EVALUATION OF SINGLE-CELL PROTEIN

IN TROUT DIETS.

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

February, 1976.

SUMMARY.

A yeast, bacteria, alga and two fungi were evaluated in pelleted trout feeds.

Pellets were produced by dry and wet pelleting processes. The latter was more convenient and applicable to the production of small quantities of water stable pellets.

An inexpensive feeding system, applicable for use in small-scale tank systems, was developed. This was adapted for controlled, satiation and demand feeding techniques. Satiation feeding appeared to be the most applicable to the experimental design.

The five products were well tolerated in compound feeds at a level of 20% and 40% fed successively for 12 and 10 weeks, during which time no apparent pathological disturbances developed. Dietary performance was related to the protein content.

Both fungi had low digestible protein contents, thus limiting their usefulness as a protein supplement in trout diets.

The alga, bacterium and yeast had a higher digestible protein content and each was subsequently tested at four levels in semi-purified diets. The protein efficiency ratio varied in a manner similar to that shown for the rat, but maximum values were obtained at higher protein levels.

The alga was palatable and the level of digestible protein was similar to that shown for several plant seed meals. It was efficiently utilized in one experiment and it could be used as a supplementary protein in trout feeds

Poor palatability of the bacterial protein was a consistent feature of the results and this may impose a limit to its usefulness in trout diets, despite the very high level of digestible protein found in this material.

The yeast was extremely palatable and highly digestible. The material was efficiently utilized in the semi-purified diets, producing an acceptable growth and feed conversion efficiency and a relatively high protein efficiency ratio. This product could be used as a major component of balanced trout diets.

ACKNOWLEDGEMENTS.

I wish to express my sincere thanks to Professor A.J.Matty for his interest and support throughout this work and to members of the technical staff of the Department of Biological Sciences for their co-operation. My thanks must also go to Mr.F.K.E.Imrie of Tate & Lyle Limited, for his encouragement and the financial assistance of his company. I would also like to acknowledge the Science Research Council for financial support.

My thanks are extended to members of the Aston Fish Culture Unit, past and present, for many interesting discussions and for their friendship.

Finally, I am especially grateful to my parents for their interest and to my wife, Kate, for her patience and understanding and for the tremendous assistance she has given throughout the research and during the preparation of this thesis.

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GENERAL INTRODUCTION

There is currently an upsurge of interest in fish farming throughout the world. The methods used are extremely diverse, being greatly influenced by local socio-economic conditions and needs. In the developed countries economic conditions generally dictate that production should be intensive and the emphasis at this time is necessarily on high priced species. In Western Europe, the production of rainbow trout, currently estimated at 60,000 tons per annum, predominates. The industry is still comparatively small, but an impressive and accelerating growth rate has occurred over the past decade. The rate of growth in the United Kingdom has been somewhat slower than in other parts of Europe, notably Denmark, France, Italy and more recently Spain, and production is currently estimated at only 2,500 tons per annum. There is, nevertheless, a growing interest in this new, emergent industry and it is attracting investment from both private individuals and large corporations.

One of the major technical limitations to the growth of the industry is that of maintaining a food supply which is adequately nutritious and reasonably priced. The choice of a particular food type depends largely on the availability, cost and the ease with which it can be handled and fed to the fish. With the exception of parts of Scandinavia, where supplies of 'trash fish' are available in quantity, the demand in Europe is predominantly for dry compound feeds. In this respect at least, fish farming is similar to any other intensive livestock production enterprise. Furthermore, fish compound feeds contain essentially the same raw materials as those of other livestock such as pigs and poultry, although in different proportions. Increasing world demand for the limited supplies of feed ingredients, particularly of protein from conventional sources, will inevitably result in shortages in the future. Compound feeds for fish generally contain large quantities of protein and are therefore particularly sensitive to increases in the price of protein commodities caused by shortages in supply. If fish farming is to emerge as a significant industry, production costs, of

which 50% - 80% may be accounted for in feed, must be kept as low as possible to ensure adequate profitability of fish farming ventures. It is therefore very relevant, at this time, to assess the potential contribution of alternative protein sources such as industrially produced single-cell proteins. Such was the aim of this study.

SECTION 1.

SINGLE-CELL PROTEIN IN PERSPECTIVE.

SALMONID DIETS

Introduction

Nutrients are required for the maintenance of body functions of all animals. Additional nutrients are required to support productive processes, of which growth is the major consideration in the context of fish culture. The ultimate objective of nutritional research is to provide feeds containing the minimum quantities of the nutrients in a balance required for optimum production. Obtaining a knowledge of the quantitative requirements of fish for these nutrients and of the value of food sources in supplying them is fundamental to this objective.

Nutrient Requirements

The publication recently of a number of reviews on fish nutrition has superceded the necessity for a detailed account in this thesis. A general review on fish nutrition has been published by Cowey and Sargent (1972). The reviews by Phillips (1970) and that of the Subcommittee on Fish Nutrition (U.S. National Research Council, 1973) have specific regard to the nutrient requirements of trout. Fish metabolism and bioenergetics have been the subject of reviews by Beamish and Dickie (1967) and Warren and Davis (1967) whilst regard for the energy requirement of fish and how this can be met using practical diets is the subject of the review by Phillips (1972). The relative merits of different energy sources have also been discussed by Halver (1970) and Cowey (1975). Protein and amino acid requirements have been reviewed by Halver (1970) and Mertz (1969, 1972), fats and fatty acids by Lee and Sinnhuber (1972) and vitamins by Halver (1972). Little work has been undertaken to determine the precise mineral requirements. Phillips (1970) and Halver (1970) briefly discuss mineral requirements such as they are known.

Despite considerable progress since 1953 when Halver was able to define a purified diet which contained sufficient nutrients to maintain the health and well-being and support the growth of salmonids for prolonged periods (Halver and Coates,1957), the nutritional requirements of fish are not well understood by comparison with domesticated terrestrial animals. The large number of nutrient variables that exist in a feed and which frequently cannot be measured with certainty, let alone be controlled, and the numerous complex relationships and interactions between the nutrient components, make the study of the requirement of any one specific nutrient difficult. In addition, a number of environmental parameters may affect the growth potential and consequently the nutritional requirements. Important environmental parameters include temperature and chemical composition of the water, the degree of oxygen saturation and the free ammonia concentration of the water, both of which will be dependent on fish loading ($kg\ m^{-3}\ min$), and the intensity and duration of illumination. Stocking density ($kg\ m^{-3}$) and space factors may be important, the situation being complicated by hierarchal effects. The effect of such environmental parameters have been considered in a number of reviews including those of Brown (1957), Warren and Davis (1967), Burrows (1972), Hastings (1969) and Hastings and Dickie (1972). In addition, the food type and level of feeding will also have a considerable bearing on the nutrient requirements and the growth performance of fish. (Paloheimo and Dickie, 1966b; Brett et al,1969).

Clearly there are many variables and controlling them is frequently no simple matter. The nutrient requirements of fish thus far reported are specific for the species under study, for the methods used and for a particular set of environmental circumstances prevalent at the time of the study.

Practical Feeds

Since much of the work on the basic nutrient requirements of salmonids has been carried out only in comparatively recent years, during the

formative years in the development of fish husbandry techniques fish culturists did not have the benefit of knowing the protein and amino acid requirements, the fat and fatty acid requirements or the vitamin requirements of their fish. Choice of feeds was therefore more a question of finding out what was available in sufficient quantity and at a price which allowed economic fish production. The available foodstuffs were tested singly or in combination in 'feeding trials' in the hatcheries, a trial and error approach.

Although complete compound feeds now form the basis of feeding programmes in most salmonid hatcheries and farms throughout the regions of the world where husbandry techniques are well-developed, there are instances where the prevailing economic conditions are such that older, more traditional methods have been retained. Salmonid diets can conveniently be divided into three categories.

1. Fresh foods

Natural feeding of trout generally starts with crustaceans, especially Cladocera, Copepoda, Malocostraceans, Isopoda, Amphipoda and water-insect larvae, followed by adult insects, worms, molluscs and other larger sized animals such as small fish, tadpoles and frogs (Ghittino, 1972).

Chemical analyses of natural food organisms have been reported by Phillips et al (1954), Hastings and Dickie (1972) and Ghittino (1972) and conversion efficiencies ranging from 2·3 to 7·1 have been reported by Phillips (1954). From such data it is clear that natural food organisms could provide a valuable source of nutrients for trout. However, natural feeding is of little consequence in most culture operations involving intensive rearing techniques. Natural feeding can be relevant in some extensive fish culture operations involving, for example, pond culture of cyprinids (Huet, 1970; Bardach, 1972) although, as with trout, high stocking densities cannot be supported without fertilization or

supplemental feeding (Szumiec, 1969; Gurzeda, 1969; Merla, 1969).

Blood, inedible viscera, unhatched eggs, wastes from poultry dressing plants, meat packing houses and fish processing plants are a few materials which separately or in combination offer a protein quality and a balance of vitamins suitable for fish production (Hastings, 1969). Fresh liver was at one time a favourite for fry in the United States (Tunison, 1940). Whilst some fish culturists still prefer to feed fresh liver occasionally to fry, there are considerable problems with feeding offal, notably difficulties of processing, storage, wastage and pollution.

Fresh fish was traditionally fed in Denmark (Rasmussen, 1967), the Federal Republic of Germany, in particular the northern regions (Tack, 1967) and also in Ireland (Piggins, 1967) and parts of Japan (Nomura and Fuji, 1969). However, refrigerated storage and transport facilities are expensive, processing is labour intensive, and as with feeding offal there can be considerable wastage and pollution. Despite these problems, there are regions where 'trash fish' supplies are available in abundance in close proximity to the fish farms and it is still apparently economical to use them. Denmark and Norway are notable examples. This form of feeding is not very relevant to the United Kingdom industry at this time since 'trash fish' is not landed in quantity and much of the rainbow trout is currently produced inland in fresh water farms where the economic advantage would be lost in view of the high cost of cold storage, transport and labour. Furthermore, it is unlikely that the additional pollutional load could be tolerated in a country in which fresh water is an obvious limiting factor.

2. Meat-meal feed mixtures

Concerned with the rising price of fresh ingredients, the Cortland researchers had been testing the addition of dry feed ingredients for some years before World War 2. After seven year's testing, Tunison (1940) described a satisfactory formula consisting of equal parts (24%)

of spray-dried skim milk powder, whitefish meal, cottonseed meal and wheat middlings. 4% salt was added as a binding material. Other ingredients were tried during and after the war because of a lack of dried skim milk powder and whitefish meal and the work has been summarised by Phillips (1970). Binding materials were necessary for this type of diet to produce the correct consistency for extrusion and to reduce leaching of nutrients and pollution. A number of binding agents have been assessed including tapioca, mucilose (psyllium seed), vegetable gums (Wolf, 1951), C.M.C., Irish Moss, Kelgin and Kelcoloid (Wood, Griffin and Snieszko, 1954). However, Combs and Burrows (1958) concluded that it would be difficult to justify the use of commercial binders except in diets which cannot be bound with salt. The use of binding agents, including gelatin, was therefore generally restricted to work with purified diets (Wolf, 1951 and Halver, 1957).

Some typical meat-meal diets for trout and salmon are shown in Appendix 1.

The preparation of meat-meal mixtures was carried out on site at the hatchery. It was a time consuming process. A logical extension in the use of this type of formulation therefore was the development of improved techniques for the food preparation. A very successful method was developed jointly by the Oregon State Game Commission and the Oregon Agricultural Experiment Station Seafoods Laboratory. The basic formula was 40% wet meat and fish products and 60% dry meal ingredients. A vitamin mix modified from the original McLaren test diet (McLaren, 1946, 1947a, 1947b) was included. Processing was carried out by adding the dry ingredients, which were blended with vitamins and anti-oxidants, to the wet ingredients. The mixture was passed through machines similar to those used in the spaghetti and macaroni manufacturing industry (Hastings, 1969). A single machine could mix a 40 : 60 mixture continuously and extrude solid or tubular pellets cut to any length. Pellets produced in this way were quick frozen and stored at low temperatures until required.

The diet, called the Oregon Moist Pellet, was developed and tested on Pacific salmon in laboratory and pilot-scale feeding trials, finally being evaluated in a series of feeding trials on a production scale (Reported in a series of the Oregon Fish Commission Research Briefs, 1951-1955 and summarised by Houblou et al, 1959).

This type of diet is still used extensively in fish culture, particularly on the West Coast of the United States.

Detailed specifications of the processing of the Oregon Moist Pellet as provided by the Fish Commission of Oregon (1968), are summarised in Appendix 1. For the wet ingredients the emphasis is on the use of marine fishery products which are available on the West Coast and found suitable for Pacific salmon. The formulation is not rigid, however, and variations can be successfully used (Hastings, 1969). Further, the diet is suitable for other species of farmed fish including rainbow trout (Phillips, 1970).

The use of moist pellets has definite advantages over the use of meat-meal mixtures. The pellets can be commercially produced thus taking the diet preparation out of the daily routine of the hatcheries. This, and the ease with which the pellets can be handled, result in labour saving and lowered costs. There is more complete food consumption which is reflected in higher conversion rates and significantly less pond fouling.

3. Dry ingredient mixtures

During the 1950's, use of meats as principal ingredients in salmonid diets became increasingly difficult because of their increasing scarcity and high cost. In addition, the methods of preparation of diets incorporating such products were tedious and time consuming, and large quantities of refrigerated storage space were required. Furthermore, it became obvious that disease vectors could be transmitted through the use of such products, particularly of raw fish (Guenther, Watson and

Rucker, 1959; Ross, Earp and Wood, 1959). Whilst this factor was largely eliminated in the preparation of the Oregon Moist Pellet by pasteurising the wet ingredients (Houblou et al, 1959) it nevertheless added impetus to the growing effort to produce a complete feed from entirely dry ingredients.

In the early 1950's several commercial dry pelleted foods came onto the market. These were composed of all dry ingredients containing 30-40% fish meal and all known vitamins at that time. The formula varied from one company to another and was kept secret. The diets were processed on equipment used in the production of livestock feeds. They had been tested on a small production scale at the Cortland Hatchery and found satisfactory only if fed with an occasional meat supplement, and to fish 3" in length or greater (Phillips, 1952). Early attempts to rear fish on dry pelleted feeds with occasional meat supplementation have also been described by Brockway (1953), Willoughby (1953)

Encouraged by this, the Cortland workers attempted to pellet different variations of the dry portion of their meat-meal mixes, called the Cortland No. 6 dry feed, and test it as a complete feed for trout. Slight changes were made to the formula to allow ease of pelleting and the production of stable pellets (Phillips, 1953). A series of experiments were then tried with various vitamin supplements based on Wolf's vitamin experiments (1951) as described by Phillips (1957). Problems were experienced (Phillips, 1957, 1959 & 1960) until the early 1960's when Phillips (1961, 1962, 1963) successfully developed an open formula trout pellet which was tested over a two year period on brown trout in production raceways. The fish could be handled without loss and were, in general, of a satisfactory appearance. No abnormal blood conditions were reported, and haemoglobin and microhaematocrit levels were normal. Furthermore, the diets were the first such to produce satisfactory formation of reproductive products by the fish, and they were therefore considered nutritionally complete.

During the years that followed these first attempts to produce complete dry feeds, development continued at the Cortland Hatchery and later at the McNenney National Fish Hatchery, South Dakota, which is now the Diet Testing Development Centre, dealing specifically with formulation and processing research of fish foods for cold water species. Changes in formulation with respect to the vitamin levels, quality of protein, choice of oil, etc. have taken place. McNenney formulations have been described by Orme (1970) but they are subject to change since this field is one of continuous development.

Other successful attempts to produce diets from entirely dry ingredients have also been described. At the Salmon-Cultural Laboratory, Washington, a programme of research involved the development of a dry diet for salmon along the same lines as that for trout at the Cortland Hatchery. Initially wet ingredients were evaluated for chinook salmon (Onchorynchus tshawytscha) and sockeye salmon (O. nerka) followed by the inclusion of dry-meal components, with raw meats and pasteurised fish products serving as supplements to the diet and finally evaluating the dry-meal fraction as a complete feed with vitamin supplementation. The work has been summarised and the formula, called the Abernathy pelleted diet, has been described by Fowler and Burrows (1971), although it is obviously flexible. It is generally simpler than the McNenney formula. The vitamin levels have been raised by inclusion of the McNenney Premix PR-25, (Fowler, 1973) to ensure a safety margin against possible stress conditions. Although originally developed for species of Pacific salmon, it has been tested with steelhead trout (Salmo gairdneri) and Atlantic salmon (Salmo salar).

Fish culturists in Asia and Europe experienced the same rise in prices and diminished availability of liver and other livestock offal as in the United States and the use of dry compound feeds increased during the 1960's. Feed production, however, is generally in the hands of commercial compounders and little information is published. The specifications are similar to those used in the U.S. but the use of protein,

fat and carbohydrate sources are largely dictated by economic considerations peculiar to each country. Development of formula feeds in Japan has been described by Nomura and Fuji (1969). The formulae tend to be simpler, being based on white fish meal as the main protein ingredient, wheat flour as the cereal component and fish oils as the lipid component.

Some typical dry feed formulations, including the McNenney diet and Abernathy diet, are shown in Appendix 1.

Processing of dry ingredients may take a variety of forms, particularly with respect to pelleting. The processing involves a number of steps including grinding, mixing, pelleting and cooling. The methods have been described by Carlson (1969) and Hastings (1969) and the industrial application by Stivers (1970) and Robinson (1970). Different pelleting processes have been used to produce fish feeds. Reference to conventional pelleting processes can be found in the above citations. The process involves the application of steam to the mix which is then compressed through a die block. Steam gelatinizes the starches and produces a harder pellet. Water stability can be improved by an expansion process (Hastings, 1969) involving the application of steam under high pressure, causing the material to expand as it is extruded through the die. Wet extrusion methods involving the addition of water may also be used where very water stable pellets are required (Szumiec, 1969). A more sophisticated process involving spraying a fine mist of water onto a meal mix as it falls onto a rotating disc causing the particles to agglomerate has been described by Decker and Pigott (1967). This method has been used for the production of pellets from the Abernathy formulation (Fowler, 1971). Although there are obvious advantages in increasing the water stability of feeds for fish, the expansion process, wet extrusion and agglomeration techniques are more expensive.

The advantages of dry pellets have been described many times. A complete diet containing supplements can be made. The pellets can be stored easily without refrigeration or large areas of space. They are

easily handled and can be fed using automatic dispensers, saving on labour requirements. There is little wastage, there being almost no loss of nutrients and consumption can be 100%. This not only improves the conversion efficiency but also results in less time being required for cleaning troughs, tanks or ponds and reduces pollution of the effluent.

Present Status of Feed Formulation

Since a feeding trial with the species in question is still the most useful method of obtaining results which have a direct application in feeding practice (Maynard and Loosli, 1969), it is not surprising that the procedures adopted 40 years ago are fundamentally unchanged today. There is now a greater variety of available foodstuffs, mainly dry, from which to choose, but a knowledge of their composition and cost, together with the experience gained over the years from the manipulation of the ingredients into formulae and their subsequent testing, remains the basis of feed compounding today.

With the possible exception of the work on vitamin requirements, which obviously had a definite and direct application to practical formulation without which the development of diets from dry ingredients would never have occurred, it is difficult to determine the extent of the impact which the accumulating knowledge of the basic nutrient requirements of fish had on feed formulation. Most of the pure nutritional research was carried out in parallel with diet development programmes and many conclusions, such as the level at which to include protein in a diet, have been reached by both methods independently. This is not to say, however, that a knowledge of the basic nutrient requirements is not useful to the formulator today. On the contrary, it provides a framework within which he can use his experience. Thus, although the approach is still one of formulating, compounding and subsequently testing diets in feeding trials, the use of numerical coefficients for the nutrient requirements by informed scientists minimises the errors and allows more

rapid progress in the development of formula feeds.

The ultimate objective of the feed formulator is to provide a complete diet that meets all the nutritional requirements of the animal for the optimisation of the productive function with which he is concerned at the minimum cost. The number of variables involved make this problem extremely complex. It is not surprising therefore to find computers being used increasingly to solve the problem. 'Computer compounding' or 'Least Cost Formulation' is a means by which a compound feed formulation can be produced from available ingredients, subject to certain constraints, at the least possible cost and in mathematical terms, is a particular example of the linear programming problem. Although it is used successfully in the formulation of feeds for poultry and pigs, it must be mentioned that the constraints are very much more well defined in such areas of livestock production reflecting the very much greater volume of data on the nutrient requirements of these species. It has already been mentioned that the nutrient requirements of fish, as far as they are known apply only for each species studied and are then only one dimensional, being correct for the methods employed and the conditions prevalent at the time of the study. Use of such values as constraints in computer formulation for fish should therefore be treated with extreme caution when formulating diets for the variable circumstances found in practice.

Conclusion

Although the study of fish nutrition is as yet in its infancy, impressive achievements have already been made, enabling food conversion ratios (dry food fed, usually containing approximately 10% moisture, per unit wet weight gained) between 1·0 and 1·5 to be widely reported. This already compares most favourably with poultry where food conversion ratios of 2·2 may be expected.

The use of dry ingredients will enable formulations to be adjusted to

take advantage of each new development in fish nutritional research. This, coupled with improved husbandry methods, could lead to a truly remarkable performance in terms of animal production in the future.

The advantages of feeding dry pellets have already been stated. They have been summarised by Phillips (1970): "For economy, ease of feeding and efficiency of food preparation, the pelleted fish feed represents the best feeding programme yet devised". Pelleted fish foods certainly offer the best opportunity for the development of an efficient,large-scale fish farming industry in most countries with highly developed economies, including Britain.

WORLD DEMAND FOR FEED PROTEINS

Introduction

The successful formulation of dry pelleted feeds during the early 1960's enabled the task of feed preparation to be taken out of the daily routine of the hatchery. Although a little more specialised, particularly with respect to the production of fry feeds, fish feed compounding was not too dissimilar to that for poultry or pigs and therefore large compound feed manufacturers were able to use existing equipment. It furthermore enabled manufacturers to use feedstuffs which they were already purchasing in quantity for their existing feed manufacturing business. The economic advantages of bulk purchase and large-scale manufacturing capacity are obvious. Since essentially the same ingredients are used in fish compound feeds as in those of other livestock, the prices are very much determined by the level of intensive livestock production throughout the world and hence the demand for feedstuffs and the level of supply of those feedstuffs. The availability and cost of raw materials for fish feed compounding must therefore be considered in this perspective.

