64[a]	1·03[ab]	0·80[b]	0·45[b]	0·223
S.G.R.[3]	1·93[a]	1·29[b]	1·07[c]	0·64[d]	0·192
F.C.R.[4]	1·00[a]	1·64[b]	1·97[c]	4·62[d]	0·881
PER.[5]	1·83	1·68	1·98	1·82	
NPU.[6]	29·54	24·93	29·17	13·07	

1. SE = Standard error from residual mean square in the analysis of variance.
2. Average weekly weight gain.
3. Specific growth rate.
4. Food conversion ratio.
5. Protein efficiency ratio.
6. Net protein utilisation.

abcd = Mean values for components with the same superscripts are not significantly different (Duncans multiple range test $p>0·05$).

Figure 3. Specific growth rate produced by the experimental diets when fed to carp (initial weight group - 8·75g). (n = 12 for each treatment).

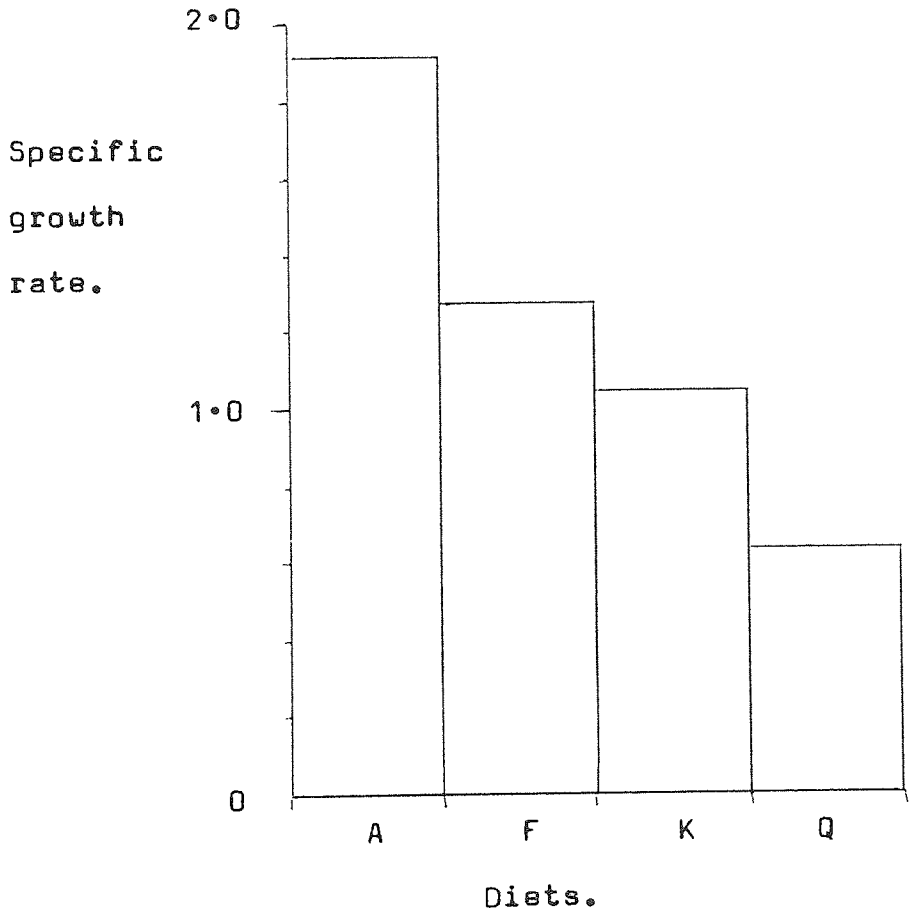


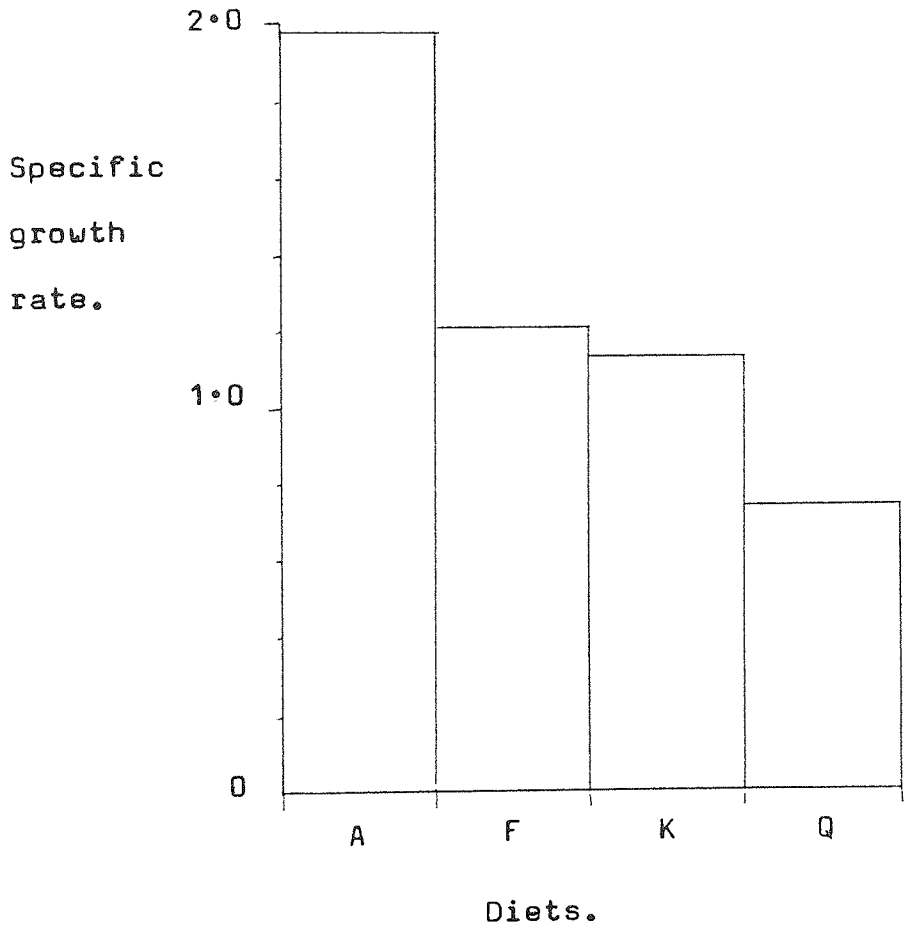
Table 1.16. Performance of the fish of initial mean weight of 13·20g fed the experimental diets for 5 weeks at 22°C. (n = 12 for each treatment).

Diets.	A.	F.	K.	Q.	±SE.[1]
Intial Wt. (g).	13·73[a]	12·54[a]	13·24[a]	13·26[a]	0·736
Final Wt. (g).	27·53[a]	19·17[b]	19·77[b]	17·35[b]	1·245
Av. Wt. Inc.[2] (g)	2·76[a]	1·33[b]	1·31[b]	0·82[b]	0·354
S.G.R.[3]	1·99[a]	1·21[b]	1·14[b]	0·77[c]	0·193
F.C.R.[4]	0·98[a]	1·68[b]	1·86[c]	4·07[d]	0·839
PER.[5]	2·01	1·56	1·29	1·19	
NPU.[6]	32·54	17·95	19·00	8·55	

1. SE = Standard error calculated from residual mean squares in the analysis of variance.
2. Average weekly weight increase.
3. Specific growth rate.
4. Food conversion ratio.
5. Protein efficiency ratio.
6. Nte protein utilisation.

abcd = Mean values for components with the same superscripts are not significantly different (Duncans multiple range test $p > 0·05$)

Figure 4. Specific growth rate produced by the experimental diets when fed to carp (initial weight group - 13·20g) (n = 12 for each treatment.)



When specific growth rate is correlated with the protein content of the diet, there was a significant ($p<0.05$) correlation for both series of fish. For the smaller fish, the correlation coefficient was 0.989 whilst for the larger fish it was 0.965.

Table 1.17. shows the results of the proximate analyses on the fish carcasses from this experiment. The figure given for each diet represents the average value for each component for fish taken from both series for each treatment. The analyses show that body fat increases with decreasing dietary protein level. Body protein content decreases with corresponding amounts as the fat level increases. There is a significant difference ($p<0.05$) in fat levels between diets A and F and between K and Q but the difference between diets F and K is not significant ($p>0.05$). For protein levels, the only significant ($p<0.05$) difference is between diet Q and all the other diets. There are differences in moisture levels between diet A and the rest which are also significant ($p<0.05$).

Table 1.17. Proximate analyses of the combined fish carcasses (n = 6 for each treatment).

Diet. / Component.	A.	F.	K.	Q.	±SE.[1]
Moisture.	75·05±0·84^{a}	72·10±0·95^{b}	72·25±1·34^{b}	72·29±1·67^{b}	0·5580
Protein.	16·12±1·58^{a}	14·81±1·71^{a}	14·70±1·56^{a}	7·22±1·08^{b}	0·6145
Fat.	6·47±0·71^{a}	10·34±1·07^{b}	10·60±1·41^{b}	18·61±2·23^{c}	0·6559
Ash.	1·85±1·46^{a}	2·25±1·24^{a}	1·89±1·18^{a}	1·39±0·88^{a}	0·4938

1. SE = Standard error.

abc = Mean values for each component with the same superscripts are not significantly different (Duncans multiple range test p>0·05).

DISCUSSION.

The results of the first experiment in which diets containing varying levels of waste bread showed that increasing the level of the waste improved the growth rates of the fish until a maximum was reached with a level of inclusion of bread of 30% of the total diet. Further increases caused a slight reduction in the rate of growth. The most likely explanation for the differences shown was the difference in the digestibility of the carbohydrate sources used.

Workers such as Chiou & Ogino (1975) have shown that the digestibility of α starch (cooked starch) remained constant regardless of the level of its inclusion in a diet whilst that of β starch (uncooked starch) decreased as its level of inclusion increased. They also showed that the digestibility of β starch was lower than that of α starch. This is supported by Inaba et al. (1963) who used rainbow trout as the test fish and breadcrumb as the α starch source. This is in agreement with part of the first experiment which showed that as the bread level (α starch) increased and the wheat middling level (β starch) decreased, growth rates increased. The peak was reached with 30% bread in the diet. This increase in growth rate may be due either to the positive effect of the α starch or the negative effect caused by the reduced digestibility of the β starch. Of the two, the

former is most likely since when β starch was removed from the diet as in the highest bread inclusion, the growth rates did not continue to increase. The possible explanation for this is that the optimum energy to protein ratio may have been reached. This would mean that diets with high levels of accesible energy would have excess for the amount of protein available. Conversion of this excess energy to body fat would then occur. However, the results of the proximate analyses are not clear enough to show that the fish fed the highest levels of bread, do have the higher body fat levels (table 1.6.).

Whilst varying digestibilities of carbohydrate sources may be the major factor causing changes in growth rates, it is possible that variations through balancing the dietary components had some effect. This especially applies to protein because differences in protein can markedly affect growth rates and interfere with the responses from the test material. In cases where the test material is not a pure source as in this experiment, it is difficult to know whether to balance the overall protein level of the diet or the protein source. The problem arises because not all proteins have the same dietary value.

Proteins are made from different combinations of about twenty amino acids of which eleven are essential for fish growth and are required in the diet. Not all proteins contain all the essential amino acids so if fish are fed only these proteins, then the growth rates can be drastically reduced. The best protein sources are those with a similar amino acid profile to the consuming animal and for fish, these are the fishmeal protein sources. Protein sources from other animals are nearly as good whilst those from vegetable sources often lack one or two of the essential amino acids and feeding with these therefore results in poor fish growth. Diets containing varying amounts of different protein sources but with the same overall protein level can therefore be seen to produce differing rates of growth.

In this experiment, it was decided to balance the overall protein level and not the protein source. Since the diets contain large amounts of fishmeal then none of the essential amino acids should be deficient even where the additional protein is supplied by vegetable sources.

Although there was a definite trend of increasing growth rates with increasing fishmeal protein levels, it is doubtful that this was due to the level of fish-meal because the increments were only in the range of

one or two percent.

To eliminate this problem in future work, it was decided that in the second experiment both the protein source and the overall protein level would be kept constant in each test diet. This was achieved with a fishmeal level of 19% and an overall protein level of 25%.

The results of this second experiment show that although the nutritional components of the diets were balanced so that each diet had a protein level of 25% and a fat level of 17%, there were significant differences ($p<0.05$) in the resultant growth rates. Growth was seen to increase as the level of pie waste decreased from 56% (diet I) to a level of 43% of the diet (diet K). Further decreases in the level of pie waste to 23% (diet N) resulted in a decline in the growth rates excluding the lowest level of 17% (diet O) which showed a very slight increase. Since the components were all balanced, the differences must be due to the source of these components. As the only ingredient to vary throughout the range of the test diets other than the experimental ingredients, were the oils then it is possible that the differences in growth rates produced by the diets, may be due to these.

There are one or two possible explanations for these differences, the first being that in the diets with the low levels of pie waste, the additional fat content was made up to the required level by the inclusion of a relatively high percentage of mixed oils. The oils may not have been as well incorporated into the diet during preparation as the fat in the pie waste and some may have been lost. This is supported by two observations. The first is that the proximate analyses (table 1.9.) show that the low pie waste diets have reduced fat levels. The other observation is that during the feeding of these diets, small slicks of oil were seen on the surface of the water in the experimental fish tanks, indicating loss of some of the oil. This suggests that the oil was not as well bound in the diet as was the fat in the pie waste. The loss of fat may explain the decrease in growth rates for the low pie waste diets due to a loss in energy and therefore in sparing potential.

The second possible explanation for the growth rate differences is that the three fats that made up the total fat content for each diet could produce different growth performances because of their varying physio-chemical composition. The fat used in the bakery products was a hard fat and mainly of vegetable origin but could contain some animal fat. The oils used in the diets with the lower levels of pie waste as the additional fat source were liquid and were of vegetable

and fish origin. Table 1.10. shows the percentage make up of these three sources for each diet. As can be seen the amount of hard fat decreases whilst the corn/cod liver oil mix increases with decreasing amounts of pie waste in the diet. The origin of the hard fat is in some doubt being either of vegetable or animal or as a mixture of the two due to commercial least cost formulation so comparison with other hard fats is difficult. Most nutritional investigations into differing fat sources have compared hard animal fats with fish oils and vegetable oils. In this work, mixtures of oils were used however comparisons are still possible.

Consideration of different fat sources involves two main factors. The first is the requirement by fish of essential fatty acids (EFA) which if deficient can cause a reduction in the rate of growth. Fish are known to have a requirement for the linolenic series of fatty acids and fish oils are a good source of these (Castell, 1979). They also have a requirement for the linoleic series although to a lesser extent. These fatty acids are found in many vegetable oils. It is possible that the hard fat is deficient in either or both of these fatty acids which may be restored by the addition of a small amount of the oil mixture. Takeuchi et al. (1978a) found that both hydrogenated fish oil and beef tallow when used as the only fat source resulted in deficiencies of EFA and also in reduced growth rate. These were restored by

supplementation with a small amount of fish oil. Similar supplementations of cod liver oil to the diets in this experiment would account for the improvement in the growth rates and would explain why the growth rate of diet J is superior to that of diet I and that of diet K is superior to that of diet J. Further increases would not result in any additional improvement as the requirement for the EFA would have been met.

The use of animal fats as a dietary energy source has been shown by many workers. Stickney & Andrews (1971) found that feeding a hard animal fat (beef tallow), a fish oil (menhaden oil) and a vegetable oil (safflower oil) to catfish resulted in equally good growth from the beef tallow and the fish oil but reduced growth from the vegetable oil. Similar results were reported by Yingst & Stickney (1980) although here soybean oil was used as the vegetable source. This was supported by Reinitz (1980) who fed similar diets to trout. Yu et al. (1977) fed increasing amounts of animal fats in diets with herring oil as the other fat source and found that all the diets produced equally good growth. However this was not unexpected since no diet contained the hard fat alone. These studies have shown that animal fats are a good fat source for use in fish diets although some supplementation with essential fatty acids is required.

Whilst there have been several studies into the potential of using hard animal fats in fish diets, not as much consideration has been given as to the use of solidified vegetable oils. One investigation was carried out by Dupree (1969) when he compared liquid corn oil, hydrogenated corn oil and beef tallow. He found that diets containing hard corn oil gave the best growth followed by the liquid corn oil and then the beef tallow, when fed to catfish. Phillips et al. (1965) reported equally good utilisation of solid and liquid cottonseed oil by brown trout. These results suggest that solid vegetable fats result in as good as, if not better growth than the liquid form of the same source. It would therefore appear that the reduced growth of the low pie waste diets was not due to the presence of the hard fat in the pie waste unless the source of fat was inferior to that of the liquid oils used. As least cost formulations are used in the manufacture of the hard fat, there is no way in which the identity of the fat source can be determined.

Another possible explanation for the observed reduction in growth rates is the presence of large amounts of liquid corn oil required to balance the quantities of hard fat from the pie waste. Although

corn oil was shown to produce good growth when used as a dietary fat source by Dupree (1969), there are reports that it can result in the depression of the rate of growth. This is because corn oil is rich in fatty acids of the linoleic series (Castell, 1979) and there is some evidence that these fatty acids supress growth rates. This has been shown to occur in catfish (Stickney & Andrews, 1971), however the situation is less clear for carp and further investigation is necessary.

The results of the two previous experiments in which different bakery wastes were evaluated for use in fish diets, have shown that these wastes have a role to play in fish nutrition. It was thought that because of the nature of these two experiments, a number of questions such as the role of the ratio of protein to energy in fish diets, had not been answered and it would be therefore worthwhile extending the experimentation to compare the best diets from each group as well as looking at the potential of a total waste diet. The results of this last experiment have raised a number of further issues which will also be considered.

Sin (1973a) observed the effect of differing dietary protein levels on the growth of young carp. He found that weight increased with increasing dietary protein up to a level of 38·4% of the diet. Further increases resulted in a decreased growth rate. Similar observations were made by Ogino & Saito (1970) in which the specific growth rate increased up to levels of 55% dietary protein, however conversion of dietary protein to body protein did not increase past a protein level of 38% of the diet. In this experiment, as protein levels increased, specific growth rates also increased up to the maximum level of 45·59% of dietary protein, however body proetin also increased so that the highest level of dietary protein produced the maximum body protein level. Dietary protein levels correlated very well with specific growth rates giving correlation coefficients of above 0·95 in both series of fish, ie the two different intial weights, showing that at higher protein levels, the growth rates are similar whereas, as the protein level was reduced, the fish of lower intail weight did not utilise the diets as well.

In order to aid comparision of the different dietary protein levels used in this experiment, it is necessary to use additional parameters such as Protein Efficiency Ratio (PER) and Net Protein

Utilisation (NPU). These give a measure of the efficiency with which fish are able to utilise different levels of dietary protein. The PER values for the larger fish show that as the dietary protein level decreases there is also a reduction in the efficiency from 2·01 to 1·19. The smaller fish have efficiencies of about 1·82 for the higher and lower protein diets (A & Q) and efficiencies of 1·68 and 1·98 for diets F and K. For both sizes of fish, diet F had a similar level of about 1·60 whereas all the other diets show different efficiencies for both fish size groups. The most efficient diet is therefore diet K for the smaller fish and diet A for the larger. The smaller fish seem to have utilised the lower protein diets more efficiently than the larger ones. For the higher dietary protein levels, protein utilisation is similar for both sizes of fish.

Although PER values give a somewhat better indication of the nutritional status of the fish with respect to dietary protein, than food conversion ratios, they do not take into account the proportion of ingested protein used for maintenance and they are based on the assumption that growth of the fish consists of tissues with identical composition in all groups. It is also possible that PER is affected by the feeding rate employed so that comparison between different experiments can be difficult (Cowey & Sargent, 1972). A method of improving the assessment of

the nutritional status of the fish is to use the apparent efficiency of deposition of dietary protein as body tissue ie Net Protein Utilisation (NPU). NPU is generally determined by the carcass analysis method of Bender & Miller (1953) and Miller & Bender (1955). Since no estimation was made of endogenous nitrogen losses, apparent NPU was used in this experiment. The NPU values for the larger fish were found to generally decrease with decreasing protein level with the exception of diet K which was superior to diet F. For the smaller fish a similar pattern was seen, except that the NPU value for diet K was as high as that of diet A. This indicates that the utilisation and deposition of protein was equally good at the lower dietary protein level and was probably due to differences in the energy level. Protein to energy ratios will be considered later. Similar results with PER values were obtained by Cowey et al. (1972) with plaice and by Zeitoun et al. (1974) with rainbow trout. In contrast, Ogino & Saito (1970) found that PER values for carp decreased with increasing dietary protein level. Cowey & Sargent (1972) suggest that this is due to carp having a lower maintenance requirement possibly due to the presence of micro-organisms in the gut. The present results have shown that carp can have similar PER patterns as other species. Steffens (1981) suggests that results will vary with different dietary protein sources. For example, Ogino & Saito (1970) used casein as the main

source of dietary protein whilst in this study, a variety of sources were used with the major contribution being made by fishmeal. Another difference that can affect all the measured parameters is that of the size of fish used.

Fish of different sizes have been shown to have differing protein requirements. Page & Andrews (1973) showed that catfish of body size 14 - 100g required 35% protein in the diet whilst fish of over 100g required only 25%. In both cases, this was only true if the energy requirement had been met. Dupree (1976) looked at many published and unpublished reports and concluded that fish of similar size have similar requirements as long as they are held in similar conditions. He suggested that fry have a requirement of 50% dietary protein, small fingerlings - 40%, large fingerlings - 35%, and large fish - 30%. Gerking (1952) suggested that the change in requirement was due to differences in protein utilisation. For blue gill sunfish, he found that 10g fish converted 33% of their dietary protein intake whereas 105g fish utilised only 5%. This was confirmed by Kitamikado et al. (1964) who suggested that this may be due to lower enzyme activity in juvenile fish. Another possibilty is that smaller fish have a higher metabolic rate than larger ones. For brook trout, fish of 1g gain approximately 100% per month whilst at 20g this is reduced to 36%

(Phillips, 1972). The energy requirement of carp of 12g is 24·48Kcal Kg^{-1} day^{-1} whereas for larger fish of 600g, this is reduced to 7·97Kcal. This may be due to the body surface area which is reduced in larger fish so decreasing heat loss (Schaperclaus, 1933).

In this experiment, the differences in the size of each group is so small that it may be impossible to explain the differences in the results by size alone.

In the calculation of correlation coefficient of dietary protein and specific growth rate, the larger fish were found to have a lower coefficient than the smaller fish. This was due to an increase in the growth rate of the larger fish at a dietary protein level of 24% through the presence of increased fat levels over the higher protein diets. This reulted in protein sparing by the fat and is evident from the NPU values as for both sizes of fish, the values for diet K exceeded those of diet F. Jauncey (1979) found that he could reduce the protein content of the diet from 45 to 29% by increasing the fat content to 18% with no effect on weight gain. Similar observations were made by Sin (1973b). Increasing dietary lipid was intially found to depress growth (Dupree, 1969) however this has not been confirmed by other workers who have increased fat levels up to 30% (Kitamikado et al., 1964). In this experiment, the presence of fat levels

of up to 17% of the diet have increased the growth by sparing the protein. This however, is only apparent in the larger fish. It is possible that the enzymes used for fat breakdown are not developed in the fish of smaller sizes. This has been suggested for fry (Bryant, personal communication) however there is no evidence to support this.

The relationship between energy content and protein content has been examined for several species of fish. This relationship is important because protein as well as providing the building blocks for new tissues, can also be utilised as an energy source. Under conditions where energy intake is limited, the dietary protein will be used as the source of energy. Conversely, an excessive energy intake at moderate protein levels will lead to deposition of fat and therefore changes in the carcass composition. Although several workers have tried to define the optimum ratio between energy and protein, close comparision is very difficult, not only because of differences in species but also because the actual calculation used is different. For example, Garling & Wilson (1976) used protein to energy ratios and Takeuchi et al. (1979) used energy to protein. Cowey & Sargent (1979) suggest that it is much better to calculate the ratio as protein energy to total energy. This is because the protein will always add to the total energy content

of the diet and so increasing the protein level will also result in an increase in the total energy content of the diet. To draw any conclusions from this work, it is necessary to compare these results with those from other studies. As no standard method has been used it has been necessary to recalculate the results of the other studies to bring them into line with this work. This has been achieved using the ratio PE/DE as recommended by Cowey & Sargent (1972). In all cases, the figures supplied by the original author have been given and these are followed by the recalculated values.

Lee & Putnam (1973) reported that maximum growth for trout was obtained with 160mg protein/Kcal at 3·0Kcal/g and 130mg protein/Kcal at 3·7Kcal/g whilst at a higher level of 4·4Kcal/g, only 91mg protein/Kcal were required. Recalculated these values are 67, 55 and 39 respectively for protein energy as a percentage of the total energy. Watanabe et al.(1979) thought their value for optimum DE/P was high for trout and in excess of all other studies but in fact when reworked, their value was 34 for an energy value of 4·5Kcal/g which is well within the levels found by Lee & Putnam (1973).

For Tilapia, Winfree & Stickney (1981) found that 34% protein at 3·2Kcal/g gave optimum growth with a ratio value of 108. Recalculated, their value is equivalent to 48. Jauncey (1982) produced results of a similar level

at 116 with 3·6 Kcal/g energy. The recalculated value is 52 however this higher value may be due to differences in the species of tilapia used in the separate studies.

Several studies have been reported on the protein to energy ratio for channel catfish. Tiemeier et al. (1965) found that 133·7mg protein/Kcal gave the optimum level for the ratio which is equivalent to a reworked value of 60, whilst Page & Andrews (1973) found that the optimum ratio was 75 or 133·1mg protein/Kcal. This gives a new value of 54. Garling & Wilson (1976) found the value to be much lower with a ratio of 88 at a dietary protein level of 24% and an energy level of 2·7Kcal/g. Recalculated the value is only 39.

For carp, the only reference to protein energy ratios is made by Takeuchi et al. (1979) who found that the optimum level of dietary protein was 31% and the optimum ratio of protein to energy was in the region of 97-116. This gives a new value of about 30 which is in keeping with the results of this experiment. Their value falls in the range given by diets F and K.

Carp would therefore appear to do well on relatively low protein, high energy diets as they are capable of utilising fat and carbohydrate as energy, so releasing the protein for growth. From the results of the other workers, it would seem that carp are the species of fish most capable of dealing with high energy diets

and this is very important in their commercial culture since the relative cost of energy is much less than protein. This work also questions whether the optimum levels of dietary protein found by some workers are correct since it can be seen that the level of energy also has an important role to play in fish nutrition.

The final aspect of this work to consider is the effect of bakery waste on fish body composition. Increasing levels of bread in the diet of carp appears to result in little change in the composition of the whole body carcass. All dietary inputs were kept constant with the exception of the carbohydrate which decreased with increasing levels of bread in the diet.

There are conflicting reports on the effects of dietary carbohydrate on fish body composition. Undoubtedly some of the variations are due to differences of species, levels of feeding, type of carbohydrate and overall diet quality (Buckley & Groves, 1979). The most deleterious effects occur with trout because under natural conditions, very little carbohydrate is found in their diet (Edwards et al., 1977). Phillips et al. (1948) reported that pathological glycogen resulted when trout were fed in excess of 12% carbohydrate. This was supported by McLaren et al. (1946) but in subsequent studies, McLaren et al. (1947) found that as long as the diet

was balanced, trout could accept up to 45% carbohydrate and suffer no deleterious effects.

In more recent work, Refstie & Austreng (1981) fed trout with increasing carbohydrate up to levels of 49% of the diet. They found that liver tissue was discoloured and the fish had higher liver weights. This is in keeping with earlier findings however they also found that high carbohydrate levels resulted in increased body moisture content. There is no evidence at all that warmwater fish suffer pathological liver conditions as experienced by trout at high dietary carbohydrate levels.

This work has shown that the dry matter content of the carcass was reduced and this is in keeping with the findings of Refstie & Austreng (1981) but in this case the reduction was not as great, however these workers also found a reduction in the body fat which was not the case here. This difference is probably due to differences in the species of fish used because there is some evidence which shows that body fat increases with increasing dietary carbohydrate levels (Ogino et al. 1976). In this study, a slight increase in body fat is apparent although no clear picture emerges from the results.

No other trends were visible from the results of the carcass analyses of the first experiment and this was probably due to the balanced nature of the test

diets. The only component that did vary was carbohydrate and the probable reason for the lack of clear results was that the amount of digestible carbohydrate remained constant although the amount of total carbohydrate did vary through the range of the test diets.

The body composition of fish fed diets containing pie production waste as in the second experiment is different to that of fish fed bread waste diets alone. The most noticeable feature is the increase in the body fat content with increasing levels of pie waste in the diet. This can also be correlated with increasing additional mixed oils. There is plenty of evidence that increasing dietary lipid results in increased deposition of body fat in catfish (Garling & Wilson, 1976; Murray et al., 1977 and Page & Andrews, 1973), carp (Sin, 1973a;b), chinook salmon (Buhler & Halver, 1961) and trout (Austreng et al.,1977; Lee & Putnam, 1973; Reinitz et al., 1978; Takeuchi et al., 1978b). In this experiment the dietary fat levels were however similar in all the diets but there may have been some preferential use of the dietary fat, the hard fat from the bakery waste being used for growth and the oils being converted directly into body fat. There is no real evidence to support this as there have been very few substitution type experiments of this kind. One that was reported was by Yu et al. (1977) who

substituted lard into diets containing herring oil and fed them to trout. The body fat levels decreased from 10•5% to 9% with a 50% substitution of lard. Similar replacements of animal fat into trout diets containing soybean oil resulted in a decrease in the body fat from 10•85% to 9•74% in work conducted by Reinitz (1980).

As well as the increase in body fat levels, there was an accompanying decrease in body protein content. This was unexpected as Buckley & Groves (1979) report that changes in body protein level are rare as the majority of variations in body composition relate to the fat content. This is usually associated with comparable changes in body moisture. There seems to be no adequate explanation for these protein changes although it may be argued that they are the result of discrepancies in the method used in the determination of the body composition. This depended on the measurement of all the components with the exception of protein which was then calculated by difference. This is similar to the technique used in the determination of nitrogen free extract which has been used by many workers for diet analysis. This method of determination by difference was used in the analysis of body composition by Brett et al. (1969) for the measurement of ash and by Caulton & Bursell (1977) who used it for protein. By

difference measurement is acceptable in the determination of fish body composition because the carbohydrate content of the body never exceeds more than 0·5% (Black, 1958). It is possible that any discrepancy in the measurement of other body components will be magnified and this would affect the protein level in the final calculation. It is unlikely that this is the case here because all the samples were determined at a similar time.

Although Buckley & Groves (1979) reported that changes in body protein were rare, there is one situation in which changes have been found to occur. Wood et al.(1957) found that wild fish of several species had consistently higher body protein contents than hatchery raised fish. This is due to differences in the amount of food obtained. Similarily, Brett et al. (1969) and Huisman et al. (1979) both found that body protein levels varied with the level of feeding. How changes in feeding level can be applied in this case is open to question.

The results of the analyses of the body composition of the fish carcasses from the final experiment showed that body fat increased with decreasing dietary protein. Body protein was also seen to change with a reduction with decreasing dietary protein. Similar observations were made by Huisman (1976) who found that protein decreased with increasing dietary energy when expressed as a percentage of lean body fat. Lee & Putnam (1973)

also found a correlation between P/DE and body protein in trout but in that case the changes in body protein level were very small.