The Present Situation

The major protein feedstuffs used in feed compounding, and therefore available for fish feeds, are meals derived from oil seeds and fish. The principal seed proteins include soya bean, cotton seed, ground nut, sunflower seed and rapeseed and 92% of the fish which are reduced to meal are oily species such as anchoveta, herring, pilchard and menhaden. By comparison, white fish meal, such as that produced mainly from fish unsold after being offered for human consumption and from fish offals, consisting largely of the heads, skeletons and trimmings left over after removal of the fillets (Abrams, 1971), contributes very little to the total world production.

World production of oilcake increased from an average of nearly 38M tonnes/annum in the period 1961-65 to 48M tonnes/annum in 1966-70 and in 1972 was estimated to have reached 56M tonnes, equivalent to nearly 24M tonnes of protein of which soya bean meal accounted for about 59% (Breslin, 1974). Approximately two thirds of the total soya bean meal and about 90% of that exported was produced in the United States of America (Arnott, 1974).

Fish meal supplies have also increased during the last 20 years. In 1958 the world production was 1,396,000 tonnes; by 1962 it had risen to 2,885,000 tonnes, largely as a result of a dramatic increase in production from Peru which made her the largest fishing nation in the world in terms of total catch, almost the whole of which was reduced to fish meal, and by 1966 the total world production had risen to 4,066,000 tonnes (Borgström, 1970). Production reached a peak of 5,323,000 tonnes in 1970 (Bellido, 1974).

The increases in the production of oil seed meals and fish meal during that time adequately matched the steadily increasing demand for protein for the feedstuffs industry and relative price stability was maintained. Thus, during the decade 1962-1972 the price of fish meal fluctuated between £50-60 per tonne and the value of soya bean meal showed a marginal downward trend from approximately £40 to £38 per tonne. (Tolan and Hearne, 1974). The prices were generally similar on a unit protein basis.

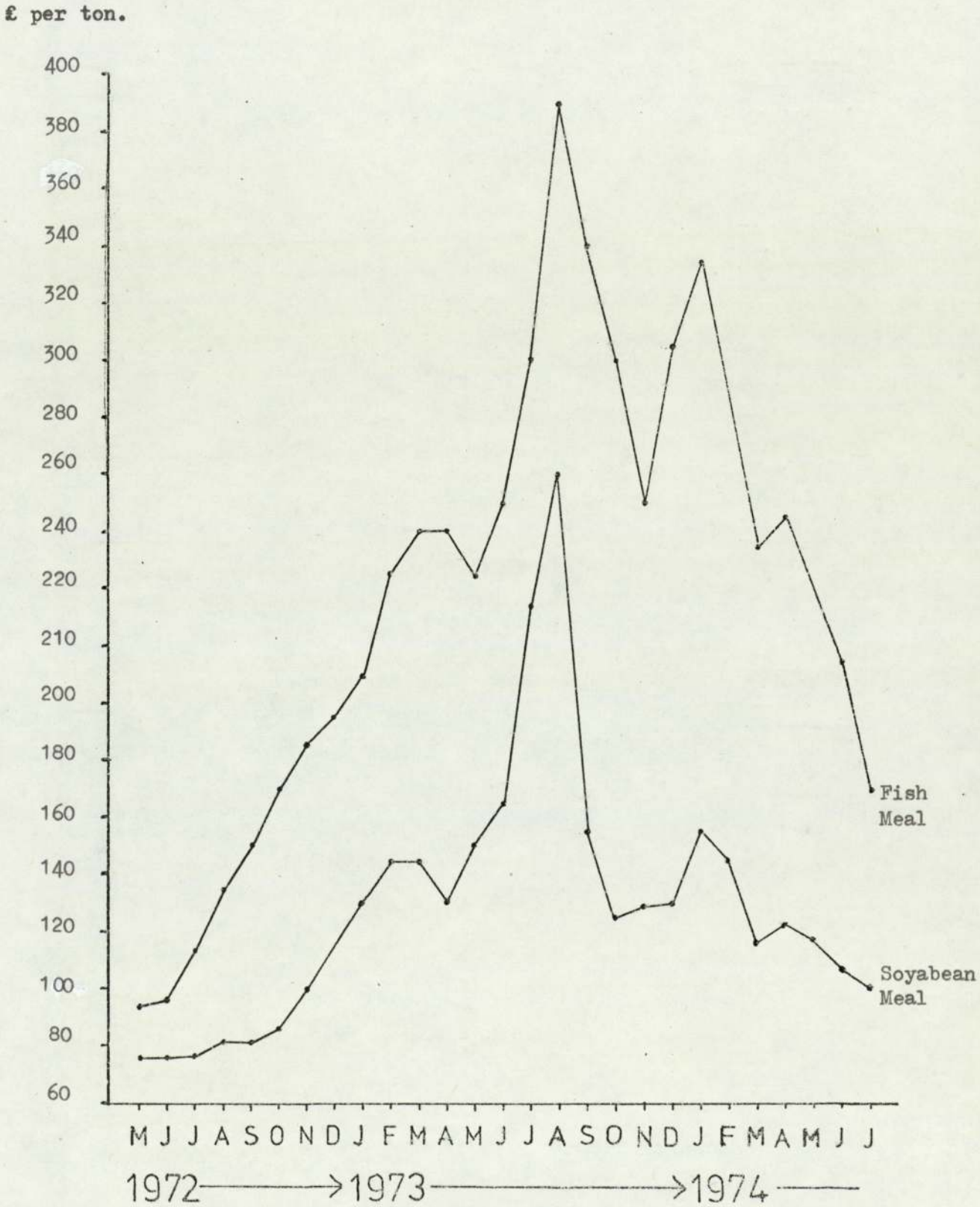
Towards the end of 1972 there was, however, a dramatic change. A number of factors were responsible for this. In the first instance, the Peruvian Fishing Industry, which by that time was supplying about 70% of the total world fish meal exports, collapsed completely. The industry is based on the vast supplies of anchoveta that rely on the cool coastal waters off the coast of Peru brought by the Humboldt current. For some time the industry had been threatened by a combination of overfishing and the warm current that drifts southward along the coast of Peru

around about Christmas time, a phenomenon christened "El Nino" by the Peruvian fisherman. During the last quarter of 1972, the Humboldt current was repulsed due to this ecological disturbance and only 47,000 tonnes of fish meal were produced,compared to 760,000 tonnes during the same period of 1971 (Winsch, 1973). The collapse of the Peruvian Fishery wiped more than a million tonnes from the total supply of fish meal in 1972 compared with the previous year (3·9M tonnes in 1972 compared with 5·1M tonnes in 1971).

In addition to this, disastrous droughts in Russia, India, Australia and Africa greatly reduced cereal, sunflower and peanut crops, and Asia had poor rice crops. The devastation of the Russian sunflower seed crop in particular led to an unexpected situation in which the Soviet Union purchased 40 million bushels of soya beans from the United States. This purchase came at a time when the United States soya bean crops were already being overstretched due to poor harvesting in 1972.

The situation was not much improved in 1973 when excessive rainfall in the U.S. delayed spring seeding and there was very little fishing activity by Peru. As a result the supply of feed proteins in 1973 fell considerably short of the demand and the market became highly volatile, a situation which was not helped by the worsening world currency problems. At the height of the problem in August 1973 the difficulties were summarised in the market activities section of the Journal of Flour and Animal Feed Milling: "With export restrictions, monetary problems, rumours, crop reports, Government statements, counter Government statements, the market remains crazy. What does remain acute, however, is the supply position. This in itself is a disaster. It does not matter what the article is, animal protein, vegetable protein, roughage, fillers, cereals and any other by-products, they are difficult to find and every-time there is a trade, one could almost say another world record is broken". The prices for fish meal and soya bean meal had increased by at least 300% over the 1972 base level, fig 1.

Fig. 1. Protein Prices.



Fishing off the coast of Peru remained sporadic throughout 1973 and 1974 and there have been as yet no signs of a complete recovery. Cereal production improved, however, in the autumn of 1973 and there was a fall off in prices of proteins generally. More recently, due to the world recession, there has been a substantial reduction in the world demand for feed proteins and this, coupled with the generally improved supply situation, has enabled seed proteins to cover the demand and deflated prices of most protein commodities are back to 1972 levels.

The high feed prices of 1973 were disastrous for livestock producers throughout the world. In many instances, serious losses were incurred. By far the largest importers of proteins are West Europe and Japan. Compound feed production in the EEC in 1973 was 58·50M tonnes (European Federation of Feed Manufacturers) and in Japan around 15M tonnes. The requirement for protein is greatest from the pig and poultry sectors of the industry. In 1972 compound feed production for pigs and poultry in the EEC amounted to about 19·1 and 16·5M tonnes respectively, and in Japan 4·7 and 9·2M tonnes respectively (CAFMNA, 1973). Clearly, it was these industries which were hardest hit during 1973.

Two important facts are revealed in such statistics. The first is the vulnerability of Japan and West Europe's current supplies of protein and therefore their livestock industries. The second is the enormous potential market for alternative protein sources which can be proved equally as effective in terms of nutritional quality and which are competitively priced. In recent years, these two facts have added substantially to the impetus of research into alternative protein sources.

Medium Term Demands

Although serious, the short term problems may be considered as transient. One cannot predict increases in supplies of fish meal in the future; indeed one cannot even be certain that production levels of con-

ventional species will ever return to the 1972 levels. However, it seems likely that much of the increased demand for protein in the immediate future will be met by an increased supply of oilseed meals. The FAO has projected a demand for 71·4M tonnes of oilcake as a protein supplement in animal feeds in 1980 and against this they have projected an increase in supply of 3·4% per year giving a total production of 75M tonnes, i.e. a slight surplus to demand (FAO, 1971). This is not to say, however, that such an increase will be achieved. According to Arnott (1974) a report was produced in the United States indicating a potential of 19M tonnes of soya bean oil meal. In addition to the United States therefore, one must look to increased production of oilseeds in the developing countries either for home extraction or for export to the developed countries where they could be subsequently extracted. A report published recently (Sturgess, 1975) suggests that the yield of soya beans is unlikely to move up very rapidly and that the price of oilseed meals by 1978/79 will have moved up to a suggested level of 120% over the 1971/72 price. It has been assumed that prices of protein feeds of animal origin will rise a little faster than this. Thus the livestock industry will be faced with a predictable, steady increase in the price of protein feed commodities, over the next five years.

Beyond this time, one cannot be sure what the supply situation will be. In some of the developing countries to whom we look for a supply of oilseed proteins, e.g. Brazil, economic development has proceeded at a rate which has enabled them to create their own livestock industries. In addition, parts of the Middle East, with the benefit of their vast oil revenue, may also be expected to create their own livestock industries. As livestock production expands and intensifies in such countries, one may expect a progressive increase in demand for feed proteins and, in some instances, a progressive swing from export to home consumption. Such developments could, in the not too distant future, create supply problems which will be reflected in much higher costs for feedstuffs.

Long Term Demands

It is more difficult to consider the broader concepts involved in the

world food supply situation and the effects changing circumstances might have on feed protein supply. There is now generally a greater awareness of the world food shortage, and particularly protein shortage, than ever before. The subject has become a daily talking point in both the popular and technical press. The problem is immense and extremely complex. An in depth study has been reported by the President's Science Advisory Committee (1967). The world demand for food protein is variously estimated at 80-90M tonnes, of which 25M tonnes should be derived from high quality protein having an essential amino acid balance similar to that of animal protein. Present production of animal protein is of that order of magnitude but more than two thirds of it is consumed by less than one third of the world population, which infers that there is a substantial gap between the supply to and demand of the remaining two thirds of the world population.

The disparity between the developed and the developing countries is obvious and it will be extremely difficult to maintain the difference in standards even at the present level since the population of the developing countries is increasing at twice the rate as it is in the developed countries. The problem has been made even more difficult in the past twelve months due to the large increases in the price of oil and its effect on the world economy.

It is fully acknowledged that consumption of animal protein in the developed countries is greater than is needed and that animal production, particularly of monogastric species such as pigs and poultry, is extravagant in terms of efficiency of protein utilisation. On the other hand, whilst proteins used for animal production could be used directly by humans in the nutritional sense, they are not currently acceptable to any extent for direct consumption. The nutritional value of a protein is nil until it is readily accepted and consumed. Although the process of feeding animals is inefficient and expensive, it is nevertheless necessary in order to upgrade the protein. Processing techniques are now being developed which will enable feed commodities to be used

directly in human foods, but they are as yet expensive and a great deal of creativity and ingenuity will be required to market these products successfully. One of the major problems facing the developing countries is that of raising their income to a level which would enable them to produce or purchase the protein that they require in a form which suits their demands.

Whilst it is unlikely that the world food problems will be solved for a very long time to come, it is perhaps inevitable that eventually there will be a swing from indirect consumption of many of the conventional proteins which are currently used in animal feeds, to direct human consumption. The extent of such a swing or the effect on world animal feed supplies cannot be predicted at this time, but it is as well to be aware that a conflict in demand for proteins could conceivably occur in the future.

Conclusion

Since fish feed compounds will generally contain 40-50% protein in the finished dry feed, they are particularly sensitive to fluctuations in the price of proteins. During the crisis of 1973, therefore, the cost of fish compounds increased dramatically. Since food generally represents 50-80% of the production costs of farmed fish, this had a substantial effect on profitability. It was unfortunate that the fish farming industry was faced with such a problem as it was beginning to gain momentum. If fish farming is to emerge as a significant industry in the future, protein supplies must be guaranteed. In view of the anticipated difficulties in maintaining a supply of conventional proteins, it is essential that an effort be made to evaluate the potential of new sources of protein as and when they become available.

THE POTENTIAL OF SINGLE CELL PROTEIN

Introduction

The President's Advisory Committee (1967) suggested ten possible ways of meeting future world demand for protein, including the production of microbial protein and algae. This was by no means a new concept. Algal protein has been consumed for centuries in the Chad Republic of Africa and yeasts and moulds were produced as human food supplements in both world wars. Whilst large-scale production of such non-conventional protein sources for feeding directly to those people in greatest need might be the ideal and ultimate objective, the problems of acceptability are even more acute than for the plant-seed proteins and processing costs are again beyond that which can be afforded by most of the under-nourished nations. There is some current interest in the production of microbial protein for specialist human markets in the West, but generally the added cost of achieving the high standards of production and quality control necessary for food products destined for the human market make it less attractive at this time, than the animal feeds market which has attracted most interest.

The concept of feeding micro-organisms to animals is not new either. Yeast (Saccharomyces spp) used extensively in the brewing industry has been sold as a by-product to the feed industry for several decades, as have fodder yeast (Candida spp) produced on a wide range of carbohydrate substrates. However, in view of the potential size of the market for new protein feeds and the current and projected difficulties of meeting the demand for protein from conventional sources, there has been a considerable increase of interest in the field of microbial fermentation and much innovation involving the production of several species of organisms on a much wider range of substrates including hydrocarbons. Aspects of the subject have been reviewed extensively in those books called "Single-Cell Proteins" edited by Mateles

and Tannenbaum (1968) and Davis (1974). Other reviews include those by Stokes (1959), Rose (1961), Bunker (1963), Snyder (1970), Alagaratnam (1971) and Worgan (1974).

Definition of Single-Cell Protein

Tannenbaum (1971) has defined single-cell protein (SCP) as "a generic term for crude or refined sources of protein whose origin is either unicellular or simple multicellular organisms, that is bacteria, yeast, fungi, algae and perhaps protozoa". According to Scrimshaw (1968), the name was coined in 1966 by researchers at the Massachusetts Institute of Technology as one which "would not have the unpleasant connotation relative to food invoked by the terms 'bacterial' or 'microbial'". Shacklady (1972), amongst others, has pointed out that the term is not an absolutely precise description since some are not truly monocellular. Nevertheless, it is a term which is sufficiently well understood to be applied without much danger of ambiguity. It is a term which has now been universally accepted in both the popular and technical press and also by the Protein Advisory Group of the United Nations.

The Potential of S.C.P. Production

One of the factors which would seem to indicate a vast potential for S.C.P. production is the range of substrates which can be utilized to support the growth of micro-organisms. Most micro-organisms are capable of utilizing simple forms of nitrogen as a starting point for the synthesis of their own cell proteins. Carbon sources which can be utilized to provide the energy source include carbon dioxide, a whole range of carbohydrates such as molasses and other agricultural by-products or wastes, wastes from food processing and wood pulping industries, organic wastes from human sewage treatment or from intensive animal production, petroleum, natural gas and by-products of the petro-chemical industry. It is convenient to discuss SCP production potential in terms of the categories of substrate utilized.

Carbon dioxide

Algae would at first sight seem very attractive for biomass production since they are autotrophic and are therefore able to grow in purely inorganic culture media using sunlight, carbon dioxide and ammonium ions as the only source of energy, carbon and nitrogen. Much of the early interest in industrial-scale algal culture was reviewed extensively in the book on this subject edited by Burlew (1953). The general review of Snyder (1970) also contains a useful section on algal production including some of the more recent developments.

There has been much interest in the production of algae for the purpose of gas exchange during inter-planetary flight (Gafford and Fulton, 1962; McDowell and Leveille, 1964 and Le Chance, 1968). In this respect, the specific high temperature strain of Chlorella 7-11-05 (Sorokin and Myers, 1953) has been extensively studied. The organism has a rapid doubling time of the order of 2-3 hours at 38^{o}C and is a good oxygen evolver and therefore suitable for regenerating atmospheres contaminated with carbon dioxide.

The possibility of growing algae in sewage oxidation ponds has also received a great deal of attention. In this respect water purification is of primary importance. In general the production of mixed algal cultures is reported although Scenedesmus appears to be the predominant species. The methods used are reported by Oswald and Golueke (1968). The use of sunlight as an energy source would seem to be the only practical means of mass culture of algae at present. The costs are prohibitive when growth is in artificial illumination. Lagoon-type culture on sewage is restricted to those areas of the world giving rise to large volumes of sewage where the supply of water is critical, where the intensity of sunlight over the whole year is adequate and where temperatures of 70^{o}C - 80^{o}C are obtained throughout the year (Gordon, 1969). Industrial production possibilities will very probably be limited to the geographical area situated between 35^{o} of latitude North and South

of the equator.

Growth may be limited by the carbon dioxide supply in addition to temperature and sunlight. In obtaining optimum growth an atmosphere of 2% carbon dioxide is required and this means use of covered tanks which adds to the expense (Senez, 1972). Other problems include contamination by other organisms and expensive harvesting since cell yields tend to be low (Worgan, 1974). In Czechoslovakia Scenedesmus quadricauda is apparently being studied mainly because of its larger, heavier cells which aid harvesting. Problems such as those described would seem to offset the use of a cheap substrate such as carbon dioxide.

A more recent development is the production of the procaryotic blue-green algae, Spirulina maxima (Clément, Giddey and Menzi, 1967). This organism proliferates spontaneously in certain areas of Lake Chad in Africa and Lake Texcocco in Mexico (Senez, 1972) and has been consumed locally in the Chad area for centuries. The organism grows at high pH, 9·5 - 10·0, which has the advantage of increasing the absorption of carbon dioxide into the culture media and also reducing the possibility of contamination. The cells grow in a spiral form and are large, enabling the biomass to be harvested and dried by simple means. Pilot plants to assess the economics of production have been established in Mexico and the South of France by the Institute Francaise de Petrole and yields of 40 - 45 ton/hectacre/year have been reported.

Shacklady (1972) has pointed out that algal culture does not seem to be either as universally applicable nor as amenable to industrialisation as the other forms of SCP production. There appears to be general agreement that the production of algal protein using present techniques could not be considered as a means of producing competitively priced proteins. The greatest hope would seem to be the production of the blue-green algae.

A second group of micro-organisms, in this case bacteria known as

Hydrogenomonas spp., have also been studied in connection with bio-regenerative life support systems in space travel (La Chance, 1968). These organisms are aerobic and autotrophic and capable of utilizing gaseous hydrogen as the energy source, carbon dioxide as the carbon source and ammonia as the nitrogen source, According to Senez (1972), cultivation of these bacteria has been envisaged and is the subject of laboratory-scale studies.

Carbohydrates

The most extensively studied carbon-energy source is carbohydrate of which there is theoretically an inexhaustible supply. Peppler (1968) has discussed the production of Saccharomyces cerevisiae, Candida utilis and Saccharomyces fragilis on molasses, spent sulphite liquor and whey respectively, along with the value of the three yeasts and debittered Brewer's Yeast (Saccharomyces cerevisiae) produced as a by-product of the brewing industry. By far the largest segment of the industry is concerned with the production of Saccharomyces cerevisiae on molasses but this protein source is the most expensive in view of the high cost of this substrate. Industrial wastes containing fermentable carbohydrates, on the other hand, can have a negative value, since they often present a disposal problem. Such is the case with the production of yeasts on spent sulphite liquor from the pulping process. Reviewing the production of SCP on waste, Forster (1973) suggests that for every 100 tons of pulp produced, about 20 tons of fermentable sugars are discharged as effluent having a B.O.D. of 25,000 - 50,000 ppm. The sugars are a mixture of hexoses and pentoses which are converted by Candida utilis into protein with a yield of 27% and a B.O.D. reduction of 60%. In some countries the benefits to environmental pollution control could be of primary importance rather than the biomass production.

Cellulose is an attractive substrate because of its abundance and ubiquity. However, it appears that no-one has yet developed a sufficiently economical method of breaking down the cellulose to fermentable sugars either chemically or biologically. The problems in-

volved have been discussed by Alagaratnam (1971). Senez (1972) reports that yeasts (Candida utilis) have been produced on hydrolysed wood for many years in several Eastern European countries, particularly the U.S.S.R. Bunker (1968) reported that an output of 900,000 tons of yeast was envisaged by the U.S.S.R. Whilst wood hydrolysates might not be the cheapest source of fermentable carbohydrate, there is sufficient evidence to suggest that in some places at least, it is considered sufficiently economical to justify large-scale production of SCP.

In addition to yeasts, mycelial fungi frequently grow on the media produced from wood hydrolysis and sulphite pulping industries. Several species of fungi including Oospora spp, Trichosporon spp, Dematium spp, Fusarium spp, Spicaria spp, Penicillium spp and Aspergillus spp have been reported to utilize the substrate,as discussed by Alagaratnam (1971). The growth of Oospora spp and Trichosporon spp was approximately the same as that of Candida yeast. Sherwood (1974) has referred to plans for commercial production of fungi on pulp wastes in Finland using Peacilomyces spp as the organism.

Neither baker's, brewer's nor food yeast are capable of utilizing starch substrates (Worgan, 1974). The Symba process (Jarl, 1969) developed in Sweden to use starch wastes involves the growth of two yeasts, Endomycopsis fibuliger and Candida utilis, in association. The former produces an excess of amylase which hydrolyses the starch to sugar which is subsequently used by Candida utilis. The harvested product consists of Candida utilis and Endomycopsis fibuliger in the ratio 9:1.

In contrast, fungi can utilize starches directly. In this respect the literature has been reviewed by Gray (1966, 1970), Alagaratnam (1971), Worgan (1974). There is currently a considerable interest in the utilization of agricultural and industrial wastes and surpluses by fungi (Litchfield, 1968; Spicer, 1973; Sherwood, 1974; Thorne, 1975; Imrie, 1975). Worgan (1974) has stressed that it is essential that micro-

organisms should be able to utilize cheap, readily available substrates if a process for producing SCP is to be economical. Agricultural and industrial wastes are frequently cheap or they may even have a negative value, but Litchfield (1968) has drawn attention to the fact that the cost of collecting, storing, transporting and pretreating complex wastes and the relative cost of their disposal by other techniques must be compared to the cost of using purified carbohydrates before a final decision can be made on the suitability of a waste material as a substrate for fungal growth. Senez (1972) has suggested that one of the problems of having an S.C.P. industry based on waste carbohydrates is that they are available in quantities which are too small or intermittent and seasonal to enable economically sized production facilities to be built. However, Thorne (1975) has pointed out that the cheaper the substrate the smaller the economic production limit becomes.

The development of a small-scale industry based on agricultural wastes in the developing countries would appear to offer considerable promise. Imrie (1975) discusses such a project in South America involving the production of filamentous fungi on citrus wastes currently being dumped with consequent pollution of the environment. The fungi would be destined for use in animal feeds. Imrie has summarised the advantages of the project: "Apart from the potential profitability of the microbial protein itself, this venture would save considerable foreign exchange, generate new industry, new jobs and additional wealth".

A large number of fungi have been screened for potential mould protein production on carbohydrates. Species which appear to offer suitable potential are Fusarium spp (Spicer, 1973), Penicillium notatum (Coppock, 1970) and Aspergillus niger (Imrie and Phillips, 1971).

Hydrocarbons

It is some seventeen years since British Petroleum announced their interest in the production of micro-organisms and their success in

growing a yeast, Candida lipolytica, on gas oils. Since that time, the interest in SCP production on hydrocarbon sources has grown considerably. Reviews on the subject can be found in the books edited by Mateles and Tannenbaum (1968), Davis (1974) and de Pontanel (1972). Reviews have also been written by Humphrey (1967, 1970) and Walker (1971).

One of the major advantages of hydrocarbons as a substrate for SCP production is the vast quantity available. Walker (1971) reported that the annual production of n-paraffin substrates appeared to be about 50 M tons in 1970 which could yield a potential protein production of 20 M tons / annum and the potential production from methane appears to be many times greater. Furthermore, they are available as non-agricultural products, their production being independent of climatic conditions or seasonal fluctuations and they are available all over the world, in many instances in countries short of protein (Norris, 1968).

The essential difference between carbohydrate and hydrocarbon substrates is that the former contribute carbon, hydrogen and oxygen to the system in a water soluble form, whereas the hydrocarbons are devoid of oxygen and almost insoluble in water (Shacklady, 1970). They take up more oxygen than carbohydrates during the fermentation process and the yields are consequently higher. Yeasts of the genus Candida utilize purified substances or crude oil with weight yields close to 100% (Senez, 1972). In addition to using more oxygen, however, heat production is double or triple that for the equivalent weight of carbohydrate-grown cells and the added expense of increasing oxygenation and cooling largely offset the advantage gained using cheap substrates (Norris, 1968).

The range of organisms capable of utilizing hydrocarbons is a further advantage. According to Walker (1971), it has been postulated that for any representative microbial culture collection, about $\frac{1}{4}$ of the organisms would be capable of utilizing hydrocarbons. Moreover, the hydrocarbon utilizing fraction would include bacteria and fungi, including yeasts. In

general terms it can be said that for gaseous hydrocarbons and others of short chain length, it is necessary to use bacteria. For longer chain hydrocarbons either bacteria or yeasts are suitable (Shacklady, 1970). There is, however, at least one report of the successful production of fungi, Graphium spp, on natural gas (Zajic, Volesky and Wellman, 1969).

Most of the initial interest was in the production of yeasts on n-paraffins or gas oils containing n-paraffins. The preferred species is Candida lipolytica, although Candida intermedia, Candida tropicalis and Candida rugosa have also been used. The most widely published processes are those of British Petroleum involving the utilization of n-paraffins and gas oils by Candida lipolytica, summarised by Shacklady (1970) and Walker (1971). In comparing the costs of the two processes, Shacklady states that on the one hand an expensive raw material, n-paraffin, but a relatively cheap and simple harvesting stage is used. On the other hand, gas oil is a cheap feedstock but the harvesting is more complex and expensive. It appears that there are situations in which either could be the preferred route. Senez (1972) has suggested that the process of cultivation on gas oil is better suited to medium-sized plants (3600 - 7000 tons/a) which could be installed in a large number of small refineries in Africa, Asia or South America. As against this, cultivation on purified paraffins appears preferable for major units producing 100,000 tons/a or more.

Whittenbury, Phillips and Wilkinson (1970) have isolated more than 100 methane-utilizing strains of bacteria. Pseudomonas methanica appears to be the organism of choice. For longer chain hydrocarbons Nocardia, Mycobacterium or Micrococcus species could be used (Johnson, 1970). Mateles et al (1967) have also isolated a thermophilic Bacillus which utilizes n-alkanes at 70°C.

The utilization of methane as a carbon substrate has been discussed by Hammer, Heden and Carnenberg (1967) and Wolnak et al (1967) and the advantages have been discussed by Ribbons (1968), Norris (1968) and

Sherwood (1974). It is available in considerable quantities at low cost. It is often a waste product of oil production and being resistant to chemical attack it is difficult to utilize. It is also too expensive to liquefy and transport. An estimated 100 M tons / yr are therefore 'flamed off' at oil production sites throughout the world. In addition, in the 1960's massive quantities appeared from the Southern North Sea province and it is therefore a most attractive substrate for European SCP production. It has a high state of purity and being volatile leaves no traces of toxic residues in the biomass produced. There are problems, however, as discussed by Senez (1972). The cost of aeration and agitation is considerably increased and the need to supply the fermenters with a combustible mixture of methane and air involves hazards which have to be guarded against and this will inevitably mean an increase in cost of production facilities. Additionally, the cultures have to be protected against contamination by other micro-organisms and bacteriophages which means having aseptic conditions which are difficult to achieve on a large scale. Shell, however, have made some progress in the utilization of methane by bacteria. They use a mixed bacterial culture and operate their process at high temperatures (Shell International Petroleum Co. Ltd., 1974). This reduces the chance of contamination and also reduces the cooling necessary resulting in a significant saving on production costs.

Alcohols

In view of the problems associated with the utilization of methane, there has been much interest in methanol as a substrate. The advantages of methanol have been described by Senez (1972) and Beatty (1974). It is easier to handle, being a low boiling point liquid with a very high degree of purity (over 99·9%). It is extremely soluble in water. The main feedstock is natural gas, although a process has been developed by ICI for the production of methanol from hydrocarbons making it available in larger quantities and more cheaply. The production of thermotolerant yeast and thermophilic bacteria from methanol has been

discussed by Cooney et al (1975). ICI's industrial process for the production of bacterial protein Pseudomonas spp, has been briefly described by Beatty (1974).

Ethanol, also synthesized from hydrocarbons, is available in smaller quantities. It is more expensive but it is apparently being utilized for bacterial production by Esso-Nestle (Senez, 1972) and by Amoco (Wayman, 1973). In both instances, the protein appears to be destined for human market outlets.

Organic wastes

There has been some interest in the utilization of farm wastes for the production of bacterial protein. Wayman (1973) suggests that it is conceptually feasible to build small plants completely integrated with a feed lot or a pig or poultry unit and that such integration offers the prospect of significant reductions in capital costs of small-scale units. General Electric are researching into bacterial protein production on farm waste.

A suggestion has been made that algae are more suitable for growing on farm wastes than any other micro-organism and mixed algae and bacterial cultures were very productive since high concentrations of glycochollate excreted by the algae feed the bacteria (Anon, 1975).

Suitability of micro-organisms for protein production

There is clearly a wide range of substrates capable of supporting the growth of micro-organisms from within the groups of algae, fungi, yeasts and bacteria. However, of the thousands of species and strains within these four groups, only a relatively small number will be useful for protein production. In the first instance organisms will be screened for qualities which satisfy the fermentation technologist, including from Enebo (1970)

1) Rapid growth on simple media
2) Efficient energy utilization
3) Tolerance towards mechanical strains during the culture process and toxic compounds in the medium.
4) Resistance to contamination
5) Simple separation (harvesting)

Consideration of the factors involved in fermentation technology and process engineering is beyond the scope of this discussion. Reference to various processes can be found in the general reviews listed previously.

Once potential organisms have been screened they will be assessed for their suitability as a feed or food. Organisms will be analysed for crude protein content, by nitrogen analysis, and protein quality determined initially by a quantitative amino acid analysis. Short term experiments will then be used to detect potential toxicity. Having established preliminary suitability, the process can be scaled-up to assess production potential and provide material for a thorough nutritional evaluation using species for which the product is intended. A successful product will satisfy the following criteria:

1) It will be acceptable and palatable
2) It will be safe for the purpose for which it is intended
3) It will have a suitable composition
4) It will make a positive nutritional contribution to the ration in which it is used

Acceptability and palatability

Most animals are not so fastidious as humans with respect to their food tastes and single-cell proteins have generally been found to be well accepted in diets of laboratory animals and livestock. In the latter case they will be used predominantly as a protein supplement in a complete compound feed containing cereals and other protein sources. Yeasts and

bacteria are generally bland and tasteless and fungi are frequently reported as having a good texture and a distinct meaty flavour. Algae are more suspect in view of their high content of pigment which can give rise to an unpleasant taste (Hintz, *et al* 1966). There are, however, several reports in the literature indicating that several species of algae are well accepted by rats and livestock including poultry, pigs and ruminants.

Safety

Toxicity in single-cell proteins may be related to toxins produced by certain species or residual contamination from the substrates utilized in their production. There are species of micro-organisms which produce potent toxins. Worgan (1974) has pointed out that neither the genera nor the species can be used as an infallible guide to the selection of suitable micro-organisms. For example, Amanita phalloides is one of the most toxic species of fungi whereas Amanita fulva is a popular edible species. Furthermore, only certain strains of the Aspergillus flavus produce aflatoxins. Among the algae there are also toxic species, notably Microcystis aeruginosa and Anabaena flosaquae (Gorham, 1964;), and there are many pathogenic bacteria which would be quite unacceptable as a food or feed source. There has also been some concern about the damaging effects of feeding bacteria and yeasts containing large quantities of nucleic acids. This concern, however, is specifically related to their application as a human food since the primates are unable to excrete large quantities of metabolised nucleic acids in the form of uric acid. Insoluble uric acid can therefore accumulate causing kidney stones(Miller, 1968). This is not the case with farm livestock which can excrete quantities of uric acid.

Little attention was paid to the toxicity of micro-organisms caused by residual contamination from the substrate until liquid hydrocarbons were considered for large-scale SCP production. The polycyclic

aromatic compounds were then implicated as being potentially carcinogenic. However, Senez (1972) has stressed that the distillation ranges of the substrates employed are from 175°C to 300°C for the purified alkanes and 250°C to 400°C for gas oil, i.e. very much lower than the boiling point of carcinogenic, polycyclic hydrocarbons, which is around 500°C. Indeed, Shacklady (1972) reported levels of 3:4 benzpyrene, benz-(ghi)perylene and dibenz-(a, h)anthracene-3, the reputed carcinogens, in a number of yeasts as determined in a survey carried out by two German researchers. They found that the combined total levels of these polycyclic aromatic compounds was actually lower in the gas oil yeast than all but two of the dietetic yeasts of eleven samples analysed. Using F.D.A. approved methods, the actual levels in hydrocarbon grown yeasts were found to be below one part per billion which, according to Senez (1972), is the limit of sensitivity of the test.

The most widely studied single-cell proteins from the toxicological viewpoint are those produced on hydrocarbon sources, and particularly the B.P. yeast proteins. When B.P. initiated their research programme, there was no precedent for the toxicological testing of new protein sources for animal feeds and a scheme was therefore worked out in collaboration with the Centraal Instituut Voor Voedingsonderzoek.

The scheme has been described by Shacklady (1972). It consisted of a) acute toxicity tests with rats; duration six weeks, dietary level of 40% yeast, contributing 25% to 28% of protein to the diet according to the source of the yeast; b) sub-chronic toxicity tests with rats; duration ninety days, dietary levels of 10%, 20% and 30% yeast; c) chronic toxicity with rats; duration two years, dietary levels of 10%, 20% and 30% yeast in diets; d) carcinogenicity test with mice; duration two years, 10%, 20% and 30% of yeast in the diet; e) carcinogenicity test with mice; duration $1\frac{1}{2}$ years, 10%, 20% and 30% yeast in the diet, and f) multiple generation tests with rats; duration approximately $1\frac{1}{2}$ years using 10%, 20% and 30% dietary levels of yeast.

The results from such studies have been reported in detail by

de Groot, Til and Feron (1970a), b); 1971) and summarized by Shacklady (1972). Such investigations have shown that B.P. yeasts are totally without toxic, carcinogenic or teratogenic effects providing adequate evidence of their safety for use in animal feedstuffs. Extensive toxicological tests have also been conducted on hydrocarbon grown yeasts in Japan (Hoshiai, 1972) and in Russia (Pokrovsky, 1968) and in both cases the conclusions reached were as for the B.P. proteins.

By comparison, there have been few reports on toxicological tests carried out on bacterial proteins. However, I.C.I. are at present conducting a research programme on their own material Pseudomonas spp grown on methanol, including chronic feeding tests and multi-generation tests to study their effect on reproduction. No toxic effects have yet been noted during the course of their testing (Beatty, 1974). Ko and Yu (1968) also report the absence of toxic effects in mice, poultry and pigs produced from feeding Pseudomonas spp, designated 5401, grown on n-paraffins. However, there is one report of the occurrence of adverse effects on the health of rats fed bacterial SCP as the sole source of protein. Agren, et al (1974), working with an unspecified bacterial protein grown on a chemically pure hydrocarbon substrate, reported arrested growth, weight losses, bleedings at nose and eyelids and increased mortality in rats fed a synthetic diet containing bacteria as the sole source of dietary protein. Histological examination also revealed severe pyelonephritis, nephrocalcinosis and testicular atrophies and haematological investigation showed a changed neutrophil:lymphocyte ratio.

There have been very few thorough investigations specifically on the toxic effects of feeding algae, although there have been several reports of feeding experiments in which a number of species have been fed to both laboratory and farm animals without causing any marked pathological disturbances. Lubitz (1962), however, noted histological changes in the salivary gland and pancreas of some of the test animals in a feeding experiment in which rats were fed Chlorella 7-11-05 at a

level of 92%.

There have also been few investigations on fungi. Jennison, Richberg and Krikzens (1957) fed 17 wood rotting Basidiomycetes, grown in submerged culture, to mice and guinea pigs and noted no acute toxicity. Again there are reports of feeding studies in which no toxic effects have been noted (Litchfield, 1968). Aspergillus niger produced by Tate & Lyle Ltd (Imrie and Phillips, 1971) and Fusarium spp produced by Rank Hovis McDougall (Spicer, 1973) are currently undergoing lengthy toxicological and nutritional investigations but as yet no results have been published. A recent report has been published in which a mould, Graphium spp, grown on gaseous hydrocarbon substrates, was fed at levels up to 100% of the diet in short term (1 week) toxicity experiments and in chronic toxicity tests at 20% and 40% levels for five months (Volesky, Zajic and Carroll, 1975). The rats appeared to be active and healthy at the end of five months. There was a slight change in the size of organs relative to the body weight but there were no gross histological changes. Rats were fed for a further five months on a stock diet and autopsied at the end of that period. No tumours were found in those rats previously fed the diet containing 40% mould. However, very few experimental animals were used in these trials.

Clearly, there are a few examples of SCP's in which freedom from toxicity has undoubtably been proven and there will be many more. Nevertheless, each new species of micro-organism produced for use in animal feeds or ultimately in human food should be thoroughly tested by procedures similar to those outlined by Shacklady (1972) for B.P. yeasts. Indeed, in 1969 the Protein Advisory Group of the United Nations appointed an ad hoc working group on SCP to study and evaluate these products more intensively. The status of SCP was broadly reviewed by the group (PAG statement No. 4, 1970) and the importance of thorough toxicological testing was emphasised. Approved guidelines for the toxicological testing of SCP destined for the human food market have been published (PAG Guidelines No. 6 and No. 7, 1970, revised 1972). Guide-

line Number 6, called "Preclinical Testing of Novel Sources of Protein" was also adopted for testing SCP destined for animal feed use. The procedures outlined in the guidelines are similar to those proposed by B.P. Proteins for testing their own products. A more recent guideline has been reported (PAG Guideline No. 15, 1974) which deals specifically with nutritional and safety aspects of novel protein sources for animal feeding. Such guidelines now clearly provide the standards which should be met by new single-cell proteins.

Composition

In view of the number of organisms involved in SCP production and the wide variation in substrates utilized, it is difficult to make generalisations about their composition. Furthermore, it has been shown that the composition of micro-organisms can be changed by altering the culture conditions. Thus Spoehr and Milner (1949) showed considerable variation in the chemical composition of Chlorella spp as a result of variations in the environmental growing conditions. The oil content was found to vary from 1·5% to 20%, protein from 9% to 58% and lipid from 4·5% to 85%. Forster (1949) has also shown than the protein content of moulds varies within extremely wide limits for any one organism grown in different culture conditions. More recently, Yamada et al (1968) have shown that the crude protein content of yeasts and bacteria cultures grown on hydrocarbons changes with pH.

In addition to protein, micro-organisms offer other useful nutrients including fats and vitamins. In the final analysis all the nutrient components must be considered when utilizing SCP in formula feeds. However, since the main interest at present is in the potential supply of protein, the following discussion will be restricted to the protein component of micro-organisms.

As can be seen from the values of crude protein shown in Table 1 for selected organisms from each of the four groups, the bacteria

generally have a higher crude protein content than yeasts which are higher in turn than algae and fungi. There is a very wide range in the latter groups and some species, notably Fusarium spp in the case of moulds and Chlorella spp and Spirulina spp in the case of the algae, can have crude protein levels as high as some of the yeasts.

The crude protein values referred to are determined from the nitrogen content x 6·25 which assumes that all the nitrogen is in the form of protein nitrogen and that 16% of the protein is nitrogen. Such assumptions can lead to large errors on both counts. There have been several reports indicating that a considerable amount of the measured nitrogen in micro-organisms will be non-protein nitrogen. Forster (1949) showed that fungi contain non-protein cell constituents such as chitin, amino sugars, purine and pyrimidine bases, nitrogen containing lipids, co-enzymes and special synthetic products such as penicillin and gliotoxin. Recently, Christias (1975) has compared four methods of estimating the protein content of four fungi and concluded that the method involving nitrogen estimation and the use of the factor 6·25 over-estimates the true protein content by 30% - 40% in all fungi studied. Blumenthal and Roseman (1957) have found the chitin content of 25 fungi to vary from 2·6% to 26·2%. Glucosamine may also represent up to 13% of fungi and Worgan (1972) has shown that the nucleic acid content of Fusarium semitectum was 5% grown at a mass doubling time of five hours. For algae, Leveille et al (1962) have reported that together the amide N + NH_3 and humin -N represent between 14% and 29% of the total nitrogen of five samples of algae. In addition, Enebo (1970) reports that Russian trials have shown that the nitrogen content of precipitated protein from the green algae Chlorella spp and Scenedesmus spp was only 12% against a theoretical 16%. This has also been shown for the extracted protein of fungi as discussed by Alagaratnam (1971).

There has been much interest recently in the nucleic acid content of yeasts and bacteria in view of the fact that such nitrogenous components cannot be easily excreted by humans. Bressani (1968) has re-

ported that the results of several studies have indicated that 8% - 13% of the total nitrogen of yeasts is due to purines, 4% to pyrimidines, 0·5% to choline, 0·5% to glucosamine and smaller proportions to other non-protein constituents. Therefore, only about 80% of the total nitrogen of the yeast cell is in the form of protein. Enebo (1970) has reported the results of one study on yeast, Saccharomyces spp, in which the true protein apparently represented only 63·8% of the crude protein value, nucleic acids accounting for 26·1% and free amino acids and peptones 10·1%. On the other hand, Worgan (1974) suggests that only 10% of the nitrogen of baker's and food yeast grown on a large scale is nucleic acid.

At least part of the reason for such differences of opinion may be due to the fact that nucleic acid content is actually variable being influenced considerably by the growth rate. Thus Worgan (1974) reports values of 10·1% and 11·9% nucleic acids for Candida utilis grown at doubling times 4·7 and 1·7 respectively and values of 12·3%, 14·2% and 18·9% for Aerobacter aerogenes at doubling times of 4·7, 1·7 and 0·8 respectively. Higher values for bacteria are generally reported. Thus Humphrey (1970) suggests levels of 15% - 20% of the nitrogen as nucleic acid nitrogen in fast growing bacteria. This seems to be indicated by the results of Palmer and Smith (1971) and may account in part for the higher crude protein values of bacteria shown in Table 1.

The usefulness of micro-organisms as a protein source in feeds will depend upon the quantity of specific essential amino acids. Amino acid profiles have been determined for many micro-organisms, a selection from each of the four groups being shown in Tables 2, 3, 4 and 5. For comparison, Table 6 includes amino acid profiles for some conventional feed proteins. Just as the protein content is dependent to some extent on environmental conditions and growth rate, so too are the amino acid profiles. Stokes and Gunness (1946) have pointed out that the amino acid composition of a micro-organism is a qualitatively and quantitatively stable characteristic of the cell under fixed growth conditions but that

amino acid content varies slightly among different strains and under different environmental conditions. Worgan (1974) has drawn attention to the considerable variation in the amino acid composition of the protein of micro-organisms reported in the literature and suggests that the differences may be due to analytical errors, the strain, the growth media or the environment. Comparing data from the literature he has shown the effect of substrate, strain and pH on two essential amino acids in a number of organisms:

Micro-organism	Substrate	Amino acid content g/100g protein	
		Lysine	Tryptophan
Candida tropicalis	Glucose	4·4	0·7
	Xylose	10·0	0·5
Candida utilis	Waste sulphite liquor	6·7	1·2
	Molasses	10·7	0·5
Escherichia coli B210	-	7·9	0·3
Escherichia coli B766	-	3·7	0·2

Micro-organism	Substrate	Amino acid content, % of cell mass	
		Lysine	Tryptophan
Pseudomonas aeruginosa	Glucose	9·7	0·3
	Xylose	4·4	0·5
	Hexadecane		
	pH 7	2·9	0·4
	pH 8	6·4	0·4

Yamada et al (1968) have also shown considerable variation in amino acid content of yeasts and bacteria grown on hydrocarbons. The time of harvest is also important. Falina, Maslova and Andreeva (1966) examined the essential amino acids of Basidiomycetes. They found highest methionine and tryptophan in mycelial hydrolysates after 24-36 hours of growth but highest lysine was found after 36-60 hours of growth.