It may be possible that these changes in the carcass composition with regard to protein were the result of an inadequate diet as Meske & Pfeffer (1979) reported that changes in protein content of the diet above a level of 20% do not affect the body protein level. This infers that dietary protein levels below 20% do affect the body composition.

Cowey & Sargent (1972) reviewed many studies in which body lipid increased with dietary energy or PE/DE ratios. For carp, Takeuchi et al. (1979) showed that body lipid increased with increasing dietary energy level. The body lipid increased from 5·8% to 9·7% with dietary energy levels of 3·0 to 4·5 Kcal/g. In this experiment, all the dietary energy levels were over 4 Kcal/g and therefore this may account for the much higher levels of fat in the carcasses.

These experiments have shown that bakery wastes appear to be a useful ingredient in fish diets. Both bread and pie waste, either in combination or separately are acceptable to the fish. They both perform as would be expected from their respective constituents. In order to achieve fast growth, it is necessary to increase the

protein level of the diet above the level that can be provided by the bakery waste alone however where this is necessary bakery waste is an excellent filler for the rest of the diet. On its own, bakery waste can be used for fish growth but it is best for use with larger fish. Ideally bakery waste would appear at its best when used as a supplementary food in extensive pond culture. It is suited for this as it is a low protein, high energy food which would complement the high protein found in the natural food from the pond.

Effects of hard dietary fat on fish growth at two environmental temperatures.

INTRODUCTION.

There are two principal requirements for dietary lipids, firstly as a major energy source as outlined earlier in this section and secondly as a source of essential fatty acids.

Certain fatty acids are essential components of cells in that they are necessary for the maintenance and functional integrity of membranes. Biomembranes contain large amounts of polar lipid and these are characterised by a high content of polyunsaturated fatty acids that confer fluidity to the membranes. This fluidity permits movement within the membrane of enzymic protein molecules, on which many of the functions of the membrane are dependent.

Membrane fluidity depends in large measure on the degree of unsaturation of the fatty acids esterified to the polar lipids. Fish live in a low temperature environment and at low temperatures, the constraints imposed in maintaining membrane fluidity are greater than at higher temperatures and can be best met by an increasing degree of unsaturation of the fatty acids. This need would be most adequately supplied by highly unsaturated ω3 (for an explanation of lipid terminology, see the addendum at the end of this section) fatty acids and quantitatively the main representatives are eicosapentaenoic

(20:5w3) and docosahexaenoic (22:6w3) acids (Cowey & Sargent, 1977).

By contrast, the major polyunsaturated acids of most terrestrial animals belong to the w6 series with arachidonic acid (20:4w6) being the principal representative. The capacity of fish to incorporate highly unsaturated fatty acids into polar lipids depends either on a dietary supply of these materials or on the ability of the fish to desaturate and elongate shorter chain acids (ultimately of dietary origin) of the appropriate configuration. In the absence of essential fatty acids from the diet, deficiency diseases will occur.

There have been several reviews about the lipid requirements of fish (Castell, 1979; Cowey & Sargent, 1972; 1979; Halver, 1975; Lee & Sinnhuber, 1972 and Sinnhuber, 1969). The main emphasis has been placed on the EFA requirements of the salmonids, in particular the rainbow trout.

Early research on homeothermic land dwelling animals showed that the w6 series of fatty acids were the essential fatty acids while the w3 series were considered non-essential (Alfin-Slater & Aftergood, 1968). The w6 series were shown to be essential to a number of species and so it became widely accepted that these were the essential fatty acids for all animals. Using this information, many researchers began supplementing fish diets with vegetable oils such as corn and sunflower oils which were very rich in linoleic acid (Buhler & Halver, 1961; Halver, 1957; McLaren et al.,

1947 and Phillips et al., 1963). It is interesting to note that although ω6 fatty acids were considered to be essential, most fish oils contained only very low levels (Aaes-Jorgenson, 1967). There is some evidence that since fish oils are rich in ω3 then it is likely that these are the essential fatty acids for fish. Thus McLaren et al. (1947) found that when cod liver oil was deleted from a diet, growth was poor. Similarly, Lee et al. (1967) found that fish oil gave superior growth to corn oil and that dietary linolenic acid (ω3) gave a positive growth response. Castell et al. (1972abc) and Watanabe et al. (1974abc) conclusively proved the EFA value of the ω3 fatty acids to rainbow trout. Castell et al. (1972) showed that the EFA requirement was met by 1% 18:3ω3 (linolenic acid) of the total diet. No combination of linolenic acid and linoleic acid (18:2ω6) resulted in as fast a growth rate as 1% 18:3ω3 alone, in fact the addition of 18:2ω6 to the diet supressed the growth rate (Yu & Sinnhuber, 1976). It is clear that rainbow trout require ω3 fatty acids, however it remains to be shown conclusively whether some dietary level of ω6 fatty acid is essential (Castell, 1979).

With warmwater fish, the evidence points to the requirement of ω3 not being as great as in rainbow trout. Dupree & Sneed (1966) initially showed that corn oil containing 18:2ω6 gave a positive growth response in channel catfish but later Dupree (1969) found that growth was actually

inhibited and that hydrogenated corn oil and beef tallow were superior to liquid corn oil. This was confirmed by Stickney & Andrews (1971) when they showed the repressive effects of 18:2w6 in catfish. Suppression of growth by unsaturated fatty acids does not seem limited to 18:2w6 as linseed oil, which is rich in 18:3w3, in the diet of catfish resulted in reduced growth similar to that produced by corn oil (Stickney & Andrews, 1972). The EFA requirements of channel catfish have not yet been properly identified but it seems certain that the requirements of this warm-water fish are less than those of the rainbow trout (Castell, 1979).

The requirements for carp seem a lot clearer than those of catfish. Watanabe et al. (1975a) intially found growth responses similar to those found in catfish. Saturated lipids gave a positive growth response, however supplements of 18:2w6 and 18:3w3 resulted in little improvement. With younger carp, it was clearly shown that there was a requirement for both w6 and w3 fatty acids (Watanabe et al., 1975b). The best growth response was obtained with 1% 18:2w6 and 1% 18:3w3 (Takeuchi & Watanabe, 1977). They also found that both 22:6w3 and w3-HUFA (highly unsaturated fatty acids) resulted in improved growth and food conversion with 0·5% w3-HUFA giving a slightly better response than that of 1% 18:3w3. Feeding and EFA deficient diet resulted in an accumulation of 20:3w9 as well as pathological symptoms

such as shock syndrome. Levels of 20:3w9 were lowered by the addition of w3 and w6 fatty acids with w3-HUFA and 22:6w3 appearing to be more effective. Farkas et al. (1977) obtained slightly different results in that they found only w3 fatty acids and not w6, effective in reducing the 20:3w9 content of carp lipids. Castell (1979) has attempted to explain the discrepancy between the results of Farkas et al. (1977) and Takeuchi & Watanabe (1977) as being the result of poor separation of the long chain fatty acids by gas-liquid chromatography.

It can be therefore seen that fish have specific requirements for certain essential fatty acids in their diets however the actual requirements are unknown since they are influenced by a number of environmental factors. For warm-water fish such as carp, the most influential of these factors is temperature. This is due in part to changes in plasticity and permeability of membranes, enzymic activation and lipid transport at different temperatures (Castell, 1979).

Temperature is an important factor when dealing with dietary lipid requirement. As mentioned earlier, fish raised at higher temperatures may have a different requirement for PUFA (polyunsaturate fatty acids), therefore this experiment was designed to investigate the effects of hard fats fed to carp at two environmental temperatures. The experiment also considers the use of hard fats such as those found in bakery wastes, as a source of energy as

well as a source of essential fatty acids. Hard fats, whether of animal or vegetable origin are mucher cheaper than fish or vegetable oils and are also more readily available. It has also been suggested that the inclusion of hard fat in the diet reduces the chances of oxidation of the lipid component in both the diet and the harvested fish (Cowey et al., 1979). If hard fat can be found to be beneficial then its presence in bakery products increases the value of the waste.

MATERIALS & METHODS.

Diets.

A diet was formulated using purified ingredients (BDH Chemicals) in order to exclude external fat sources. From this, seven experimental diets were derived containing varying amounts of solid and liquid fats (Table 1.18.). The hard fat was obtained from Warburtons Ltd of Bolton and was of unknown origin since the fat used in the bakery industry is based on least cost formulations and is prepared from variable mixtures of different fats, the majority being of vegetable origin. The liquid fraction was made up from a 50:50 mixture of corn oil and cod liver oil. The diet containing only liquid oils constituted the control diet.

Table 1.18. Composition of the experimental diets.

Base diet.

Ingredient.	%
Casein	30
Dextrin	10
Corn starch	20
Cellulose	18
IVY[1]	4
Binder[2]	3
Fat*	15

1. IVY vitamin & mineral mix, Edward Baker Ltd.

2. Carboxymethyl cellulose.

* Fat inclusion in the base diet.

Diet.	Hard fat (%)	Oils (%)
1.	15	-
2.	12·5	2·5
3.	10	5
4.	7·5	7·5
5.	5	10
6.	2·5	12·5
7. - control	-	15

The diets were prepared as outlined in the General Methods, however after preparation the pellets were dried in a drying cabinet rather than frozen.

Experimental systems.

Two recirculating systems of eight tanks each were stocked with 12 fish per tank. The temperature of one system was maintained at $25^{o}C$ and the other at $15^{o}C$.

Feeding.

Diets were fed at a rate of 1% of body weight day^{-1} of dry feed. This was calculated on a weekly basis. Feeding occured twice daily. The experiment ran for 42 days.

Analyses.

Fatty acid analyses of the diets and fish were conducted using a PYE 304 Gas-liquid chromatograph. The lipids were extracted from wet samples in the case of the fish and dry in the case of the diets. They were extracted for 15 minutes with hot isopropanol and subsequently macerated with a ground glass homogeniser. Particulate material was removed by centrifuation and the pellets were further rinsed with isopropanol:chloroform (1:1) and chloroform. The lipid extracts were reduced in volume, taken up in chloroform, washed with three changes of 0·88M potassium chloride and dried under a stream of nitrogen. In order to reduce autoxidation, solvents with 5g w/v butylated hydroxytoluene were used. Methyl esters

Table 1.19. Fatty acid composition of the dietary fats (g/100g fatty acid.)

Fatty acids.	Hard fat.	Corn oil.	Cod liver oil.
14:0	40•70	26•43	36•86
15:0	0•98	-	1•11
16:0	17•43	7•87	9•74
16:1	9•30	-	9•90
16:2	0•84	-	0•54
18:0	5•21	1•57	2•05
18:1	16•48	18•28	18•99
18:2	2•37	44•65	1•54
18:3	0•77	0•81	-
20:1	3•07	-	7•62
20:2	1•86	-	0•42
22:1	-	-	4•20
22:2	-	-	6•55
others	0•99	0•39	0•48

Table 1.20. Fatty acid analyses of the experimental diets (g/100g fatty acid.)

Fatty acid.	1.	2.	3.	4.	5.	6.	7.
14:0	13•41	10•14	8•57	7•38	5•81	3•87	1•55
15:0	0•73	0•39	0•43	0•46	0•38	0•20	0•21
16:0	30•67	27•75	24•13	21•22	18•54	15•53	13•48
16:1	17•57	15•51	13•19	11•55	9•24	6•94	6•11
16:2	3•12	1•41	1•11	1•12	0•20	0•26	0•30
18:0	6•64	7•35	6•10	5•03	4•26	3•27	2•58
18:1	19•55	23•82	24•85	26•37	28•25	29•56	30•72
18:2	0•38	7•69	16•11	20•84	27•56	35•08	40•02
18:3	1•34	-	-	-	-	-	-
20:1	5•82	5•80	5•12	5•35	5•12	5•03	4•67
others	0•77	0•14	0•39	0•68	0•64	0•26	0•36

Percentages are of the total fat content which amounts to 15% of the diet.

of fatty acids were prepared for gas liquid chromatography in the manner of Morrision & Smith (1964). Tentative identification of unknown fatty acid derivatives were made by comparing the log of the retention time with those from a mixture of authentic standards. Identification was confirmed by co-chromatography with known standards. The fatty acid analyses of the dietary ingredients are shown in table 1.19. and of the diets in table 1.20.

RESULTS.

In all the treatments the fish soon became accustomed to the experimental diets. Those at 25°C consumed all that was given whereas those at 15°C ate much more slowly occasionally allowing some pellets to go to waste.

For both temperature regimes, the growth indices are shown in table 1.21. At 25°C, the specific growth rate can be seen to increase with increasing levels of the liquid oils until the total fat content of the diet is represented by oils and then the growth rate tailed off slightly. None of the specific growth rates are significantly different ($p>0.05$) from each other. Food conversion ratios do not follow the specific growth rate pattern but appear to be random, however like specific growth rates, there are no significant differences between them ($p>0.05$). Average

Table 1.21. Performance of carp fed the test diets for 42 days at 25°C and 15°C.

(n = 12 for each treatment.)

Diets fed at 25°C.

Diet.	1.	2.	3.	4.	5.	6.	7.	±SE.
Intial Wt.	$5 \cdot 55^{a}$	$7 \cdot 20^{a}$	$5 \cdot 97^{a}$	$6 \cdot 56^{a}$	$5 \cdot 84^{a}$	$7 \cdot 41^{a}$	$5 \cdot 87^{a}$	0·513
Final Wt.	$6 \cdot 61^{a}$	$8 \cdot 91^{bc}$	$7 \cdot 37^{ab}$	$8 \cdot 24^{abc}$	$7 \cdot 59^{ab}$	$9 \cdot 61^{c}$	$7 \cdot 58^{ab}$	0·627
Av.Wt.Inc.	$0 \cdot 18^{a}$	$0 \cdot 31^{a}$	$0 \cdot 26^{a}$	$0 \cdot 30^{a}$	$0 \cdot 30^{a}$	$0 \cdot 41^{a}$	$0 \cdot 29^{a}$	0·048
S.G.R.	$0 \cdot 43^{a}$	$0 \cdot 55^{a}$	$0 \cdot 56^{a}$	$0 \cdot 58^{a}$	$0 \cdot 64^{a}$	$0 \cdot 69^{a}$	$0 \cdot 63^{a}$	0·078
F.C.R.	$3 \cdot 31^{a}$	$3 \cdot 27^{a}$	$2 \cdot 93^{a}$	$2 \cdot 95^{a}$	$2 \cdot 47^{a}$	$2 \cdot 17^{a}$	$2 \cdot 47^{a}$	0·382

Diets fed at 15°C.

Intial Wt.	$5 \cdot 57^{a}$	$6 \cdot 34^{a}$	$6 \cdot 79^{a}$	$6 \cdot 60^{a}$	$6 \cdot 28^{a}$	$6 \cdot 31^{a}$	$6 \cdot 17^{a}$	0·544
Final Wt.	$6 \cdot 19^{a}$	$7 \cdot 10^{a}$	$7 \cdot 67^{a}$	$7 \cdot 50^{a}$	$7 \cdot 30^{a}$	$6 \cdot 84^{a}$	$7 \cdot 11^{a}$	0·601
Av.Wt.Inc.	$0 \cdot 09^{a}$	$0 \cdot 16^{a}$	$0 \cdot 23^{a}$	$0 \cdot 19^{a}$	$0 \cdot 23^{a}$	$0 \cdot 15^{a}$	$0 \cdot 21^{a}$	0·029
S.G.R.	$0 \cdot 27^{a}$	$0 \cdot 30^{a}$	$0 \cdot 37^{a}$	$0 \cdot 35^{a}$	$0 \cdot 37^{a}$	$0 \cdot 33^{a}$	$0 \cdot 37^{a}$	0·064
F.C.R.	$7 \cdot 87^{a}$	$6 \cdot 02^{a}$	$4 \cdot 49^{a}$	$4 \cdot 53^{a}$	$4 \cdot 66^{a}$	$5 \cdot 04^{a}$	$5 \cdot 22^{a}$	1·222

S.G.R. = Specific growth rate. F.C.R. = Food conversion ratio. SE = Standard error.
Av.Wt.Inc. = Average weekly weight increase.
Statistic used was Duncans multiple range test. Superscripts of the same letter are not significantly different ($p < 0 \cdot 05$).

weekly weight increases are similar to the food conversion ratios. At 15°C, the specific growth rates increase with increasing dietary oils until a level of 33% of the total fat content. At this level a maximum response is attained and the rest of the diets produced growth rates of similar levels. There is no significant difference ($p>0.05$) between any of the diets. The other indices mirror the results of the specific growth rates and show no significant differences ($p>0.05$). Comparison of the diets performance at both temperatures can be seen in table 1.22. Significant differences ($p<0.05$) appear between the temperatures in the diets with over 50% liquid oil content. This can be seen for all the measured indices.

Tables 1.23. and 1. 25. show the proximate analyses of the composition of the fish carcasses from temperatures of 25°C and 15°C respectively. Although some significant differences ($p<0.05$) do occur, there appears to be no general trend for any of the components at both temperatures. The only possible conclusion is that for both temperatures there would seem to be a lower fat content in the fish fed diets with higher oil levels. Tables 1.27. and 1.29. show the results of the proximate analyses of the composition of the fish guts from both temperatures. The trends that can be seen from these results is that the fat content of the guts from the fish held at 15°C are much lower than those from the higher temperature.

Table 1.22. Comparision of indices for test diets fed at 25°C and 15°C for 42 days. (n = 12 for each treatment).

Diets.	Temperature.	Av.Wt.[1]	S.G.R.[2]	F.C.R.[3]
1.	25°C	0·18[a]	0·43[a]	3·31[a]
	15°C	0·09[a]	0·27[a]	7·87[a]
2.	25°C	0·31[a]	0·55[a]	3·27[a]
	15°C	0·16[b]	0·30[a]	6·02[a]
3.	25°C	0·26[a]	0·56[a]	2·93[a]
	15°C	0·23[a]	0·37[a]	4·49[a]
4.	25°C	0·30[a]	0·58[a]	2·95[a]
	15°C	0·19[b]	0·35[a]	4·53[b]
5.	25°C	0·30[a]	0·64[a]	2·47[a]
	15°C	0·23[b]	0·37[b]	4·66[b]
6.	25°C	0·41[a]	0·69[a]	2·17[a]
	15°C	0·15[b]	0·33[b]	5·04[b]
7.	25°C	0·29[a]	0·63[a]	2·47[a]
	15°C	0·21[b]	0·37[b]	5·22[b]

Figures with different superscripts show a significant difference ($p<0·05$). Statistic used were t and d tests. The test to be used was determined by using the F variance ratio.

1. Average weekly weight gain.
2. Specific growth rate - mean weekly values.
3. Food conversion ratio - mean weekly values.

Tables 1.24. and 1.26. show the results of the fatty acid analyses on the fish carcasses for both temperatures. Although some difficulty in separation was encountered, the trends that are apparent are that at 15°C, the levels of 18:0 in the carcasses increase with increasing liquid oils in the diet. At 25°C, the trend is reversed. Levels of 18:1 are much higher at 25°C as are those of 16:0. Tables 1.28. and 1.30. show the same results but for analyses of the fish guts. Levels of 16:0 are again higher at 25°C, whilst 14:0 is higher at the lower temperature. 18:0 and 18:1 are reversed with high levels of 18:0 at the lower temperature and 18:1 at the higher temperature. The trends shown by some of the fatty acids both in the diets and the fish bodies are illustrated in figures 5 - 8.

Table 1.23. Proximate analyses of fish carcasses of fish fed the experimental diets at 25°C. (n = 6 for each treatment).

Diet.	1.	2.	3.	4.
Moisture.	$78 \cdot 02 \pm 0 \cdot 85^{a}$	$79 \cdot 25 \pm 1 \cdot 23^{a}$	$79 \cdot 34 \pm 1 \cdot 64^{a}$	$77 \cdot 83 \pm 1 \cdot 25^{a}$
Protein.	$10 \cdot 47 \pm 1 \cdot 84^{ab}$	$7 \cdot 58 \pm 2 \cdot 69^{b}$	$7 \cdot 82 \pm 2 \cdot 61^{b}$	$11 \cdot 74 \pm 2 \cdot 27^{a}$
Fat.	$9 \cdot 02 \pm 2 \cdot 21^{abc}$	$11 \cdot 23 \pm 2 \cdot 75^{a}$	$10 \cdot 63 \pm 3 \cdot 81^{a}$	$6 \cdot 63 \pm 1 \cdot 17^{bc}$
Ash.	$1 \cdot 99 \pm 0 \cdot 84^{b}$	$1 \cdot 43 \pm 0 \cdot 84^{b}$	$1 \cdot 70 \pm 1 \cdot 06^{b}$	$3 \cdot 63 \pm 1 \cdot 60^{a}$

Diet.	5.	6.	7.	±SE.
Moisture.	$78 \cdot 68 \pm 0 \cdot 67^{a}$	$78 \cdot 68 \pm 1 \cdot 08^{a}$	$78 \cdot 24 \pm 0 \cdot 44^{a}$	0·4871
Protein.	$6 \cdot 88 \pm 4 \cdot 96^{b}$	$11 \cdot 55 \pm 2 \cdot 72^{a}$	$8 \cdot 78 \pm 1 \cdot 03^{ab}$	1·1526
Fat.	$9 \cdot 53 \pm 4 \cdot 66^{ab}$	$5 \cdot 39 \pm 2 \cdot 84^{c}$	$10 \cdot 32 \pm 1 \cdot 15^{a}$	1·2590
Ash.	$1 \cdot 57 \pm 1 \cdot 14^{b}$	$3 \cdot 86 \pm 0 \cdot 91^{b}$	$2 \cdot 16 \pm 0 \cdot 91^{b}$	0·4731

SE = Standard error.

abc = Mean values of each component with the same superscripts are not significantly different (Duncans multiple range test, $p < 0 \cdot 05$).

Figure 5. Trends in fatty acid composition - 16:0.

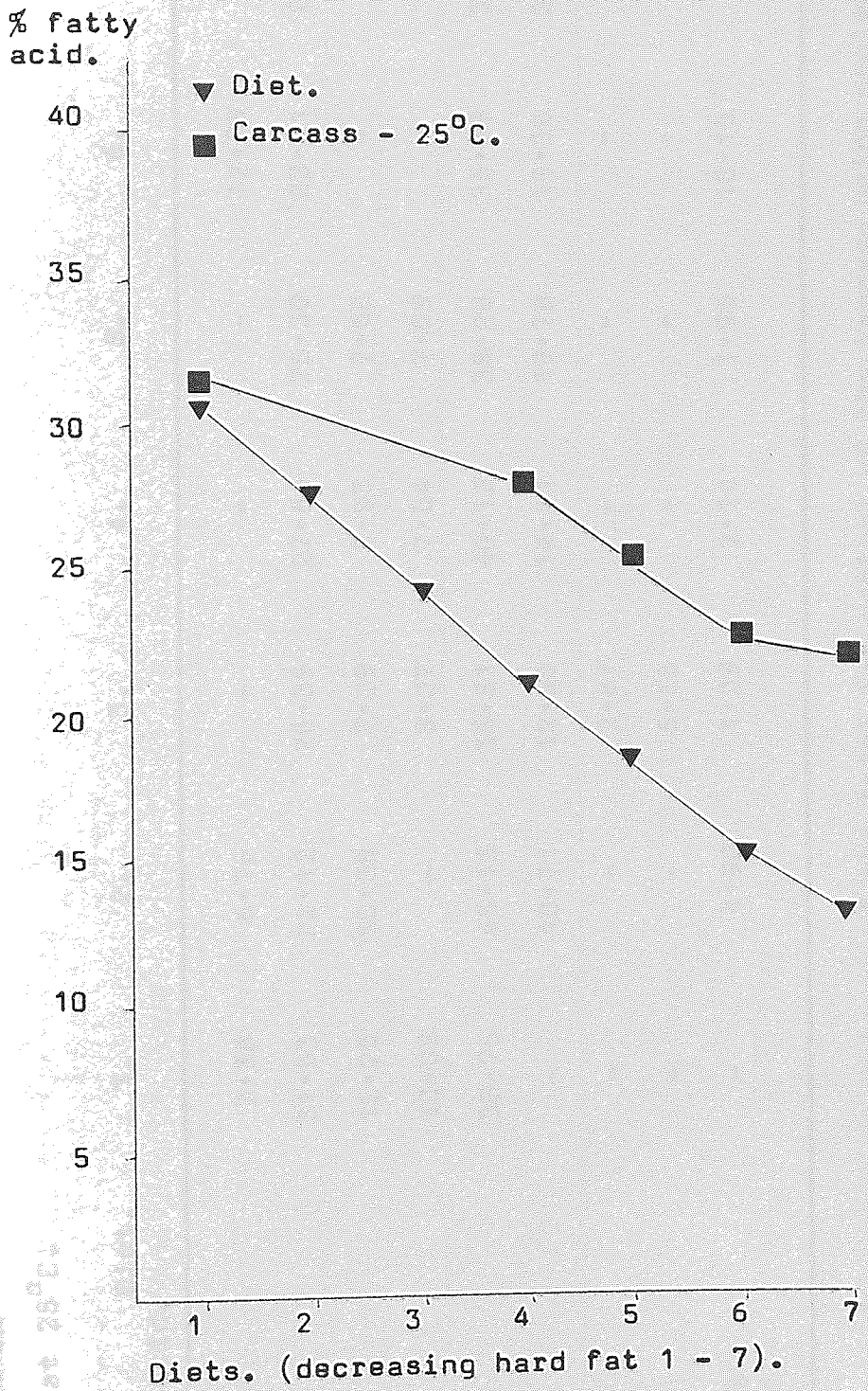


Table 1.24. Fatty acid composition of the fish carcasses of fish fed the experimental diets at 25°C.

Fatty acid. \ Diet.	1.	2.	3.	4.	5.	6.	7.
14:0	7•38	6•78	-	-	-	12•78	2•60
16:0	31•53	22•12	26•39	27•84	25•30	23•41	22•36
16:1	12•25	10•36	7•29	7•95	7•60	-	6•37
18:0	10•08	-	8•37	7•54	7•09	-	5•59
18:1	38•76	45•13	35•51	40•12	38•28	35•28	36•00
18:2	-	10•71	12•60	14•41	19•78	18•38	22•39
20:0	-	-	2•87	-	-	-	3•95
22:0	-	-	5•58	-	-	-	-
others	-	4•90	1•39	2•14	1•95	10•15	0•74

Figures given are percentages of the total fat content of the carcass using pooled samples.

Table 1.25. Proximate analyses of the fish carcasses of fish fed the experimental diets at 15°C. (n = 6 for each treatment).

Diet.	1.	2.	3.	4.
Moisture.	$76 \cdot 17 \pm 1 \cdot 42^{a}$	$76 \cdot 46 \pm 1 \cdot 22^{a}$	$75 \cdot 42 \pm 1 \cdot 86^{a}$	$76 \cdot 25 \pm 4 \cdot 46^{a}$
Protein.	$12 \cdot 75 \pm 1 \cdot 96^{bc}$	$5 \cdot 83 \pm 2 \cdot 78^{d}$	$10 \cdot 84 \pm 2 \cdot 59^{c}$	$7 \cdot 24 \pm 1 \cdot 67^{d}$
Fat.	$6 \cdot 36 \pm 2 \cdot 36^{c}$	$15 \cdot 58 \pm 4 \cdot 46^{a}$	$11 \cdot 08 \pm 3 \cdot 80^{b}$	$14 \cdot 97 \pm 5 \cdot 02^{a}$
Ash.	$4 \cdot 22 \pm 0 \cdot 97^{a}$	$1 \cdot 58 \pm 0 \cdot 98^{c}$	$2 \cdot 15 \pm 0 \cdot 75^{bc}$	$1 \cdot 03 \pm 0 \cdot 79^{c}$

Diet.	5.	6.	7.	±SE.
Moisture.	$76 \cdot 15 \pm 1 \cdot 47^{a}$	$76 \cdot 20 \pm 1 \cdot 29^{a}$	$75 \cdot 95 \pm 1 \cdot 89^{a}$	0·9894
Protein.	$15 \cdot 19 \pm 1 \cdot 82^{ab}$	$15 \cdot 41 \pm 1 \cdot 73^{a}$	$11 \cdot 55 \pm 1 \cdot 86^{c}$	0·8847
Fat.	$4 \cdot 83 \pm 1 \cdot 75^{cd}$	$4 \cdot 19 \pm 1 \cdot 51^{d}$	$8 \cdot 45 \pm 2 \cdot 34^{bc}$	1·3458
Ash.	$3 \cdot 32 \pm 1 \cdot 80^{b}$	$3 \cdot 73 \pm 1 \cdot 09^{a}$	$3 \cdot 53 \pm 1 \cdot 47^{ab}$	0·4863

SE = Standard error.

abcd = Mean values for each component with the same superscripts are not significantly different (Duncans multiple range test $p < 0 \cdot 05$).

Figure 6. Trends in fatty acid composition - 18:1.

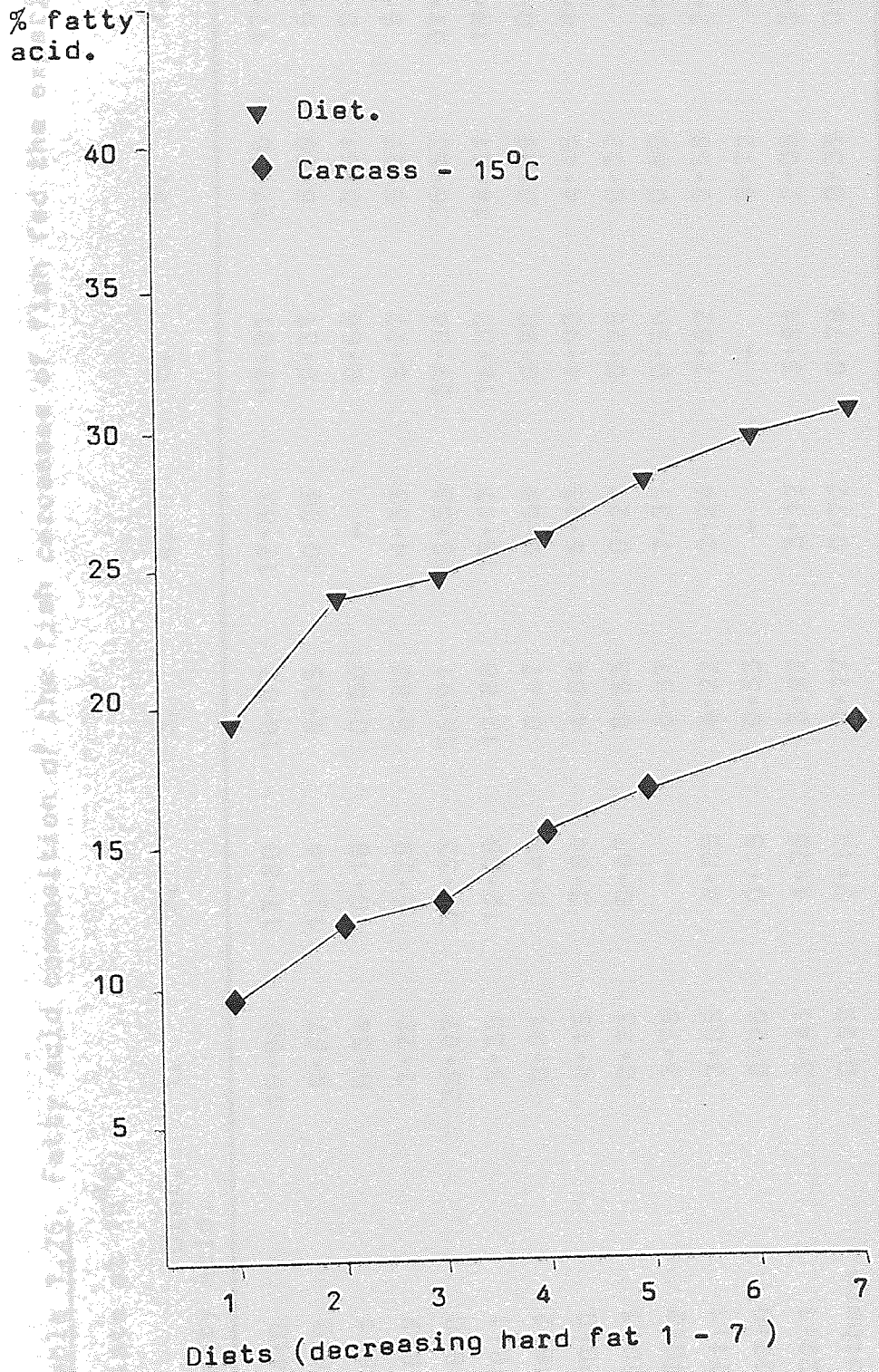


Table 1.26. Fatty acid composition of the fish carcasses of fish fed the experimental diets at 15°C.