Despite such difficulties, examination of the amino acid profiles shown in the tables reveals some general trends. Yeasts are deficient in methionine compared to the whole egg protein or fish meals. They are comparable with soya bean meal,also deficient in methionine. This deficiency has been reported several times in the literature. Chiao and

Peterson (1953) examined the content of S-amino acids, methionine and cystine, in twenty yeasts and found all deficient. Nelson et al (1960) studied the methionine content of 271 yeasts and found a range 0·4 - 1·7 g methionine/100g crude protein.

By comparison, bacteria generally have a higher methionine content as can be seen. Thus Mondolfo and Hounie (1951) reported that the methionine and cystine deficiency noted in yeasts does not occur in bacteria. Anderson et al (1958) report that although several bacterial species do not lack methionine, these same species are limiting in tryptophan. From data of Sheehan and Johnson (1971) for a mixed bacterial culture, methionine was again comparatively high but lysine very low. The lower lysine content of the protein of bacteria compared to that of yeasts is also shown by the data of Palmer and Smith (1971). According to Walker (1971), even lower values have been reported for two methane utilizing bacteria. There are exceptions to these generalisations, however. Thus, reference to Table 3 shows comparatively low methionine contents of the two Pseudomonas spp produced on hydrocarbons (Ko and Yu, 1968). Two bacterial samples examined by Palmer and Smith (1971) also had lower methionine values. The Shell bacteria grown on methane (Tol an, 1974) and the bacteria grown on methane reported by Norris (1968) have comparatively high tryptophan levels, and one of the bacteria grown on hydrocarbons (Ko and Yu, 1968) has a very high lysine content.

Methionine and cystine deficiencies have also been reported in seven moulds by Skinner and Muller (1940). That fungi are generally deficient in the S-amino acids has also been shown by Rhodes et al (1961) and by examination of the data compiled by Litchfield (1968). According to reports by Spicer (1973) and Imrie and Phillips (1971), two fungi which are being considered for industrial production (Fusarium spp - Rank Hovis McDougall and Aspergillus niger - Tate & Lyle Ltd) seem to have a higher methionine content. However, Worgan (1974) reports that methionine is deficient in Fusarium semitectum, and most recently two

further species Fusarium oxysporum and Fusarium moniliforme have also been shown to be deficient in methionine (Christias, 1975 - see Table 4). Christias has also shown low methionine contents for two strains of A. niger, confirming earlier reports of Stokes and Gunness (1946) for this species. Generally fungi have lower amino acid contents than yeasts and bacteria.

The picture is somewhat more confusing for the algae. Gordon (1969) has suggested that from several reports it appears than the S-amino acids, particularly methionine, are lower in Chlorella and that this seems to be a common feature of green algae examined to date. However, the data of Lubitz (1962) shows a reasonably high methionine content and the data of Leveille et al (1962), for a mixture of algae containing Chlorella spp and Scenedesmus spp indicates a very high methionine content. Gordon (1969) further suggests that blue-green algae generally have a higher content of essential amino acids and much more favourable methionine levels. Spirulina maxima, however, does not appear to have an adequate methionine content (Clément et al, 1967). Generally, the balance of amino acids in algae is not as favourable as in the yeasts and bacteria.

Protein Quality

Although chemical analysis frequently gives an indication of the likely value of a feedstuff, more important than the total content of the various nutrients is their availability as determined in biological assays using laboratory animals and target livestock for which the feedstuffs may ultimately be produced.

Many methods for estimating protein quality have been described and the subject has been thoroughly reviewed several times. Those of Block and Mitchell (1946), Albanese (1959), Rice and Beuk (1953), Carpenter (1963), Morrison (1964), Morrison and Rao (1966), Woodham (1960) and the Agricultural Research Council (1970) are pertinent. Unfortunately, many experiments involving the evaluation of SCP have not involved

use of standard procedures and comparison of the results is difficult. In addition, results are frequently reported in the literature without reference to the methods employed. However, an attempt is made to review reports on the nutritional value of yeasts, bacteria, algae and fungi, as determined using laboratory animals, under those headings.

Yeasts

Bressani (1968) has reviewed the work in which the nutritional value of food and fodder yeasts has been evaluated. Some of the values for Digestibility, Biological Value (B.V.) and Protein Efficiency Ratio (P.E.R.) have been summarised in Table 7. Baker's and Brewer's Yeast appear to have a higher protein quality than Candida utilis. The improvement of B.V. and P.E.R. after supplementation with methionine is clear evidence that this amino acid is limiting. Bressani comments that it is interesting to see that methionine supplementation improved digestibility, a phenomenon that usually does not occur upon addition of deficient amino acids to proteins.

A digestibility value of only 64·6% has been reported by Mitsuda et al (1967) for whole cells. The low digestibility was related to the cell wall component since extracted protein had a digestibility of 96%. The results for B.V. and P.E.R. and Net Protein Utilization (N.P.U.) for whole yeast, extracted yeast and extracted yeast supplemented with methionine are shown in Table 8. The B.V., N.P.U. and P.E.R. were higher for the extracted protein and there was a further improvement with methionine supplementation, although the protein was still not equal in value to casein.

Several yeasts grown on hydrocarbons have also been studied. Shacklady (1970) reports values for digestibility, B.V. and N.P.U. of the B.P. yeasts grown on n-paraffins and gas oil compared to soya protein isolate and dried whole egg (Table 9). The digestibility was very high compared to that reported for the food and fodder yeasts

Tables 7 and 8. The B.V. and N.P.U. were also higher and better than soya protein isolate. Methionine again improves B.V. and N.P.U. values. The values after supplementation were considerably higher than those for casein reported by Mitsuda et al (1967) and also higher than dried whole egg before it was supplemented with methionine. Walker (1971) has reported that the available lysine of B.P. yeast determined by the chemical method of Roach, Sanderson and Williams (1967) is in excess of 95%. Available methionine determined by microbiological methods has also been reported to be very high.

Other hydrocarbon grown yeasts have not performed quite so well as those produced by B.P. Pillai et al (1972) have reported the digestibility of a yeast grown on hydrocarbon substrates by the Regional Research Laboratory, Jorhat in India, at only 68% although this increased to 86% with methionine supplementation, a phenomenon already reported previously for yeasts (Bressani, 1968). Supplementation with 0·4% methionine improved P.E.R. from 1·6 to 2·1 which was still below casein at 2·5. Similar results have also been reported for hydrocarbon grown yeast produced at the Indian Institute of Petroleum (Narayanaswamy et al 1971). P.E.R. increased from 1·03 to 2·25 on supplementation with 1·6g methionine/100g protein but the values were less than those for skim milk at 3·16.

Palmer and Smith (1971), working in an independant research laboratory, have evaluated four yeasts grown on hydrocarbons. The results are shown in Table 10. Again the results for N.P.U. and B.V. are not so impressive as for the B.P. yeasts but, with the exception of Y5, the digestibility was very high. N.P.U. and B.V. increased again with supplementation.

All of these results substantiate the suggestion made earlier, on the basis of chemically determined amino acid levels, that methionine is limiting and that when supplemented, yeast proteins have a high nutritional value.

Bacteria

There is comparatively little data on the nutritional value of bacteria.

Tannenbaum (1968) has reported results for digestibility and B.V. of Bacillus Megaterium before and after extraction and N.P.U. values have been calculated from their data (Table 11). Clearly the relatively low digestibility of the bacterium is not due to any inherent characteristic of the protein but to the fact that nitrogen in various cell wall structures is not digestible. The biological value of the whole cells and the cell contents was not substantially different and was low compared to the supplemented casein control diet.

Results for the bacteria Hydrogenomes eutropha have been reported by La Chance (1968) and are shown in Table 12. The digestibility was in this case very high and the biological value was similar to that for casein. Methionine has been indicated as limiting in this species.

Palmer and Smith (1971) have reported results for two bacteria produced on hydrocarbons (Table 13). The digestibility values were again very high, comparable to the B.P. yeasts and the yeasts determined by Palmer and Smith shown in Table 10. Methionine supplementation increased the B.V. and N.P.U. values indicating again that methionine was limiting. The N.P.U. levels were generally lower than those determined for the yeasts indicating a poorer protein quality for these bacterial proteins.

Algae

There have been several studies on the nutritional value of algae. Lubitz (1962) reports a protein digestibility of 86% for Chlorella 7-11-05. P.E.R. was 2·19 compared to 3·30 for casein but there was an increase to 2·90 with 2% methionine supplementation. Geoghegan (1953) reported

a value of 1·84 for P.E.R. of Chlorella and Erchul and Isenberg (1968) report values of 1·41 and 1·54 for the P.E.R. of laboratory grown Chlorella. Leveille et al (1962) found P.E.R. for Chlorella pyrenoidosa to be only 0·94. Such differences may be due to differences in the techniques used. Harvesting can have a marked effect as shown by Erchul and Isenberg (1968). The P.E.R. of Chlorella was found to decrease from 1·54 to 0·78 when it was heated prior to drying.

The high digestibility values reported for Chlorella 7-11-05 by Lubitz (1962) would appear to be the exception rather than the rule for green algae. Cook (1962) has reported values shown in Table 14 for digestibility, B.V. and N.P.U. for a mixture of Scenedesmus quadricauda and Chlorella spp in the ratio 10:1, grown in sewage oxidation ponds. The digestibility was low although it improved after 30 minutes boiling. Erchul and Isenberg (1968) have also reported values, shown in Table 15, for seven sewage-grown algae, the predominant species being Chlorella and Scenedesmus. The wide variations in P.E.R. are due mainly to differences in digestibility.

The blue-green alga Spirulinus maxima has a relatively high digestibility and biological value as shown in Table 16 from Clément et al (1967). In contrast to the results of Cook (1962), the digestibility did not increase on cooking and the B.V. and N.P.U. decreased. The variable results for digestibility of algae may be related to differences in the chemical composition of the cell wall of different species. La Chance (1968) has discussed attempts to verify this. As reported earlier for the yeasts (Mitsuda et al, 1967) and bacteria (Tannenbaum, 1968) extracted protein appears to be far more easily digested than whole cells, although it appears that the protein value is still low compared to casein due to a deficiency in methionine as for the yeasts and bacteria (La Chance, 1968).

There have been fewer studies on amino acid supplementation of algal proteins. Leveille et al (1962) showed several algal species and

mixtures of species to be deficient in methionine. Methionine supplementation increased P.E.R. The increase was dramatic for Spongiococcum excentricum supplemented with 0·5% methionine. The increase was from 0·34 to 1·22.

Fungi

There have been very few investigations of the quality of fungal protein, although there have been several reports of feeding experiments of one type or another (Litchfield, 1968). As mentioned earlier, the protein content is generally very low and there are several reports, summarized by Litchfield, in which fungal proteins have failed to support the growth of rats or mice. One study in which the digestibility and B.V. of fungal protein was determined has been described by Fink et al (1953). They found that the digestibility of Penicillium notatum was only 58%. The B.V. was 59·8% and the calculated N.P.U. was 34·7%. This is considerably lower than that for yeasts and bacteria. However, as reported earlier, there has been some recent interest in commercial production of Aspergillus spp and Fusarium spp and it is interesting to note that Worgan (1974) lists N.P.U. values for A. niger and F. semitectum as 54·4 and 60. It would seem, therefore, that the protein quality of these species of fungi is better than that of the yeasts produced on conventional carbohydrate substrates and comparable with the yeasts and bacteria produced on hydrocarbons.

The fungal proteins have been shown to be deficient in S-amino acids, like the yeasts, bacteria and algae. Thus Skinner and Muller (1940) showed improved growth with Aspergillus spp and Penicillium spp when supplemented with cystine or methionine. Fink and Schlie (1956) also showed improved growth of Psalliota bispora supplemented with methionine and cystine.

Single-Cell Protein in Compound Feeds

In the final analysis, single-cell proteins must be shown to provide

some nutritional benefit when included in the rations of target animals. In this respect, they will be used as a protein supplement rather than as the sole protein component of the feed. The nutritional value of two mixed proteins is frequently better than either alone as has been shown frequently, including studies with algae reported by Cook et al (1963) and with yeasts reported by Bressani (1968). Maximum benefit will be obtained if the amino acids of the SCP complement those of the basal ration to which they are added.

There are a number of reports in the literature showing the usefulness of SCP in compound feeds for several classes of livestock but direct comparison of the results is impossible in view of the differences in levels used and in the composition of the basal ration. Reports by Shacklady (1974), Hoshiai (1972), Ko and Yu (1968), ICI Proteins Ltd (Information booklet) and D'Mello and Whittemore (1973) contain information on yeast and bacterial protein as feed components. Meffert (1961), Leveille et al (1962), Hintz (1966), Grau and Klein (1957), Beremski et al (1973), Okumura and Tasaki (1973) report trials on algae. There have been very few large scale trials with fungal protein. Trials are currently being carried out to assess the value of Fusarium spp and Aspergillus niger. Some results for A. niger have been reported (Imrie and Phillips, 1971).

The usefulness of a few species from each group of micro-organisms has been shown. The emphasis of the studies has been on poultry and pigs in view of the potential size of the markets for compound feeds for these animals. The yeasts and bacteria would seem to offer the greatest potential in view of the higher digestibility and relatively high B.V. They have been reported to be comparable with soya bean meal and fish meal.

Single-Cell Protein in Fish Feeds

Only the yeasts have received any real attention as a potential ingredient for fish feeds. Brewer's yeast has been included routinely in

salmonid diets for many years. The yeast is included at levels up to 5% as shown in the formulations listed in Appendix 1. Churchill (1952) evaluated torula yeast (Candida utilis) grown on sulphite liquor in the United States at 10% and 20% levels in brook trout diets otherwise composed of liver or carp and pork melts. When included in liver diets there was no advantage in terms of growth but when included in diets composed of carp and pork melts, better growth was obtained. Brewer's yeast at the same levels also increased the growth but torula yeast produced better growth than brewer's yeast at both levels. Since the diet containing carp and pork melts was lower in some of the B complex vitamins than the suggested requirements, the increase in growth of fish receiving diets supplemented with yeast could have been related to the higher content of B complex vitamins in the yeast.

Yeasts produced on hydrocarbons have been evaluated in several species of fish in Japan (Hoshiai, 1972). 'Petro-protein yeast' has been used at the rate of 15% and 30% of a compound rainbow trout feed, in isonitrogenous replacement of white fish meal and corn starch. In two further diets it was included at the 30% level but it was supplemented with methionine at levels of 0·2% and 0·4% in the diet. The weight gain in 105 days was greater than the control on diets containing 15% and 30% yeast and 30% yeast + 0·4% methionine but slightly lower for 30% yeast and 0·2% methionine. Feed efficiency was greater for all diets containing yeast protein. Supplementation with methionine did not appear to substantially increase growth or feed efficiency. In the case of supplementation at the 0·2% level, growth and feed efficiency was slightly reduced. Improved efficiency was also reported for carp fed diets containing up to 45% petro-yeast and similar results have apparently been achieved for eels, ayu, yellow tail, sea bream and prawns.

B.P. yeast (Candida lipolytica) produced on n-paraffins has also been studied as a potential feed ingredient for rainbow trout by Andruetto, Vigliani and Ghittino (1973). The yeast was used at 10%, 20% and 30% levels in place of equal proportions of casein and gelatin of a control

diet composed of 54% casein, 15% gelatin, 7% maize oil, 2% cod liver oil, 8% dextrin, 9% alpha-cellulose plus vitamin and mineral supplements. The diets were prepared as dry pellets and fed at a rate of 2-3% live body weight per day for four months. During that time palatability of diets containing the various levels of yeast was considered normal compared with the controls. In one experiment, fish of an average size of 100g grew to an average of 210g in the course of four months at a constant temperature of 8-9^{o}C. The conversion factor was 1·8 on average. Histological examination of the livers was negative for regenerative or neoplastic processes although the hepatocytes were at times vacuolated and in some instances small accumulations of ceroid, representing a non-lethal form of Liver Lipoid Degeneration, was found. Such vacuol ation is commonly found in trout livers intensively fed and the ceroid deposits could have been related to complex factors already existing before the tests. Otherwise no differences were noticed on any of the diets containing 10%, 20% and 30% yeast protein.

Cowey et al (1974) have fed B.P. yeast protein supplemented with methionine as the sole source of protein in the diets of plaice. The diet contained 83% by weight of yeast contributing 50g of protein per 100g of diet. The yeast was supplemented with methionine prior to use at a rate of 10·0g L-methionine /kg. In addition to yeast, the diet contained alpha-cellulose, vitamin and mineral premixes, maize oil, cod liver oil and a binder. The growth rate of plaice fed this diet was less than that of fish fed a similar diet containing 50% protein derived from white fish meal, and considerably less than fish fed a diet containing 50% protein derived from freeze-dried cod muscle. The growth rate was, however, better than that of fish fed a diet containing 50% protein derived from Promine-D, an extracted soya protein, supplemented with 19g L-methionine/kg. During the ten week period Protein Efficiency Ratios of 1·23, 1·78, 1·29 and 0·89 were obtained for diets containing 50% protein derived from B.P. yeast, freeze-dried cod, white fish meal and Promine-D respectively. Corresponding N.P.U. values were found to be somewhat similar. These were 0·38, 0·42, 0·35 and

0·34 respectively for the four diets listed above indicating that there was little difference in their relative nutritional value at the dietary protein levels used. Digestibility of the yeast was high, 90%, as has been shown for poultry and pigs. It was comparable to freeze-dried cod, 91%, and white fish meal, 86%, used in this experiment.

The yeast cell wall is composed mainly of glucan, mannan, protein and chitin and according to Hibino et al (1974) the enzymes contributing to the digestion of yeasts are proteinase, chitinase, p-1, 3-glucanase. The levels of these enzymes in a number of species of fish were determined by Hibino et al. Peptic activity and tryptic activity were very high in the eel and ayu respectively and present in rainbow trout. Chitinase activity was found in ayu, carp, rainbow trout and eel being very high especially in the eel and rainbow trout. B-1, 3-glucanase activity was very high in the ayu and carp and also present in trout. It was interesting to note that chitinase activity, in particular, increased in the eel and rainbow trout when the diets contained yeast. It seems likely that yeasts will be highly digested in several species of fresh-water fish in addition to plaice. The encouraging growth and food conversion results obtained with rainbow trout fed diets containing yeasts would seem to indicate a reasonable digestibility of yeast by this species.

Two trials in the United States have recently been carried out to evaluate dried activated sludge recovered after the treatment of industrial wastes. Orme and Lemm (1973) report feeding trials in which sludge from paper processing wastes has been incorporated into the diets of rainbow trout and Windell, Armstrong and Clinebell (1974) report trials in which sludge obtained from treatment of brewing wastes has been incorporated into diets of rainbow trout.

The sludge from the paper processing industry is composed mainly of mixed bacterial species with a crude protein content of 42·3%. The dried sludge was incorporated into a standard compound feed (the McNenney formula - Appendix 1) at 25% and 50% levels replacing some

of the conventional ingredients. The amount of other ingredients was adjusted to balance the protein level at 40% and the fat at 9%. The three diets containing 0%, 25% and 50% dried sludge contained 32%, 20% and 15% herring meal respectively. The material could be processed easily into trout pellets. The sludge was considered to be palatable. No abnormalities or deficiences were noted during the 285 days of feeding. There were no indications of any adverse effects as shown by examination of external characteristics as well as internal organs, and mortality was similar and low in all groups. Growth and feed efficiency was, however, reduced as herring meal was decreased and sludge increased in the feeds. Growth rates for the three diets were 18·2, 13·7 and 12·1% wet wt. gain/day and corresponding food conversion ratios were 1·45, 1·58 and 1·72.

The material referred to by Windell et al as brewer's single-cell protein was not brewer's yeast but was the activated sludge produced during the treatment of brewery waste and subsequently spray-dried. It was very easily incorporated into compound feeds. The material containing 44·4% crude protein was substituted approximately isonitrogenously for some conventional feed ingredients in a standard trout compound feed, at levels of 10%, 15% and 50%. The material replaced 10% brewer's yeast in one diet, 10% fish solubles and 5% wheat germ in a second diet and several ingredients including 10% herring meal in the diet containing 50% of the material. The protein and oil content of the diets were similar. The diet containing 50% was supplemented with 0·6% methionine and 0·5% arginine. In a 140 day feeding trial, no palatability differences were observed between the control diet and those containing 10% and 15% of the SCP but groups of fish fed the diet containing 50% displayed a slight decrease in appetite after several weeks. There was no significant difference in weight gain or feed conversion of fish fed the control diet and those containing 10% and 15% SCP, but the growth and feed conversion of fish fed the diet containing 50% SCP was significantly lower. As shown by external examination, there were no signs of abnormalities or deficiencies in all groups except for some loss of dorsal fin in the group to which 50% SCP

was fed. The protein digestibility was 76·2% compared to 99·5% and 94·6% for two samples of herring meal and 86·4% for brewer's yeast. It was suggested that the lower digestibility may be due to processing.

There are no similar detailed studies on the nutritional value of other micro-organisms for fish although Singh and Bhanot (1970) report tests on diets containing several species of algae (Zygnema, Sirogonium or Mougenia spp) added to diets containing potato starch, terramycin, yeast and salt and fed to carp (Cyprinus carpio). Mougenia spp gave a growth rate comparable to the natural planktonic food of the carp and it was suggested that algae could provide a useful source of food. Chlorella ellipsoidea, however, has been reported to have a digestibility of only 57% when fed to goldfish in a diet containing 80% Chlorella and 20% potato starch (Nose, 1960).

Conclusion

Industrial-scale culture of micro-organisms for protein production offers considerable promise. A large number of micro-organisms are capable of efficient utilization of a wide range of substrates. Production in an industrial process offers an opportunity for a considerable degree of control over the environmental conditions and hence the growth rates and ultimate composition of the product. In optimum conditions, the growth rate of micro-organisms is impressive. Humphrey (1969) reports mass doubling times for bacteria and yeasts of the order of 20 - 120 minutes, and for moulds and algae, 2 - 6 hours. This compares with 1 - 2 weeks for grass and some plants, 2 - 4 weeks for chickens, 4 - 6 weeks for hogs and 1 - 2 months for cattle. Industrial production has the further advantages of compactness, requiring very little space, and is independent of the normal vicissitudes of conventional agriculture. Production can be predictable and harvesting is routine.

The composition of the biomass produced is very variable but several micro-organisms from the bacteria, yeasts, fungi and algae have been

shown to provide a useful supply of high quality protein capable of being utilized in the feed of several classes of livestock, being palatable, nutritious and non-toxic. Such feedstuffs should therefore readily find their way into the animal feedstuff market if the price is competitive with conventional feedstuffs.

A number of companies have announced their involvement in this field as shown in Table 17. Opinions and approaches may vary as will the economic conditions in different countries. The large oil and chemical companies are developing large-scale processes involving high capital investment and relying on a very sophisticated technology. A contrasting approach is that of Tate & Lyle Ltd involving low capital investment and simple technology principally directed to the developing countries. In some instances, for example the production of fungi on sulphite liquor in Finland, environmental considerations may be of primary importance, the process being developed in order to satisfy pollution control standards.

Economic viability will depend on the price of the substrate, the capital cost of the plant, the efficiency of the operation including the production of the biomass, the harvesting and its preparation into a form suitable for use and the price of the conventional sources of protein. Only those companies involved in production can assess the economic viability of their own operations. Micro-organisms offer considerable potential as a feed source and the demand for protein is evident. It remains to be seen whether technologies can be developed which will enable production at a price which can compete with that of conventional proteins and at the same time produce the profitability required by the corporate investors.

SECTION 2.

FISH-HOLDING FACILITIES AND THE ROUTINE MAINTENANCE OF STOCKS.

STOCKING TANKS

Circular stocking tanks were made by Cago Ltd from $\frac{1}{8}$" thick polypropylene sheets to the following specifications: 3' diameter and 15" deep, with the base shelving slightly to a $\frac{3}{4}$" bore P.V.C. pipe at the centre, welded flush with the inside of the base. The tanks were free standing.

A 14" length of 3" diameter P.V.C. pipe was placed over the central drain hole to prevent it being blocked after a fish mortality. It was held in position by a $\frac{3}{4}$" P.V.C. pipe inserted through the wall of the tank, positioned across the diameter and slotted into two grooves as shown in Plate 1 . A number of slots were cut in the wall of the 3" pipe at the base to allow faecal material to be washed into the drain.

The $\frac{3}{4}$" pipe consisted of two sections of equal length fitted into a socket in the centre. Four small holes were drilled in each section. When connected to the mains water supply, water 'jetted' from the holes. Each section of pipe could be swivelled in the socket so as to direct the water jets at any angle to the water surface in the tank. Inflow was directed to create a 'circular flow pattern', which, with the gradually shelving base and central drain, created a self-cleaning action. This type of inlet was believed to aid in oxygenation of the water and prevent the formation of 'dead spaces' where waste products could accumulate. The rate of water movement was determined by the flow and pressure of water entering the tank and the angle at which the jetted water entered the main body of water within the tank. Flow/ pressure and the angle of the jets were adjustable.

A 1" diameter rubber tube was connected to the $\frac{3}{4}$" P.V.C. pipe welded to the tank in the centre and was looped beneath the tank. The open end was clamped. The level of water in the tank was maintained by positioning the clamped open end of the tube at any required level. Water 'overflowed' from the open end.

The tanks were covered by $\frac{1}{4}$" diamond nylon mesh fastened to a flange around the circumference of the tank.

PLATE 1.

FISH SUPPLY AND TRANSPORT ARRANGEMENTS

The fish were hatchery reared rainbow trout (Salmo gairdneri, Richardson) purchased from Vortex Limited, Donnington, Nr Stow on the Wold. As required, about 1000 fry or fingerling fish were obtained and transported to the laboratory in five plastic bins lined with heavy duty polythene bags filled with hatchery water. Each one was 'gased' with oxygen until a good pressure was present inside the bags when the air tube and bag were tied off tightly to maintain an oxygen saturated space above the water. An oxygen cylinder was transported with the fish and the bags were occasionally 'gased' during the $1\frac{1}{2}$ hr. journey from the hatchery to the laboratory. This method was quite satisfactory for transporting the fish in the hatchery water which originated from a spring supply and was generally in the temperature range 9-12°C.

To avoid excessive mortalities due to the shock of a complete change in water temperature and chemical composition following transportation of new stocks from the hatchery, the fish were transferred to the stocking tanks in the hatchery water in which they had been transported. This was aerated by small air pumps and tap water was trickled into the tanks slowly so that the fish would have a chance to acclimatise to the new environment.

The quality of Birmingham tap water is very suitable for rearing rainbow trout. Trout have been successfully grown from fry to three year old fish. Chlorine levels are low and have not been a problem at any time.

Once a complete change of water had been made, the water flow was appropriately adjusted to the required level.

ENVIRONMENTAL PARAMETERS

The environmental requirements of rainbow trout and the relationships between stocking densities (kg m^{-3}), fish loading (kg m^{-3} min), fish numbers and temperature have been the subject of a number of reviews, as mentioned previously (Section 1 pg4).

The first and second limiting factors for the maintenance of fish at high densities in tanks are oxygen and ammonia respectively. The fish loading in the stocking tanks was such that oxygen never became limiting. The required quantity of oxygen to maintain a concentration 6-7 mg l^{-1} was supplied entirely from the incoming fresh water which was 100% saturated. The oxygen content of the effluent was occasionally checked using an E.I.L. oxygen meter. Additional aeration by pumping was available for emergency situations.

Since, under normal circumstances, oxygen never became a limiting factor, it is unlikely that ammonia would have been limiting. Total ammonia appears to be of no consequence unless it is dissociated. Free ammonia is the toxic moiety. The degree of dissociation is dependent on pH and temperature, increasing with decreasing $[H^+]$ and increasing with temperature. Free ammonia has been considered safe (on the basis of physiological parameters) up to a concentration of 0·025 mg l^{-1} (Lloyd & Orr, 1969). For most hatchery circumstances, it would appear that 1·0 ppm total ammonia would not inhibit growth and that 0·5 ppm has been considered a safe level (Burrows, 1972). Total ammonia was measured occasionally in the stocking tanks. The levels of total ammonia have in no case been considered to be excessively high and the free ammonia is unlikely to have been limiting.

FEEDING

The stock fish were fed at all times on commercial feeds produced by Cooper Nutrition Products Ltd. The size of the feed used was that recommended by the manufacturers for each size category of fish. The specifications given by the manufacturers were as follows:

	Oil	Protein	Fibre	Vitamins i.u./kg		
	%	%	%	A	D	E
Salmon Starter	7·5	58·0	1·5	20000	2000	36
Salmon Growers	6·5	46·0	3·5	18000	2000	30
Trout Floating	4·5	40·0	4·5	18000	2000	30

The fish were fed two or three times daily either to satiation or at a fixed rate suggested in tables supplied by the manufacturers. In the latter case, the rate of feeding was adjusted according to the size category of the fish and the water temperature.

SECTION 3.

EVALUATION OF SINGLE-CELL PROTEIN IN COMPOUND FEEDS 1. THE EFFECT OF SUBSTITUTING A YEAST AND TWO FUNGI FOR SOME CONVENTIONAL INGREDIENTS.

INTRODUCTION

A prerequisite for the successful nutritional evaluation of any novel feedstuff is that it should be palatable when incorporated into a feed formulation and that it should be non-toxic. The object of the initial experiment was to assess the feasibility of substituting three single-cell proteins for some conventional ingredients of a trout formulation and to develop techniques for future nutritional evaluation.

EXPERIMENTAL DIETS

Ingredient Availability

Two moulds and a yeast were initially available. The moulds were freeze-dried Aspergillus niger(M1), grown on extracted sugars from the carob bean and supplied by Tate & Lyle Ltd, and Penicillium chrysogenum, a by-product of a penicillin production process, supplied by Glaxo Laboratories. The yeast was Candida lipolytica grown on n-paraffins and supplied, under the brand name 'Toprina', by B.P. Proteins Ltd.

The trout formulation which was considered most applicable was the McNenney PR6-25 composed entirely of dry ingredients (Orme, 1970) as shown in Appendix 1. The ingredients used for the diet, however, were those that could be readily obtained. White fish meal was substituted for the herring meal and dehydrated grass meal was substituted for alfalfa meal. The remaining ingredients were similar to those listed in the McNenney formula and included wheat middlings, extracted soya bean meal, delactosed whey, distiller's solubles, corn gluten meal, brewer's yeast, soya bean oil and vitamin and mineral mixes. With the exception of the corn gluten meal (Globe Prairie) supplied by C.P.C. (United Kingdom) Ltd, all ingredients were supplied by Cooper Nutrition Products Ltd.

All feedstuffs, including the single-cell proteins, were kept in plastic

bags stored in a dry place.

Ingredient Analysis

A minimum of 1 kg of each feed ingredient was mixed in a Hobart mixer for five minutes and a 100g sample taken as representative of the homogeneous mix. This was passed through a small bench hammer mill and the sample then stored in screw-capped jars for subsequent analysis.

All analyses were determined at least in duplicate with further replicate samples if consistent results were not obtained. The methods used were as follows:

1) Moisture

Weighed samples of each ingredient, approximately 2g, were dried in aluminium dishes placed in a drying oven, set at 100°C, for 12 hours. The dishes containing the samples were allowed to cool in a desiccator and reweighed. Moisture was reported as a percentage of the original sample.

2) Ash

Weighed samples, approximately 2g, were ashed in porcelain crucibles placed in a muffle furnace set at 600°C for 2 hours, cooled in a desiccator and reweighed (A.O.A.C. Methods of Analysis, 1970). The ash content was reported as a percentage of the original sample.

3) Crude Protein

Total nitrogen content was determined by the microkjeldahl procedure. The material was digested by concentrated sulphuric acid in 30 ml microkjeldahl flasks using a catalyst mixture of 32 parts potassium sulphate, 8 parts copper sulphate and 1 part powdered selenium. The distillation procedure was carried out in a Markham Distillation Apparatus. Ammonia released from the digested sample by the addition

of 4 mls of 40% sodium hydroxide was carried over with the distillate, collected in 5ml saturated boric acid and titrated directly against 0.01N HCl using Tshiro's indicator (0·08% methyl red and 0·02% methylene blue dissolved in ethanol). The crude protein content was reported as the percentage nitrogen content multiplied by the factor 6·25.

4) Crude oil

Crude oil was determined by extraction with petroleum ether (40-60°C B.P.) in a Soxlet Extraction Apparatus (Fertilizer and Feeding Stuffs Regulations, 1968). Oil was reported as a percentage of the original sample.

The results of the analyses are shown in Table 18.

Determination of the moisture, ash and oil content was relatively straight forward. N determinations, however, showed some variation between duplicates. The microkjeldahl determination is not very satisfactory for the determination of the N content of feedstuffs in view of the small size of the sample required and the heterogeneity of certain materials such as fish meal. Although the fine grinding helped to some extent, a number of replicate determinations were required.

The crude protein content of the two moulds was low compared to the white fish meal and the yeast.

Feed Formulation

Each of the three single-cell proteins was substituted on a weight for weight basis for components of the control McNenney diet. No attempt was made to balance the nutrient components for this trial.

Only a limited quantity of freeze-dried Aspergillus niger was available. In view of the low protein content, it was substituted for the de-

lactosed whey and distiller's solubles of the control diet at a level of 18% in the feed.

Penicillium chrysogenum, also low in protein, was substituted for those ingredients in one diet and for approximately 50% of the fish meal, also at a level of 18% of the finished feed, in another diet.

A larger quantity of B.P. 'Toprina' was available and was subsequently substituted for the delactosed whey and distiller's solubles at a level of 18% in the finished feed of one diet and also for approximately 50% and 100% of the fish meal, at a level of 18% and 35% of the weight of the finished feed respectively, in two further diets.

The formulations of the experimental diets are shown in Table 19. The mineral and vitamin mixes are shown separately in Tables 20 and 21 respectively.

Ingredient Preparation

Prior to preparing the compound feed mixtures, the coarser ingredients were ground in a Christie Norris laboratory hammer mill fitted with a 1 mm screen. These included the fish meal, wheat middlings, soya bean meal, corn gluten meal, dried grass meal and the freeze-dried Aspergillus niger-M1. The remaining ingredients were considered fine enough without grinding.

1kg of the mineral mix was prepared from the component chemicals (Table 20), which were separately weighed on a microbalance. The sodium chloride was weighed first. The salts weighing less than 5g in the total mix were added to portions of the sodium chloride in a mortar and were finely ground and mixed. All the portions were then mixed with the remaining salts in a Hobart mixer.

1·5kg of the vitamin premix was prepared according to the McNenney

specifications (Table 21). Some of the vitamins supplied by C.N.P. Ltd did not have 100% activity and the quantities were adjusted to give the required potency specified in the table. With the exception of the choline chloride and ascorbic acid, individual vitamins were weighed on a microbalance and added to half of the complement of the wheat middling carrier in a bowl, rough mixing by hand after each new addition. The remainder of the wheat middling was then added and the whole mixed in the Hobart mixer for 15 minutes. The choline chloride and ascorbic acid were added at the time of mixing the major feed ingredients.

Ingredient Mixing

5kg of each diet was prepared. The feed ingredients were weighed on a Metler top loading balance (accuracy 0·1g) starting with the smallest quantities first, adding each to the bowl of the Hobart mixer and mixing for a few minutes after each new addition. When all the ingredients had been added, the whole was mixed for a further 15 minutes. The oil was then added slowly in a fine stream while the mixer was running.

The 5 kg batches were each put in to plastic bags which were then placed in polyethylene bins for transport.

Pelleting

Pelleting was carried out using a California Laboratory Pellet Mill, at the Beecham Research Laboratories, Tadworth, Surrey. Steam was not available at the time. Pellets were prepared by compression through a $\frac{1}{8}$'' die ring. The pellets were cut to approximately $\frac{1}{8}$'' length. The pellets were collected in large plastic bags within the polyethylene bins. The bags were left open long enough for the pellets to cool and were then tied off.

Pellet Quality

All diets were of a natural colour with no added pigments or dyes. They were therefore light brown with little variation in colour between the formulations. The pellets produced in the compression process were generally shiny on the outside with a soft, friable texture. To a limited degree, the binding capacity of the diet mixture was reflected in the ease with which it pelleted. During the experiment, the dust was removed from the pellets by sieving. The dust was collected and the total taken as a crude measure of the binding capacity of the feed formula. The results are shown in Table 22.

The results were in accordance with observations during the trial that pellets CO2 and CO5 were slightly harder and pellet CO8 slightly softer than the others. Whilst slight variations in the conditions during pelleting may have been possible to some extent, it was felt that the differences in the texture and composition of the ingredients was largely responsible. Thus, for example, high levels of the B.P. 'Toprina' substituted for fish meal reduced the quality of the pellets. This has also been shown by Andruetto et al (1973). The very fine, dry, dusty texture of the B.P. 'Toprina' is responsible for the difference and special texturising of this material or special processing of the feed mix may be required for successful utilization of large quantities in compound feeds. The application of steam during the pelleting process would probably have resulted generally in an improved pellet quality.

Water Stability

Water stability is a most important factor to consider in any assessment of pellet quality related to fish feeds. The concept and its importance has been discussed by Hastings (1970) and methods of measuring pellet quality have been described by Hastings (1964) and Hepher (1969).

A method for assessing pellet stability in water, which appeared to

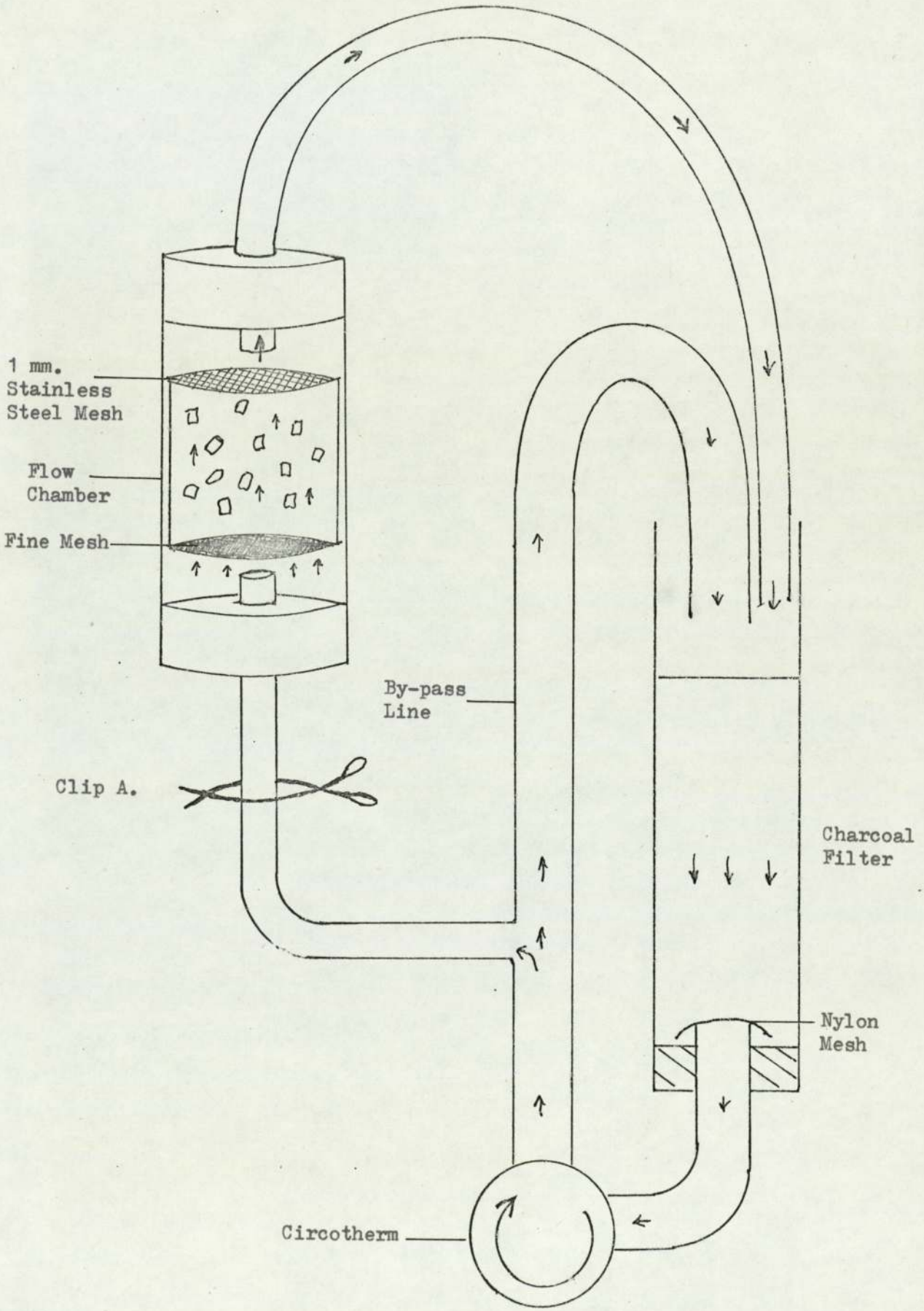
relate more closely to the conditions to which the pellets may be subjected to in the experimental tanks, was developed. This involved subjecting the pellets to gentle agitation in flowing water. For this purpose a small flow chamber was made as shown in Fig 2. This was supplied with water at a variable flow rate in an upward direction. The quantity of water required to be passed through the chamber was considerably less than the quantity pumped. It was drawn off the by-pass line by varying the diameter using the clip A shown in the diagram. Water leaving the by-pass line and the flow chamber was passed into a charcoal filter from where it was repumped back around the circuit. A constant temperature was maintained by the chiller-circulator.

A small pellet holder was made for insertion into the flow chamber. This fit tightly so that the water had to pass through it. A very fine stainless steel mesh was fitted into the bottom of the holder and a 1mm mesh into the top. 5g quantities of pellets were placed inside the holder, which was then inserted into the flow chamber. Flow was adjusted by altering the aperture in the by-pass line as described. Water passed in an upward direction through the chamber. This created a gentle 'joggling' movement of the pellets and carried away material (less than 1mm) which broke away from the pellets as they disintegrated. The particles were prevented from being recycled by the charcoal filter.

The assessment was carried out on duplicate samples at a flow rate of 200ml/min and a temperature of 11^{o}C which corresponded with the ambient tap water temperature at the start of the feeding trial. The results are shown in Table 22.

The water stability of the experimental feeds was similar with the exception of diet CO5. The poor stability of this feed was surprising in view of the fact that the pellets seemed to be harder than the others and there were less fines produced during the pelleting process and subsequent handling. None of the experimental feeds approached the quality of the Cooper Nutrition Products' expanded pellet which was very hard and very water stable.

Fig. 2. Water Stability Testing Apparatus.



Diet Composition

The water content of the diets was determined using the method described for the feed ingredients. The composition of each diet was then determined from the analytical data on the ingredients and corrected for the change in water content.

The results are shown in Table 23.

GENERAL METHODS PHASE I

Experimental Facilities

Some thought was given to future research demands when the experimental facility was designed for the initial feeding trial, since one of the objectives was to evaluate methodology and the design of experimental systems.

It was envisaged that part of the evaluation programme would involve the estimation of the Biological Value of the test proteins. The problems associated with balance studies in fish have been discussed by Atherton and Aitken (1971) and a convenient method for collecting faecal material during the balance period was described. The design outlined below was based on that work.

At the time the experiments were considered, space and water were strictly limited. The site for the proposed experimental system was a small area between two offices in a research laboratory. The area was subjected to normal laboratory conditions of lighting and heating, and disturbance from staff and students gaining access to the staff offices. The laboratory was not designed as a wet laboratory and had minimal plumbing services. These factors were considered when the system was designed.

The completed system (Plate 2) consisted of two 8' x 18" x 9" P.V.C. troughs, strengthened with metal strips along the length and fibreglassed externally (made to specification by Cago Ltd). The troughs were stacked one above the other on a 'Bartangle' framework and a Churchill 'circotherm' (chiller-circulator) was positioned at a height midway between them. Inlet and outlet pipes were positioned in the troughs in such a way that, when connected to the appropriate parts of the circotherm, water could be drawn from the bottom trough, pumped to the top trough from where it could overflow back to the bottom trough (Fig 3).