Diet. / Fatty acid.	1.	2.	3.	4.	5.	6.	7.
14:0	19·96	17·80	18·97	17·63	17·81	17·16	17·09
16:0	9·67	12·14	9·35	10·06	8·37	6·89	6·47
16:1	0·34	0·18	0·56	-	0·66	0·31	0·28
16:2	7·22	5·29	5·83	4·33	5·27	5·27	5·43
18:0	36·97	37·33	36·91	33·33	37·04	36·53	39·31
18:1	9·35	12·06	12·88	15·17	16·37	14·31	19·37
18:2	0·91	0·49	0·47	0·59	0·90	0·39	0·74
20:0	4·15	3·84	4·04	5·23	4·93	4·45	4·23
20:1	0·73	0·47	0·53	0·27	0·25	0·32	-
20:2	3·75	-	0·97	7·31	0·96	0·60	0·92
21:1	3·05	4·05	4·22	2·24	2·82	2·58	2·26
22:0	0·26	0·15	0·53	-	-	8·72	-
22:1	3·41	4·06	3·97	3·12	3·84	2·08	3·66
others	0·23	2·14	0·77	0·72	0·78	0·39	0·24

Figures given are percentages of the total fat content of the carcass using pooled samples.

Table 1.27. proximate analyses of fish guts of fish fed the experimental diets at 25°C. (n = 6 for each treatment).

Diets.	1.	2.	3.	4.
Moisture.	76·89±2·21^{a}	78·91±8·18^{a}	76·29±2·54^{a}	78·89±3·65^{a}
Protein.	8·73±0·38^{a}	8·12±0·48^{b}	6·56±0·07^{c}	5·04±0·15^{e}
Fat.	12·56±1·44^{ab}	10·95±2·96^{b}	14·85±3·02^{a}	14·69±3·70^{a}
Ash.	1·32±0·38^{abc}	1·52±0·48^{ab}	1·80±0·07^{a}	0·97±1·15^{cd}

Diets.	5.	6.	7.	±SE.
Moisture.	77·01±3·63^{a}	81·94±3·40^{a}	80·50±5·29^{a}	1·9952
Protein.	5·73±0·18^{d}	6·26±1·02^{c}	6·25±0·24^{c}	0·1564
Fat.	15·63±1·79^{a}	10·50±0·21^{b}	12·41±2·77^{ab}	1·0310
Ash.	1·13±0·18^{bcd}	0·80±1·02de	0·34±0·24^{e}	0·1564

SE = Standard error.

abcde = Mean values of each component with the same superscripts are not significantly different (Duncans multiple range test $p<0·05$)

Table 1.28. Fatty acid composition of the fish gut of fish fed the experimental diets at 25°C.

Fatty acids. \ Diets.	1.	2.	3.	4.	5.	6.	7.
14:0	6•54	6•20	4•62	4•07	4•24	1•18	1•93
16:0	25•57	23•99	19•64	20•81	20•13	20•33	14•90
16:1	15•79	13•73	10•50	11•14	9•67	7•87	6•53
18:0	4•97	5•68	4•88	4•60	5•22	5•51	3•72
18:1	33•59	32•58	44•12	40•41	38•03	36•53	38•62
18:2	3•88	8•52	11•46	12•65	17•67	23•27	22•36
20:0	3•13	3•50	3•11	3•93	4•56	-	5•35
20:1	-	1•78	-	-	-	-	-
20:2	0•47	1•17	-	0•71	-	5•29	-
22:0	1•45	2•35	1•39	1•36	-	-	1•02
22:1	-	-	-	-	-	-	2•44
22:2	-	-	-	-	-	-	1•05
others	4•91	0•50	0•28	0•32	0•48	0•02	2•08

Figures given are percentages of the total fat content of the fish gut using pooled samples.

Figure 7. Trends in fatty acid composition - 16:1.

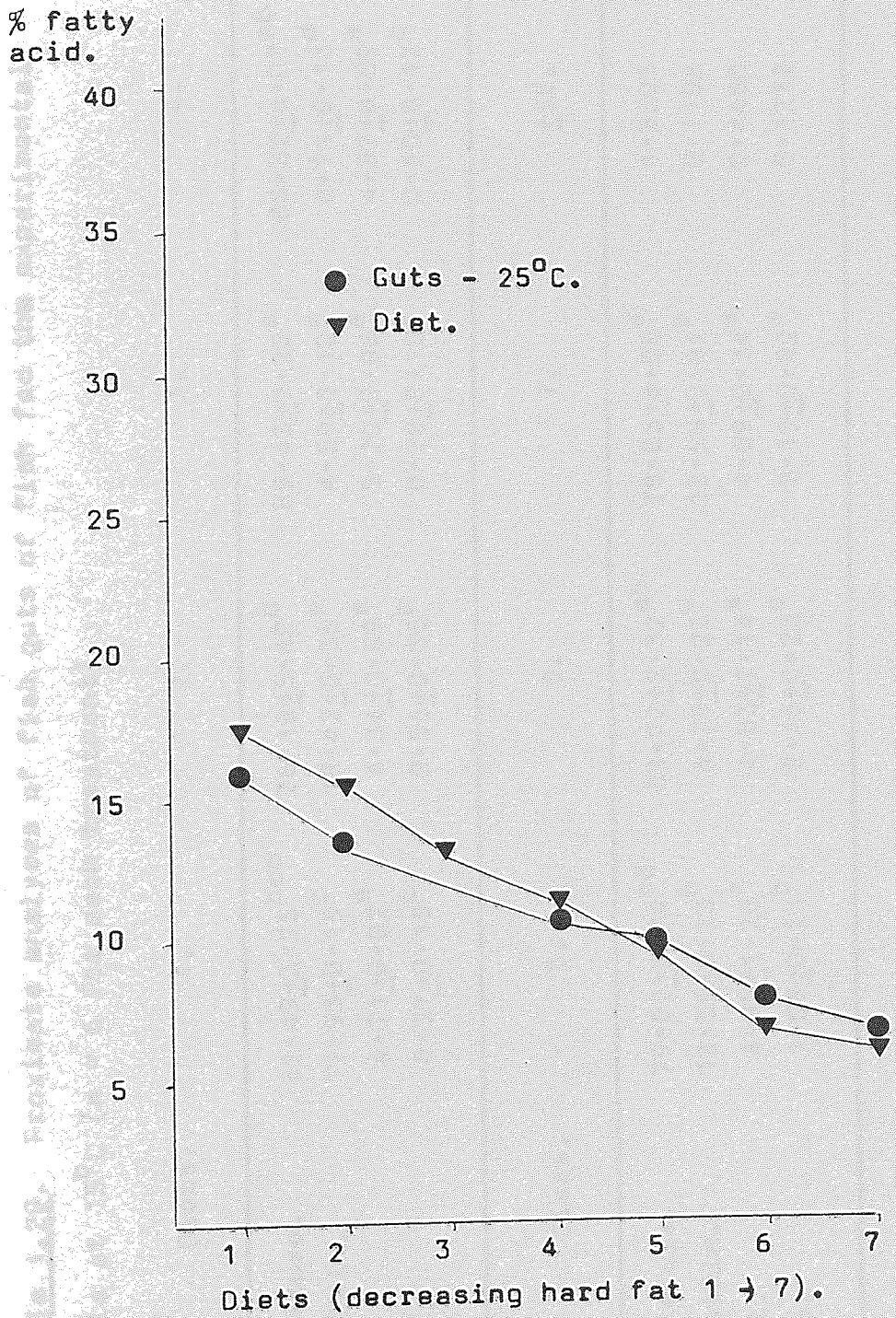


Table 1.29. Proximate analyses of fish guts of fish fed the experimental diets at 15°C (n = 6 for each treatment).

Diets.	1.	2.	3.	4.
Moisture.	$81 \cdot 49 \pm 3 \cdot 25^{bcd}$	$76 \cdot 18 \pm 5 \cdot 40^{d}$	$89 \cdot 43 \pm 2 \cdot 20^{a}$	$85 \cdot 32 \pm 4 \cdot 07^{ab}$
Protein.	$12 \cdot 93 \pm 0 \cdot 19^{c}$	$18 \cdot 42 \pm 0 \cdot 35^{a}$	$4 \cdot 59 \pm 0 \cdot 10^{e}$	$8 \cdot 71 \pm 0 \cdot 13^{d}$
Fat.	$4 \cdot 71 \pm 0 \cdot 02^{a}$	$4 \cdot 34 \pm 0 \cdot 69^{a}$	$5 \cdot 20 \pm 0 \cdot 86^{a}$	$4 \cdot 87 \pm 0 \cdot 69^{a}$
Ash.	$0 \cdot 37 \pm 0 \cdot 19^{c}$	$0 \cdot 56 \pm 0 \cdot 35^{c}$	$0 \cdot 28 \pm 0 \cdot 10^{c}$	$0 \cdot 60 \pm 0 \cdot 13^{c}$

Diets.	5.	6.	7.	±SE.
Moisture.	$79 \cdot 92 \pm 5 \cdot 74^{cd}$	$89 \cdot 72 \pm 2 \cdot 32^{ab}$	$78 \cdot 83 \pm 4 \cdot 05^{d}$	1·8505
Protein.	$13 \cdot 55 \pm 0 \cdot 13^{b}$	$4 \cdot 79 \pm 0 \cdot 23^{e}$	$13 \cdot 58 \pm 0 \cdot 81^{b}$	0·1198
Fat.	$4 \cdot 95 \pm 1 \cdot 28^{a}$	$4 \cdot 53 \pm 2 \cdot 14^{a}$	$4 \cdot 89 \pm 0 \cdot 34^{a}$	0·4460
Ash.	$1 \cdot 08 \pm 0 \cdot 13^{b}$	$0 \cdot 46 \pm 0 \cdot 23^{c}$	$2 \cdot 19 \pm 0 \cdot 82^{a}$	0·1212

SE = Standard error.

abcde = Mean values of each component with the same superscripts are not significantly different (Duncans multiple range test $p < 0 \cdot 05$)

Figure 8. Trends in fatty acid composition - 18:2.

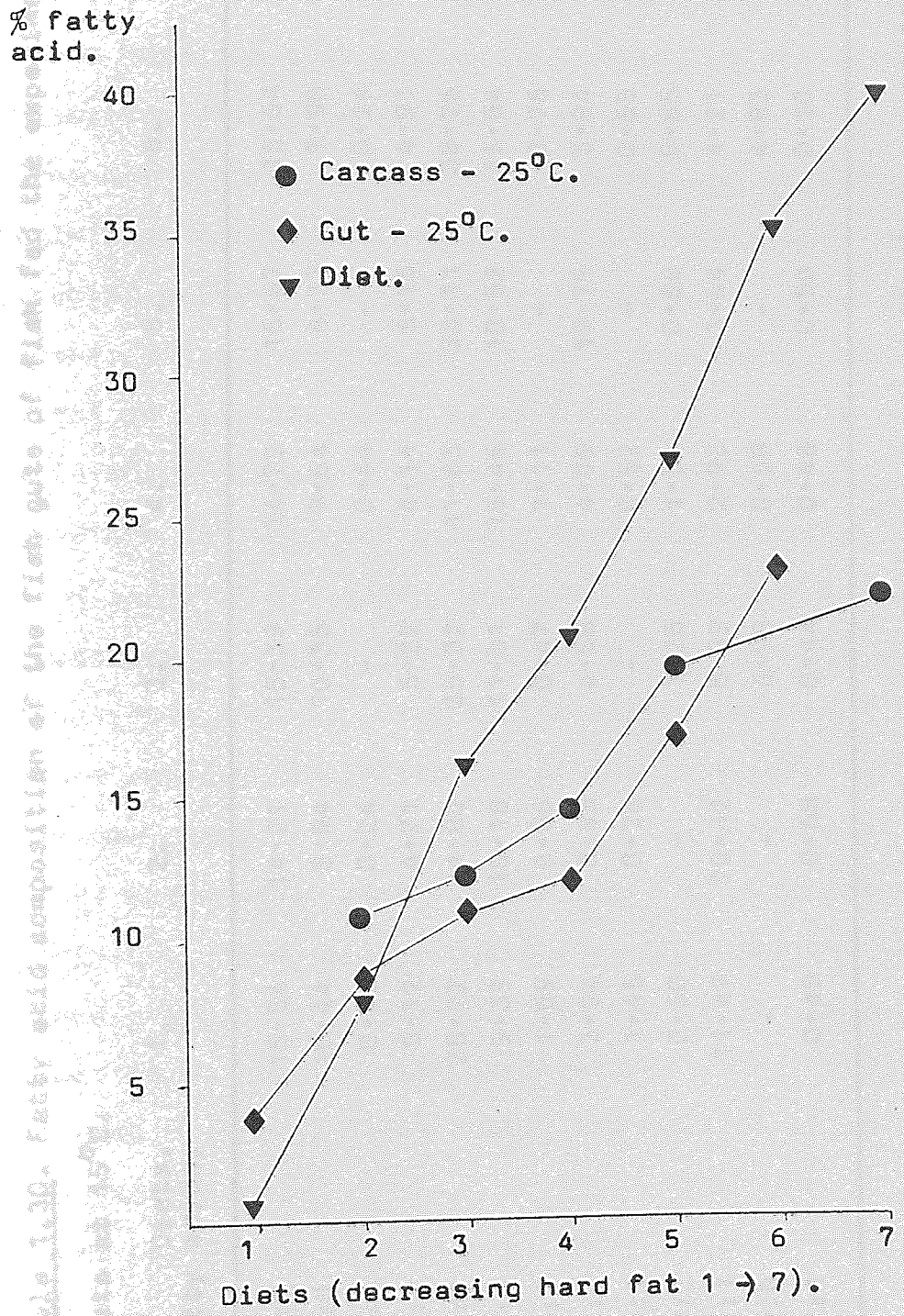


Table 1.30. Fatty acid composition of the fish guts of fish fed the experimental diets at 15°C.

Fatty acids. Diets.	1.	2.	3.	4.	5.	6.	7.
14:0	15•54	14•21	18•39	14•72	16•67	15•64	12•92
16:0	10•44	8•94	10•35	9•64	8•37	7•90	6•06
16:1	0•22	0•24	-	0•44	-	0•24	0•24
16:2	5•12	4•71	5•22	4•14	5•15	4•91	4•22
18:0	38•72	34•80	35•32	41•83	33•11	38•26	38•28
18:1	9•39	10•18	11•01	16•46	10•52	15•54	20•35
18:2	1•09	0•66	0•47	1•11	-	0•76	0•86
20:0	3•24	3•85	4•58	4•09	19•79	3•94	3•97
20:1	1•45	0•26	-	0•52	-	0•65	0•80
20:2	0•60	-	1•25	1•54	0•88	0•85	0•66
21:1	11•47	20•42	5•02	2•93	3•64	4•21	2•65
22:0	-	-	6•98	0•58	-	4•83	6•68
22:1	0•45	0•68	0•44	0•48	0•50	0•20	0•25

Figures given are percentages of the total fat content of the fish gut using pooled samples.

DISCUSSION.

This study has raised a number of separate issues regarding the use of hard fats in fish nutrition. These include hard fat utilisation, the effect of environmental temperature and fatty acid requirement.

The use of hard fats, both of animal and vegetable origin has been suggested by several workers as a cheap source of dietary energy and essential fatty acids for a number of different fish. In this experiment, two main sources of fat were used. These were a hard fat of unknown origin and a mixture of corn oil and cod liver oil. The test diets incorporated varying levels of these fats so that a range of diets were tested. The results showed that at both temperatures, the hard fat alone produced the worst growth rates. The addition of increasing levels of the oil mixture resulted in increasing growth rates especially at the higher temperature whilst at the lower temperature, a plateau was reached after the addition of limited amounts of the oils. This would suggest that the hard fat was not as good a source of dietary fat as the oil mixture either because it was not as well utilised for energy or because it lacked essential fatty acids, the absence of which, supressed growth.

Hard fat of animal origin has been used by Reinitz (1980) as a fat source in trout diets. Up to 50% of the fat in the diet was replaced by animal fat and no adverse

effects were observed and in fact at 20°C, a combination of fats gave a slightly better performance than either of the fat sources alone. This is similar to the findings of this experiment where at the higher temperature, the best growth rates were the result of mixed fat sources. Similar results were also found in trout where swine fat was used as the fat source at levels up to 50% of the total dietary fat (Yu et al., 1977). Mugrditchian et al. (1981) fed mixtures of fats including animal fat to chinook salmon with a maximum level of about 60% of the total fat source. The performances of the diets containing the animal fat were as good as those of the control. Catfish have been used for many studies of this nature. Dupree (1969) found that beef tallow when used as the fat source resulted in only poor growth. Conversely, Stickney & Andrews (1971; 1972) found that beef tallow resulted in good growth, producing better results than diets containing safflower oil. Similar results were obtained by Yingst & Stickney (1980) when beef tallow was compared with soybean oil. Murray et al. (1977) found that a mixture of animal fat and fish oil gave better growth than either on their own. They also found that when the two fat sources were added to corn oil, then growth supression occured.

The use of hard fats of vegetable origin has been rarely considered, mainly because of the lack of naturally

occuring examples. Hydrogenated vegetable oils have only been used in one study. Dupree (1969) found that hydrogenated corn oil when included in diets for catfish gave superior growth to diets containing liquid corn oil.

Temperature has been the subject of several investigations since there appears to be a link between it and the utilisation of various fats as well as possible changes in the essential fatty acid requirement of certain fish as environmental temperature varies. Reinitz (1980) showed that mixtures of fats gave a better growth performance than each fat alone at temperatures of 11^{o}C and 20^{o}C when fed to rainbow trout. The difference between the diets was more noticeable at the higher temperature. For catfish, Stickney & Andrews (1971) showed that the good growth rates shown by hard fats were obtained at all temperatures between 20^{o}C and 30^{o}C with increasing growth rates as the temperature increased. Similar results were obtained by Murray et al. (1977) where mixtures of fats gave better growth than single fat sources alone. This occured at both temperatures used, 23^{o}C and 28^{o}C and as before the gap between the different treatments was larger at the higher temperature. In a trial where a single experimental treatment was used at a wide range of temperatures, growth rates were seen to increase to an optimum level of 30^{o}C and then they tailed off (Andrews & Stickney, 1972).

Temperature has also been seen to affect the apparent absorbability of animal fat. At two temperatures, 23°C and 28°C, the absorbability was much lower at 23°C. At both temperatures, the absorbability decreased with increasing levels of animal fat in the diet up to a maximum level of 15%.

With regard to fatty acid composition and requirement, the interactions of hard fat with liquid oils at different environmental temperatures has produced mixed results. Table 1.19. shows the composition of the three fat sources and it can be seen that myristate (14:0) was rich in all three fats. The hard fat also had high levels of palmitate (16:0) and oleate (18:1) whilst corn oil had high levels of oleate (18:1) and linoleate (18:2) and cod liver oil mainly oleate (18:1). The corn oil as expected has high levels of ω6 fatty acids in the form of 18:2ω6, whilst the cod liver oil appeared to lack any of the ω3 fatty acids however this may be the result of poor separation of the fatty acids by the GLC. Comparison with other analyses of different fish oils showed that levels of 18:3ω3 were also very low with an average level of under 1% however in other fish oils ω3 fatty acids are also represented by high levels of 20:5ω3 and 22:6ω3. In this analysis, the ω3 fatty acids of longer chain length were absent and again the reason was most likely due to poor separation. It is highly probable that although

there appears to be no trace of w3 fatty acids in the cod liver oil sample, there should be in fact, a substantial amount present. A similar problem of poor separation and identification occured in the analysis of the test diets which bear no relationship with the analyses of the individual oils and fats shown in table 1.19. Some examples of this are that the level of 18:2 in diet 7 (Table 1:20) is 40% yet if the levels shown in table 1:19 are correct then the level should only be 23%. The level of 18:3 in diet 1 is much higher than that of diet 7 yet the latter should have a high amount of this fatty acid as fish oils are a rich source although the analysis of cod liver oil (table 1:19) does not show this. Finally the levels of 14:0 in all the diets can be seen to be low yet all the fat samples analysed in table 1:19 show very high levels.

Although the results of the analyses of the fat samples and the test diets are in doubt, certain trends can be observed. These are a decrease in the levels of 14:0, 16:0 and 16:1 and an increase in the level of 18:1, all with increasing levels of mixed oils in the diets. Levels of 18:2w6 were also seen to increase with increasing levels of dietary oils. This was not surprising since the increased levels of corn oil would contribute the major proportion of this w6 series fatty acid. Liquid corn oil has been known to cause a supression of growth in catfish (Dupree, 1969) because of its high levels of linoleic acid (18:2w6) and

Stickney & Andrews (1971) found the same supression with safflower oil which supplied linoleic acid at a level of 7·3% of the total diet. Similar supression was found for rainbow trout (Lee et al., 1967; Higashi et al., 1966; Castell et al., 1972a;b and Yu & Sinnhuber, 1972) and for salmon (Buhler & Halver, 1961; Nicolaides & Woodall, 1962 and Tinsley et al., 1971).

In this study, the level of 18:2ω6 was at a maximum in the diet containing 100% oils as the fat source and amounted to 3% of the total diet. This is not as high as the level of Stickney & Andrews (1971) however it represents a considerable amount of linoleic acid in the diet. This level of 3% did not appear to have any supressing effect on growth of carp in this experiment because at the higher temperature of 25°C, growth rates increased with increasing levels of linoleic acid. Only the diet containing the highest level of 18:2ω6 produced a slightly reduced growth rate. At the lower temperature of 15°C, growth rates reached a plateau with 10% hard fat and 5% oils. This may be due to either a lower metabolic rate at the lower temperature or it may be due to suppression of the growth rates by the higher levels of linoleic acid in the higher oil diets. This differentiation at the two temperatures is not unique since Murray et al. (1977) found growth suppression tended to be more pronounced at lower environmental temperatures. In this experiment, the lower temperature was considerably lower than that used by Murray et al. (1977) so the effect should be even more pronounced.

In those test diets where dietary fat was made up predominately from hard fat, the growth rates were reduced in comparison with the growth rates of the high oil diets. The reason for this may not be a suppression of growth similar to that caused by linoleic acid but rather a deficiency of another essential fatty acid such as linoleneic (18:3w3). Although the analyses of the dietary fat samples used in this experiment showed either very low levels or no linoleneic acid, cod liver oil as with other fish oils should contain a relatively high level. The addition of cod liver oil to the diets should provide enough 18:3w3 to prevent any EFA deficiency and so improve growth rates of the diets with no cod liver oil. Supplementation with small amounts of linoleneic acid has been shown to improve growth rates in carp (Takeuchi & Watanabe, 1977).

The results of the body composition analyses on both carcasses and guts are slightly confused. No trends are visible at all. The reason for this is unknown. Differences in the protein levels are due to the cumulative effects of differences in the fat and moisture levels. The only possible explanation is sampling error. Discrepancies also occur in the fatty acid analyses and these may have occured for similar reasons.

In other studies, it has been found that the fatty acid composition of the fish carcasses was usually influenced by the dietary fatty acids. An example of

this was shown by Stickney & Andrews (1971) where fish fed diets with safflower oil which is rich in w6 fatty acids, had high carcass levels of 18:2w6. Fish fed menhaden oil which is rich in w3 fatty acids produced high carcass levels of 20:5w3 and 22:6w3. Temperature has little effect on the fatty acid composition of the fish carcasses (Murray et al., 1977).

With regard to the actual body composition, the main findings of other studies is that body lipid increases with increasing temeperature (Andrews & Stickney, 1972) since at higher temeperatures, the fish metabolism is higher and this leads to a higher rate of fat deposition.

This experiment has covered most of the potential factors that affect fish growth with regard to fats. Due to difficulties encountered in the analyses of both fatty acid composition and fish body composition, the effects of hard fats as found in bakery products, on fish growth, require further investigation.

ADDENDUM.

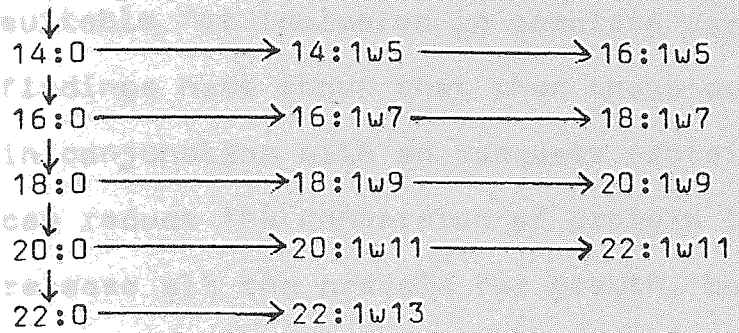
A note on the nomenclature and metabolic pathways of fatty acids.

Fatty acids can be described by using a numerical system. In this the first number identifies the number of carbon atoms, the second number, the number of double bonds in the molecule and the first number which follows the omega symbol, the position of the first double bond from the methyl group. Linolenic acid would therefore be written 18:3w3. This represents a molecule which has 18 carbon atoms with three double bonds. The first double bond to occur in the chain, when counting from the methyl end, would be on the third carbon atom.

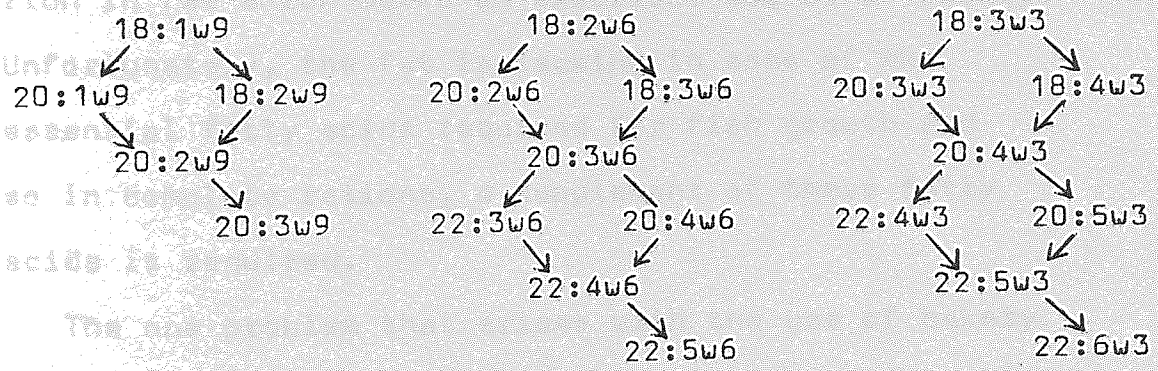
Animals have requirements for different fatty acids. The type of fatty acid depends on the animal under consideration. Fish have a requirement for fatty acids of the w3 series which means that for fish growth to occur, the fatty acids must have the first double bond on the third carbon atom. Fish also have a requirement for the w6 series fatty acids although to a much lesser extent. In this case the first double bond is on the sixth carbon atom.
The pathways exhibited by fatty acids are shown in figure 9.

Figure 9. Fatty acid conversion pathways. (From Castell,1979).

Saturated and monoenoic fatty acids.



Polunsaturated fatty acids.



CONCLUSIONS - Section 1.

The four experiments contained within this section have demonstrated that bakery wastes in the form of either bread waste or meat pie production waste are suitable for inclusion in complete carp rations. The findings have shown that when these wastes are used in conjunction with an adequate protein source, they can reduce the conversion of protein to energy and so release all the protein for growth. However when used on their own, they do not contribute greatly to the growth rates of the fish. The pie waste as well as containing large amounts of carbohydrate is also rich in fat which makes an excellent source of energy. Unfortunately, the fat is lacking in some of the essential fatty acids required for fish growth and so in complete rations, a supplement of these fatty acids is required.

The one problem that arises with the use of bakery wastes is their variability in composition. If such a product was to be used in commercial diets, then much of the variation may be evened out by the use of large quantities.

SECTION 2.

The use of bakery wastes in supplementary diets.

INTRODUCTION.

Production of salmonid fish in tanks relies totally on feeding with complete rations. These fish diets, since they contain the maintenance and growth factors necessary for the fish, are very expensive, often costing over 50% of diets production costs (Collins & Delmondo, 1976). By comparison, warmwater fish are grown in ponds where natural food is present, the cost of which is very low. However, yields of fish grown on natural foods alone are low and often are insufficient to cover the fixed costs of the pond area (Tal & Hepher, 1967). The profitability of these systems depends on the use of supplementary feeds which are simpler and less expensive than complete diets (Hepher, 1979). These feeds are designed to supplement the natural food in the pond.

Supplementary feeds have two roles to play in the feeding of pond fish. They are primarily used to alter the nutritional status of the pond. The dry weight protein level of natural feeds is about 50% (Mann, 1961; Schaperclaus, 1963) whereas the protein requirement of carp is only 35-40% (Kaneko, 1969; Sin, 1973a; 1973b; Shiloh& Viola, 1973) which means that when carp are feeding, much of the natural protein goes to waste through conversion to energy. Feeding high energy supplements releases all the protein for growth so obtaining the maximum production for the minimum cost.

If the number of fish in the pond is increased above the carrying capacity, then the natural protein level will become limiting and to maintain maximum growth, the inclusion of additional protein in the supplemental feed will be necessary. The larger the standing crop of fish, the larger the deficit of natural protein and the higher the level of additional protein required (Hepher, 1979). This is the second role of supplemental feeds.

Supplementary feeds can therefore take many forms ranging from a total energy feed to one containing some additional protein. Traditionally in carp culture, the term supplementary feed refers to the energy type of ration with the protein type only being used where there are high stocking densities. This is a much more intensive type of carp farming.

There is a wide range of energy type of supplemental feed which are used in carp culture. The most popular are cereals such as wheat, barley, oats and maize. Others include cottonseed, soybean, lupin and other pulse feeds. Animal products can also be used but their use is limited by local availability. The common factor of all these feeds is that they are low in protein but rich in carbohydrate.

As part of the nutritional component is already present in the pond, calculation of the supplementary portion can be very difficult because it is important to maintain the

correct nutritional balance. This is made especially hard as the natural portion is continually changing as conditions vary. Such conditions are temperature, fertility and water quality and all three can have a great influence on the production of the natural food.

Methods of estimation of the amount of supplemental feed necessary varies throughout the world although they are usually based on similar factors. In some cases it is necessary to have an estimate of the standing crop in the pond. These estimates are most easily obtained by sampling the pond. Tal (1956) recommends biweekly seining, a recommendation echoed by Tiemeier et al. (1964). From the estimate of average fish weight, the amount of feed is calculated for the total number of fish in the pond. This estimate is open to two sources of error. The first is that the sample may not be representative of the total population. Swingle (1967) found that at least 100 fish were necessary for an accurate sample from ponds of 0·5 ha to 10 ha and even then, average weights have been found to be 8 - 19% different from the true weights. The second possible source of error is in the number of fish in the true population. With very large ponds and differing species of fish, mortality rate can vary from 0 - 35% of the total number of fish. Carp survival is usually around 97-100%.

A different method of estimating the standing crop is by monitoring the amount of food previously fed into the pond, ie by expected growth rates. For this approach it is necessary to know the approximate conversion factor for each food type. The conversion factor is calculated using the equation: Feed fed/Weight increase. This is the same as food conversion rate but when using the equation for extensive pond culture, it also takes into account the presence of natural food.

Estimations using this equation are also open to error since there is no way of ensuring that the food is converted at the same rate as which it is being estimated. This is due to the influence of several factors which can affect the conversion value. The most obvious of these is temperature, however natural food availability probably has more effect since, if the natural food is low, then the supplementary food will not be converted as efficiently and fish growth will be consequently low.

Swingle (1967) illustrates the estimation of standing crop by two methods: seine netting and feeding. He also compares these two methods with the final weight obtained on draining the pond. These findings are shown in table 2.1. Three examples are shown. The accuracy of both methods illustrated is about equal. Ideally, estimates obtained from both methods can be used together.

Table 2.1. Three examples of the accuracy of estimating the standing crop by two methods. Figures are in Kg/ha. (From Swingle, 1967).

Estimate.	Seining.	Feed.	Draining.
Example A.			
1	235	253	
2	433	578	
3	692	690	
4		1170	1303
Example B.			
1	183	249	
2	366	378	
3	501	708	
4		1204	1185
Example C.			
1	251	320	
2	531	480	
3	847	892	
4	1288	1252	1193

Once an estimate of the standing crop is obtained, it is then possible to calculate the amount of supplementary feed required to feed the fish. In America and some other areas of the world, supplementary feed rate is based on a percentage of the body weight. This can be kept constant throughout the growing period or can be varied with temperature.

When feeding a constant percentage rate, although the rate is maintained throughout the season, the actual amount of food will increase with increases in the weight of the standing crop.

When temperature is also considered, then the rate is varied as the temperature changes. An example of this type of feeding pattern is given by Szumeic (1977) and is shown in Table 2.2.

Table 2.2. Feeding schedule based on body weight and temperature (From Szumeic, 1977).

Temperature.	% Feed rate.
15-18^{o}C	2
18-21^{o}C	3
21-24^{o}C	4
24^{o}C-	5

In other parts of the world, feeding rates are calculated for the whole of the growing season. An estimate of the food necessary for the fish to attain a predetermined weight is obtained by multiplying the expected weight gain by the food quotient or conversion factor for the pond. This estimate of the required food is not distributed evenly throughout the growing season but instead is divided into unequal monthly portions. This is because the carp, being a warmwater fish, increases its feeding activity with increasing environmental temperature. The feed is distributed in amounts that are proportional to the monthly temperature. Smaller amounts of food are fed at the begining of the year not only because of low temperatures but also because the fish are small as compared with their expected final weight and therefore the feed is fed proportionally (Huet, 1972). Such a schedule is shown in Table 2.3.

Feeding schedules can be further adapted to allow for fish of different marketable sizes. This is achieved by calculating the expected growth rates and then dividing the food into differing unequal amounts. The general trend is to produce a smaller fish, to feed larger amounts of food at the begining of the year whilst to produce larger fish, the majority of the feed is fed towards the end of the year. A table demonstrating this principal was developed by Walter (1934). There is however no indication of the amount of feed to be fed to each size and whether

Table 2.3. Feeding schedule for carp as used in Japan. (From Hora & Pillay, 1962).

Month.	Monthly amount fed as annual %.	Essential food.	Daily frequency.
Jan.	0		
Feb.	0		
Mar.	0		
Apr.	1	Wheat,pupae,soy waste.	1
May.	4	Mixed foods.	1-3
Jun.	15	Mixed foods,pupae.	3-6
Jul.	20	Silkworm pupae.	3-7
Aug.	30	Silkworm pupae.	5-9
Sep.	20	Silkworm pupae.	5-7
Oct.	9	Pupae,wheat,beans.	2-5
Nov.	1	Pupae,wheat,beans.	1
Dec.	0		

it is different or the same. Feeding reduced amounts of feed would seem a more efficient method of controlling the final weight of the fish rather than by distributing the same amount of food at differing times. Walters feeding schedule is shown in Table 2.4.

Flexibility is necessary in a schedule such as Walters so that feeding can be extended or shortened as changes in the environmental temperature occur. Feed can be distributed as many times as preferred, frequent feeding giving better conversions but increased labour costs (Huet, 1972).

The most complex feeding schedules are used when extensive culture is highly intensified. This happens in countries where growing seasons last for nearly the whole year due to naturally high water temperatures. An example of this is Israel where due to the warmer water, fish are stocked into ponds in numbers which are in excess of the normal carrying capacity of the pond. This results in a severe shortage of natural feed. To counter this, a higher protein supplement is supplied in the form of a pellet. This is complementary to the standard supplementary feed. Maximum growth is therefore produced when water temperatures are at their peak. The feeding schedule, such as that of Marek (1975), includes fish size, density and water temperatures amongst its variables. A small part of the whole schedule is shown in Table 2.5.

Table 2.4. Feeding schedule as developed by Walter (1934).
(From Huet, 1972).

Monthly feed as annual %.	Relationship between weight at time of stocking and weight at harvest as 1 to :					
	For C2 (fish of two summers).				For C1 (fish of one summer).	
	2.	2·5.	3.	4.	10.	20.
May.	15	13	11	9	-	-
June.	20	18	16	14	10	-
July.	25	24	23	21	20	25
August.	30	32	33	36	45	50
September.	10	13	17	20	25	25

Table 2.5. Complex feeding schedule (From Marek, 1975).

Size (g).	Density in thousands/ha. 2-4	4-6	6-8	- 50
20-50	-/1=1	-/2=2	1/1=2	
50-100	-/2=2	-/3=3	1/3=4	in g/day/fish at 24°C.
100-200	1/5=6	2/6=8	4/5=9	
1200				

where Pellets/Sorghum = Total

so 1g / 3g = 4g

1g of pellets is fed with 3g of sorghum to give 4g of feed in total. This table is for fish at 24°C. For temperatures 18-20°C, 50% of the amount shown is given, whilst for temperatures between 20-24°C, 70% of that shown is fed.

It can be seen that the methods involved in the feeding of pond fish are much more difficult than the conventional feeding of salmonid species. With those it is usually sufficient to consult a simple feeding chart and then distribute the feed to the fish. In extensive culture there is a considerable amount of 'feel' involved with the farmer being able to tell by experience the state of

the state of the pond and the amount of input required to maintain maximum production.

It is for these reasons that evaluation trials in ponds are extremely difficult. There is no sure way of ensuring that the feed is producing a response or whether the response is the result of some external influence. Only many reruns of any trial will sort this out and in this work, because of the time limits imposed, it is impossible to repeat the experiments.

The aim of this section is to look at the potential of bakery waste as a supplementary feed in extensive carp culture. Bakery wastes have been used in the past as fillers in complete rations for a variety of domestic animals (see section 1) but the only suggestion for their use in fish diets was by Schaperclaus (1933) who suggested that they would make a good supplementary feed for pond fish. He made no attempt to quantify his suggestion.

Bakery wastes would appear to fulfill the requirement of a supplementary feed as they are high in carbohydrate and fat which both supply energy. The waste is low in protein which makes it cheap to use.

The section consists of two trials, the first being a simulation experiment of pond conditions in the laboratory. The second being a pond trial on a working commercial farm. The trials were designed to find the optimum level of supplementary feeding to maximise fish production.

Tank simulation of pond feeding for the evaluation of bakery waste diets as supplementary feed.

MATERIALS & METHODS.

Diets.

Diets A and Q from earlier experiments were used as the test diets. Diet A was a commercial trout diet (Edward Baker Ltd, Omega no 4) which had been repelleted as a moist diet. Diet Q was a pellet comprising of 50% waste bread and 50% pie production waste. The proximate analyses of the two diets are shown in Table 2.6.

Experimental system.

A water recirculation system of eight tanks was maintained at 22^{o}C. Five tanks were each stocked with 15 fish of an average weight of 32g.

Feeding.

The aim of this experiment was to simulate the supplementary feeding of carp in ponds, however in order to feed at a supplementary level it is necessary to have another food source to supplement. In ponds this is the natural food which has a protein content of about 50%. In this experiment, diet A is used instead. This diet has a similar protein level to that of natural food. The difficulty lies in the calculation of the amount of the 'natural feed' to feed.

Table 2.6. Proximate analyses of the test diets (%).

	A.[1]	Q.[2]
Protein.	47·0	7·4
Fat.	8·0	9·4
Ash.	10·0	3·9
Nitrogen free extract.	27·0	41·4
Moisture.	8·0	37·7

1. Diet A - High protein commercial trout food (Edward Bker Ltd) represents natural food. Analysis is of original pellet before replleting as a moist diet with an average moisture content of 35%.

2. Diet Q - High energy pellet, 100% bakery waste represents supplementary feed. Pellet differs from original diet Q in that it lacks mineral & vitamin additives.

In ponds, natural food levels vary with changing conditions. In this trial, it was decided to eliminate many of these independent factors and instead link the amount of feed to be fed to the body weight of some control fish. This would increase as the experiment progressed and to some extent this does happen in a pond due to the continual cropping of the natural food and its reestablishment through fertilisation. Even so, towards the end of the season, levels of natural food do fall off in a well managed pond.

The control fish (tank 1) were fed at 1% of their body weight per day of dry diet A. This was adjusted to allow for moisture content. It was hoped that 1% was just over the maintenance requirement for the fish at 22^{o}C and that they would show slight growth. The amount fed was recalculated every week by weighing the fish. Half the amount of food fed to these fish was then fed to the other four tanks; this then represented the 'natural food' of the pond. The supplementary food (diet Q) was fed at three levels, 1%, 2% and 3% of body weight/day and this was also calculated weekly. The final tank received no supplementary feed. Supplementary feed was fed on only six days a week and natural food on seven. The five treatments were as follows:-

1. 1% body weight/day/dry diet A. - control.
2. 50% of amount fed to 1.
3. 50% of amount fed to 1 + 1% body weight/day/dry diet Q.
4. 50% of amount fed to 1 + 2% body weight/day/dry diet Q.
5. 50% of amount fed to 1 + 3% body weight/day/dry diet Q.

Treatments 1, 2 and 3 were fed twice a day and 4 and 5, three times a day. If the first three treatments were fed three times a day, then the amount of food given was so small that only one or two fish received the most of the feed. The experiment ran for 84 days.

RESULTS.

The performance of each treatment is shown in Table 2.7. It can be seen that the specific growth rates have increased with increasing amounts of supplementary feed. The same increase has also resulted in a decrease in the food conversion ratios. All the specific growth rates were significantly different ($p<0.05$) from each other whereas the food conversion ratios were not ($p>0.05$). The best performance was obtained by feeding 3% supplementary feed. This treatment had the lowest overall protein level of the total dietary input (Table 2.8.), although in fact the fish received the largest amount of protein.

Table 2.7. Performance of carp fed on the test diets for 84 days at 22°C. (n = 15 for each treatment).

Treatment. Feeding rate.	1. Control.	2. 0%	3. 1%	4. 2%	5. 3%	±SE.
Intial weight.	$32 \cdot 24^{a}$	$31 \cdot 87^{a}$	$31 \cdot 70^{a}$	$31 \cdot 35^{a}$	$31 \cdot 89^{a}$	1·461
Final weight.	$69 \cdot 07^{b}$	$46 \cdot 32^{a}$	$63 \cdot 38^{b}$	$82 \cdot 13^{c}$	$86 \cdot 08^{c}$	4·185
Av.Wt.Inc.[1]	$3 \cdot 07^{bc}$	$1 \cdot 20^{a}$	$2 \cdot 64^{b}$	$4 \cdot 23^{cd}$	$4 \cdot 52^{d}$	0·421
S.G.R.[2]	$0 \cdot 90^{a}$	$0 \cdot 44^{b}$	$0 \cdot 91^{c}$	$1 \cdot 14^{d}$	$1 \cdot 17^{e}$	0·084
F.C.R.[3]	$1 \cdot 97^{a}$	$1 \cdot 30^{a}$	$1 \cdot 97^{a}$	$1 \cdot 98^{a}$	$2 \cdot 50^{a}$	0·294

SE = Standard error.

1. = Average weekly weight increase. 2. = Specific growth rate.

3. = Food conversion ratio.

Statistic used was Duncans multiple range test. Superscripts of the same letter are not significantly different ($p<0 \cdot 05$)

Figure 10. Specific growth rate v Dietary protein level for supplemental diets.

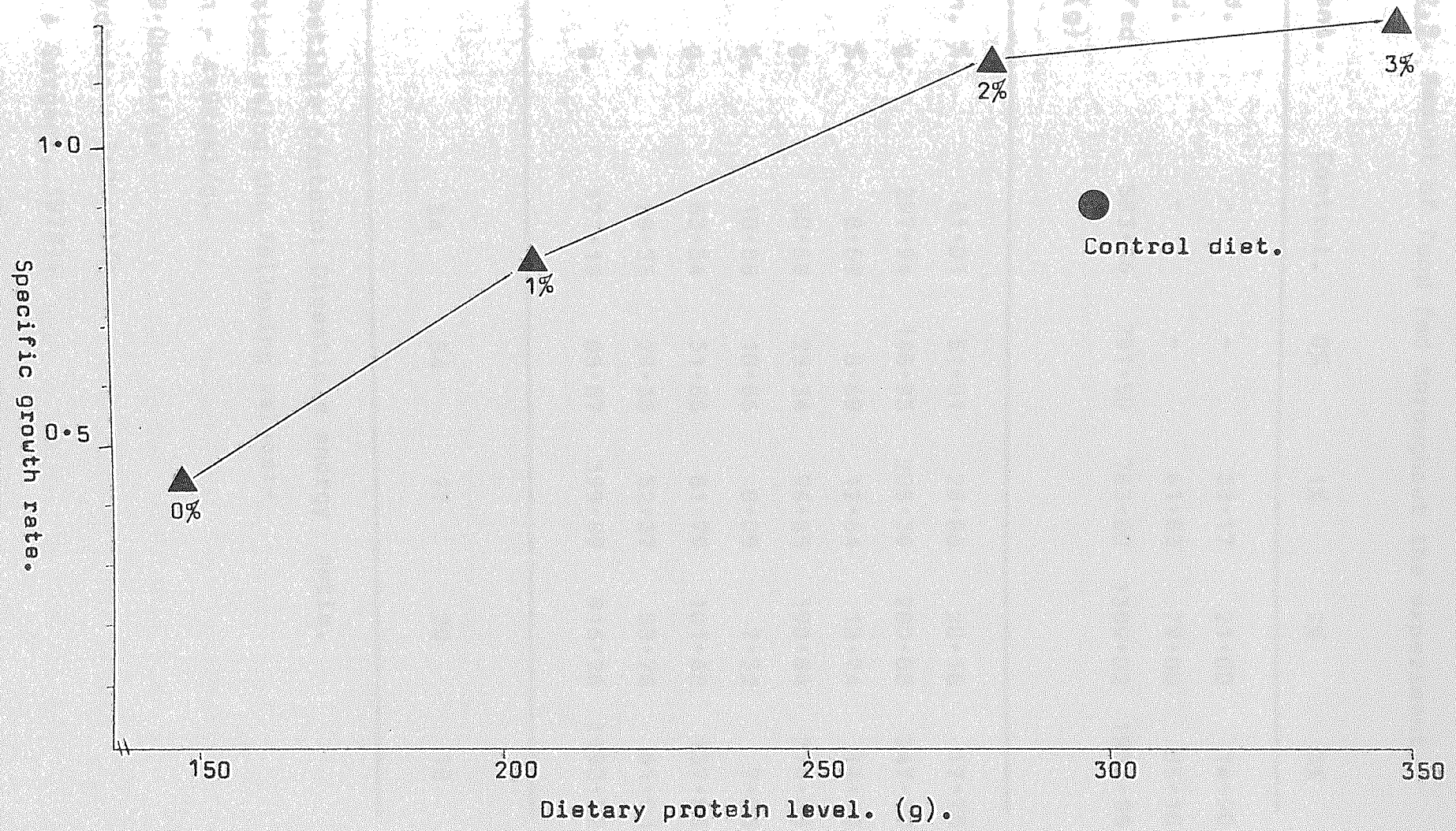


Table 2.8. Amount of food fed throughout the experiment.

Treatment.		Control.	0%	1%	2%	3%
% A fed.		-	-	38·17	21·00	14·90
% Q fed.		-	-	61·83	79·00	85·10
Total fed A + Q (g).		583·16	291·50	763·80	1389·02	1955·95
Protein.	%.	51·01	51·01	26·86	20·16	17·78
	g.	297·94	148·93	205·36	280·08	347·82
Fat.	%.	8·69	8·69	12·64	13·74	14·13
	g.	50·69	25·34	96·55	190·84	276·33
Ash.	%.	10·86	10·86	8·09	7·32	7·05
	g.	63·34	31·66	61·79	101·68	137·85
N.F.E.	%.	29·35	29·35	52·37	58·76	61·03
	g.	171·19	85·57	399·99	816·26	1193·72
PE/DE[1]		54	54	27	20	18

1. Digestible protein: Digestible energy ratio.

Calculated using the following values:-

Protein - 4·5kcal/g.

Fat - 9·0kcal/g.

Carbohydrate - 4·0kcal/g.

(Cowey & Sargent, 1979).

The specific growth rate can be linked with the weight of protein fed (Figure 10.) and at the higher levels of feeding, it can be seen that there was little effect in raising the specific growth rate. It can also be seen that the control diet falls well below the line. This is an indication of the effectiveness of protein sparing by supplementary feeds.

The difference between the treatments can also be seen in Figure 11 which shows the change in the amount of 'natural food' that each tank received. As the fish grew fast, then the amount of diet A (natural food) dropped and conversely when the fish grew slowly, then the amount of diet A increased.

Table 2.8. also shows the total amount of each feed mix as was fed to the different treatments. The table shows that although the treatment with the highest level of feeding received the most protein, it also received excessive amounts of dietary energy and therefore has a very low protein to energy ratio.

This is reflected in the body composition of the whole fish carcasses which are shown in table 2.9. As the feeding level is increased, body fat levels also increase and the difference between the four treatments is significant ($p<0.05$). The largest difference occurs between 1 and 2% feed rates. The increased fat levels are met by a decrease in body moisture, the levels of which are also significant ($p<0.05$).

Figure 11. Rate of change of amount of diet A fed to the experimental fish as a percentage of their body weight. (Diet A = 'Natural food').

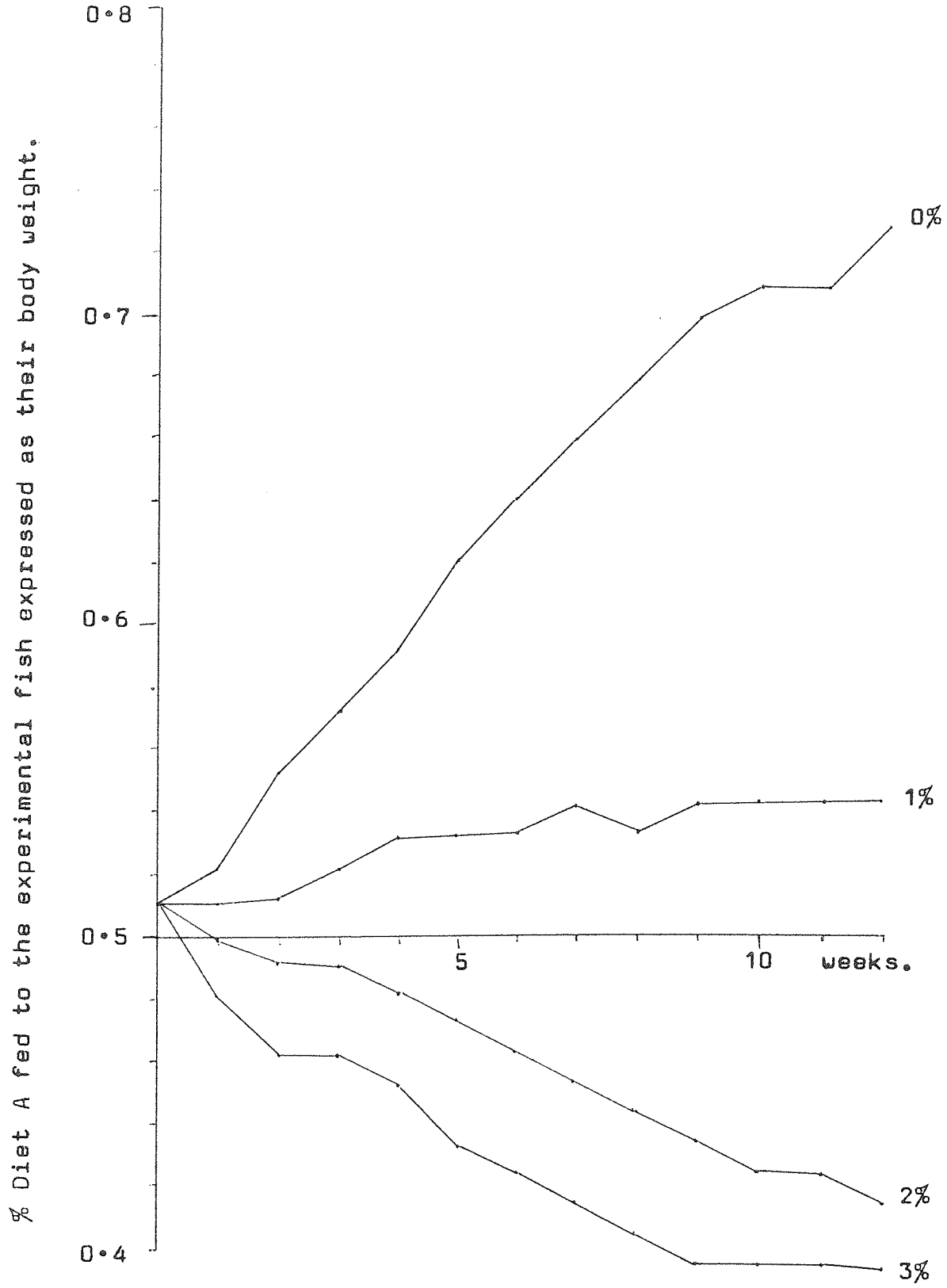


Table 2.9. Proximate analyses of whole fish carcasses from carp fed experimental diets at 22°C for 84 days. (n = 6 for each treatment.

Diet.	Control.	0%.	1%.
Moisture.	79·03±1·34^{a}	79·22±0·92^{a}	72·74±1·51^{b}
Protein.	12·80±1·69^{a}	13·01±0·98^{a}	13·69±0·78^{a}
Fat.	5·55±1·69^{a}	5·65±1·45^{a}	11·88±1·82^{b}
Ash.	2·12±2·18^{a}	1·62±0·78^{a}	1·18±0·36^{a}

	2%.	3%.	±SE.
Moisture.	67·86±1·43^{c}	65·60±1·46^{d}	0·6029
Protein.	12·38±0·97^{a}	13·60±1·52^{a}	0·5072
Fat.	18·18±1·38^{c}	19·38±2·23^{c}	0·7787
Ash.	1·13±0·67^{a}	0·91±0·34^{a}	0·4509

SE = Standard error.

abcd = Mean values of each component with the same superscripts are not significantly different (Duncans multiple range test $p<0·05$).

The use of bakery wastes as a supplementary feed in extensive carp ponds.

MATERIALS & METHODS.

Experimental ponds.

A pond of approximately 0·1 ha (0·25 acres) was divided into four sections of 20m by 17m using chicken wire supported by wooden posts. The dividers were dug into the pond bottom to prevent the fish from digging underneath and entering another section. The pond was filled to about a depth of one metre after lime (300Kg/ha) and fertiliser (animal manure,10 tonnes/ha) had been applied to the pond bottom. The pond was left alone for 3 weeks to allow a natural food population to develop before the fish were introduced.

Experimental fish.

Each of the four sections were stocked with 150 fish of average weight, 133g.

Diets.

A standard pellet prepared totally from bakery waste and containing 50% waste bread and 50% pie production waste was used in all the treatments. The proximate analysis of the feed was protein - 7%, fat - 9%, ash - 4%, N.F.E. - 40% and moisture - 40%. This varied slightly from batch to batch through differences in the nature of the waste.

Feeding.

The four sections were labelled A,B,C & D. The feeding of the experimental feed was carried out as follows:-

A - 2%. B - 3%. C - 0%. D - 1%.

This was varied with temeprature due to reduced feeding at low temperature and increased feeding at higher temperatures. A final feeding table was prepared as follows:-

	Section.			
	A.	B.	C.	D.
Temperature.				
$\rightarrow 10^{o}C$	-	-	-	-
$10- 18^{o}C$	1%	1·5%	-	0·5%
$18- 24^{o}C$	2%	3%	-	1%
$24^{o}C \rightarrow$	-	-	-	-

These percentages are a percentage of the body weight of the fish. This was calculated by monthly seining of the pond in order to sample the fish. This was backed up with estimates of fish weights calculated from the conversion of the feed already fed. Food fed was calculated on a dry weight basis and then converted to allow for the moisture content of the feed. Feed was fed on only six days a week. The experiment ran for 182 days.

Measurements.

Temperature, oxygen, pH and ammonia were all measured daily and recorded on a record sheet together with general comments on the running of the trial and overall weather conditions.

Additional note.

The conditions for running the trial were maintained as long as no major problem arose. If this should happen, then the decision on what action to take was left to the farm manager.

RESULTS.

Tables 2.10. - 2.15. present the results of the pond trial which ran from 23rd April 1981 until 21st October 1981 with feeding starting on May 5th and finishing on October 6th. Table 2.10. shows the amount of food fed to each section every month over the experimental period together with the final amount, both in moist and dry form.

Although the food fed was in the ratio of 1%, 2% and 3% of the weight of the standing crop, the total amount is in fact in excess of these figures due to differential feeding with some rates being more efficient than others.

Table 2.10. Monthly feeding to each section (Kg).

Section.	C.	D.	A.	B.
Feeding %.	0%.	1%.	2%.	3%.
April.	-	-	-	-
May.	-	4·1	7·4	11·8
June.	-	6·8	13·2	21·6
July.	-	13·5	29·3	48·8
August.	-	15·0	34·5	57·5
September.	-	8·5	19·7	24·0
October.	-	1·6	3·7	6·2
Total (moist).	-	49·5	107·8	169·9
Total (dry).	-	33·0	71·9	113·3

Figure 12 shows the average water temperatures upon which the feeding rates were based. The temperatures can be seen to follow the typical pattern of a British summer in that it is totally unpredictable. Water temperatures rarely exceeded $20^{o}C$ so growth rates were never as high as was expected.

Table 2.11. shows the estimated weights of the standing crop throughout the experimental period. Difficulties were encountered in accurately sampling the sections so a variety of methods were used to obtain the estimation. The estimates of the final weights were not that far from the actual final weights obtaibed so the methods employed were fairly succesful.

Table 2.12. presents an estimation of the total productivity of each section and of the total pond. This was calculated by multiplying the weight increase of the no feed section by 4 to give the production from the whole pond.

Table 2.13. shows the growth indices for each section. As accurate sampling was extremely difficult, there are few data available for statistical analyses. The growth indices are therefore lacking in this area however this is not the case for the final fish weights and as can be seen, there are significant differences between each section ($p<0.05$).

Figure 12. Average weekly water temperatures of the experimental pond during 1981.

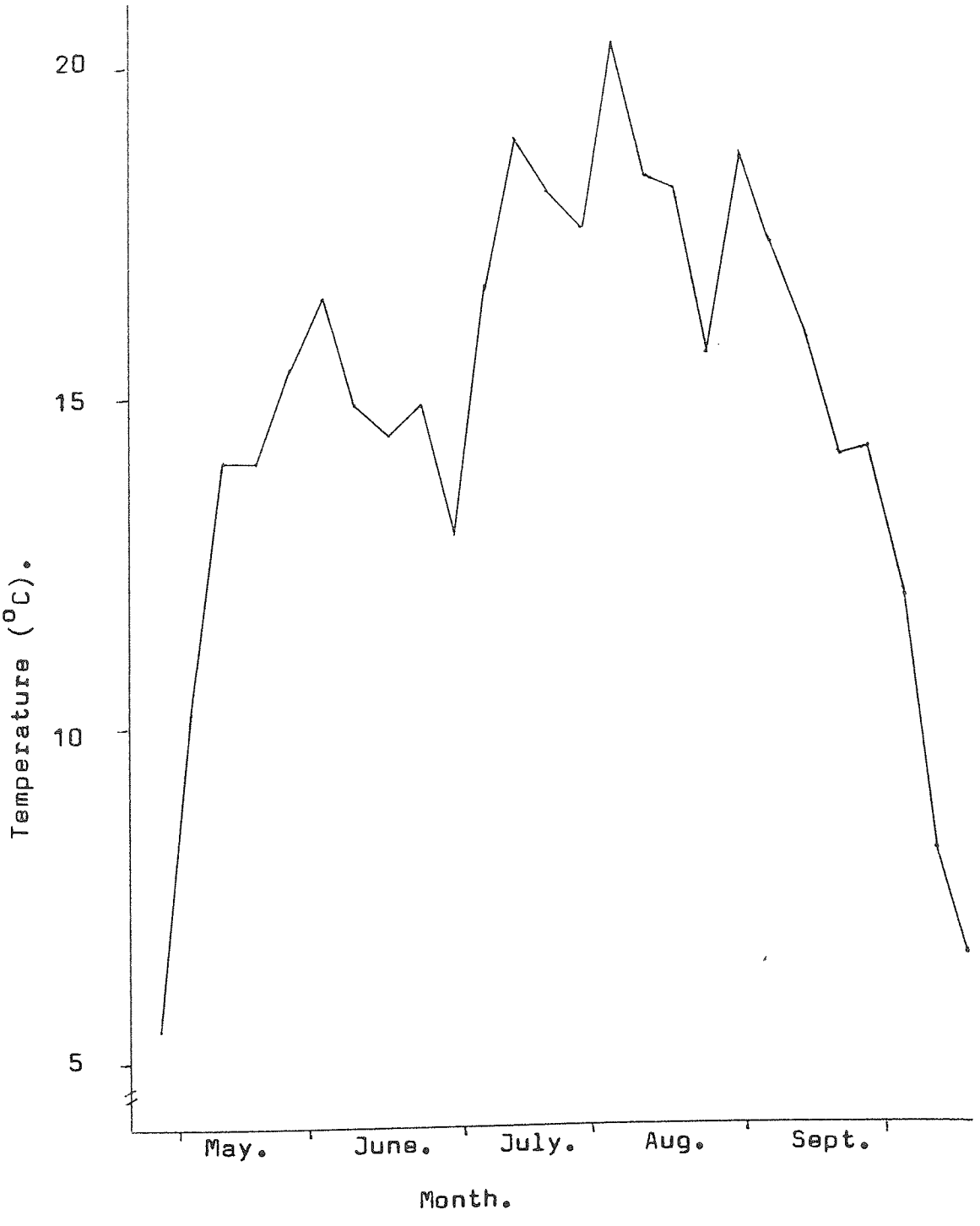


Table 2.11. Estimation of fish weights from throughout the pond trial (g).