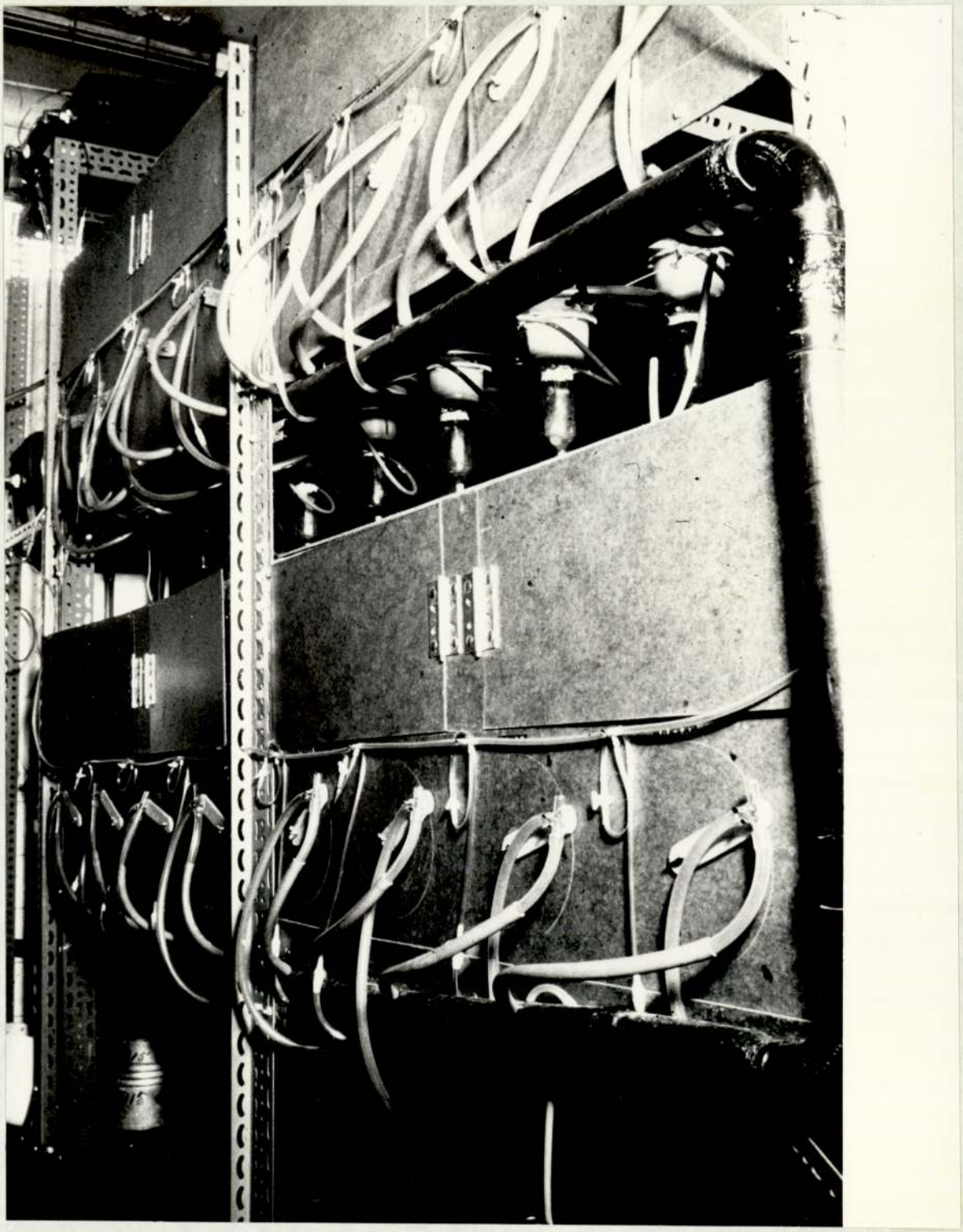
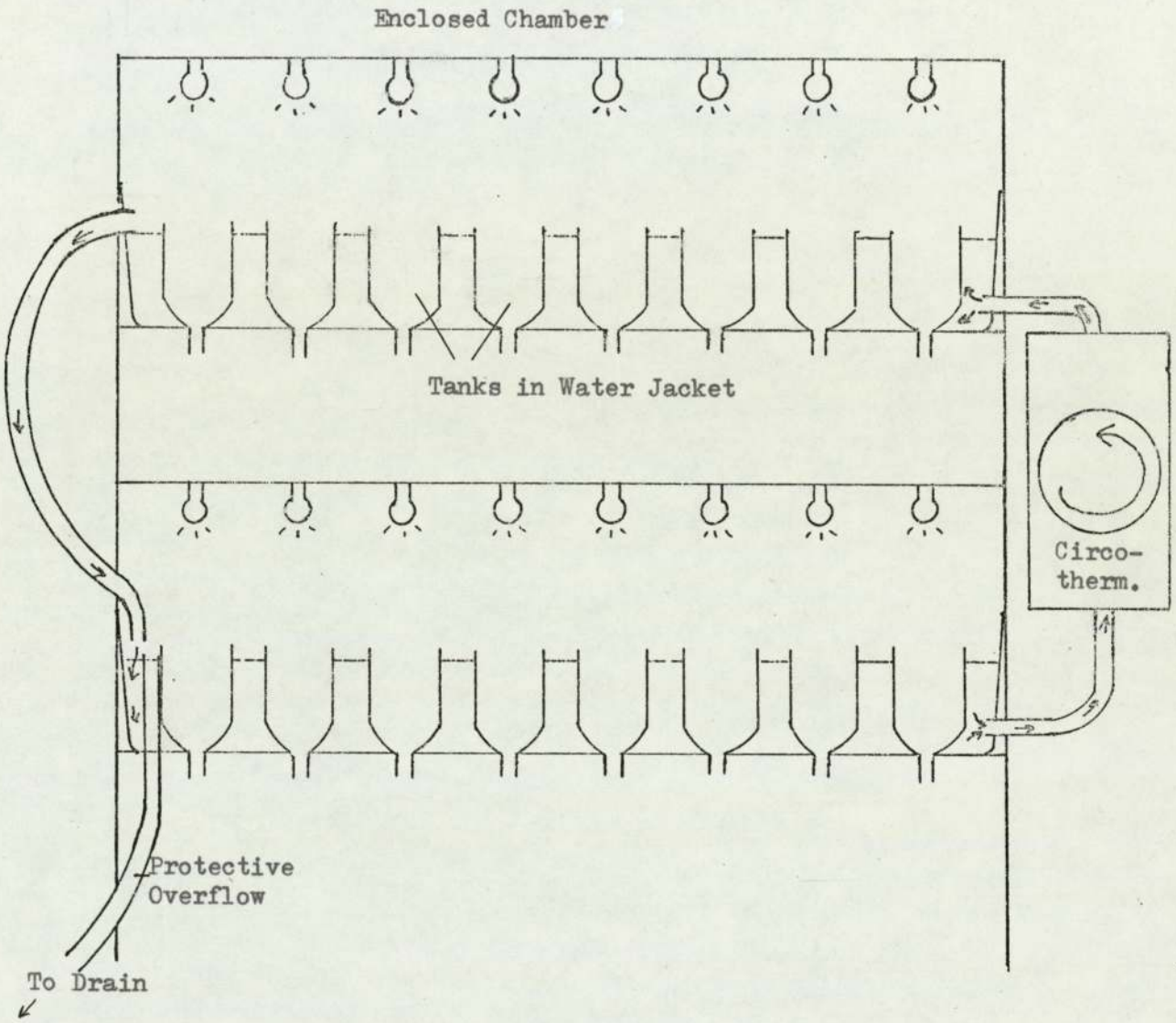


PLATE 2.

Fig. 3. Nitrogen Balance System.



The circotherm could be set to the desired temperature, thus providing a temperature controlled water jacket for the tank system. A precautionary overflow pipe was built into the lower trough and connected to the drainage system.

The tops of 4 gallon high density polyethylene bottles, 10·25" in diameter and cut 10" from the top, provided tanks with an approximate capacity of 10 litres. 8 'tanks' were fitted into each trough. The neck of each was inserted through holes cut in the bottom of the troughs to accommodate faecal traps beneath. A washer each side of the trough bottom provided a convenient seal when the bottle top was screwed up tightly. The traps were made to specification from glass tube, $2\frac{1}{2}$" diam x 5" long drawn to a $\frac{1}{4}$" outlet. To prevent the collection of faecal material in the 3" diameter bottle top, the continuity of the sloping walls was maintained using a plastic funnel and hose connector, the latter of which was inserted directly into the trap through a cork bung. The design details are shown in Fig 4.

The plumbing system was designed to allow for both open and closed circuit operation of each tank. On open flow the tanks were supplied with water continually from two lavatory cisterns connected to the domestic water mains. Each was set 5' above the tanks it served, ensuring the same constant head of water for each of the two rows of 8 tanks. In each system, water passed to the tanks through a series of 8 inter-connected 5' lengths of $\frac{1}{2}$" glass tubing, immersed in the water baths, and then to a $\frac{1}{2}$" ring main. The glass tubing was to serve as a heat exchanger, in an attempt to equilibriate the temperature of the inflowing water to that of the water bath set by the thermostatically controlled circotherm. Inlet to each tank was through a $\frac{1}{4}$" tube from the ring main, connected to a glass bend which was inserted through the wall of the tank and positioned to give a circular flow pattern.

The outlet from the tank was via the trap and $\frac{1}{2}$" glass bend, shown in Fig 4, which was connected to a water levelling device, shown in

Fig. 4. Nitrogen Balance Tank - Construction Details.

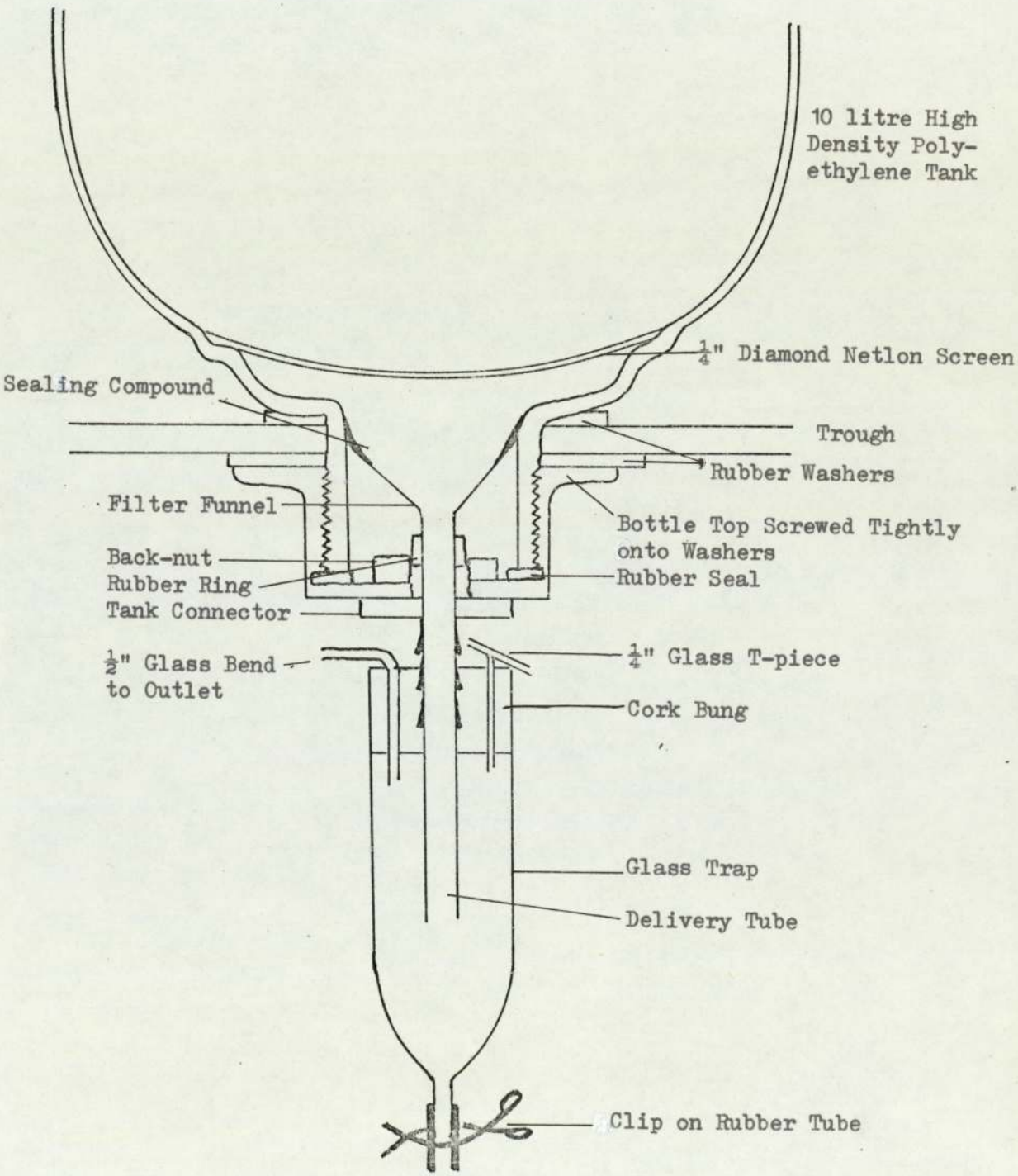


Plate 2 and represented diagramatically in Fig 5. The wooden strip to which the levelling T-piece was attached could be turned through 360° thus raising or lowering the level of water in the tank to any desired volume. Waste water passed into a 4" drainage system made from old vulcathene pipes, welded to provide a flow of waste water to a drain cup plumbed into the University system.

For 24 hr. sampling of faeces and metabolites, a closed circuit circulation system, as used by Atherton and Aitken (1971), was incorporated. Water was recirculated from the trap to the tank by means of an air-lift mechanism, connected to an arm of the T-piece inserted through the cork, shown diagramatically in Fig 5. Air was supplied to each tank from an aerostyle pump via a ring main. To switch from open flow to closed circuit, water flow was turned off and an airtap to each tank opened.

Finally, a connection was made from the second arm of the T-piece direct to the main drain to enable the tank to be emptied completely without moving the position of the levelling device. This was normally clamped.

The tanks were covered by a fine nylon mesh stretched tightly over the rim and held by a band of elastic tape (Plate 3). Access could be gained without removing the cover, by inserting a plastic netlon cylinder at one point, plate 4. Fish could be fed or netted through this access point. The covers were necessary to prevent fish from jumping out. The trough was covered by a plastic sheet to reduce evaporation from the water jacket, and to prevent fish and/or food being dropped into it.

Each 8 tank system was completely enclosed by hardboard sheets, fixed to the 'Bartangle' frame (Plate 2). A distance of 1' was left between the upper and lower levels, and 1' 6" between the lower level and the floor. This accommodated the traps and in addition provided a working surface. Access to the tanks was provided by 4 hinged doors,

Fig. 5. Nitrogen Balance System - Outlet Plumbing.

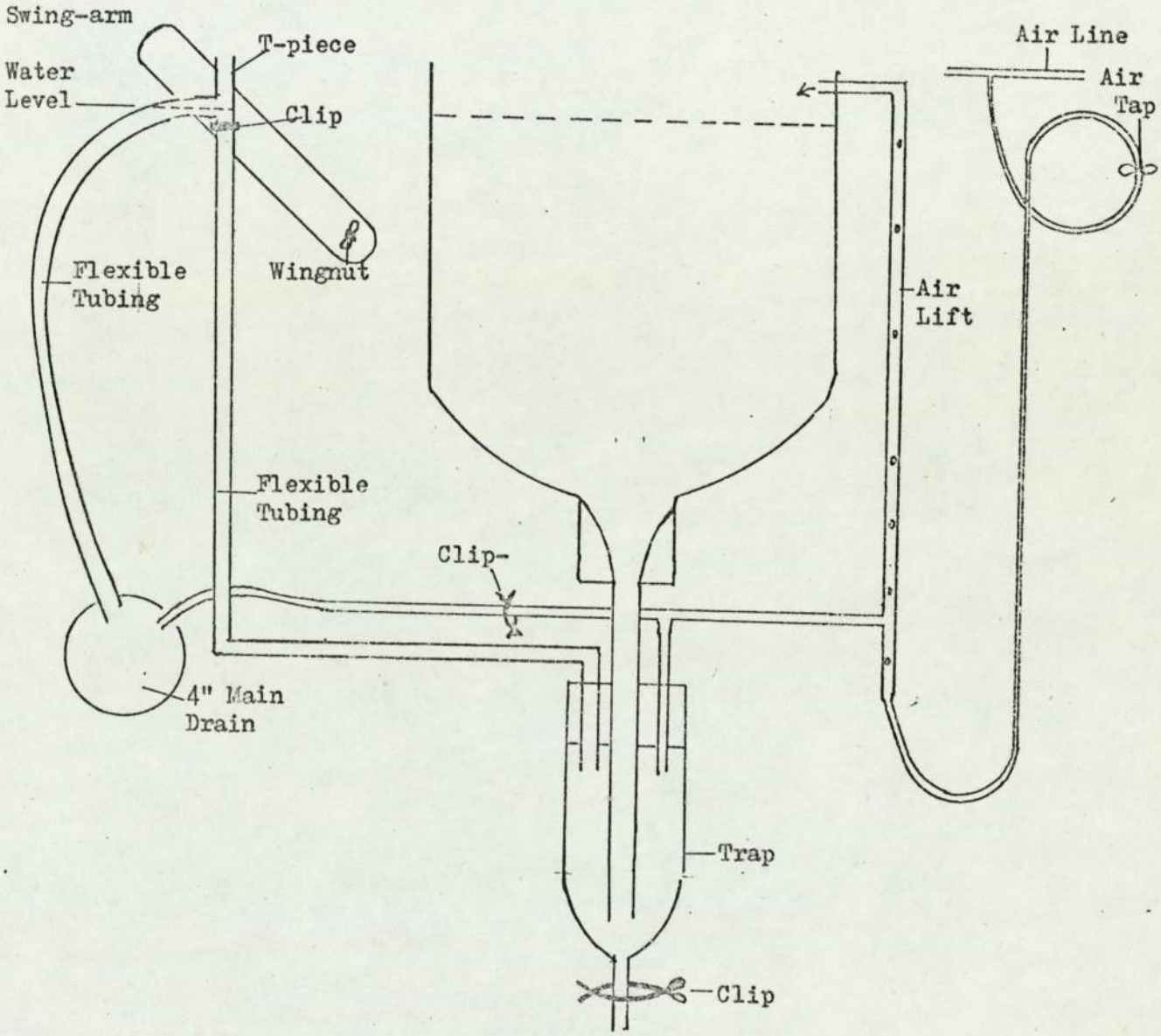


PLATE 3.

PLATE 4.

each 12" x 18" set into the front face. The enclosure was built in an attempt to provide a more constant environment by keeping disturbance to a minimum.

Lighting was supplied to each tank by a 6 watt bulb, set 1' above it. As a safety precaution bulbs were screwed into MES holders set into a hardboard enclosure. The wiring was completely protected by the enclosure which was bolted to the roof of the system. A further precaution was the use of a low voltage supply, transformed from the mains. A 12 hr. lighting regime was maintained by a 24 hr Venner timer.

The system was tested with flow for a number of weeks until all immediately apparent technical problems were solved. A feeding trial proposed to last 12 weeks was commenced in the system on open flow. During this time it was envisaged that the balance technique could be assessed.

Experimental Animals

448 rainbow trout of similar size (16-17g) were selected from the stocking tanks. The selected fish were then randomly sorted into 16 groups of 28. Each group of fish was weighed in total using a wet method. This involved 'taring' a large beaker of water on a triple beam balance and then pouring a bucket of water containing the entire population of 28 fish into a fish net. The fish were retained in the net for a standard 15 second drain time with gentle shaking of the net to allow water to drain off the fish before adding them to the 'tared solution balance'. The weight of the fish was read off to the nearest gram.

The fish were weighed every two weeks in this manner.

Environmental Parameters

The flow rate was adjusted to approximately 250ml/minute and the

'swing arm' outlet was set to give a volume of 10 litres in each tank. The compressor was switched on to ensure an adequate aeration within the tanks. The circotherm was set to run at 12°C. The temperature in each tank was recorded daily.

The stocking density at the beginning of the trial varied from 45·5 - 47·8 kg m^{-3} and the loading varied from 1820 - 1912 kg m^{-3} min.

Feeding Methods

Introduction

For all types of feeding experiments it is desirable, and for most it is essential, to keep some record of food consumption. In designing comparative feeding trials it is necessary, therefore, to decide which feeding regime to use.

The more commonly used methods in livestock feeding experiments have been discussed by Maynard and Loosli (1969). Generally it is necessary to decide between group feeding and individual feeding and between ad libitum feeding and some form of controlled feeding.

Group versus individual feeding

The relative merits of group and individual feeding in relation to the design of comparative feeding trials have been discussed by Crampton (1959). Individual feeding has the advantage of increasing the effective number of experimental observations with which the significance of observed differences between treatments, especially where these are small, can be determined. A further advantage is that it preserves the identity of individuals (Maynard and Loosli, 1969). This is useful in the event of the occurrence of mortalities when the data relating to dead individuals can be eliminated from the experimental results completely. Unfortunately, in many instances, the limitation of the use of

individual feeding is set by the availability of space and equipment and the operational costs, rather than by experimental design considerations.

Ad libitum versus controlled feeding

The second consideration is whether to feed the individuals or groups as much as they will readily consume, called ad libitum feeding, or whether to control the food intake to a predetermined level.

Ad libitum feeding 'gives unbiased results for direct practical applications in terms of the food, species and function under study' (Maynard and Loosli, 1969), and is the most commonly used procedure in farm animal investigations. In some tests, however, the method is limited by the fact that differences in palatability rather than nutritional value may be responsible for observed differences in production.

Controlled feeding has been proposed as a solution to this problem. Osborne and Mendel (1916 and 1917) pioneered the use of controlled feeding in their experimental design. In their experiments, the level at which to feed during the period of controlled feeding was predetermined in a preliminary experiment. However, in a form of controlled feeding referred to as 'paired feeding', described by Mitchell and Beadles (1930), experimental animals are selected in pairs, one of the pair being fed the control feed and the other the test feed, both in exactly the same quantities determined by the individual consuming the least. This technique has been criticised, however, on the grounds that the faster growing individuals in experiments in which controlled feeding is used would be penalised by virtue of their having a proportionately higher metabolic demand as their weight increased above that of the slower growing individuals. A refinement of the technique, which resolves this problem, involves the use of feeding scales based on the weight of the animal at any time (Carpenter and Porter, 1952; Sherwood and Weldon, 1953), but the method is much more tedious than the ad

libitum technique. A further criticism of controlled feeding methods is that the level consumed by the group eating the least would not allow the full benefit of the better rations to be expressed.