Section.	C.	D.	A.	B.
Feeding %.	0%.	1%.	2%.	3%.
Date.				
15/4/81**	134•8(150)	131•4(150)	132•1(150)	134•8(150)
15/5/81*	141•4 (51)	159•7 (28)	134•8 (52)	145•1 (42)
11/6/81*	169•3 (12)	162•6 (5)	171•8 (55)	193•0 (30)
15/7/81*	193•9 (11)	225•8 (9)	203•2 (5)	216•4 (25)
29/7/81+	-	271•2	240•5	260•2
16/8/81+	-	328•4	290•3	318•6
18/8/81*	-	-	-	269•5 (8)
31/8/81+	-	367•4	324•5	358•5
22/10/81**	293•5 (150)	317•6 (150)	334•8 (150)	350•8 (150)

Weight given is of a single fish in grams.

Figures in parenthesis represent the number in the sample.

Superscripts represnt the method of estimation.

* = seine netting.

** = average weight of 10 fish measured at the start and end of the trial.

+ = estimation by food conversion.

Table 2.12. Estimation of natural productivity from the experimental pond.

Section.	C.	D.	A.	B.
Feeding %.	0%.	1%.	2%.	3%.
Total fish weight inc. (Kg).	23·80	27·93	30·41	32·41

Total natural productivity = 4(wt increase from section C).

= 4(23·80Kg).

= 95·22Kg/ pond.

= 380·88Kg/acre.

= 952·20Kg/ha.

Table 2.13. Performance of carp during the experimental pond trial.
(n = 15 for each treatment - 15 weighings of 10 fish each).

Section.	C.	D.	A.	B.	±SE
Feeding %.	0%.	1%.	2%.	3%.	
Intial weight.	134·79[a]	131·40[a]	132·08[a]	134·79[a]	3·98
Final weight.	293·49[a]	317·58[b]	334·82[c]	350·83[d]	5·21
Weight gain.	158·70	186·18	202·74	216·04	
Specific growth rate.	0·41	0·46	0·49	0·50	
Food conversion ratio.	-	1·18	2·36	3·49	

abcd = Mean values of each component with the same superscripts are not significantly different (Duncams multiple range test $p<0.05$).

Specific growth rates were seen to increase with increased feeding rates whereas food conversion ratios decreased.

Table 2.14. shows the contribution made by the supplementary feed in the conversion of dietary protein to body protein. This is illustrated by protein conversion ratios which not surprisingly follow the pattern of the whole pond conversion ratios.

Table 2.15. shows the proximate analyses of the body composition of the whole fish carcasses. Moisture levels in the body and also ash levels decreased with increasing feeding rate whilst protein and fat levels increased. Protein levels were not significantly different ($p>0.05$) however fat levels were significant ($p<0.05$) from each other.

Table 2.14. Performance of protein conversion indices.

Section.	C.	D.	A.	B.
Feeding %.	0%.	1%.	2%.	3%.
Total fish wt inc (Kg).	23·80	27·93	30·41	32·41
Dry food fed (Kg).	-	33·00	71·90	113·30
Protein in food (kg).	-	3·63	7·91	12·46
Conversion from food.	-	8·00	10·88	13·17
Total fish protein inc (Kg).	4·18	3·12	3·85	4·25
Protein from food (Kg).	-	0·46	0·84	1·13
Total protein conversion.	-	1·16	2·05	2·93
Protein conv from food.	-	7·87	9·46	11·05

Table 2.15. Proximate analyses of fish from the experimental pond trial. (n = 6 for each treatment).

Feeding %.	0%.	1%.	2%.	3%.	±SE.
Moisture.	$76 \cdot 01 \pm 3 \cdot 32^{b}$	$74 \cdot 91 \pm 2 \cdot 47^{ab}$	$71 \cdot 76 \pm 2 \cdot 41^{a}$	$71 \cdot 60 \pm 1 \cdot 65$	1·141
Protein.	$13 \cdot 76 \pm 2 \cdot 90^{a}$	$11 \cdot 19 \pm 4 \cdot 08^{a}$	$12 \cdot 65 \pm 2 \cdot 62^{a}$	$13 \cdot 12 \pm 1 \cdot 39^{a}$	1·268
Fat.	$6 \cdot 60 \pm 3 \cdot 38^{c}$	$8 \cdot 24 \pm 2 \cdot 87^{bc}$	$10 \cdot 96 \pm 2 \cdot 34^{ab}$	$13 \cdot 42 \pm 2 \cdot 18^{a}$	1·189
Ash.	$3 \cdot 12 \pm 2 \cdot 65^{ab}$	$5 \cdot 16 \pm 3 \cdot 52^{a}$	$4 \cdot 12 \pm 2 \cdot 86^{ab}$	$1 \cdot 35 \pm 1 \cdot 16^{a}$	1·099

SE = Standard error.

abc = Mean values for each component with the same superscripts are not significantly different (Duncans multiple range test $p<0 \cdot 05$).

DISCUSSION.

Both the pond and tank trials have produced similar results although the response was greater from the trial conducted in the tanks due to the greater control with which it was maintained. The results of the tank trial have shown that supplementary diets do have an important role to play in the nutrition of pond fish. Although the experiment was only a simulation of pond conditions, the inclusion of the energy components into the dietary input raised the growth rates significantly ($p<0.05$). This is in keeping with what would be expected in a conventional pond and in fact the results are similar to those of the pond trial.

The addition of supplementary feed at 1% to the 'natural feed' resulted in nearly a doubling of the specific growth rate. Further increases were seen when the feeding rate was increased to 2%, however raising the rate further to 3% had little extra effect. These improvements in growth rates amounted to 84% for the 1% feeding rate over the 0%, 159% for the 2% feeding rate and 166% for the 3%. These can be compared with the same figures for the pond trial which were 14.7%, 27.7% and 36.1%. For both experiments, the biggest gain was made by increasing the feeding rate from 1% to 2%. Little extra benefit was gained by raising the feeding level any further. In terms of actual weight

gains, the results of increasing the feeding rate are best shown by the pond trial. Natural productivity was found to be 952 Kg ha^{-1} and this was raised to 1117 Kg ha^{-1} by feeding 1% supplementary feed. 2% feeding produced 1217 Kg ha^{-1} and 3%, 1302 Kg ha^{-1}. Although the feeding rates were doubled and trebled, the growth rates did not match these increases. This was not unexpected as the diets did not contain high levels of the growth components, only those giving energy. The effect of these is to maximise the growth available from the natural feed and in this, the feed has been successful. The provision of excess energy as in the 3% feeding regime had no effect on growth and this can be demonstrated through the protein to energy ratio. This can only be shown in the tank trial as it is impossible to measure the overall protein value of the pond trial as the majority of the protein was tied up in the natural food. The ratios for the three feeding levels were as follows:- 1% - 27, 2% -20 and 3% - 18. The most effective growth was shown by the 2% feeding regime which had a ratio of only 20. This can be compared with work earlier in this thesis which showed that the optimum value for carp is in the region of 30. It must be assumed that PE/DE can change if the protein level is limiting.

The results shown by the tank trial were encouraging because of their similarity to the pond experiment. The trial was difficult to run because of the complex nature of the situation it was designed to simulate. The simulation was restricted to the estimation of the natural feed. If the estimation had been too high, then the fish would have had excess protein and the benefit of the supplementary feed would not have been apparent as the excess protein would have probably been used for energy rather than growth. In the opposite situation, where there is too little 'natural food', protein would have been limiting and any weight increase would be due to deposition of body fat rather than flesh.

The ideal situation for this simulation was where the fish were just growing with the protein only just becoming limiting. This was found to occur at half the food fed to the control fish although towards the end of the trial, this was seen to change as illustrated in Figure 11.

The analyses of the fish body composition support the view that at the higher feeding level, the energy component is in excess. This is apparent because of the high levels of body fat present in fish from both trials. At the lower feeding level, the energy is used more efficiently with the result that there is less body fat deposited. This is in keeping with the findings of Brett et al. (1969); Murray et al. (1977); Nijkamp

et al. (1974) and Sin (1973a; 1973b). Nijkamp et al. (1974) also demonstrated that a reduction in moisture to compensate for the extra body fat, an observation which was also made in this work.

The levels of fat in the body, especially those of the fish from the pond trial, are not high when compared with other pond fish. Meske (1973) reported that pond fish have often been found with body fat levels of up to 20%. The fish from this experiment are therefore well within this accepted limit.

There was a slight change observed in body protein level and a similar change was seen by Huisman et al. (1979) when fish were fed differing levels of the same diet.

These experiments have shown that bakery wastes can be used successfully in the extensive pond culture of carp. However supplementary feeding of bakery wastes only works when the 'natural feed' is constantly present and this depends on the natural productivity of the pond. The estimate of pond productivity is to some extent only an approximation since it is unknown how much nutrient drift occured between the sections. This was bound to occur since the divisions only prevented the fish from mixing and not the water and therefore also the nutrient supply.

It is probable that the supplementary feed supplied even more to the total nutrient supply than is suggested by the growth indices. This is because the figure for natural productivity seems very high, especially for Britain. Although to some extent the size of fish in the pond is important, Schaperclaus (1933) gives a maximum figure for pond productivity of 400 Kg/ha for fertile waters; this compares with 952 Kg/ha found in this experiment. The whole concept of productivity is so dependent on external factors that it is difficult to draw any conclusions from the high figure found here. However because the differential is so wide, the conclusion that must be drawn is that the feed did add to the natural productivity and the true figure will probably be lower than that given.

This raises a number of other issues which may have had some effect on the results of this experiment. The first is the question of using divided ponds for this trial. Due to commercial pressure on the farm, it was only possible for one pond to be allocated for experimental use during the summer of 1981 so either the experiment would have to be abandoned or a divided pond used instead. The use of divided ponds for experimentation has been discussed by Wohlfarth & Moav (1967), who compared the production from a series of divided and adjacent ponds in an experiment on fish stocking densities. They found

that the variance of differences was much higher in adjacent ponds than in those with a division. They stressed that this was only true in experiments with differing stocks of fish and when considering work that involved fertilisation or nutrition studies such as this one, then the interactions may be significantly different between the sections. This has to be borne in mind when considering this work although there is also the opposing view that the interactions between sections are not as great as the differences which may occur when separate ponds are used. This is illustrated by the fact that the pond next to the experimental pond in this work, which is structurally identical to the pond used, is consistently warmer by about $1^{o}C$ than the experimental pond. However, even small differences in temperature like this are known to produce significant differences in growth throughout a growing season. In this case, the differences are due to slight shading by distant trees. Even without such a physical cause for the differences, variations between a series of ponds was measured by Buck et al. (1970) who found significant differences in replicate experiments.

Another controlling factor is that of stocking density and this can drastically affect the growth rates because of the demand on protein supply.

The cultivation of fish in ponds is usually thought of as always being a form of extensive farming but this is not always the case since if stocking levels are very high, the demands on the protein supply become excessive and supplementary protein has to be added into the system. This can reach extreme situations in which the fish crop out all the natural food and therefore all the available protein. The protein requirement of the fish has then to be met from external sources and the system becomes intensive.

There are few suggestions as to the correct level of stocking when the literature is consulted since every pond has its own potential productivity and the stocking density depends on this level. Recommendations as to actual figures are therefore very difficult to make. Even if productivity could be successfully estimated, stocking levels are still very difficult to predict since no forecast of the total productivity for any year can be really accurate as the physical conditions which govern the productivity can change from year to year. Despite these problems, there have been some suggestions for approximate numbers of fish to stock. On a three year growing cycle, German farmers usually stock second year fish,ie those approaching

market size, at a density of between 300 and 800 per hectare. These fish are an average weight of between 200 and 500 g. The exact weight will govern the final density. After the third summer, the fish should reach a market size of 1 - 1·5 Kg (von Lukowicz, 1974).

In warmer areas of Europe, efforts have been made to reduce the growing season to two years which means that the final year fish will be smaller. In order to offset the reduction in growth and produce fish of market size at the end of the season, the stocking density has to be reduced. Such a reduction in density has a positive effect on individual fish growth. Walter (1934) and van Oven (1957) carried out experiments to monitor the effects of stocking density on fish growth and they found that when plotted on a log-log scale, a straight line was produced. This reaches a maximum point beyond which individual growth rates quickly drop to zero. This level is the carrying capacity of the pond which is the point at which the maximum weight of fish can be sustained (Hepher, 1969a).

In this experiment, the fish used were early C2 (second summer fish) and therefore are similar to the first year fish of a two year growing cycle. Due to commercial pressures, the stocking density was high with 6000 fish/ha stocked and consequently

the individual growth rates of the fish were low. If the density had been reduced to 2000 fish/ha, then the final weights for individual fish in each section would have been 0 - 611g, 1 - 690g, 2 - 740g and 3 - 783g. These figures are estimates only and the final weights if such a stocking density had been used would probably be a lot lower. A further reduction to the levels as suggested by von Lukowicz (1974) would have produced fish of 0 - 1·72Kg, 1 - 1·73Kg, 2 - 2·16 Kg and 3 - 2·3Kg. It can be seen that stocking density can be very important and a balance between it and other factors has to be found to produce realistic growth rates.

Of major importance to this experiment is that of sampling technique especially in the estimation of the standing crop. As mentioned in the introduction of this section, Swingle (1967) illustrated the difficulty of estimating the weight of fish in a given pond with examples of estimation by seining and food quotients. He compared these with the final weights of the fish found by draining the pond and found that both methods gave results of equal accuracy. As he stated, both methods when used together probably give the best overall estimate of the standing crop. In this study, it can be seen that the number of fish caught in each seining

decreased as the experiment progressed (Table 2.11.). It is possible that the seine net was too light for the size of fish or that as the pond was netted, the fish learnt to bury themselves into the soft mud at the bottom of the pond and so evade capture.

Whatever the reason for the failure to capture the fish, the problem of escaping fish is a major one, so much so that there have been a number of studies outlining the problem and suggesting solutions eg, by the use of genetic manipulation. One of these is the work of Wolhfarth et al. (1975) who examined the different strains of domestic carp and their ability to escape from the seine net. Whatever the reason for the reduced captures, the problem of accurate estimation by netting increased with the progession of the experiment. Estimation by food quotient was used instead however this was not a major problem as the estimates used by this method did reflect the state of the standing crop. For future experiments of a similar nature, it would seem best to use both methods together as suggested by Swingle (1967) and not rely on either one at any one time.

CONCLUSIONS - Section 2.

This section comprises of two experiments which although have the same objectives, were conducted under totally different conditions.

The first experiment was a trial to measure the effects of a high energy ration made from bakery wastes when introduced into a controlled system where protein levels were only just limiting. The results indicate the beneficial effect of the suplementary feed and also show that the effects diminish when the feeding level is increased.

The second experiment was in principle a repeat of the first although it took place in a traditional carp pond which is a partially uncontrolled system. This experiment was subject to influences from several external factors. The results were however very similar to those of the first experiment and showed that bakery wastes make an excellent supplementary feed for use in extensive pond culture.

The nutritional profile of the waste is ideal for a role of a supplementary feed and the variability that makes it unsuitable for use in complete rations, poses no real problem when used as a supplementary feed as the variability will be masked by the size of the ponds.

SECTION 3.

The development of a stable pellet from raw bakery waste.

INTRODUCTION.

There are many types of fish culture taking place in the world today. These are divided principally into two categories, intensive and extensive. Fish raised in intensive culture, ie in small confinement at high density must recieve all their nutrients and energy from specially prepared complet diets (Nose, 1979).

Up to thirty years ago most hatchery production diets consisted of fish or slaughter house waste. By the 1950s more economical meat meal diets were devised by blending dry meals with meat (Phillips, 1956). These were developed into nutritionally balanced diets such as that as the Oregon Moist Pellet (OMP) (Hublou, 1963). Further research resulted in the development of successful formulae for dry diets. Although dry diets were developed later than semi-moist pellets, they will be considered first here.

The first successful dry pellet used in raising trout was described by Phillips et al. (1964). This was a complete balanced diet of known formulation. A typical formula for a high protein diet for trout would be that of the Abernathy diet which contains fishmeal, cotton-seed meal, dried whey, wheat germ, soybean oil and a vitamin supplement. The protein level of the finished pellet would be about 45% (Fowler & Burrows, 1971).

For warmwater fish, low protein diets have been developed because the fish are able to obtain most of their protein requirements from natural feeds. Warmwater fish diets therefore tend to be of a supplemental type (Nose, 1979). There are some cases where warmwater fish are grown in intensive culture and for these fish, complete rations have been developed usually from the same type of ingredients as used in trout rations. The protein content of these diets is about 32% (NAS, 1977).

Dry diets can be presented in various forms such as hard pellets, expanded pellets, crumbles, blocks and agglomerates (Hastings, 1969). The most widely used form is the hard pellet. These are produced using a feed pelleting mill which involves the following processes:- conditioning of the feed mixture with steam, compression by rolling the feed through holes in a die ring, cutting the extruded pellets, cooling followed by drying. The conditioning process adds about 5-6% water through the use of steam and raises the temperature to 85^{o}C. Compression and extrusion increases the temperature further to 90^{o}C. Extruded pellets can be air dried within 5 minutes (Hastings, 1969).

Some species of fish do not adjust to feeding on hard pellets and prefer something softer. Although hard

pellets were developed after moist and semi-moist pellets, the softer pellets are still very popular amongst fish culturists.

Semi-moist pellets were developed in order to improve conversion, decrease cost and improve the water quality of the culture system over wet foods (Hastings, 1969). Tunison (1940) developed the first meat-meal diet which was known as the Cortland No 6 formula. The meal portion contained fishmeal, dried skimmed milk, cottonseed meal and wheat middlings. This, when mixed with meat could be extruded through a ricer and could be stored for several days at 3^{o}C. The best known semi-moist diet was produced by Hublou (1963) and is known as the Oregon Moist Pellet (OMP). The basic formula consisted of 40% meat and 60% meal. Average moisture content was between 35-40% and the diet was frozen until required.

Many variations have been made from this diet such as those of Crawford & Law (1972) in which different fish species were used for the wet part of the diet. The moist pellet has the advantage that it can utilise wastes generated by the fish industry without any supplementary processing and so it can be adapted to utilise protein sources that have so far been discarded (Nose, 1979).

Production of semi-moist pellets is similar to that of dry pellets except the mixture has a high

moisture content. This means that the mixture cannot be used in a pelleting mill as these cannot cope with diets of high moisture contents. Instead the mixture is pelleted in a spaghetti production machine (Ellis, 1969) or in the mincer attachment of a food mixer (Solber, 1976). Once long worm-like strands are formed they can be cut to the required length and quick frozen for storage in a freezer (Phillips, 1956; Hastings, 1969). In order to avoid high costs of storage of frozen feeds, semi-moist diets used in commercial situations are generally made on site and are used within a day or two of manufacture (Webber & Huguenin, 1979).

It can be seen that the production of a viable finished pellet from an experimental test product can be undertaken in a variety of ways however, the physical nature of the test material often dictates the type of pellet that can be produced especially when commercial requirements are considered.

Hard pellets are the simplest to make, requiring only the use of a pelleting machine however all the ingredients have to be dry for the machine to work. This excludes diets made from bakery wastes as the moisture content is far in excess of the maximum that can be used in a pelleting mill. Hard pellets can alternatively be made from moist ingredients

by using a simple mixing and mincing machine however the resulting pellets have to be dried to remove the excess moisture. Alternatively the ingredients could be dried first before pelleting in a mill. Both these methods would be suitable for pelleting bakery wastes however the cost of drying the ingredients or the pellets, exceeds the value of the waste.

Production of a finished pellet within the constraints of not using heat for drying restricts the finished product to a semi-moist or moist pellet. Of these, the former shows the most promise since moist diets ususally have a much higher moisture content than those found in bakery wastes.

It is for these reasons that semi-moist pellets would appear to be the most suitable medium for presenting bakery wastes to the fish. There are a number of problems that prevent the waste from being directly used in a semi-moist pellet. The first of these is the removal of inedible parts of the waste. Pie production waste is a mixture of dough, meat filling and aluminium foil trays. The latter constitute part of the wrapping of the finished meat pie. During production, the rest of the ingredients are added to the foils so that they are always present.

The second problem is to remove any excess moisture from the waste without the use of expensive heat. Any

reduction in the overall moisture would be of benefit to the final product in order to reduce storage problems.

The third problem is that of storage. Food quickly deteriorates with passing time. This process can be slowed by cooling or freezing or by the addition of anti-microbial agents. Some semi-moist foods such as petfoodscan be stored for long periods of time and such a capability would be an advantage for a supplementary feed.

The final question to be considered is the stability of the finished product once it has been distributed to the fish in the water. Carp are slow eaters and the pellets may remain on the bottom for a number of hours before they are consumed. The pellets should be resistant to disintegration for as long as possible.

Thus, it can be seen that there are a number of difficulties to overcome before a working pellet can be produced. These aspects are considered in this section.

Elimination of inedible material from pie wastes.

INTRODUCTION.

Meat pie waste is a variable mixture of pie filling, uncooked pastry and aluminium foil cases. The variability of the mixture depends on the stage at which the waste is discarded. The aluminium foil waste results from a breakdown in production and can consist of aluminium foil cases alone or with variable amounts of filling. The foil waste represents only a small percentage of the total waste produced, the majority being either only pastry or only filling.

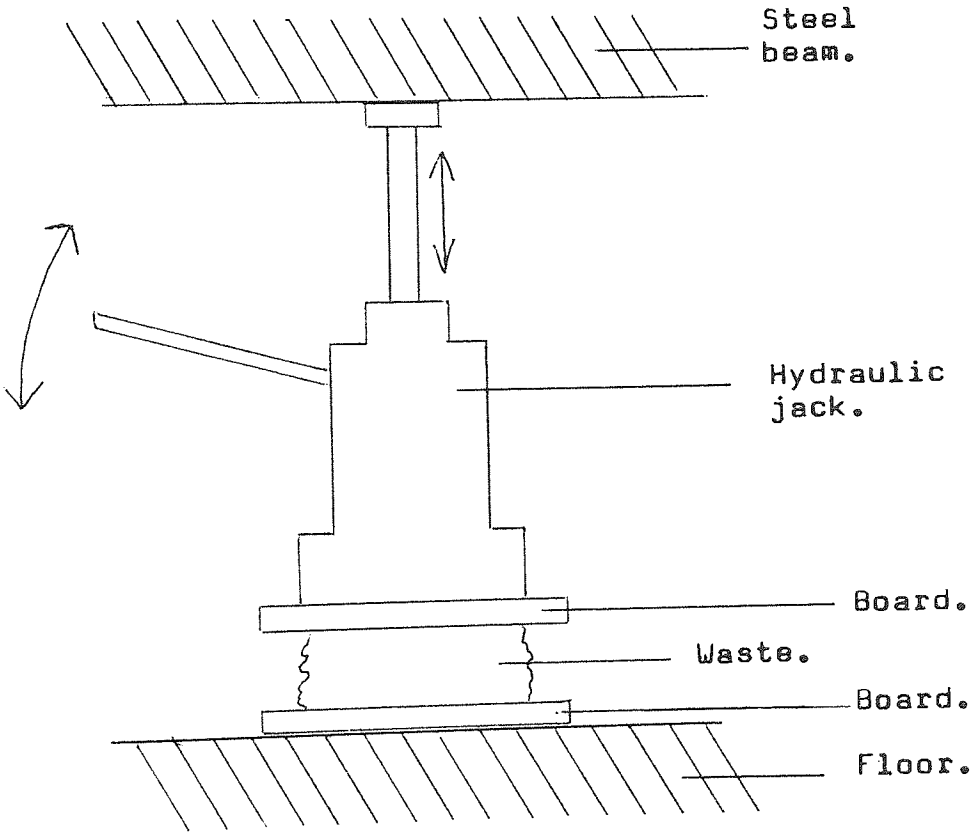
The present experiment aimed to determine whether the edible portion of the foil waste could be recovered. A number of preliminary trials were held to find the best method of removing the foil from the waste. One idea was to make the waste into a type of silage by the addition of 3%w/w formic acid. The foil dissolved in the acid, however associated problems occured which were impossible to overcome, such as toxicity. A second idea was to shred the waste to such a small size that the fish would ingest them and then after passage through the gut, excrete them. It was found that the foil would not shred into small enough pieces and that the edges left were very sharp and so would rip the tissues of the gut as it passed through.

A final idea was to press the mixture with such a force that the edible portion of the waste would be extruded from the foil and leave it behind. This experiment is designed to measure the effectiveness of such a system.

MATERIALS & METHODS.

A simple hydraulic press was constructed by placing a one ton hydraulic car jack under a horizontal steel beam which was firmly attached to a wall. Two wooden boards (30x30x1cm) were located underneath the base of the jack and between them, a preweighed sample of waste was placed (Figure 13). The jack was pumped by its handle so that pressure was applied to the waste causing the more flowing elements to be forced out. This was continued until no further movement of the waste could be observed. The pressure was then released and the remaining mixture of foils and pie were removed and weighed. The foils were separated out and washed clean and then weighed.

Figure 13. Arrangement of hydraulic jack for pressing pie waste to extract aluminium foil cases.



RESULTS.

The results of the pressing trial can be seen in Table 3.1. The table shows the weight of the foils as a percentage of the pre- and postpressing weights of the pie waste. Although the foils account for a greater proportion of the volume of the waste, they only account for 4% of the prepressing weight and 8% of the post-pressing weight. This increase in the percentage weight is significantly different ($p<0.05$) and amounts to 100% increase. The amount of the edible pie waste recovered is about 50%, the remaining 50% remains caught within the foils.

DISCUSSION.

The extraction of the aluminium foils from the pie waste is a major problem which is most effectively solved by manual removal. This however, is a very labour intensive operation as well as being hazardous. Mechanical separation is very difficult as the foils are randomly spread throughout the waste and are often bent around each other. Removal by pressing allowed for the pie to be squeezed out from within the bent foil cases, although a large proportion remained behind. One advantage of the pressing method

Table 3.1. Results of the trial to extract foil trays from the bakery waste by pressing with a hydraulic jack.

Pressing.	Weight of foil as % of the waste.
Prepressing.	$4 \cdot 03 \pm 0 \cdot 52^{a}$
Postpressing.	$8 \cdot 46 \pm 2 \cdot 07^{b}$

ab = Mean values of six replicates of the components with the same superscripts are not significantly different (Students t - test $p < 0 \cdot 05$)

is that the fraction that is expelled by the pressure of the jack tended to be the filling which is of a higher nutritional value than the less mobile pastry. Although pressing appears to be quite effective, there is some question whether a scaled up version would be equally efficient, since the resistance of the mixture to pressure will be increased with increasing amounts of waste. Also open to question, is whether the foil waste is worth recovering as it represents only a small portion of the total waste. At the time of writing, no unit is in operation and all separation remains manual.

Reduction of moisture in waste bread.

INTRODUCTION.

Previous work dealing with the preparation of intermediate moisture feeds has always involved the incorporation of a moist and a dry ingredient to produce an intermediate moisture product (Solberg, 1979). The dry ingredient soaks up the excess moisture from the moist ingredients and this binds the two components together. With fish diets made from one hundred percent bakery waste, this does not occur since the two major ingredients are both intremediate moisture products with an average moisture content of 38%. The waste is however very variable in consistency due to the unpredictability of production breakdowns which cause the wastage. This difficulty applies mainly to the pie waste which can range from all filling, which has a high moisture content of up to 75%, to all pastry, which has a low moisture content of 25%. It is therefore possible that the pie portion of the diet can be classed as a moist or wet ingredient to be mixed with it to remove excess moisture. As the bread which is the other main ingredient is also semi-moist, it would

help if it could be dried to some extent. The use of heat for the drying of waste feeds is expensive and uneconomic however it was found that bread left at room temperature for a day or two lost a lot of its moisture and when used in the preparation of the diets, mixing was found to be a lot easier. This applies only to supplementary diets made from 100% bakery wastes since in complete diets, the addition of other dried meals aided the mixing process.

This experiment is designed to show that excess moisture can be removed from waste bread without the need for expensive high grade heat.

MATERIALS & METHODS.

A standard large white sliced loaf of bread was divided into three portions of six slices each. The first portion was placed into an oven at 105^{o}C to measure the moisture content by drying to constant weight. The second portion was weighed and then spread out onto a tray and left exposed at room temperature. After 24 hours, the slices were dried in the oven as before. The third portion was also left at room temperature but a fan was sited next to it so that air was blown over them for 24 hours. The slices were then measured for moisture content.

Table 3.2. Moisture content of sliced bread dried in air. (n = 6 for each treatment).

Type of bread.	Moisture content (%).
Fresh.	$39 \cdot 71 \pm 0 \cdot 32^{a}$
Air dried for 24 hours.	$23 \cdot 54 \pm 0 \cdot 36^{b}$
Blow dried with air for 24 hours.	$15 \cdot 96 \pm 0 \cdot 23^{c}$
±SE.	0·1509

SE = Standard error.

abc = Mean values for components with the same superscripts are not significantly different (Duncans multiple range test $p<0 \cdot 05$).

RESULTS.

The results of this experiment are shown in Table 3.2. The moisture content can be seen to be significantly lower ($p<0.05$) for both methods of drying. The moisture content of the slices was reduced by 16% by leaving them exposed at normal room temperature and a further 7% reduction was gained by using a fan to blow air over the slices resulting in a total reduction of 23% over the moisture content of fresh bread.

DISCUSSION.

The effect of air drying bread can be seen to be very significant ($p<0.05$) with a 40% reduction by simple air drying and a 60% reduction by blowing unheated air over the slices. A reduction in the moisture content of bread is advantageous when preparing bakery waste pellets containing large amounts of pie waste because the dried bread absorbs the excess moisture and aids binding. The economics involved in the use of bakery wastes dictates that heated drying is impossible, so simple air drying becomes a realistic cheaper alternative.

Preservation of bakery waste diets.

INTRODUCTION.

Fish feeds are classed by their moisture contents into three types, dry - moisture content less than 20%, intermediate - moisture content between 20% and 50%, and moist - moisture content over 50% (Solberg, 1979). An intermediate moisture food is one that is moist enough to be ready to eat and yet dry enough to be shelf stable (Kaplow, 1970). For shelf stability, intermediate moisture foods require to be stored without refridgeration or freezing in order to avoid the growth of micr-organisms. Due to their high moisture content as compared with dry feeds, intermediate moisture feeds are very suseptible to microbial growth. Reduction of moisture levels to avoid the growth of these organisms is a very old method of preserving the food.

Many of the earliest preserved foods were sun dried to achieve the necessary reduction in water level or were mixed with ingredients that reduced the water activity, eg salt (Karel, 1976). The actual level of moisture is not that important in classifying intermediate moisture feeds since there are foods with only 10% moisture which are not microbiologically stable whilst there are others with 25% which are. Water activity is therefore a much better method for

defining intermediate moisture foods.

Micro-organisms require an abundant supply of water to grow. Depriving these organisms of a sufficient amount of water is an effective way of prohibiting their growth. One method of measuring the amount of water available for growth is by calculating the water activity. This is defined as the vapour pressure of water above that of the feed (Kaplow, 1970). Solberg's (1979) classification of feeds using moisture content can be applied to water activity also. Thus, moist feed has a water activity (aw) of 1·0-0·9, intermediate moisture feed, an aw of 0·9-0·6 and low moisture feeds, an aw of 0·6-0·0 (Leistner & Rodel, 1976). Most organisms occuring in feeds, occur at high water activities, ie above 0·9, however yeasts can usually tolerate lower levels of about 0·88 and moulds at about 0·8. Leistner & Rodel (1976) give a list of the water activities for the multiplication of most micro-organisms associated with foods. The lowest level given is for a mould which can grow at a water activity of 0·62. This means that only dry feeds will escape the need for some form of preservative to prevent the growth of micro-organisms. However, in many cases there is a constraint on the use of dry diets either for economic reasons or that the fish species grown will not adapt to dry diets. For these reasons, the use of preservatives in fish diets is still necessary.

The requirements of such preservatives are that they inhibit or retard the growth of micro-organisms or any deterioration of the food due to the presence of micro-organisms (Tilbury, 1980) as well as being palatable and non toxic to the fish. One method of preventing the growth of such micro-organisms is by the use of humectants which lower the water activity whilst allowing high moisture contents. Such compounds include polymers, glycerol, sugar and salt, however these all present problems with taste (Karel, 1976). One compound which has shown promise as a humectant is propylene glycol (Kaplow, 1970). It also has the added property of being an anti-mycotic agenet which will resist bacterial growth although not that of moulds and yeasts. The use of anti-mycotic agents is another method of preventing the growth of micro-organisms. Potassium sorbate has been widely used especially together with propylene glycol, so that they both constitute an anti-mycotic system that protects a product against moulds and yeasts (Kaplow, 1970). A reduction in pH to a slightly acid level of about 5-6 also helps in the inhibition of bacterial growth, but not the growth of moulds and yeasts (Leistner & Rodel, 1976) therefore the pH of the feed should be as low as palatability permits.

Most of the work on intermediate moisture feeds has been carried out on human and pet foods, although there have been some attempts at preserving moist fish feeds. Crawford et al. (1973) used potassium sorbate as an anti-mycotic agent in a modified Oregon Moist Pellet for chinook salmon. Results were encouraging but not that good. Potassium sorbate was included in the diet at a level of 0·8% of the total. This did effectively inhibit microbial growth when the diet was stored at 70°F but feed consumption was reduced. This was mainly due to enzyme mediated reactions occuring in the food. Preliminary trials with carp fed bakery waste diets treated with propylene glycol and/or potassium sorbate showed that the fish were reluctant to accept the feed and it was therefore concluded that these types of preservative were unsuitable for this kind of diet.

Other recent developments in the preservation of moist rations involve the use of fermentation techniques which result in a high quality product with a shelf life of about 30 days at ambient temperatures (Webber & Huguenin, 1979). Details of this system are not yet published. There are however two other presevation systems which are worth considering for use in fish diets. The first of these is preservation by acid as mentioned earlier.

The use of acids for the preservation of feedstuffs, both for animal and human consumption is widespread. For the latter, strict controls are enforced by government regulations in order to prevent adulteration of foods. These regulations are relaxed where animal feedstuffs are concerned and certain acids that are prohibited for human consumption are widely used in animal feeds. Formic acid is an example of such an acid. For human use, acetic and citric acids carry no restriction concerning their use and sorbic and benzoic acids are allowed but carry some restrictions. Propionic acid has a limited use in its salt form as an anti-mycotic agent in bread and flour confectionary (Preservatives in food, Regulations 1979 SI No 752).

In animal feedstuffs, acids were first used in the 1920s in Finland, when a mixture of sulphuric and hydrochloric acids were used to treat green fodder. Work on the treatment of trash fish with acids started in Sweden in 1936. Treatment with acids resulted not only in the preservation of these materials, but also caused a change in their physical state. The natural enzymes in the material helped by the acidic environment, broke down the material to form a liquid product called silage. Little is known about the process of liquifaction but the result is a change in the nutritional components of the

material. Many different acids or combinations of acids have been used but formic is found to work at a higher pH and requires no neutralisation (Tatterson & Windsor, 1974; 1975). Although bakery wastes are cooked and therefore contain no viable enzymes, the concept of acid preservation was an attractive idea because of the ease of application.

The other potential preservation system is based on very old methods and that is preservation by salt. This early method of lowering water activity was known to the Ancient Greeks. An example of its use was the salting of meat on sailing ships (Tilbury, 1980). After the discovery of micro-organisms, the effect of salt on microbial growth was found to correlate well with its use to preserve foods (Measures & Gould, 1976). Rockwell & Ebertz (1924) showed that salt dehydrates any biological material that it comes into contact with, so explaining how a reduction in water activity is achieved. The salt acts as a humectant and can be used in a variety of ways, either at low concentrations (2-4%) when it works effectively in combination with low temperatures or with acid. It can also be used at higher concentrations when it is used in such preservation techniques as brining (Sinskey, 1980). One problem that often occurs when using humectants such as salt is that of associated taste problems (Karel, 1976).

Salt is extremely useful as a preservative because of its ease of handling. Aother similar preservative in common use is calcium propionate. Calcium propionate is a permitted food preservative which is used as an anti-mould agent in bread and flour confectionary (Sawyer & Crosby, 1980) and is therefore already present in the waste although in very small quantities.

Both these preservatives are readily available and easy to work with. These experiments are designed to investigate the potential of a number of preservatives for use in bakery waste diets either on their own or in combination. Both acceptability to the fish and prevention of microbial growth are considered.

Palatability of bakery waste diets preserved with acids.

MATERIALS & METHODS.

Diets.

Standard bakery waste diets were prepared from 35% air dried bread and 65% pie waste (equivalent to 50% moist bread and 50% pie waste) and with a proximate analysis of:- protein - 7%, fat - 9%, ash - 4%, N.F.E. - 40% and moisture - 40%. During preparation, acid was added as a fixed percentage of the pie waste. A variety of acids were used so that a whole range of diets was prepared (Table 3.3.).

The pH of each diet was measured by mixing a fixed weight of pellet in a measured quantity of deionised water. The approximate pH was determined using litmus paper and the values are shown in Table 3.3. The pellets were stored in a freezer and removed daily for feeding.

Determination of acceptability of the diets.

Each type of pellet was fed to a number of fish over a one or two day period. The pellets were either accepted or rejected by the fish. In the case of rejection, the fish were seen to injest the pellet and then spit it out. The diets that were accepted are shown in Table 3.3.

Table 3.3. Total range of acid preserved diets prepared for testing and pH values.

% inclusion in pie waste.	0·1	0·5	1·0	2·0	3·0
% inclusion in diet.	0·065	0·325	0·65	1·30	1·95
Acid.					
Formic acid.	5·1*	4·8	4·5	3·8	3·5
Acetic acid.	5·1*	4·9	4·8	4·6	4·2
Hydrochloric acid.	5·1*	4·8*	3·5	-	-
Sulphuric acid.	5·0*	3·5	-	-	-

* = Diets accepted by the fish and used in the feeding trial.

Fish and feeding.

Six fish were used in each treatment. The feed was fed at a rate of 1% of body weight day^{-1} dry feed equivalent for six days a week.

The diets fed are shown in table 3.3. and they were stored in the freezer and removed on the day of feeding.

The experiment ran for 42 days.

RESULTS.

In all the treatments, the fish soon became accustomed to accepting the diets and consumed nearly all that was given even though the diets had a very strong acidic odour.

The results of the trial are shown in Table 3.4. For all the indices, none of the treatments were significantly different from each other ($p<0.05$). The best growth was shown by the diet containing 0.1% hydrochloric acid which together with the 0.1% acetic acid diet performed better than the control although the differences are very small. Diets containing formic acid and the highest level of hydrochloric acid exhibited the worst growth. Specific growth rates and food conversion ratios followed the same pattern as the average weekly weight gains.

Table 3.4. Palatability of acid preserved bakery waste diets fed at 18°C.

(n = 6 for each treatment).

Diets.	0.	0·1%F.[1]	0·1%A.[2]	0·1%Cl.[3]	0·5%Cl.[3]	0·1%S.[4]	±SE.
Intial Wt.	111·24[a]	111·63[a]	112·58[a]	112·83[a]	115·78[a]	114·85[a]	13·338
Final Wt.	122·07[a]	119·09[a]	124·88[a]	126·43[a]	124·22[a]	126·87[a]	14·302
Av.Wt.Inc.	1·80[a]	1·18[a]	2·05[a]	2·27[a]	1·41[a]	2·00[a]	0·367
S.G.R.	0·22[a]	0·15[a]	0·25[a]	0·27[a]	0·17[a]	0·23[a]	0·043
F.C.R.	4·38[a]	6·63[a]	4·39[a]	4·07[a]	8·52[a]	4·32[a]	1·754

1, F=Formic acid. 2, A=Acetic acid. 3, Cl=Hydrochloric acid. 4, S=Sulphuric acid.

Av.Wt.Inc = Average weekly weight increase. S.G.R. = Specific growth rate.

F.C.R. = Food conversion ratio. SE = Standard error.

a = Mean values for each component with the same superscripts are not significantly different (Duncans multiple range test $p<0·05$).

Palatability and microbial stability of bakery waste diets preserved with salt and/or calcium propionate.

MATERIALS & METHODS.

Diets.

Preliminary work with carp fed salt enriched bakery waste diets showed that levels above 5% salt were not tolerated by the fish. A range of diets was therefore prepared with salt levels of between 3% and 5%. Diets were prepared from 35% air dried bread and 65% pie waste (equivalent to 50% bread and 50% pie waste). Two additional diets were made, one with calcium propionate at 1% and one with a combination of 3% salt and 0·5 calcium propionate. A control diet was prepared without any additional preservative. The full range of diets was as follows:-

1. Salt - 3%
2. Salt - 4%
3. Salt - 5%
4. Calcium propionate - 1%
5. Calcium propionate - 0·5% /Salt - 3%
6. Control.

The diets were stored in a freezer until required.

Fish.

Six tanks were stocked with six fish each of average weight of 135g.

Feeding.

The fish were fed 1% of their body weight/day of dry feed equivalent for six days a week. At the beginning of each week, a quantity of each diet, enough for the equivalent of three weeks feeding, was removed from the freezer and stored at room temperature. This was used to feed the fish. At the end of the week, the remaining food was packed in a polythene bag and stored at room temperature. A second quantity was then removed from the freezer and treated in the same manner. This was repeated for a total of four weeks, so that at the end of the period, four bags of food remained. The fifth weeks food was then taken from the first bag which had been held at room temperature for four weeks. During the following weeks, the remaining bags of food were used so that in this second run, the food that was used was always four weeks old. The experiment ran for a total of 56 days and was divided into two runs of 28 days each, the first feeding fresh food and the second, food that was four weeks old.

Measurement of microbial growth.

A sample of each diet was sent to the microbiology section of Warburtons Ltd, Bolton for analyses of microbial activity. The food was transported frozen and when the trial was started, the food was thawed and left at room temperature. A sample was removed from each bag of food at regular intervals and a measurement of microbial activity was taken by recording the total viable plate count on Agar plates at $37^{o}C$.

RESULTS.

The performance of each diet is shown in Table 3.5. The results of the specific growth rate index for the first run (fresh food) showed no significant ($p>0.05$) difference between any of the diets. The best growth was exhibited by the diet containing 3% salt/0.5% calcium propionate and the next best by the diet with 5% salt. For the second run (four week old food) there were again no significant differences ($p>0.05$) between the treatments but this time, the best growth was shown by the diet with 5% salt. These results are mirrored by the food conversion efficiencies and the average weekly weight gains. A comparision of the results of the

Table 3.5. Palatability of bakery waste diets preserved with salt and calcium propionate fed at 18°C. (n = 6 for each treatment).

Diets.	0.	1%P.[1]	3%S /·5%P.	3%S.[2]	4%S.[2]	5%S[2]	±SE.
Intial Weight.	132·99[a]	131·22[a]	136·28[a]	138·10[a]	132·66[a]	138·21[a]	19·190
Final Weight.	140·69[a]	139·24[a]	151·00[a]	147·76[a]	143·48[a]	146·31[a]	20·093
Av.Wt.Inc.(Run 1).	1·79[a]	1·55[a]	1·96[a]	1·47[a]	1·59[a]	1·65[a]	0·577
Av.Wt.Inc.(Run 2).	0·71[a]	0·90[a]	0·88[a]	0·46[a]	0·61[a]	1·07[a]	0·685
S.G.R. (Run 1).	0·20[a]	0·16[a]	0·19[a]	0·15[a]	0·16[a]	0·17[a]	0·058
S.G.R. (Run 2).	0·07[a]	0·09[a]	0·08[a]	0·05[a]	0·06[a]	0·10[a]	0·067
F.C.R. (%) (Run 1).	26·91[a]	23·69[a]	27·50[a]	20·65[a]	23·25[a]	21·50[a]	8·795
F.C.R. (%) (Run 2).	9·56[a]	12·95[a]	11·87[a]	6·33[a]	8·79[a]	14·91[a]	9·777

1. P = calcium propionate. 2. S = salt. SE + Standard error.

Run 1 = fresh food. Run 2 = 4 week old food.

a = Mean values for each component with the same superscripts are not significantly different (Duncans multiple range test $p<0·05$).

Figure 14. Stability of preserved pellets. 0/0·1% HCl.

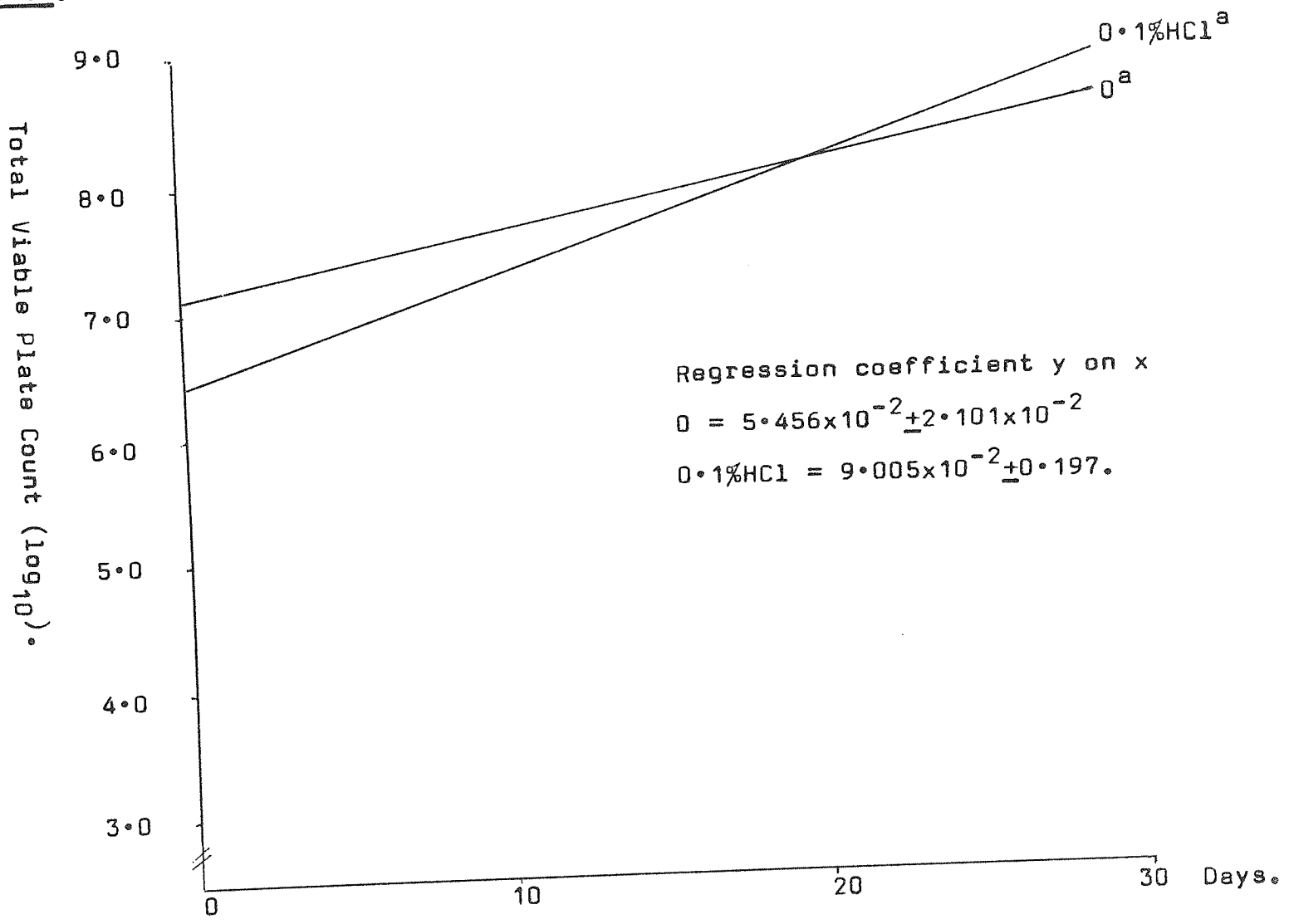


Figure 15. Stability of preserved pellets. 0/3% salt.

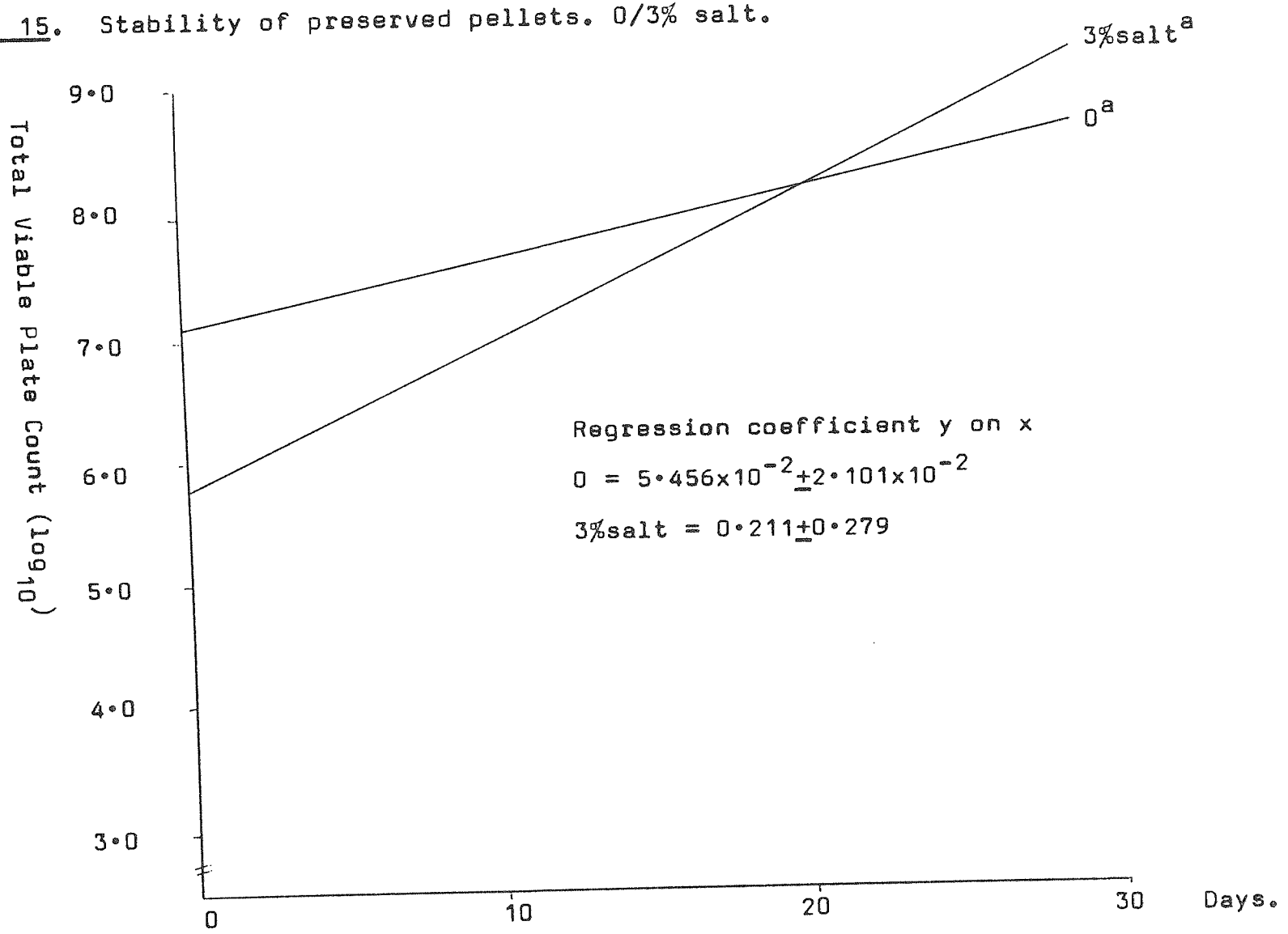


Figure 16. Stability of preserved pellets. 0/4% salt.

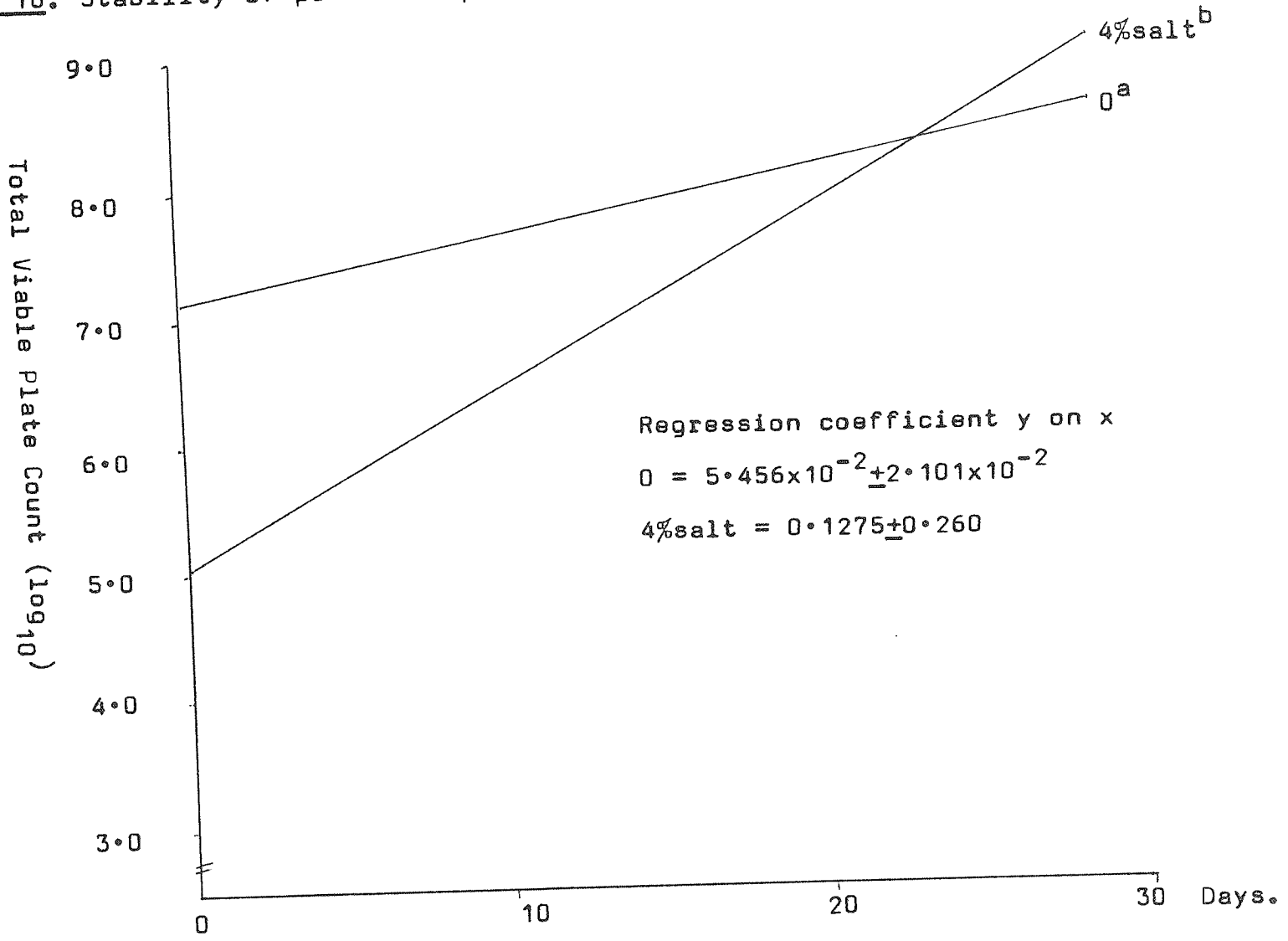


Figure 17. Stability of preserved pellets. 0/5% salt.

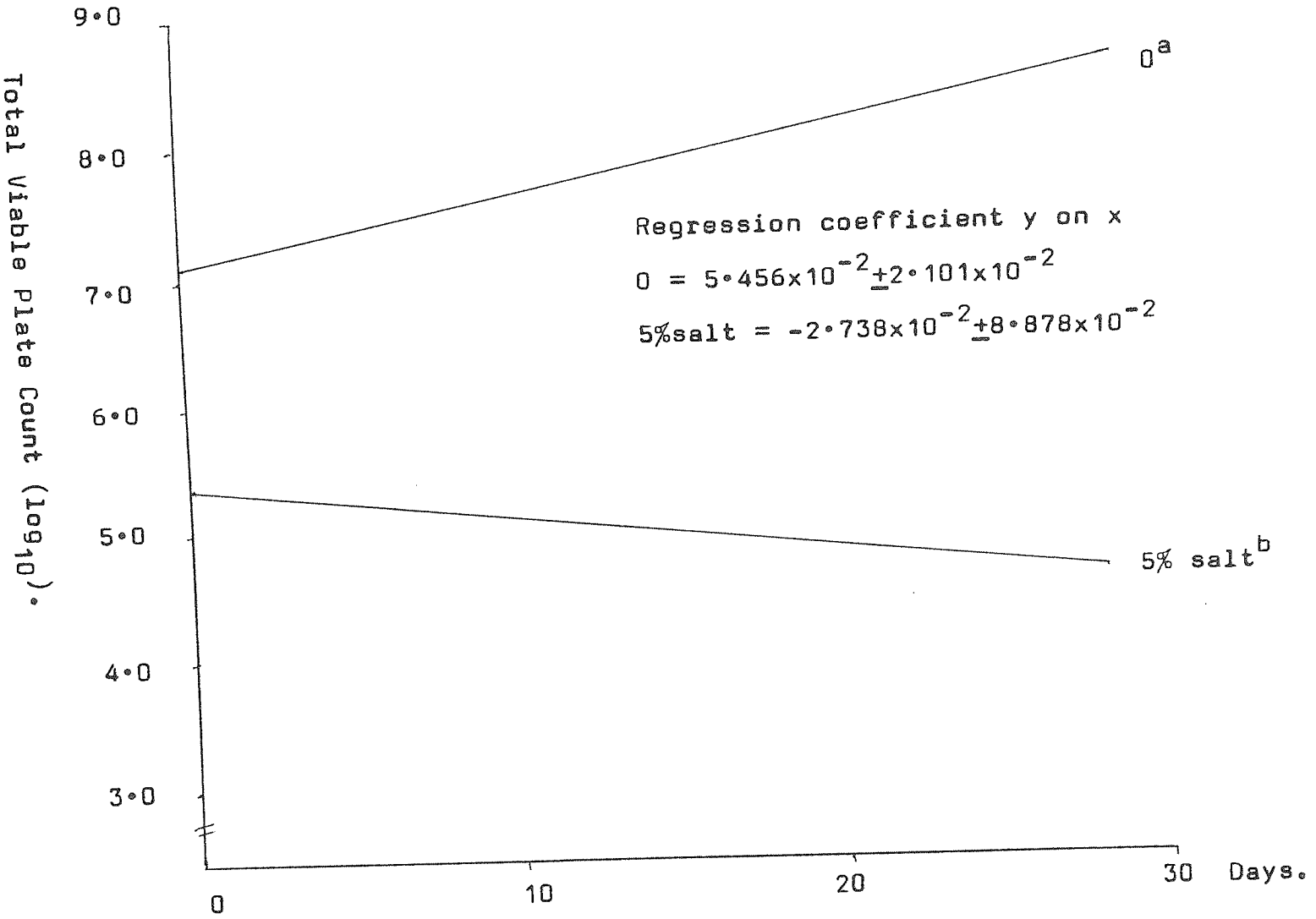


Figure 18. Stability of preserved pellets. 0/3% salt- 0·5% calcium propionate.

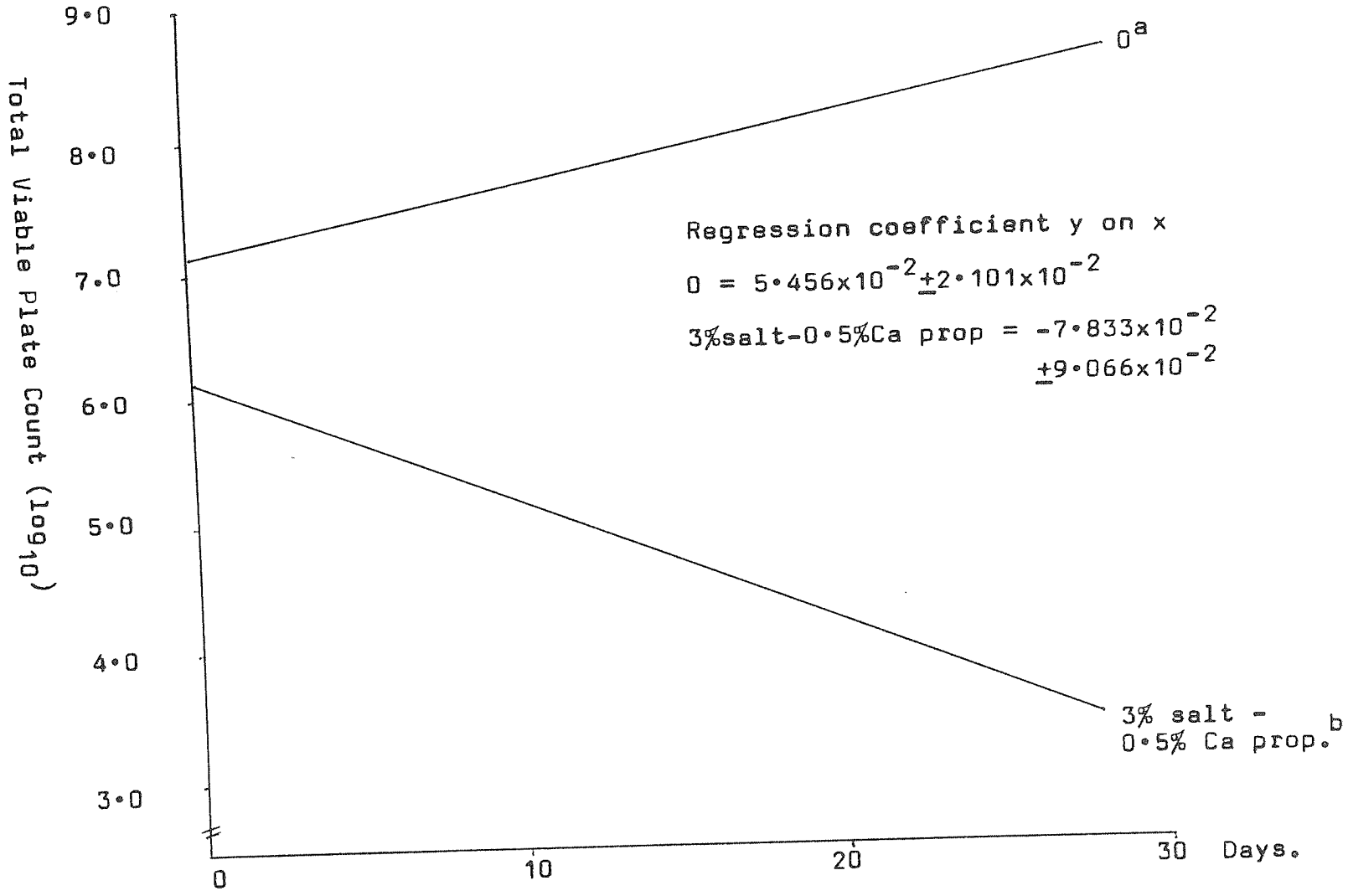


Figure 19. Stability of preserved pellets. 0/1% Calcium propionate.