A more recent alternative approach to resolving the difficulty in interpreting results of experiments involving the use of the ad libitum technique was suggested by Carpenter (1953). He proposed the use of a mathematical expression to determine an 'appetite quotient' which would enable the experimenter to decide whether observed differences in food consumption were the result of differences in palatability or whether they reflected differences in the demand for metabolizable energy.

It is clear that there are problems inherent in the application of both ad libitum and controlled feeding methods in experimental feeding trials. The choice will ultimately depend on the objectives of the trial. It has been concluded, Maynard and Loosli (1969), that 'there are many problems concerning which the use in separate experiments of both ad libitum and controlled feeding will give much more information than either procedure alone. The effective investigator must select his method in accordance with his problem, frequently employing more than one method, and finally, he must interpret his results with full consideration of the advantages and limitations of the method used'.

Feeding methods in comparative fish feeding trials

The use of individual feeding in fish nutrition studies is generally limited to those involving nitrogen or energy balance, where fish are maintained in individual containers, as described, for example, by Atherton and Aitken (1970), Smith (1971), Smith (1974). The use of this technique is otherwise severely restricted by limited space, tank numbers or water availability. For most practical purposes involving diet testing, large numbers of fish will be kept in tanks where individual feeding is neither possible nor even desirable. In this instance food is

administered to the whole group. The efficiency with which the food is utilized can only be determined for the group. If the statistical validity of observed differences is to be determined, then one must use as many replicates of the group as possible. Unfortunately, this itself is frequently limited by physical considerations such as space and water availability.

The choice between ad libitum feeding and controlled feeding in fish studies is complicated by the fact that the animals are maintained in an aquatic environment. Whilst ad libitum feeding may be achieved conveniently and simply in the case of poultry or pigs for example, by filling troughs with a measured quantity of food and allowing the animals to feed from this at will, food consumption being the difference between the starting weight and that measured at the end of the day, this cannot be achieved in the aquatic environment. In fish feeding experiments, therefore, 'ad libitum' feeding has referred to a technique in which the fish are fed to satiation a stated number of times per day. In this circumstance feeding must be carried out with the utmost care and fish must be observed closely during feeding to avoid food wastage.

More recently, 'demand feeding systems' have been developed in which fish knock some form of trigger in the water and receive food in so doing. This method provides an opportunity for the fish to feed at all times and thus feeding may be considered to be truly ad libitum. Unfortunately, however, unlike the situation in which pigs or poultry are feeding from troughs, food which is dispensed from the feeder and unconsumed will be wasted. If this occurs to any extent during a feeding trial, the immeasurable waste will cause erroneous results giving, for example, low food conversion efficiency data.

By comparison, controlled feeding is simple and can be achieved by hand or automatically where dry foods are used. The actual quantity of food administered is generally based on some form of the feed tables developed for meat-meal mixtures by Deuel et al (1952), where the

daily level of feeding is adjusted to a percentage of the body weight, calculated for a number of size categories of fish and a range of temperatures.

Haskell (1959) modified Deuel's original equation and produced a method by which the feeding rate is determined from values for expected conversion, as estimated from previous hatchery records, and the daily increase in length of the fish. Butterbough and Willoughby (1967) modified the Haskell equation for use under a variety of hatchery conditions and subsequently produced a feeding chart. This has been adapted to a feeding slide rule by Piper (1970).

Conclusion

Controlled feeding, using scaled levels as given in feeding tables, appeared to be the simplest and most appropriate for the initial feeding trial. Such methods were used extensively in feeding trials carried out at the Cortland Hatchery during a diet evaluation programme (Phillips et al, 1953-1963). The tables of Deuel et al (1952) designed for meat-meal mixtures were adjusted by Phillips et al (1961) for use with dry diets containing a proportionately higher caloric content. Feed tables published by compound feed producers have been similarly adjusted. Tables supplied by Cooper Nutrition Products Ltd were used in this instance.

Each of the eight experimental feeds were allocated to two tanks, one to a tank on the bottom part of the system and the other to a tank on the top part. Feeding was carried out seven days a week. The daily feeding rate was adjusted to the percentage body weight recommended in the C.N.P. feeding tables and based on the temperature, the size of the fish and the total weight in each tank. Feed was accurately weighed into stoppered plastic bottles daily and feeding was carried out twice per day, allowing the fish approximately equal portions of the weighed food on each occasion. Feed was presented through the access point of

each tank cover previously shown in Plate 4. Pellets were presented as slowly as possible in an attempt to ensure complete consumption. Unfortunately, on many occasions this was not achieved.

The feeding rate was adjusted each time a new set of fish weights were obtained.

RESULTS PHASE I

General Observations

By the end of 28 days it was apparent that the experimental system was not suitable for a long term feeding trial. The system failed in a number of the design features:

1. The design of the faecal trapping mechanism made the system unsuitable for long term studies. The trap was too small, particularly with respect to the outlet from which collection was made. The clip frequently 'pinched' the rubber tubing tightly closed forming a seal which was difficult to break. Faecal material often adhered in the trap forming small clumps which could not pass through the $\frac{1}{4}$" outlet. There was no way of isolating the trap from the 10 litre tank since the hose connector connected the tank directly to the trap. As the water was removed from the trap during emptying, it was replaced simultaneously from the tank via the delivery tube. This caused upwelling within the trap making faecal collection extremely difficult.

2. Corks, as opposed to rubber bungs, were chosen for the traps because of their low cost. However, such a choice proved a false economy. A large proportion of the joints were found to leak, a problem that could not be solved by the use of wax or vaseline. The joints were white-sealed as a last resort. This was not 100% efficient, and caused further problems later in view of its permanancy!

3. The air lift tubes were too small, so that the volume of air to that of water could not be adjusted to give a smooth flow of water during closed circuit circulation. Occasionally, unconsumed pellets entering the traps were drawn up the open end of the air-lift tube in the trap, causing blockages in the air-lift system.

4. The temperature control system was inadequate. The circotherm was unable to maintain the set temperature and served only to create a slight temperature gradient between the inlet and outlet ends of the water bath.

5. The enclosure, and particularly the doors, were far too small and not enough space was left between upper and lower systems providing inadequate working space.

As a result of the inadequacy of the faecal trap design, in particular, the emptying procedure was both tedious and time consuming to the extent that the general maintenance of the system began to occupy a considerable proportion of the working day. On occasions, it was not possible to give adequate attention to such maintenance as was required. One of the consequences was the build up of faeces and waste food in the traps. This in turn created an environment which was suitable for the growth of filamentous fungi,enhanced by floculation of the solubilised protein and suspended particulate matter from the waste food. The resulting 'mat' then acted as a strainer thus adding further to the build-up of faecal material, filamentous fungi, algae, bacteria and even protozoa. The result is shown in plate 5. This made it impossible to empty the traps by the normal outlets. Emptying could only be achieved by backflushing, and then with difficulty. This obviously disturbed the fish as clumps of material were carried into the tanks. On occasions, the 4" main drains were blocked as a result of emptying the traps when in this condition. This caused a considerable degree of flooding within the laboratory!

PLATE 5.

Performance Data

Despite these problems, however, the trial was continued in this system until the 60th day although no attempt was made to measure the fish weights between the 28th and 60th days. The fish were fed on the fixed feeding regime during this period. Unfortunately, it was apparent that consumption was frequently not complete as shown by the appearance of food in the faecal traps.

The growth data for each diet treatment is shown in Table 24. The mean temperature is also shown. Conversion efficiency data is not reported in view of the fact that an inestimable quantity of food was not consumed during the trial period.

Daily mortalities appeared to be caused by cannibalism. 'Fin nipping', in some instances, was extremely severe. In some fish removed, the caudal fin was absent and on a few occasions the vertebral column was exposed. Fish were obviously stressed in this system. There did not appear to be any relationship between the mortalities and the diets. The excessive losses in tank 10 were inexplicable, but the heavy losses in tanks 4 and 14 were the result of 'systems problems' which resulted in oxygen deficiency within these tanks.

The stocking and loading parameters at the end of 60 days are shown in Table 25. In an attempt to determine if the cause of the general stress was related to the environmental parameters, the oxygen content, pH and ammonia levels of the effluent from each tank was determined. Oxygen was measured using an E.I.L. oxygen meter, pH was measured using a Dynacap pH meter and total ammonia was determined using an E.I.L. ammonia probe. The results are shown in Table 26.

A high oxygen content was maintained in all tanks by the air lift and was not considered to be limiting in any tank, being greater than 70% in

all cases. The degree of dissociation of the ammonia was determined from the relationship $NH_3^{\circ} = \frac{[NH_4^{+} + NH_3^{\circ}]}{1 \cdot 0 + \text{antilog}_{10}\,(pka-pH)}$ using a computer programme (Watson, in prep), knowing the pH, temperature and total ammonia. Although variable, as shown in Table 25, the free ammonia in all cases was very much lower than the 0·025mg/1 considered as a safe level for rainbow trout (Lloyd & Orr, 1969) and was in no case considered to be limiting.

It was not clear to what extent the apparent stress was caused by other factors such as tank size, the light intensity or the rate of feeding. It was obvious, however, that the trial could not be continued for any length of time in that system. A second 16 tank system was therefore installed out of doors, in a transparent plastic greenhouse, in an attempt to keep the trial running.

GENERAL METHODS PHASE II

Experimental Facility

In view of the limited time available for the installation of this system and the necessity therefore of using readily available materials, the design was kept as simple as possible. The bottom halves of the polythene bottles used in the balance system were used as tanks giving an approximate volume of 10 litres. Since the bottom of the tank was ridged, it was impractical to use a central drain system. The method used simply provided 'overflow' drainage from the bottom of the tank. Inlet was by way of a rubber tube connected to a 'ring main' at one end, and clipped to the side of the tank at the other end. Flow was controlled using a Hoffman clip. The ring main was served from a header tank into which water was jetted to ensure adequate oxygen saturation and drive off residual chlorine. No additional aeration was provided, the oxygen being supplied only from the fresh inflowing water. A plastic bag, slit at the bottom and held firmly around the top of the tank was suspended from two string lines, providing a very useful means of preventing fish jumping out, but at the same time, allowing easy observation of the fish, particularly during feeding periods. The lighting was natural daylight.

The trial was continued in this new system by transferring the fish on the 61st day after the start of the trial on the experimental diets.

Experimental Animals

Fish were retained, as far as possible, in the same groups, although the number was reduced to 20 per tank. Due to the heavy mortalities in tanks 4, 10 and 14, it was necessary to redistribute the fish. Thus the 28 fish from tank 13 which had been on the same diet as those in tank 4 were divided between these two tanks. The number of fish in tank 10 was made up from excess fish of tank 7 also on the same

diet and the number of fish in tank 14 was made up from excess fish of tank 3 again on the same diet. In this way there was no change in diet allocation, all fish receiving exactly the same diets as during the first 60 days. The number of fish in tanks 4, 13 and 14, however, was only 14, compared to 20 in the remaining tanks.

The weighing technique was changed from that previously described. Fish were weighed individually rather than in groups. The technique involved anaesthetising the fish with MS222 (Sandoz). A solution of 50ppm of MS222 was found to be satisfactory. This concentration was sufficient to lightly anaesthetise the fish within a few minutes and was tolerated for periods up to 20 minutes without causing mortalities. Fish recovered quickly in fresh aerated water. Whilst under anaesthesia, fish were individually removed from the water, rolled gently on a damp cotton wool pad to remove excess water and weighed on a top loading balance (accuracy 0·1g) 'tared' with a damp cotton wool pad on the pan. The weights were recorded directly onto an electrical adding machine fitted with a print-out roll.

Environmental Parameters

The flow rate was adjusted to approximately 250ml of water / min. The volume of water in the tank was fixed by the position of the overflow pipe. This was approximately positioned to give a tank volume of 10 litres. Temperature was ambient mains water temperature which was recorded daily.

The total weight of fish in each tank at the start of Phase II and the stocking and loading factors are shown in Table 27.

Feeding

Fish were fed a controlled quantity of food twice daily, seven days a week as previously described. The quantity was determined from the

C.N.P. feeding tables based on temperature, total weight of fish in each tank and the size category. The commercial control feed was from a new bag of Cooper Nutrition Products' 'Beta' Floating Trout Food, Size No. 4.

RESULTS PHASE II

General Observations

Surprisingly, the tanks were self-cleaning. In this respect the combination of bottom drainage and the fish constantly stirring the water was adequate. The tanks, however, drained directly onto the floor thus causing a gradual build-up of the 'sewage-type' fungus mentioned previously. In addition the outside of the tanks became progressively coated in algae. The system was therefore regularly hosed and the floor periodically disinfected.

Problems with water flow were also experienced. Whilst the method of attaching the inlet-lines to the tanks was simple and therefore attractive, occasionally a fish would jump at the inflow tube and displace it from the clip. On one occasion this resulted in complete loss of fish in one tank. Since the fish depended almost entirely on the incoming water for oxygenation, they were stressed within 15 minutes of loss of water flow. Further, the water availability at this site was strictly limited and it was difficult to balance the flows to ensure an equal and adequate supply of running water to each tank.

Performance Data

The growth data for each diet is shown in Table 28 and conversion efficiency data is shown in Table 29.

During this phase of the experiment diet CO1, i.e. Cooper Nutrition Products' Beta Trout Food, was not consumed very well and consequently growth and conversion efficiency was low. This was related to the use of a new bag of pellets. After 28 days the fish were transferred back to the older stock diet and there was a substantial improvement in one tank and a partial recovery in the other. Therefore the data for the 'commercial control' has not been included in Tables 28 and 29.

In some tanks the growth rate and conversion efficiency clearly began to fall 30 days from the start of Phase II of the experiment. This was particularly marked in tanks 2, 10 and 12. Careful observation of the fish showed a diminished response to food. The fish in these circumstances showed all the signs of oxygen stress which included a decreased appetite and consequently greater food wastage. Oxygen deficiency in some of the tanks was confirmed by measurement with an oxygen meter. The pH and total ammonia was also measured. The results are shown on Table 30 along with the approximate stocking densities and loading factors at this time.

The stocking density and loading factor in many of the tanks was considered excessively high. The loading in some tanks was greater than 4kg per litre flow per minute. In tanks 2 and 10 oxygen was below 50%. The performance has already been shown to have fallen off substantially in these tanks. The oxygen content was below 60% in several other tanks and was therefore likely to have become limiting. Despite these observations, mortalities occurred only in tanks 1 and 5, with 2 and 1 fish respectively.

Total ammonia was low and in view of the low pH and temperature, the free ammonia was likely to have been well below the limiting levels. No attempt was made to compute the values in this case.

DISCUSSION

The environmental conditions during much of the experiment were

obviously not very satisfactory. Nevertheless, some basic trends were apparent. The mean weights for each diet have been plotted for the two phases of the experiment, as shown in Fig **6**.

Differences in the growth of fish on the different diets was evident in both phases of the experiment. The mean percentage increase in live weight of fish on each diet in phase I is shown in Table 24. The values have been plotted against the protein content of the finished feed from Table 23. The % increase in weight generally increased with increasing protein content in the finished feed as shown in Fig **7**.

The growth rate increased substantially in phase II of the experiment. The most likely explanation of this is that the actual food consumption rate increased, there being very much less wastage of food in the flat bottomed tanks used in phase II.

The quantitative laws governing the growth of fish have been the subject of a review by Weatherly (1972), among others. Specific growth rate can be derived from growth data using the expression

$$YT = Yt.e^{g(T-t)}$$

where YT is the final size (at time T) and Yt is the initial size (at time t); T and t are given in time units, T being later than t; e is the base of the natural logarithm; g is a growth rate known variously as specific, instantaneous, geometrical, multiplicative, etc.

The constant g x 100% has been defined as the specific growth rate of the fish expressed as the instantaneous rate of gain in weight per unit weight (percent change in weight / time) and is more conveniently written by the equation

$$G = \frac{\log_e YT - \log_e Yt}{T - t} \times 100$$

from Brown (1957)

In the early stages of the life of fish under controlled laboratory

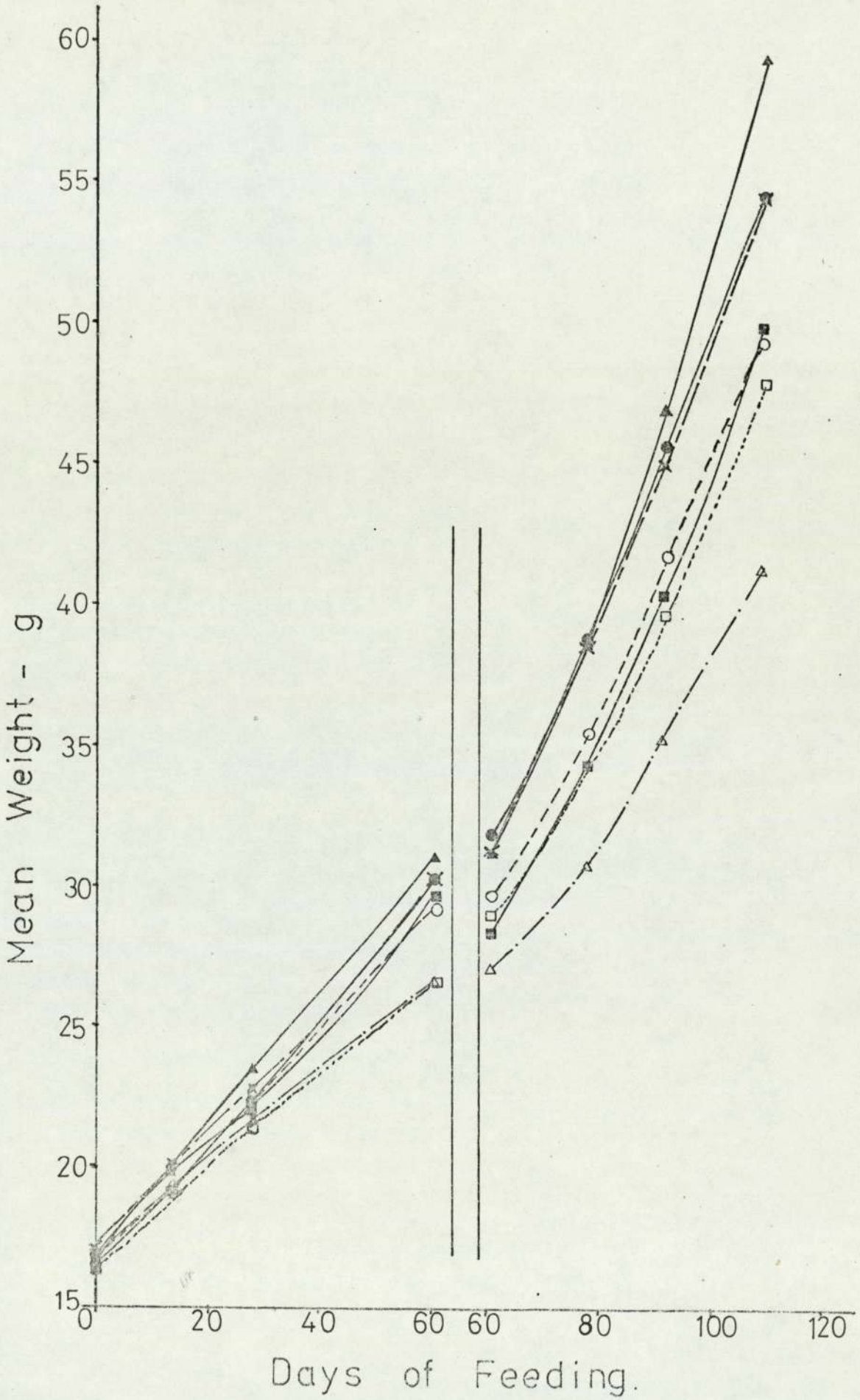
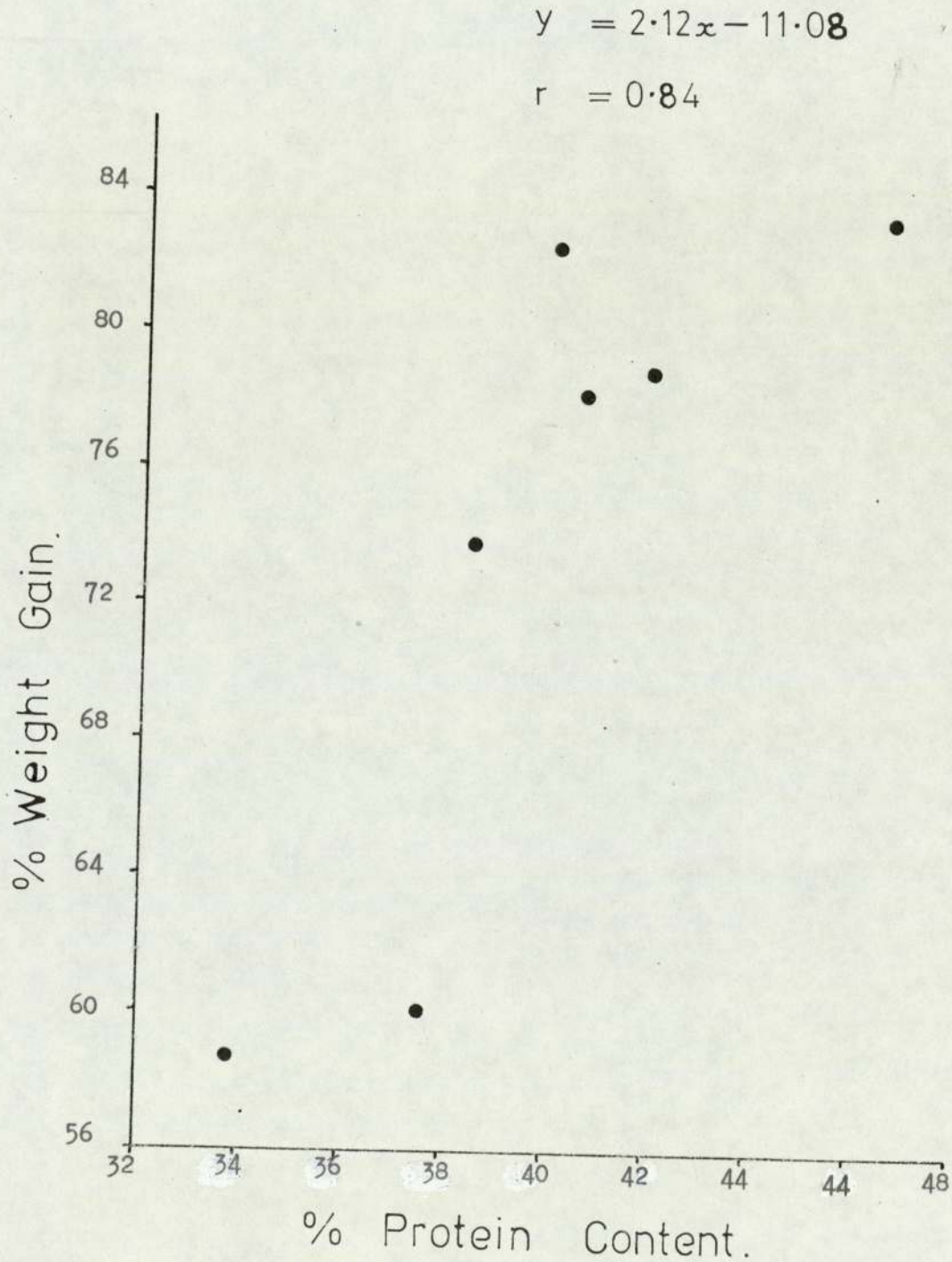

60
55
50
45
40
35
30
25
20
15
Mean Weight - g
0
20
40
60
60
80
100
120
Days of Feeding.