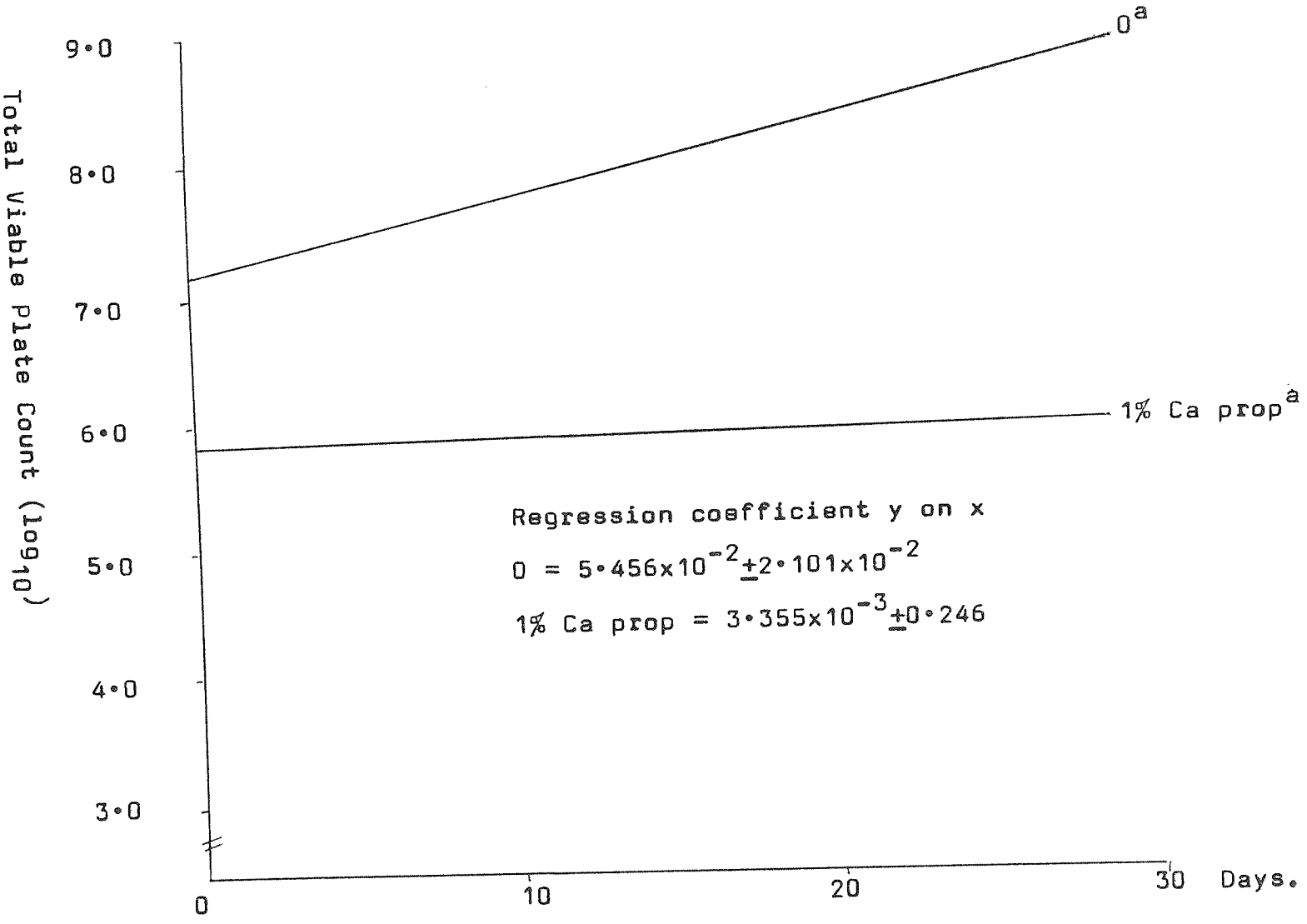
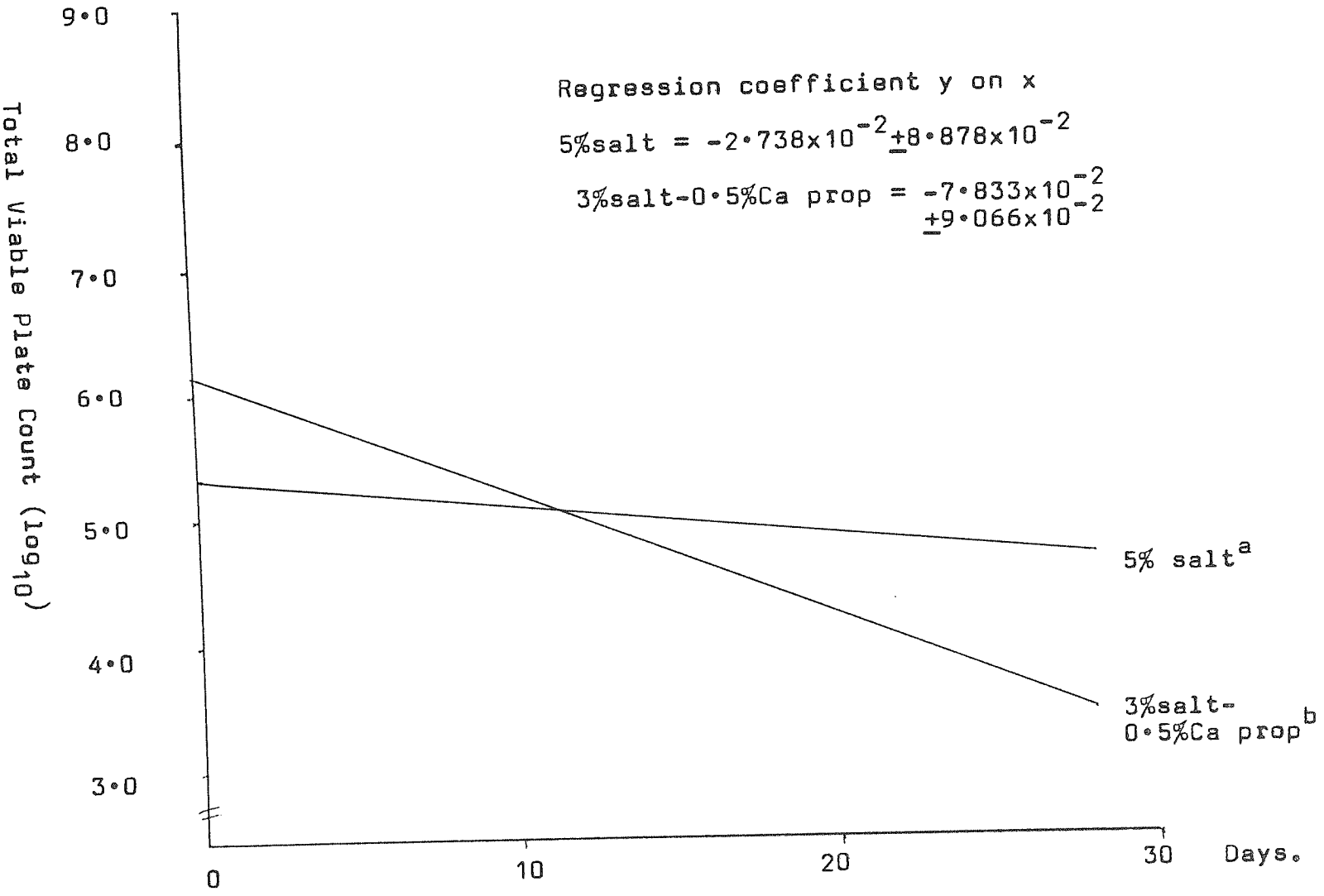


Figure 20. Stability of preserved pellets. 5% salt/3% salt-0·5% Ca Propionate.



specific growth rates for both runs shows there is a decrease in performance when the food is changes from fresh to four weeks old although the decrease is only significant ($p<0.05$) for the diet containing the combination of preservatives (Table 3.6.).

Figures 14 - 20 show the microbial stability of the pellets when protected by the different preservatives. The figures show graphically the total viable plate count against time. The best retardation of microbial count was achieved by 3% salt/0.5% calcium propionate and 5% salt. These are shown in figures 17 and 18. There is also a significant difference ($p<0.05$) between the performance of the 5% salt and the 3% salt/0.5% calcium propionate preservatives, with the combination of salt and calcium propionate retarding microbial growth most effectively.

Table 3.6. Comparison of specific growth rates of diets preserved with salt and/or calcium propionate when fed fresh or 4 weeks old to carp (n = 6 for each treatment).

Preservative.	Fresh food.	4 week old food.
0.	0.205^{a}	0.070^{a}
1% Ca propionate.	0.165^{a}	0.090^{a}
3% salt/ 0.5% Ca propionate.	0.192^{a}	0.082^{b}
3% salt.	0.147^{a}	0.050^{a}
4% salt.	0.162^{a}	0.062^{a}
5% salt.	0.170^{a}	0.102^{a}

ab = Mean values for each component with the same superscripts are not significantly different (Students t - test $p<0.05$).

DISCUSSION.

In all the treatments, the performance of acid preserved diets was about the same. Hydrochloric and acetic acids showed the best growth and therefore would seem the most acceptable to the fish, however at the levels of acid incorporation that were used, the effects of the acid on preservation was negligble. The pH of the diets was above 5 with the exception of 0·5% hydrochloric acid and this is not really low enough to aid preservation (Tatterson & Windsor, 1974). The true level of acid was lower than the incorporation levels since the acid was added to the pie waste only as this deteriorates much more rapidly than the bread. Carp appear not to be able to accept such diets either because of the low pH or more likely due to the taste of the acid. In work where trout were fed low pH diets, taste would not have been such a problem since the diets were effectively ensiled which would mask the taste of the acid (Crampton, Personal communication).

Diets for carp containing only small amounts of acid as a preservative appear not to be palatable to the fish and for this reason, the use of acids in bakery wastes diets seems unacceptable. A better proposition as far as preserving bakery wastes diets is concerned is the use of a salt/calcium propionate system as the preserving agents.

Unlike some preservatives, such as acids, salt and calcium propionate are simple to use and do not present any toxicity problems to the user. The palatability trial using these additives in fish diets showed that the fish did not reject any of the inclusion levels used. Higher levels, when used in a preliminary trial were rejected as predicted by Karel (1976) due to taste problems. The best growth rates were achieved by the fish fed the diets with the highest level of salt and the combination of the two preservatives, which suggests that the preservatives did not affect the acceptability of the pellet and therefore food coversion efficiency. The reduced growth of the fish fed diets containing less salt was most likely due to the reduced effect of the preserving agent which resulted in a deterioration of the food through microbial activity (Tilbury, 1980).

On the second run with four week old food, the deteriration may have been due to oxidation of the fat content or similar changes rather than microbial growth. This is because the growth rates of all the fish fed the whole range of diets decreased,yet the total viable plate counts indicated that microbial activity decreased in some of the diets after the 4 week period. The deterioration of food for reasons other than microbial activity is quite possible

especially since the pie wastes contain high levels of fats which can easily go rancid at room temperature.

The total viable plate counts showed that the growth of micro-organisms was only retarded in the diets containing 5% salt and 3% salt/0·5% calcium propionate. This corresponds with the results of the feeding trial as both these diets performed well. Increasing salt level reduces microbial growth until the problem of taste makes the use of that preservative impossible. A reduction in the level of salt is made possible if necessary by the addition of a small amount of calcium propionate. This obviously acts against some of the micro-organisms that are not affected by salt alone. The most likely of these are moulds since calcium propionate is used as an anti-mould agent in bread (Sawyer & Crosby, 1980). Although the addition of calcium propionate is desirable, it is not practical at present due to its high cost. The market price of calcium propionate is £735/tonne whereas the price of salt is only £42/tonne. The slight loss in effective preservation by using salt alone is compensated by its low cost. The economics in this case takes priority because of the extreme low price of the rest of the ingredients in the diet. Salt is therefore the most realistic preservative for use in this system.

The addition of salt could however lead to other problems since both sodium and chloride are important in the osmoregulation of fish. These elements can either be derived from the aquatic environment or from the food. Most fishmeals contain substantial amounts of sodium chloride and yet it is common for commercial feeds to include as much as 4% extra (Lall, 1979). There is some evidence from terrestrial species which suggests that high dietary salt levels can cause depression of growth. Thus, Zaugg & McLain (1969) found that salt enriched diets (1·5 - 12%) caused reduced growth rates and lower feed efficiencies in young coho salmon. They suggested that the elimination of these electrolytes involved a considerable expenditure of energy. Shaw et al. (1975) fed Atlantic salmon with diets that were sprayed with as much as 12% salt and found no growth depression. The conflicting results of these studies may be due to differences in species, age or method of salt application. In the second study, the salt was sprayed on and may have been lost when the pellets entered the water so reducing the amount the fish injested. The salt in the first study was added during manufacture so was spread throughout the feed. Salt at such levels was not tolerated by carp in this study and therefore any depression of growth was very reduced.

Water stability of bakery waste pellets containing different binding agents at various levels of inclusion.

INTRODUCTION.

One of the major problems associated with the development of intermediate moisture diets for fish is that the food be required to hold together once it is introduced into the water. Adequately bound feeds allow the fish to receive the feed mixture in the same composition as when it was fed into the pond or tank and so reduce loss to a minimum (Hepher, 1969a). The stability of a pellet is of considerable significance, both to prevent unfavourable conditions by fouling due to disintegration of the pellet, and to achieve an economically sound conversion rate. For slow feeders such as carp, stability is particulary important (Meyers et al., 1972).

Early work with binders for trout pellets concentrated on vegetable products. Wolf (1951) tried materials such as tapioca, mucilose (psyllium seed) and vegetable gums, however none were really successful for binding synthetic diets. The most satisfactory binding system that he found was a gelatin and cooked potato starch mixture which proved very complicated to prepare. Most production diets therefore utilised a combination of beef

spleen and salt to produce a rubbery mass that bound well. For experimental use, spleen was useless as it tended to affect growth responses due to the many nutritional variables it introduced (Wood et al., 1954). Phillips (1956) mentioned the use of salt as a binder on its own but only in conjunction with other parts of the diet such as spleen since it is the combination of the two that exerts a binding effect that holds the dry feed particles in physical union.

Further work introduced the potential for manufactured products to act as binders. Wood et al. (1954) tried carboxymethyl cellulose (CMC) and found it much better than spleen. Thain & Urch (1973) used hydroxypropyl methyl cellulose (HMPC) and found it to be an effective binder whilst alginates were tried by Meyers et al. (1972) for crustacean diets and these were stable for up to 48 hours. Starch within the diet also produced a good bind when gelatinised by Meyers et al. (1972).

Methods to measure stability have been developed by Hastings (1964) and modified by Hepher (1969b). A different method was used by Smith (1975) but it is more suited to fast water systems. This experiment was designed to test the stability of semi-moist bakery waste pellets with and without a number of differing binding agents at several levels of inclusion.

MATERIALS & METHODS.

Diets.

A standard bakery waste pellet was prepared using 35% air dried bread and 65% pie waste (equivalent to 50% moist bread and 50% pie waste). During mixing, a measured amount of binding agent was added so that a whole series of pellets were made with levels of inclusion from 1% to 5% for each binder. The binders used are as follows:-

1. F383A, alginate binder. Alginate Industries, London.
2. Celacol HPM450P,celacol gum. British Cellanese.
3. Jalan B37,starch binder. Laing National.
4. Capsul,dextrine binder. Laing National.

A control pellet was made with no binder. The pellets were prepared in the usual manner using a mincer attachment with a 5mm die.

Equipment.

A rectangular frame of 58x100mm was made from 9x13mm wood. One rectangle of aluminium mesh, mesh size 2x1mm, was stapled to the bottom of the frame and a second was cut to act as a lid but was not attached to the frame. Thin wire handles were stapled to each side of the frame so that the height of the handles from the base was 100mm. Two rubber bands were placed around the length of the frame to hold the mesh lid in place

and two pices of lead, 40x10mm were placed on the lid but under the rubber bands to act as ballast. Six of these frames were constructed and these were suspended from hooks attached to two wooden bars which were placed across the top of a plastic aquarium (38x15x19cm) which was filled with water to a depth which allowed the frames to be well submerged when they were in position. The aquarium was placed on the base of a magnetic stirrer with the flea in the water. The stirrer was switched on at half speed to simulate water movements.

Method.

A weighed quantity of pellets were placed in each of the six preweighed mesh frames. An extra sample was dried to constant weight in an oven at 105^{o}C to measure the moisture content of the pellet. At time zero, the six frames were submerged in the tank with the aid of the lead ballast. After every hour, one frame was removed and placed in the oven. This was repeated until all six frames were in the oven and these were then dried to constant weight. From these measurements, the loss in weight was calculated for all the frames. These were averaged out to give a single value for each treatment. The method was repeated for each binder at all levels of inclusion.

RESULTS.

Table 3.7. shows the results of the pellet stability experiment. The figures given are the average percentages of the dry diet lost after immersion for hourly periods of up to six hours. The alginate binder, F383A, exhibits a slight increase in the loss with increasing levels of inclusion. The other binding agents display no pattern, but all remain at about the same level. There is no significant difference ($p>0.05$) between any of the levels of inclusion for each of the binders with the exception of the dextrine binder, Capsul, at levels of 2%, 4% and 5%. At these levels, there is a significant difference ($p<0.05$) from the other levels but not from each other. Although there is some significance, the loss is still within the range of loss displayed by the other binding agents. With one or two exceptions, all the binders produced worse results that were worse than those produced by the pellet with no binding agent.

DISCUSSION.

The use of well bound pellets is desirable since unstable pellets lose their advantage over unpelleted feed and may cause unfavourable conditions in the pond (Barthelmes, 1966). The incorporation of a binding agent into a bakery waste pellet has had little

Table 3.7. Stability of bakery waste pellets containing different binders at various levels of inclusion at 15°C. (n = 6 for each treatment).

Binder.	Level of inclusion (%). 0.	1.	2.	3.	4.	5.	±SE.
F383A.[1]	8·18[a]	9·56[a]	10·05[a]	10·45[a]	10·42[a]	11·68[a]	1·011
Celacol.[2]	8·18[a]	8·05[a]	9·46[a]	9·42[a]	8·76[a]	9·83[a]	0·939
Jalan B37.[3]	8·18[a]	8·66[a]	7·46[a]	9·45[a]	8·69[a]	8·75[a]	0·864
Capsul.[4]	8·18[a]	9·81[a]	10·07[ab]	7·81[a]	11·57[b]	9·22[ab]	0·851

Mean values represent the loss in dry weight averaged from the six samples (%).

1. F383A = Alginate binder, Alginate Industries. 2. Celacol = HPM450P Celacol gum. British Cellanese. 3. Jalan B37 = Starch binder, Laing National. 4. Capsul = Dextrine binder. Laing National.

±SE = Standard error.

ab = Mean values for each component with the same superscripts are not significantly different (Duncans multiple range test $p<0·05$).

benefit in the formation of a well bound feed. If anything, the inclusion of a binder has worsened the natural binding potential of the dietary ingredients. Pellets without a binding agent resulted in less loss than most of the bound pellets at all levels of inclusion with the exception of only one or two and in most cases these differences are not significant ($p>0.05$). The reason for this may be the amount of cooked starch already present within the bakery waste pellet. Dietary starch has been suggested as a binder by Meyers et al. (1972) but only in the preparation of expanded pellets where raw starch is gelatinised during expansion and case hardening. The latter process takes place in a high temperature drier which also affects the state of the starch.

In fish diets which contain only small amounts of dietary starch, cooked starch has sometimes been included as a binder. Wolf (1951) used cooked starch together with gelatine and found he got a good bind. Heinen (1981) found that 3% cornstarch added to the diet worked well when use in moist rations but was not as successful when the diets were dried. Added starch used as a binding agent in this experiment did not increase the bind produced by the dietary ingredients.

Alginates are a popular binding agent and are often used in the prepartion of fish diets. Wood et al. (1954) found alginates unsatisfactory to bind synthetic diets however Meyers et al. (1972) found that the stability was achieved fro up to 48 hours. This is due to the alginate having the unique ability to react with polyvalent ions such as calcium, to form a gel or solution with a high viscosity. This reaction has been described by Andrew & MacLeod (1970). Long term stability of alginates was found to occur by Heinen (1981) when used in diets designed for crustaceans. Solberg (1976; 1979) also found alginates to be suitable binders for moist rations containing large amounts of trash fish. Other binders such as carboxyl methyl cellulose have been found to be effect for synthetic diets (Wood et al., 1954) and also moist diets (Heinen, 1981). Solberg (1979) showed HPMC to be the least successful of the binders tested, however Thain & Urch (1973) found it to be the most effective.

It would seem that the type of binder used and the level of inclusion is dependent on the type of ingredients and the amount of binder incorporated. Only testing each diet would prove the amount of stability achieved. In the case of bakery waste diets, they appear to form a successful bind on their own and the addition of binders only decreases the binding

capability and this worsens with increasing levels of inclusion.

Measurement of the amount of bind within a pellet is difficult and different workers have tackled the problem in a variety of ways. Meyers et al. (1972) do not mention their method of measurement but only state that their pellets lost about 2% during the first 60 minutes immersion in water. Thain & Urch (1973) refer only to agitation to determine effective binding whilst Wood et al.(1954) measured the degree of bind by noting the length of the pellet worm as it was extruded during manufacture. The longest length at which the worm broke represented the most effective degree of binding. Heinen (1981) tested pellets in a beaker of water which was oscillated 100 times per minute. Pellets were considered stable if they could be removed intact or broke only into very large pieces.

The method used to measure the stbility of the pellets in this experiment was a modified method of Hastings (1964) and Hepher (1969b). Hepher considered that Hastings method was too simple and did not take into account time, water movement and temperature. He also thought the mesh size of the frame used was too small, however this problem is difficult to overcome since pellet size has also to be taken into account. In this ezperiment, the percentage loss of the pellet may have

been increased if the mesh size had been enlarged, however it is all relative since if the mesh size had been only just smaller than the pellets, then probably the complete sample of pellets would have been lost. It was considered that as long as all the pellets were treated in the same manner, then mesh size was not that important. If dry pellets had been used then it is possible that definite flakes would have become detached, however it was observed that the moist pellets did not lose definite flakes but instead tended to gain water and the collapse whilst still holding together, so unless the mesh size was exceptinally large, the pellets would not fall through.

The general conclusion of this experiment was that the use of binders in bakery waste diets is unnecessary as a successful bind was achieved by the dietary ingredients alone.

CONCLUSIONS - Section 3.

This section has been concerned with the practical problems involved in the production of a bakery waste pellet. The results of the experiments have shown that on a small scale the problems can be overcome.

The biggest problem has been the removal of the foil trays from the pie waste. To some extent this can be solved by the use of a simple press. In the long term, it may be possible to separate the foil contaminated waste at the bakery as it constitutes only a small percentage of the total waste.

When the pie waste is very moist, mixing a semi-moist pellet can be difficult due to the excess moisture. The addition of a drier meal helps in the removal of this moisture and experiments with air drying have shown that enough moisture can be removed from bread slices to make this feasible.

Preservation of the sem-moist diet is important to prevent any deterioration. This is essential if the diet is to perform well. Preservation by ensiling is popular with salmonid diets yet carp were reluctant to accept the low pH diets. Although the acids preserved the diets well, their use was unrealistic.

Salt by contrast, preserves the diets to a lesser extent but is acceptable to the fish. Its use is much more feasible in practical diets especially

as it is much easier to handle than acids. The performance of the salt can be increased by the incorporation of calcium propionate, a commonly used preservative in the bakery industry, into the mixture. There is some question as to whether the extra benefit gained by its use is worth the extra cost.

Water stability is important when dealing with carp in ponds because the pellets can remain for some time on the bottom before being consumed. The starch present in the waste has been shown to provide sufficient bind that it is unnecessary to add additional binding agents.

The experiments in this section have shown that a practical pellet made from bakery wastes is possible and the only additive found necessary was salt.

GENERAL CONCLUSIONS.

The work covered in this these has encompassed many aspects of applied fish nutrition from intensive culture, where complete dietary requirements have to be met, to low productivity extensive culture, where only parts of the dietary needs are catered for. As well as meeting the nutritional demands of the fish by the use of bakery waste as a dietary source, this work also considered the practical problems involved in the production of a well bound product which can be offered to the fish from raw bakery waste.

This thesis has been presented in three sections covering complete diets for intensive use, supplemental pellets for extensive use and pellet technology. These in total represent a changing attitude to carp culture in this country.

Intially, the problem set for the research for this thesis related only to the application of bakery wastes as a potential nutritional source for carp. This involved an evaluation of the waste fed to fish held in intensive recirculation systems. This limited the type of trial to one that used diets where the waste consisted of only part of the fish ration. It was therefore possible that the results of any evaluation could be masked by the interactions of the other dietary components, in particular the protein level. However, the third

experiment has shown that protein to energy ratios are more important since the expensive protein component can be reduced if the energy required by the fish is met from other sources. Admittedly, overall protein level is still important if it is limiting in the diet and then it becomes the most crucial factor.

Another example of dietary interactions that affect an evaluation trial is that of protein source. Even with equal protein levels, two diets can produce different growth responses. This can be the result of using different protein sources. For reasons such as these, drawing definite conclusions from small scale tank trials is very difficult. In the past, other workers have produced data for the presentation of optimum levels of various components. Such optimum levels are really only relevant for the particular conditions that were used in that one experiment. In the evaluation of new feed sources, such data on optimum levels are even more confusing. In this present study, it would appear that carp seem able to tolerate relatively high levels of bakery waste in their diets without any ill-effects. Long term experimentation with a practical diet that has been specially formulated for one set of defined conditions would confirm the potential value of the bakery waste as a feed source. The reluctance to confirm that value in this thesis is due to the variability of

the dietary components in the waste. The problem of variability is due to the make-up of the separate parts of the waste. These are dependent on the problems encountered on the production line at the bakery. Long term studies which were impossible to organise for this work would result in an average value for any data generated.

Not only were bakery wastes difficult to evaluate, but they also resulted in problems of presentation. This again was due to the variability of the waste, especially in moisture content. This problem was magnified by the constraint placed on this research of not being able to use additional heat to remove the moisture for the production of a dry pellet. The problem of moisture had to be solved either by the reduction of excess moisture through the use of drier ingredients which soaked up the extra water or through the use of alternative drying methods. In the end, a combination of the two was considered the best option even so, the removal of all the moisture was not possible.

The presence of intermediate moisture levels meant that preservatives were needed to reduce the possibility of harmful microbial growth. Varying methods of preservation were tested and the final outcome was that the most realistic system was considered to be the

addition of 5% salt to the diet. Better results could be obtained with more expensive preservatives but these were considered to be uneconomic in a system which relied on inexpensive wastes as the sole nutrient source. It was also found that extra binding agents were not necessary in the production of a finished package as the carbohydrate in the waste had a good binding potential.

The most promising use of bakery wastes is in feeds designed for extensive pond culture. These are primarily supplementary feeds although in situations where the stocking density is in excess of the carrying capacity, additional protein can be incorporated. In the extensive pond trial carried out for this thesis, commercial pressure meant that the fish were stocked at a relatively high density which in turn led to protein shortages. The fish did not therefore grow as well as expected although the results did show that some benefit was gained from feeding supplementary bakery wasye diets. The high energy portion of the feed relieving the pressure on the protein supply by sparing the protein for growth. This is a much more effective way of utilising protein for fish growth since cheap dietary components are used to provide the energy rather than expensive protein.

That bakery wastes are cheap, there is no doubt. Prices quoted for the wastes used in this research are £50/ton for the waste bread and £10/ton for the pie waste. Both these prices are ex-bakery. The expense incurred in using these wastes is in delivery and processing. Delivery is especially expensive since the wastes contain large quantities of moisture and it is this that is expensive to transport.

Processing is also rather expensive and especially if complete diets are manufactured since the composition has to be kept constant in order to balance the nutritional requirements of the fish. This is not so much a problem when bakery wastes are used as supplementary feed since much of the nutritional requirement is obtained by the fish directly from the pond.

The addition of bakery wastes into extensive ponds, especially wastes containing a large proportion of meat, raises another problem associated with processing.

Meat wastes are often used for feeding domesticated farm animals. This waste has to be treated by maintaining the waste at 100^{o}C for not less than 60 minutes. This has to be carried out by law and this is enforced by Statutory Instrument no 1936, The diseases of animals (waste food) order. 1973. This order requires that the farmer be licenced to carry out the treatment of waste

food for feeding to farm animals. The order lists all the animals to which it applies and fish are not included. This means that at present, it is permitted to feed waste meats and other food directly to fish without prior treatment. If waste food becomes a staple part of fish feeds, then it is possible that the order may be extended to include fish. This would probably make the use of waste foods uneconomic as part of the attraction of the wastes at present is the low cost. Treatment would add substantially to the cost and this would limit its use as other feed sources became more attractive.

This research has shown that the most promising role for bakery wastes is in the nutrition of extensively cultured fish. Not long ago, there was little regard for extensive culture in this country but lately this has changed with the realisation that intensive culture with its high capital investment, is not the only economic way to grow fish.

REFERENCES.

AAES-JORGENSEN, E. (1967) Fish oils as a source of essential fatty acids. In 'Fish oils'. Avi Publishing Co. Westport Conn. pp300-321.

AGRICAPITAL GROUP. (1978) Our Daily Bread. BSSRS. 37p.

ALFIN-SLATER, R.B. & L. AFTERGOOD. (1968) Essential fatty acids reinvestigated. Physiol. Rev. 48 : 758-784.

ANDREW, T.F. & W.C. MACLEOD. (1970) Application and control of the algin-calcium reaction. Food Product Development. 4 (5) : 99-104.

ANDREWS, J.W. & R.R. STICKNEY. (1972) Interactions of feeding rates and environmental temperature on growth, food conversion and body composition of channel catfish. Trans. Am. Fish. Soc. 101 : 94-99.

A.O.A.C. (1975) Official methods of analysis of the Association of Official Analytical Chemists. W. HOROWITH. (ed) 12th edition. A.O.A.C. Washington D.C.

ARRINGTON, L.R. (1965) Animal feeds from many sources - Dried bakery products. Sunshine State Res. Rep. 10 : (1).

AUSTRENG, E., S. RISA, D.J. EDWARDS & H. HVIDSTEN. (1977) Carbohydrate in rainbow trout diets 11. Influence of carbohydrate levels on the chemical composition and feed utilisation of fish from different families. Aquaculture. 11 : 39-50.

BAKER, F.S. JR. (1964) Dried bakery products in a steer fattening ration. Fla. Agr. Exp. Sta. Ann. Rep. 337p.

BARDACH, J.E., J.H. RYTHER & W.O. MCLARNEY. (1972) Aquaculture. The farming and husbandry of fresh-water and marine organisms. Willey-Interscience 868p.

BARTHELMES, D. (1966) Verschiedenarhge Fuuterungsuersuche mitt dem 1965 bereitgestellten pellertierten Eiweissfutter fur Karpfen. Dt. FischZtg Radebeul. 13 (5) : 130-135.

BENDER, A.E. & D.S. MILLER. (1953) A new brief method of estimating net protein value. Biochem. J. 53 vii.

BEN-GERA, I. & A. KRAMER. (1969) The utilisation of food industry wastes. Advances in Food Research. 17 : 77-152.

BLACK, E.C. (1958) Energy stores and metabolism in relation to muscular activity in fishes. In. 'The investigation of fish problems'. P.A. LARKIN. (ed). H.R. Macmillan lectures in fisheries. University of British Columbia, Vancouver. 397p.

BROWN, E.E. (1977) World Fish Farming: Cultivation and Economics. Avi Publishing Co. Westport Conn. 397p.

BROWN, M.E. (1957) The Physiology of Fishes. Vol 1. Academic Press.

BRETT, J.R., J.E. SHELBOURN & C.T. SHOOP. (1969) Growth rate and body composition of finglering sockeye salmon in relation to temperature and ration size. J. Fish. Res. Bd. Can. 26 : 2363-2394.

BUCK, D.H., C.F. THOITS III & C.R. ROSE. (1970). Variation in carp production in replicate ponds. T. Am. Fish. Soc. 99 (1) : 74-79.

BUCKLEY, J.T. & T.D.D. GROVES. (1979) Influence of feed on the body composition of finfish. Proc. World. Symp. on Finfish Nutrition & Fishfeed Technology. Hamburg 1978. Vol 11. Berlin 1979. 335-343.

BUHLER, D.R. & J.E. HALVER. (1961) Nutrition of salmonid fishes IX. Carbohydrate requirements of chinook salmon. J. Nutr. 74 (3) : 307-318.

CASTELL, J.D. (1979) Review of lipid requirements of finfish. Proc. World. Symp. on Finfish Nutrition & Fishfeed Technology. Hamburg 1978. Vol 1. Berlin 1979. 56-84.

CASTELL, J.D., D.J. LEE & R.O. SINNHUBER. (1972a). Essential fatty acids in the diet of rainbow trout; lipid metabolism and fatty acid composition. J. Nutr. 102 : 93-100.

CASTELL, J.D., R.O. SINNHUBER, D.J. LEE & J.H. WALES. (1972b) Essential fatty acids in the diet of rainbow trout; Physiological symptoms of fatty acid deficiency. J. Nutr. 102 : 87-92.

CASTELL, J.D., R.O. SINNHUBER, J.H. WALES & D.J. LEE. (1972c) Essentail fatty acids in the diet of rainbow trout: Growth, feed conversion and some gross deficiency symptoms. J. Nutr. 102 : 77-85.

CAULTON, M.S. & E. BURSELL. (1977) The relationship between changes in condition and body composition in young Tilapia rendalli. Boulenger. J. Fish. Biol. 11 : 143-150.

CHIOU, J.Y. & C. OGINO. (1975) Digestibility of starch in carp. Bull. Jap. Soc. Sci. Fish. 41 (4) : 465-466.

COWEY, C.B., J.W. ADRON, R. HARDY, J.G.M. SMITH & M.J. WALTON. (1979) Utilisation by rainbow trout of diets conatining partially rendered hide fleshings. Aquaculture. 16 : 199-209.

COWEY, C.B. & J.R. SARGENT. (1972) Fish Nutrition. Adv. Mar. Biol. 10 : 383-492.

COWEY, C.B. & J.R. SARGENT. (1977) Lipid nutrition in fish. Comp. Biochem. Physiol. 57B : 269-273.

COWEY, C.B. & J.R. SARGENT. (1979) Nutrition. In Fish Physiology Vol VIII. Bioenergetics and Growth. HOAR, W.S., D.J. RANDALL & J.R. BRETT. (eds) Acedemic Press. pp1-69.

COLLINS, R.A. & M.V. DELMONDO. (1976) Comparative economics of aquaculture in cages, raceways and enclosures. FAO Tech. Conf. on Aquaculture. Kyoto. FIR/AQ/conf/76/R.37. 11p.

CRAWFORD, D.C. & D.K. LAW. (1972) Mineral composition of Oregon moist pellet production formulations. Prog. Fish. Cult. 34 : 126-130.

DAMRON, B.L., P.W. WALDROUP & R.H. HARMS. (1965) Evaluation of dried bakery product for use in broiler diets. Poultry Science. 44 : 1122-1126.

DUNCAN, D.B. (1955) Multiple range and F tests. Biometrics. 11 (1) : 1-42.

DUPREE, H.K. (1969) Influence of corn oil and beef tallow on the growth of channel catfish. Bur. Sport. Fish. Widl. Tech paper 27. 13p.

DUPREE, H.K. (1976) Studies on nutrition and feeds of warmwater fishes. Proc. 1st Conf. on Aquacultural nutrition. Oct 1975. 65-84.

DUPREE, H.K. & K.E. SNEED. (1967) Response of channel catfish fingerlings to different levels of major nutrients in purified diets. US. Fish. Widl. Ser. Tech.paper 9. 21p.

EDWARDS, D.J., E. AUSTRENG, S. RISA & T. GJEDREM. (1977) Carbohydrate in rainbow trout diets 1. Growth of fish of different families fed diets containing different proportions of carbohydrate. Aquaculture. 11 : 31-38.

ELLIS, J.N. (1969) Short review of trout feeding equipment in the United States of America. EIFAC Tech. paper 9. Symposium on new developments in carp and trout nutrition. Rome 1968. 66-70.

FARKAS, T., I. CSENGERI, F. MAJOROS & J. OLAH (1977) Metabolism of fatty acids in fish 1. Developments of essential fatty acid deficiency in the carp. Aquaculture. 11 : 147-157.

FOWLER, L.G. & R.E. BURROWS. (1971) The Abernathy salmon diet. Prog. Fish. Cult. 33 : 67-75.

FURUKAWA, A. (1975) Diet in the yellowtail culture. Proc. 1st Conf. on Aquacultural Nutrition. Oct.1975. 85-104.

GARLING, D.L. & R.P. WILSON. (1976) Optimum dietary protein to energy ratio for channel catfish fingerlings. J. Nutr. 106 : 1368-1375.

GERKING, S.D. (1952) The protein metabolism of sunfishes of different ages. Physiol. Zool. 25 (4) : 358-372.

GHITTINO, P. (1979) Formulation and technology of moist feed. Proc. World Symp. on Finfish Nutrition & Fishfeed Technology. Hamburg 1978. Vol 11. Berlin 1979. 37-49.

GLUDE, J. (1977) NOAA Aquaculture plan. NOAA US Dept. Commerce. Washington D.C. 41p.

HALVER, J.E. (1957) Nutrition of salmonid fishes IV. An amino acid test diet for chinook salmon. J. Nutr. 62 : 245-254.

HALVER, J.E. (1975) Nutritional requirements of cold water fish. Proc. 9th Conf. Nutr. Mexico. 3 : 142-157.

HASTINGS, W.H. (1964) Fish food processing research. Feedstuffs. 36 (21) : 13.

HASTINGS, W.H. (1969) Fish food processing. EIFAC Tech. paper 9. 23-42.

HEINEN, J.M. (1981) Evaluation of some binding agents for crustacean diets. Prog. Fish. Cult. 43 (3) : 142-145.

HEPHER, B. (1969a) The development and manufacture of carp pellets in Israel. EIFAC Tech. paper 9. 43-47.

HEPHER, B. (1969b) A modification of Hastings method for determination of water stability of fish feed pellets. EIFAC. Tech. paper 9. 48-54.

HIGASHI, H., T. KANEKO, S. ISHII, M. USHIYAMA & T. SUGISHASHI. (1966) Effects of ethyl linoleate, ethyl linolenate and ethyl esters of highly unsaturated

fatty acids on essential fatty acid deficiency in rainbow trout. J. Vitaminology. 12 : 74-79.

HORA, S.L. & T.V.R. PILLAY. (1962) Handbook on fish culture in the Indo-Pacific region. FAO Fisheries Biology Tech. paper 14.

HUBLOU, W.F. (1963) Oregon pellets. Prog. Fish Cult. 25 (4) : 175-180.

HUET, M. (1972) Textbook of Fish Culture. Fishing News Books. 436p.

HUISMAN, E.A. (1976) Food conversion efficiencies at maintenance and production levels for carp and rainbow trout. Aquaculture. 9 : 259-273.

HUISMAN, E.A., J.G.P. KLEIN BRETELER, M.M. VISMANS & E. KANIS. (1979) Retention of energy, protein, fat and ash in growing carp under different feeding and temperature regimes. Proc. World Symp. on Finfish Nutrition & Fishfeed Technology. Hamburg 1978. Vol 1. Berlin 1979. 175-179.

HUNT, P.C. (1971) The Barbel. Proc 5th Br. Coarse Fish Conf. 89-92.

INABA, D., C. OGINO, C. TAKAMATSO, T. VEDA & K. KUROKAWA. (1963) Digestibility of dietary components in fishes 11. Digestibility of dietary protein and starch in rainbow trout. Bull. Jap. Soc. Sci. Fish. 29 (3) : 242-244.

JAUNCEY, K. (1979) Growth and nutrition of carp in heated effluents. PhD Thesis.University of Aston in Birmingham.

JAUNCEY, K. (1982) The effects of varying dietary protein level on growth, food conversion, protein utilisation and body composition of juvenile tilapias (Sarotherdon mossambcus). Aquaculture 27 : 43-54.

KANEKO, T.M. (1969) Composition of food for carp and trout. EIFAC Tech. paper 9. 161-168.

KAPLOW, M. (1970) Commercial development of intermediate moisture foods. Food Technology. 24 : 889-893.

KAREL, M. (1976) Technology and application of new intermediate moisture foods. In.'Intermediate Moisture Foods'. DAVIES, R., G.G. BIRCH & K.J. PARKER. (eds). 4-27.

KIRK, W.G. & F.M. PEACOCK. (1969) Blended dried bakery products in steer fattening rations. Univ. Florida. Agr. Exp. Sta. Circ. No S 197. 10p.

KITAMIKADO, M., T. MORISHITA & S. TACHINO. (1964) Digestibility of dietary protein in rainbow trout ll. Effect of starch and oil content in diets and size of fish. Bull. Jap Soc Sci. Fish. 30 : 50-54.

KORNEGAY, E.T. (1974) Blended dried bakery products for growing and finishing swine. Feedstuffs. 46 (15): 23.

LALL, S.P. (1979) Minerals in finfish nutrition. Proc. World Symp. on Finfish Nutrition & Fishfeed Technology. Hamburg 1978. Vol 1 Berlin 1979. 85-97.

LEE, D.J. & G.B. PUTNAM. (1973) The response of rainbow trout to varying protein/energy ratios in a test diet. J. Nutr. 103 : 916-922.

LEE, D.J., J.N. ROEHM, T.C. YU & R.O. SINNHUBER. (1967) Effect of w3 fatty acids on the growth of rainbow trout. J. Nutr. 92 : 93-98.

LEE, D.J. & R.O. SINNHUBER. (1972) Lipid requirements. In.'Fish Nutrition'. HALVER, J.E. (ed) Academic Press. pp149-186.

LEISTNER, L. & W. RODEL. (1976) The stability of intermediate moisture foods with respect to micro-organisms. In. 'Intermediate Moisture Foods'. DAVIES, R., G.G. BIRCH & K.J. PARKER. (eds) 120-133.

MCLAREN, B.A., E.F. HERMAN & C.A. ELVEHJEM. (1946). Nutrition of rainbow trout. Studies with purified rations. Arch Biochem. 10 : 433-441.

MCLAREN, B.A., E.F. HERMAN & C.A. ELVEHJEM. (1947) Nutrition of rainbow trout. Studies with practical rations. Proc. Soc. Exp. Biol. Med. 65 : 97.

MANN, H. (1961) Fish culture in Europe. In. 'Fish as Food'. BORGSTROM, G. (ed) Academic Press. pp 77-102.

MAREK, M. (1975) Revision of supplementary feeding tables for pond fish. Bamidgeh. 27 (3) : 57-64.

MEACHAM, T.N. & H.R. THOMAS. (1965) Utilisation of dried bakery products by early weaned pigs. Livestock research 1964-1965. Progress report. Animal Sciences Dept. VPI & SU. p56.

MEASURES, J.C. & G.W. GOULD. (1976) Interactions of micro-organisms with the environment of IMF. In. Intermediate Moisture Foods. DAVIES, R., G.G. BIRCH & K.J. PARKER (eds). Applied Science Publishers p281-297.

MESKE, CH. (1973) Aquakultur von Warmwasser-nutzfischen. Stuttgat Ulmer. 163p.

MESKE, CH. & E. PFEFFER. (1979) Influence of source and level of dietary protein on body composition of carp. Proc. World. Symp. on Finfish Nutrition & Fishfeed Technology. Hamburg 1978. Vol 11 Berlin 1979. 393-400.

MEYERS, S.P., D.P. BUTLER & W.H. HASTINGS. (1972) Alginates as binders for crustacean rations. Prog.Fish. Cult. 34 (1) : 9-12.

MILLER, D.S. & A.E. BENDER. (1955) The determination of the net utilisation of proteins by a shortened method. Br. J. Nutr. 9 : 382-388.

MORRISION, W.R. & L.M. SMITH. (1964) Preparation of fatty acid methyl esters and dimethyl acetates from lipids with boron fluoride methanol. J. Lipid Res. 5 : 600-608.

MUGRDITCHIAN, D.S., R.W. HARDY & W.T. IWAOKA (1981) Linseed oil and animal fat as alternative lipid sources in dry diets for chinook salmon. Aquaculture. 25 (2-3) : 161-172.

MURRAY, M.W., J.W. ANDREWS & H.L. DE LOACH. (1977) Effects of dietary lipids, dietary protein and environmental temperature on growth, feed conversion and body composition of channel catfish. J. Nutr. 107 : 272-280.

N.A.S. (1977). Nutrient requirement of warmwater fishes. National Academy of Sciences. Washington D.C. 78p.

NICOLAIDES, N. & A.N. WOODALL. (1962) Impaired pigmentation in chinook salmon fed diets deficient in essential fatty acids. J. Nutr. 78 : 431-437.

NIJKAMP, J.H., A.J.H. VAN ES & E.A. HUISMAN. (1974) Retention of nitrogen, fat, ash, carbon and energy in young chickens and carp. In.'Energy metabolism of farm animals'. MESKE, CH., H.J.LANTISCH & J.R. REICHEL. (eds) University of Hohenhiem. W. Germany. p277-280.

NOSE, T. (1979) Diet composition and feeding techniques in fish culture with complete diets. Proc. World Symp. on Finfish Nutrition and Fishfeed Technology. Hamburg 1978. Vol 1. Berlin 1979. 283-296.

OGINO, C., J.Y. CHIOU & T. TAKEUCHI. (1976) Protein nutrition in fish VI. Effects of dietary energy sources on the utilisation of proteins by rainbow trout and carp. Bull. Jap. Soc. Sci. Fish. 42 : 213-218.

OGINO, C. & K. SAITO. (1970) Protein nutrition in fish 1. The utilisation of dietary protein by young carp. Bull. Jap. Soc. Sci. Fish. 36 : 250-254.

OSBORNE, T.B., L.B. MENDEL & E.L. FERRY. (1919) A method of expressing numerically the growth promoting value of protein. J. Biol Chem. 37 : 223-229.

PAGE, J.W. & J.W. ANDREWS. (1973) Interactions of dietary levels of protein and energy on channel catfish. J. Nutr. 103 : 1339-1346.

PEO, E.R. (1965) Bakery product fed as a skim milk replacement. Feedstuffs. 37 (12) : 9.

PHILLIPS, A.M. (1956) The nutrition of trout 1. General feeding methods. Prog. Fish. Cult. 18 : 113-118.

PHILLIPS, A.M. (1972) Calories and energy requirement. In. 'Fish Nutrition'. HALVER, J.E. (ed) Academic Press. pp1-28.

PHILLIPS, A.M. & D.R. BROCKWAY. (1956) The nutrition of trout 11. Protein and carbohydrate. Prog. Fish. Cult. 18 (4) : 159-164.

PHILLIPS, A.M. & D.R. BROCKWAY. (1959) Dietary calories and the production of trout in hatcheries. Prog. Fish. Cult. 21 (1) : 3-16.

PHILLIPS, A.M., D.L. LIVINGSTON & H.A. POSTON. (1963) The effect of diet mixtures and calorie source on growth, mortality, conversion and chemical composition of brook trout. Prog. Fish. Cult. 25 : 8-14.

PHILLIPS, A.M., D.L. LIVINGSTON & H.A. POSTON. (1964) Effect of protein and calorie levels and sources on the growth of brown trout. Fish.Res.Bull. 28 : 11-19.

PHILLIPS, A.M., D.L. LIVINGSTON & H.A. POSTON. (1965) The effect of three supplemental fats on the growth rate and body chemistry of brown trout. Cortland hatchery report. No 33. Fisheries Res. Bull. 28 State of NY Cons. Dept. Albany.

PHILLIPS, A.M., A.V. TUNISON & D.R. BROCKWAY (1948) The utilisation of carbohydrate by trout. Fish. Res. Bull. 11. 44p.

PIEPER, A. & E. PFEFFER. (1979) Carbohydrates as possible sources of dietary energy for rainbow trout. Proc. World Symp. on Finfish Nutrition & Fishfeed Technology. Hamburg 1978. Vol 1. Berlin 1979. 209-219.

POTTER, L.M., J.R. SHELTON & M. KELLY. (1971) Effects of zinc bactracin, dried bakery products and different fishmeals in the diets of young turkeys. Poultry Science. 50 (4) : 109-115.

RASMUSSEN, C.J. (1969) Experiments on the efficiency and cost of dry food in relation to wet food in Danish trout culture. EIFAC Tech. paper 9. 55-62.

REFSTIE, T. & E. AUSTRENG. (1981) Carbohydrate in rainbow trout lll. Growth and chemical composition of fish from different families fed four levels of carbohydrate in the diet. Aquaculture. 25 : 35-49.

REINITZ, G. (1980) Acceptability of animal fat in the diets of rainbow trout at two environmental temperatures. Prog. Fish. Cult. 42 (4) : 218-222.

REINITZ, G., L.E. ORME, C.A. LEMM & F.N. HITZEL. (1978) Influence of varying lipid concentrations with two protein concentrations in diets for rainbow trout. T. Am. Fish Soc. 107 (5) : 751-754.

ROCKWELL, G.E. & E.G. EBERTZ. (1924) J. Infect. Dis. 35 : 573.

ROY, R. (1976) Wastage in the UK food system. Earth Resources Research Publication. 42p.

RUMSEY, G.L. (1973) The protein situation in fish feeds and feeding. American Fishes & US Trout News. 18 (7) : 8-11.

RYCHLY, J. & L. SPANNHOF. (1979) Nitrogen balance in trout 1. Digestibility of diets containing varying levels of protein and carbohydrate. Aquaculture. 16 : 39-44.

SAWYER, R. & N.T. CROSBY. (1980) Detection and analysis. In. 'Development in Food Preservatives - 1'. TILBURY, R.H. (ed) Applied Science Publishers. p75-109.

SEN, P.R., N.G.S. RAO, S.R. GHOSH & M. ROUT. (1978) Observations on the protein and carbohydrate requirements of carp. Aquaculture. 13 : 245-255.

SEWELL, R.F. (1966) Starter feed for pigs improved by addition of dried bakery product. Feeds Illustrated. March issue.

SCHAPERCLAUS, W. (1933) Textbook of pond culture. U.S. Widl. Ser. Fish. Leaflet 311.

SCHAPERCLAUS, W. (1963) Das Verhaellniss von Naturzuwachs zu Futterzuwachs im Karpfenabwachsteich sowie seine Auswirkung auf den Gesamtzuwachs den Futterquotienten und die Deschaffenheit der Karpfen. Z. Fisch. 11 N.F. (3/4) : 265-300.

SHAW, H.M., R.L. SAUNDERS, H.C.HALL & E.B. HENDERSON. (1975) Effects of dietary sodium chloride on growth of Atlantic salmon. J. Fish. Res. Bd. Can. 32 : 1813-1819.

SHILOH, S. & S. VIOLA. (1973) Experiments in nutrition of carp growing in cages. Bamidgeh. 25 (1) : 17-31.

SHIMENO, S., H. HOSOKAWA, H. HIRATA & M. TAKEDA. (1977) Comparative studies on carbohydrate metabolism of yellowtail and carp. Bull. Jap. Soc. Sci. Fish. 43 (2) : 213-217.

SHIMENO, S., H. HOSOKAWA & M. TAKEDA. (1979) The imporatance of carbohydrate in the diet of a carnivorous fish. Proc. World Symp. on Finfish Nutrition & Fishfeed Technology. Hamburg 1978. Vol 1. Berlin 1979. 127-143.

SIN, A.W. (1973a) The dietary protein requirement of young carp. Hong Kong Fish. Bull. 3 : 77-82.

SIN, A.W. (1973b) The utilisation of dietary protein for growth of young carp in relation to variation in fat intake. Hong Kong Fish. Bull. 3 : 83-88.

SINGH, R.P. & T. NOSE. (1967) Digestibility of carbohydrate in young rainbow trout. Bull. Freshwater Res. Lab. Tokyo. 17 : 21-25.

SINNHUBER, R.O. (1969) The role of fats. In. 'Fish in Research'. NEUHAUS, O.W & J.E. HALVER (eds). Academic Press. pp 245-261.

SINSKEY, AJ. (1980) Mode of action and effective application. In. 'Developments in Food Preervatives - 1'. TILBURY, R.H. (ed) Applied Science Publishers. pp 111-136.

SMITH, P. (1976) Evaluation of single-cell protein in trout diets. PhD thesis. University of Aston in Birmingham.

SOLBERG, S.O. (1976) Soft food pellets for trout. Nutritional requirements of trout and some preliminary results. Meddelelse fra Forsogsdambruget. No 56. Dept of Agriculture and Fisheries for Scotland. Translation no 1986.

SOLBERG, S.O. (1979) Formulation and technology of moist feed - moist pellets. Proc. World Symp. on Finfish Nutrition & Fishfeed Technology. Hamburg 1978. Vol 11. Berlin 1979. 41-45.

SPINELLI, J., C. MAHKEN & H. STEINBERG. (1979) Alternative sources of protein for fishmeal in salmonid diets. Proc. World Symp. on Finfish Nutrition & Fishfeed Technology. Hamburg 1978. Vol 11. Berlin 1979. 131-142.

STEFFENS, W. (1981) Protein utilisation by rainbow trout and carp. A brief review, Aquaculture. 23 : 337-345.

STICKNEY, R.R. & J.W. ANDREWS. (1971) Combined effects of dietary lipids and environmental temperature on growth metabolism and body composition of channel catfish. J. Nutr. 101 (21) : 1703-1710.

STICKNEY, R.R. & J.W. ANDREWS. (1972) Effects of dietary lipids on growth, feed conversion, lipid and fatty acid composition of channel catfish. J. Nutr. 102 : 249-258.

SWINGLE, H.S. (1967) Estimation of standing crops and rates of feeding fish in ponds. FAO Fisheries Reports. 44 (3) : 416-423.

SZUMEIC, J. (1977) Some experiments on intensive farming of common carp in Poland. In 'Advances in Aquaculture' Fishing News Books. pp 157-161.

TAL, S. (1956) A critism of the methods of calculation of carp feeding. Bamidgeh. 8 (3) : 44-51.

TAL, S. & B. HEPHER. (1967) Economic aspects of fish feeding in the Near East. FAO Fisheries Reports. 44 (3) : 285-290.

TAVERNER, J. (1600) Certaine experiments concerning fish and fruite. Reprinted in 1928 with an introduction by Eric Parker.

TAKEUCHI, T. & T. WATANABE. (1977) Requirement of carp for essential fatty acids. Bull. Jap. Soc. Sci. Fish. 43 (5) : 541-551.

TAKEUCHI, T, T. WATANABE & C. OGINO. (1978a) Use of hydrogenated fish oil and beef tallow as a dietary energy source for carp and rainbow trout. Bull. Jap. Soc. Sci. Fish. 44 (8) : 875-888.

TAKEUCHI, T., M. YOKOYAMA, T. WATANABE & C. OGINO. (1978b) Studies on nutritive value of dietary lipid in fish 13. Optimum ratio of energy to protein for rainbow trout. Bull. Jap. Soc. Sci. Fish. 44 (7) : 729-732.

TAKEUCHI, T, T. WATANABE & C. OGINO. (1979) Optimum ratio of dietary energy to protein for carp. Bull. Jap. Soc. Sci. Fish. 45 (8) : 983-987.

TATTERSON, I.N. & M.L. WINDSOR. (1974) Fish silage. J. Sci. Fd. Agric. 25 : 369-379.

TATTERSON, I.N. & M.L. WINDSOR. (1975) Fish silage. Torry Advisory Note. no 64.

THAIN, B.P. & M.J. URCH. (1973) Marine fishfeed - problems in an intensive system. Fish Farming International. 1 : 106-110.

TIEMEIER, O.W., C.W. DEYOE & S. WEARDEN. (1964) Supplemental pellet feeding of channel catfish. Rep. Prog. Kans. Un. 97 10p.

TIEMEIER, O.W., C.W. DEYOE & S. WEARDON. (1965) Effects on growth of fingerling channel catfish of diets containing two energy and two protein levels. Trans. Kansas Acad. Sci. 68 : 180-186.

TILBURY, R.H. (1980) Developments in Food Preservatives - 1. Applied Science Publishers. 165p.

TINSLEY, I.J., J.J.B. SADDLER, H.M. KRUEGER & R.R. LOWRY. (1971) Interactions in the metabolism of polyunsaturated fatty acids in the coho salmon. Int. J. Biochem. 2 : 345-348.

TUNISON, A.V. (1940) Trout feeds and feeding. Cortland NY, State Conservation Dept. NY Experimental Hatchery. 1-10.

VAAS-VAN OVEN, A. (1957) Experiment on different stocking rates of the common carp in nursing ponds. Proc. Indo-Pacif. Fish. Coun. 7 : 13-34.

VON LUKOWICZ, M. (1974) Raising carp from fry to marketable size. Two Lakes Training Course. 4-6 Oct. 1974.

WALLACE, H.D. (1965) Dried Bakery Product as a feed for swine. Feedstuffs. 37 (25) : 52.

WALTER, E. (1934) Richtlinien zur Karpfenfutterung. Sammlung Fischereilicher Zeitfragen. J. Neuman, Neudamm. Hf. 12. 2. Aufl. 20p.

WATANABE, T., I. KUBUYASHI, O. UTSE & C. OGINO. (1974a) Effect of dietary linoleate on fatty acid composition of lipids on rainbow trout. Bull. Jap. Soc. Sci. Fish. 40 : 387-

WATANABE, T., C. OGINO, Y. KOSHIISHI & T. MATSUNGA. (1974b) Requirement of rainbow trout for essential fatty acids. Bull. Jap. Soc. Sci. Fish. 40 : 493-499.

WATANABE, T., F. TAKASHIMA & C. OGINO. (1974c) Studies on nutritive value of dietary lipids in fish 1. Effect of dietary methyl linolenate on growth of rainbow trout. Bull. Jap. Soc. Sci. Fish. 40 (2) : 181-188.

WATANABE, T. ET AL. (1975a) Effect of dietary methyl linoleate and linolenate on growth of carp 1. Bull. Jap. Soc. Sci. Fish. 41 : 257-262.

WATANABE, T., T. TAKEUCHI & C. OGINO. (1975b) Effect of dietary methyl linoleate and linolenate on growth of carp - 11. Bull. Jap. Soc. Sci. Fish. 41 : 263-269.

WATANABE, T., T. TAKEUCHI & C. OGINO. (1979) Studies on the sparing effect of lipids on dietary protein in rainbow trout. Proc. World Symp. on Finfish Nutrition & Fishfeed Technology . Hamburg 1978. Vol 1. Berlin 1979. 113-125.

WEBBER, H.H. & H.E. HUGUENIN. (1979) Fish feeding technologies. Proc. World Symp. on Finfish Nutrition & Fishfeed Technology. Hamburg 1978. Vol 11. Berlin 1979. 297-316.

WINFREE, R.A. & R.R. STICKNEY. (1981) Effects of dietary protein and energy on growth, feed conversion efficiency and body composition of Tilapia aurea. J. Nutr. 111 (6) : 1001-1012.

WING, J.M. (1964) Dried bakery products in dairy cattle rations. Fla. Agr. Exp. Sta. Ann. Rep. 94-95.

WING, J.M. (1965) Preliminary evaluation of dried bakery product in dairy cattle rations. Feedstuffs. 37 (18) : pp 38 & 128.

WOLF, L.E. (1951) Diet experimentation with trout. Prog. Fish Cult. 13 : 17-24.

WOLHFARTH, G.W. & R. MOAV. (1967) The relative efficiency of experiments conducted in undivided ponds and in ponds divided by nets. FAO Fisheries Reports. 44 (3) : 487-492.

WOLHFARTH, G.W., R. MOAV, G. HULATA & A BEILEI. (1975) Genetic variation in seine escapability of the common carp. Aquaculture. 5 : 375-387.

WOOD, E.M., P.J. GRIFFEN & S.F. SNIESZKO. (1954) Synthetic binding of trout diets. Prog. Fish. Cult. 16 : 19-24.

WOOD, E.M., W.T. YASULAKE, A.N. WOODALL & J.E. HALVER. (1957) Nutrition of salmonid fishes 11. Studies on production diets. J. Nutr. 61 : 479-488.

YINGST 111, W.L. & R.R. STICKNEY. (1980) Growth of caged channel catfish fingerlings reared on diets containing various lipids. Prog. Fish. Cult. 42 (1) : 24-26.

YU, T.C. & R.O. SINNHUBER. (1972) Effect of dietary linolenic acid and docosahexanoic acid on growth and fatty acid composition of rainbow trout. Lipids. 7 : 450-454.

YU, T.C. & R.O. SINNHUBER. (1976) Growth response in rainbow trout to dietary z3 and w6 fatty acids. Aquaculture. 8 : 309-317.

YU, T.C., R.O. SINNHUBER & G.B. PUTNAM. (1977) Use of swine fat as an energy source in trout rations. Prog. Fish Cult. 39 (2) : 95-97.

ZAUGG, W.S. & L.R. MCLAIN. (1969) Inorganic salt effect of growth, salt water adaption and gill ATPase of Pacific salmon. In 'Fish in Research'. NEUHAUS, O. & J.E. HALVER (eds). Academic Press.

ZEITOUN, I.H., D.E. ULLREY & P.I. TACK. (1974) Effects of water salinity and dietary protein levels on total serum protein and hematocrit of rainbow trout fingerlings. J. Fish. Res. Bd. Can. 31 : 1133-1134.