Fig. 7. Correlation of % Weight Gain and % Protein Content During Phase I.



conditions, growth follows an exponential curve and specific growth rate is constant. Specific growth rate therefore provides a convenient method of comparing the growth of fish in laboratory experiments carried out in controlled conditions over short periods of time.

Log_e values for the mean weight of fish on each diet have been determined and recorded as shown in Table 31. The values have been plotted in Fig 8 . Despite a slight fall in temperature in Phase II of the experiment, specific growth appeared to be reasonably constant between the 61st and 92nd days of the experiments in all diets and indeed up until the 110th day for 4 of the 7 test diets (omitting the C.N.P. results). The fall in specific growth rate between the 92 - 110th days for the remaining three diets is related to the worsening environmental conditions in some of the heavier stocked tanks. Specific growth rate for each diet has been determined for the short period, 61 - 92 days, in which it was constant (Table 31). The values have been plotted against the protein content of the finished feeds as shown in Fig 9 . The results generally confirm those obtained in Phase I. Specific growth rate increased with protein content of the feed. The correlation was improved, undoubtedly as a result of the improved experimental conditions.

Delong et al (1958) found that the protein content for optimum growth in Chinook salmon (Onchorynchus tschawytscha) at $8^{o}C$ was 40% and more recently Satia (1974) has confirmed that the optimum protein content for rainbow trout is somewhat similar. The curve of growth rate against protein content does appear to begin to plateau above 40%. This was more marked during Phase I of the experiment. As one might expect this also applied to the conversion efficiency over this period (from Table 29) which was correlated with protein content as shown in Fig 10.

The caloric content of the feeds was not measured. It is possible that differences in caloric content of the diets influenced the results obtained for growth and conversion efficiency. However, the most

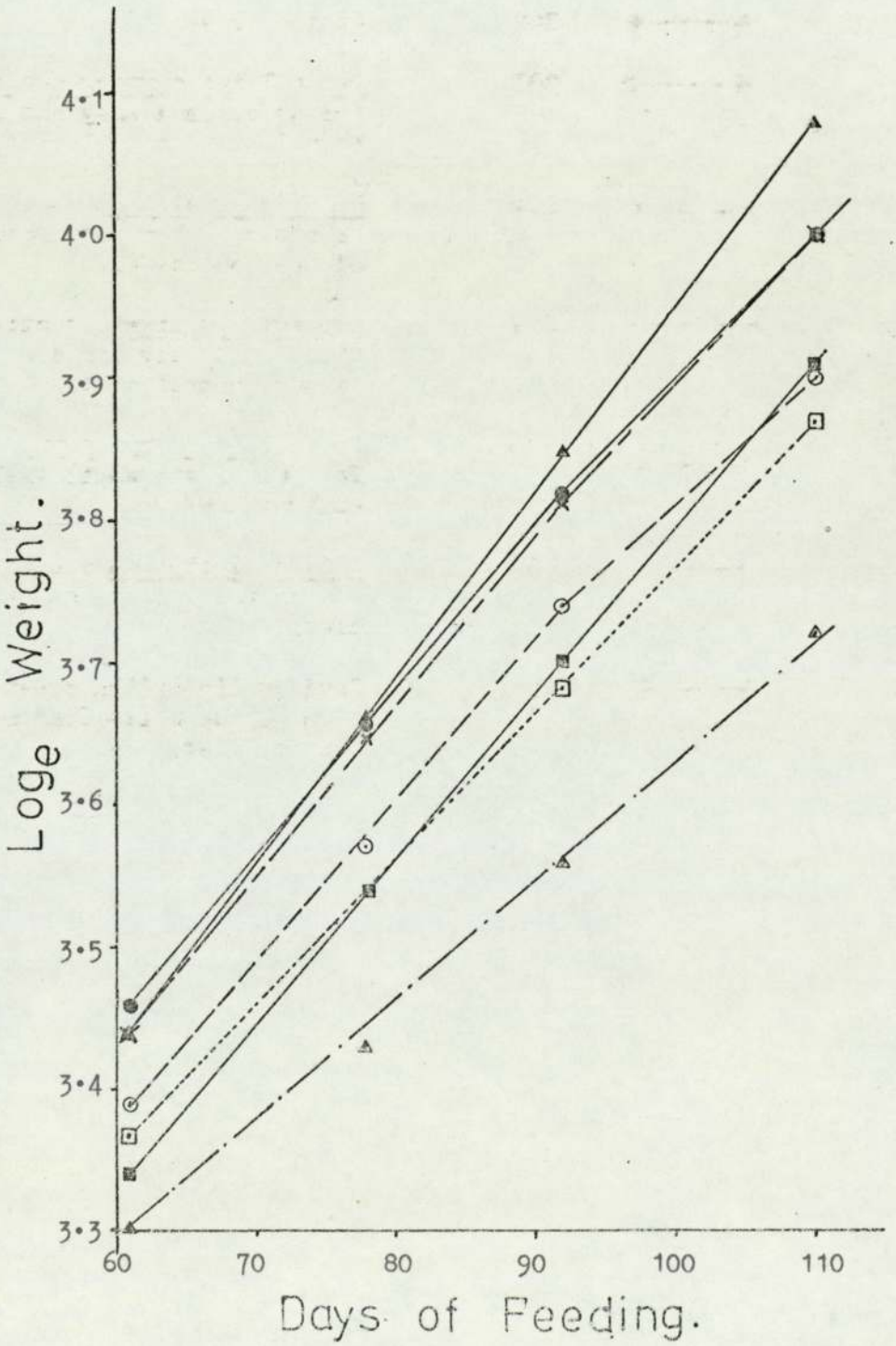

4·1
4·0
3·9
3·8
3·7
3·6
3·5
3·4
3·3
Log$_e$ Weight.
60
70
80
90
100
110
Days of Feeding.

Fig. 9. Correlation of Mean Specific Growth Rate and % Protein Content.

$y = 0{\cdot}04x - 0{\cdot}33$

$r = 0{\cdot}95$

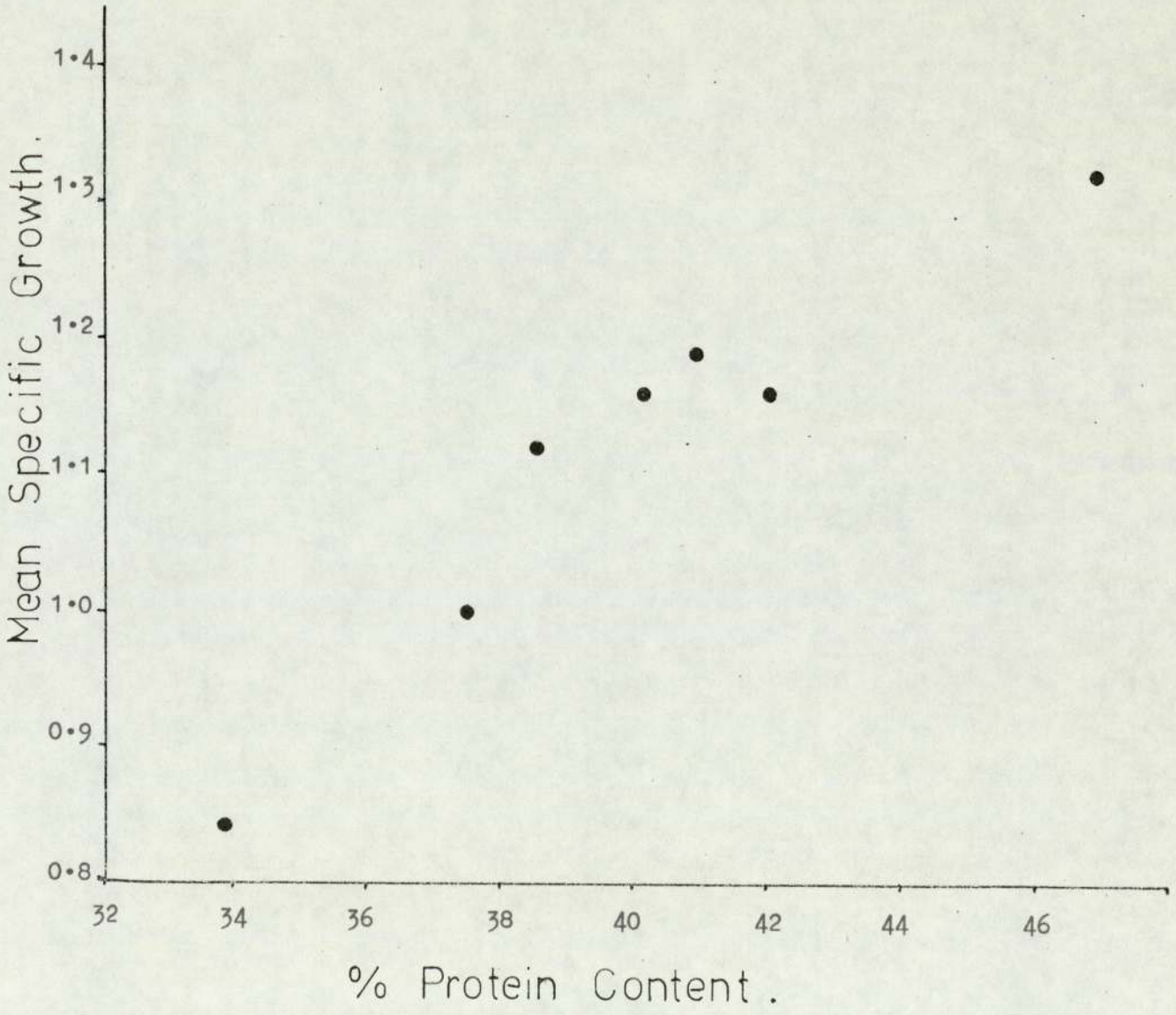
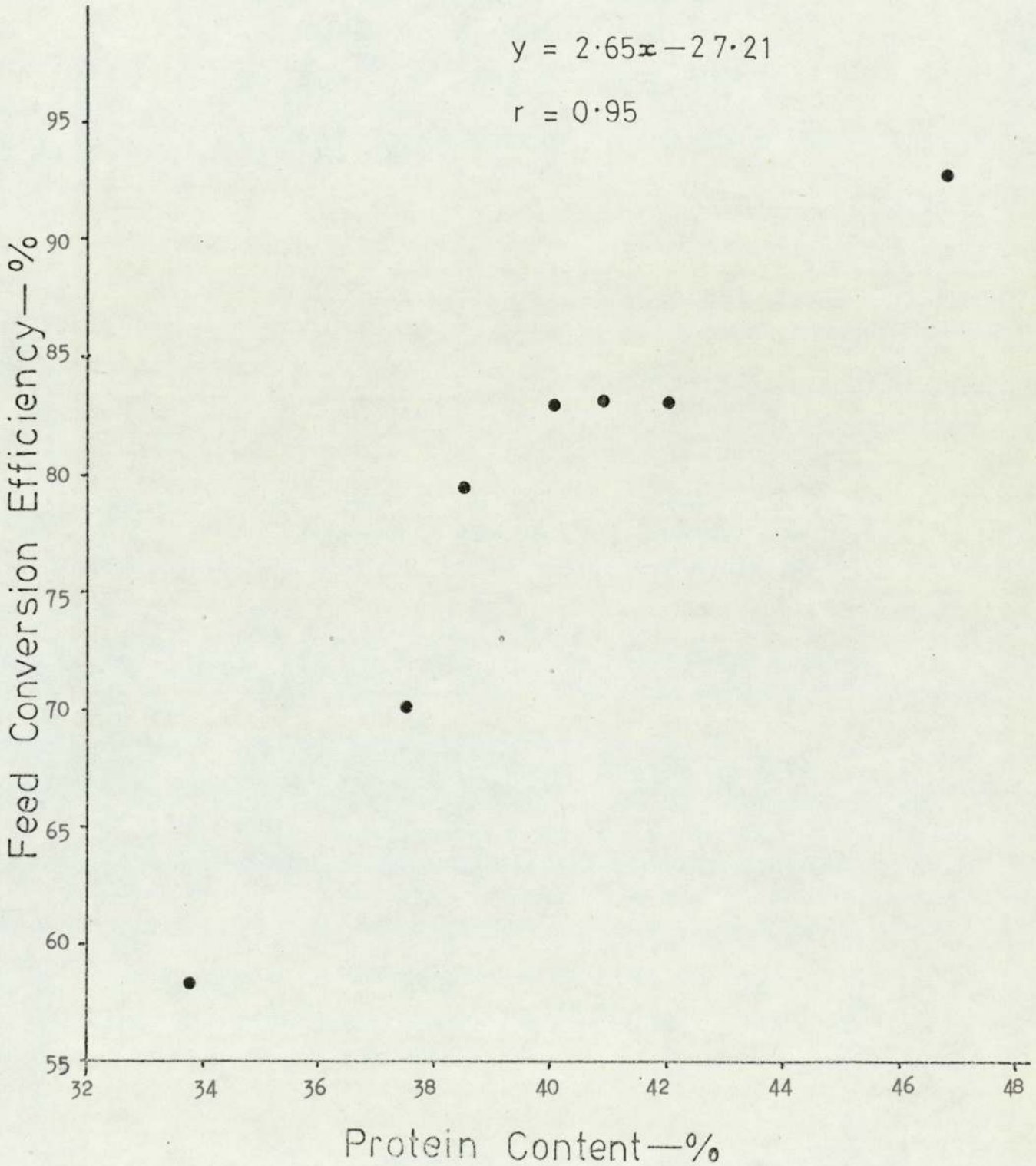


Fig. 10. Correlation of % Feed Conversion Efficiency and % Protein Content.



likely source of differences in available caloric content of the diets was the variation in protein content and in view of the earlier findings of Delong et al (1958) and Satia (1974) it is unlikely that the general conclusion would be affected.

CONCLUSION

There was obvious scope for improvement in the experimental design both with respect to the general facilities and to the methods used. Diet preparation methods could be improved to produce a more stable pellet. Different feeding methods also warranted attention. The shortcomings of the experimental tank system are obvious. There was no opportunity to assess the balance techniques in the conditions prevalent during Phase I. There was improvement in conditions in Phase II initially but increased water flow and additional aeration was required for a longer term study.

Despite the problems, there was an opportunity to evaluate the 3 single-cell proteins. They seemed to be well tolerated in the diets of rainbow trout, there being no loss of growth and conversion efficiency provided that the protein content of the feed was not lowered. Generally, the performance of the fish in terms of growth and food conversion efficiency appeared to be related to the protein content of the finished feed.

SECTION 4.

DESIGN OF IMPROVED FACILITIES AND METHODOLOGY

FOR THE EVALUATION OF COMPOUND FEEDS.

EXPERIMENTAL FACILITIES

The experimental facility incorporating the 10 litre tanks used in Phase II of the experiment described in Section 3 was redesigned in an attempt to overcome the problems previously experienced. The number of tanks was extended to 28, installed in two rows of 14, one each side of a passageway between two buildings. The passageway was not in general use and the site therefore provided relative isolation protected by buildings on each side and at both ends.

The simplicity of the system was maintained. Each row of 14 tanks was raised from the ground on a 'Bartangle' framework. The principle of the drainage system was retained, i.e. the tanks 'overflowed' from the bottom, but each outlet tube was moved to the side and connected to a T-piece, made from plastic tubing, inserted through the tank wall (fig 11). Waste water, carrying faecal material, flowed from the tubes directly into 4" open gutters sited behind each row of tanks. These conveniently drained directly to a grid on each side of the passage.

Each row of 14 tanks was supplied with water independently by a 'ring main' connected by a $\frac{1}{2}$" plastic hose line from a header tank in the laboratory. Each individual tank was supplied from the ring mains by a $\frac{1}{4}$" tube connected to a bend inserted tightly through the tank wall to prevent their displacement. Flow was controlled by a Hoffman clip on each inlet line.

In view of the problems caused by oxygen depletion in the initial trial, an additional design feature of the modified system was the incorporation of a small air lift mechanism similar to that described for the nitrogen balance system.

The plastic bags previously used were replaced by $\frac{1}{8}$" diamond netlon cylinders, attached around the rim of each tank using nylon line,

providing easy access and viewing during feeding, as before.

Finally, each row of tanks was enclosed by plastic sheeting attached to the 'Bartangle' framework. This provided protection from the elements and inquisitive animals (rats, cats and birds). Clear sheeting was used on the top so that the fish could be observed without folding it back. The complete system is shown in Plates 6 .

The problems previously experienced were largely overcome. The system was very much cleaner by virtue of the improved drainage facility, the replacement of the plastic bags with plastic mesh and because the tanks were raised off the ground. The system was very easy to maintain. The tanks were generally self cleaning except when very small fish were used. In this case the tanks were siphoned daily using a "tank vacuum", designed as shown in Fig 12, mounted on a trolley for easy manoevering between the rows of tanks. The 'overflow' tubes and airlift tubes were occasionally cleaned by direct insertion of a small test tube cleaning brush.

A few problems remained, particularly with respect to water flow. It was very difficult to maintain an equal flow rate to all tanks. Imbalances occurred, the flow rate increasing to some tanks at the expense of others. Frequent daily adjustment was necessary. Further, blockages occurred as a result of algae "sloughing" from the walls of the $\frac{1}{2}$" ring main. The additional facility provided by the air lift mechanism provided a safety factor in the event of either of such occurrences reducing flow to any of the tanks thereby causing a depleted oxygen supply.

In view of the relative success of this simply designed system, a slightly larger version, consisting of six 20 litre tanks, was installed for longer term studies.

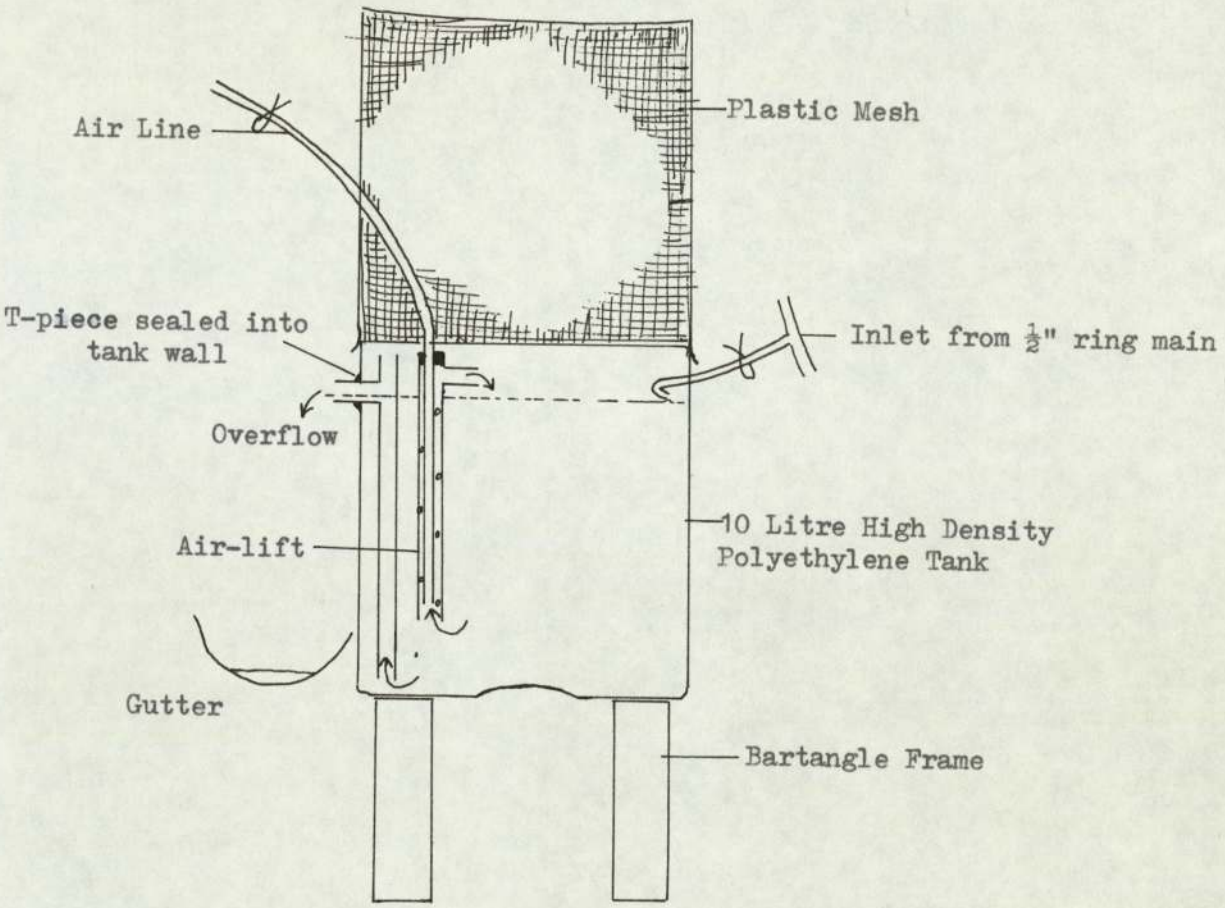
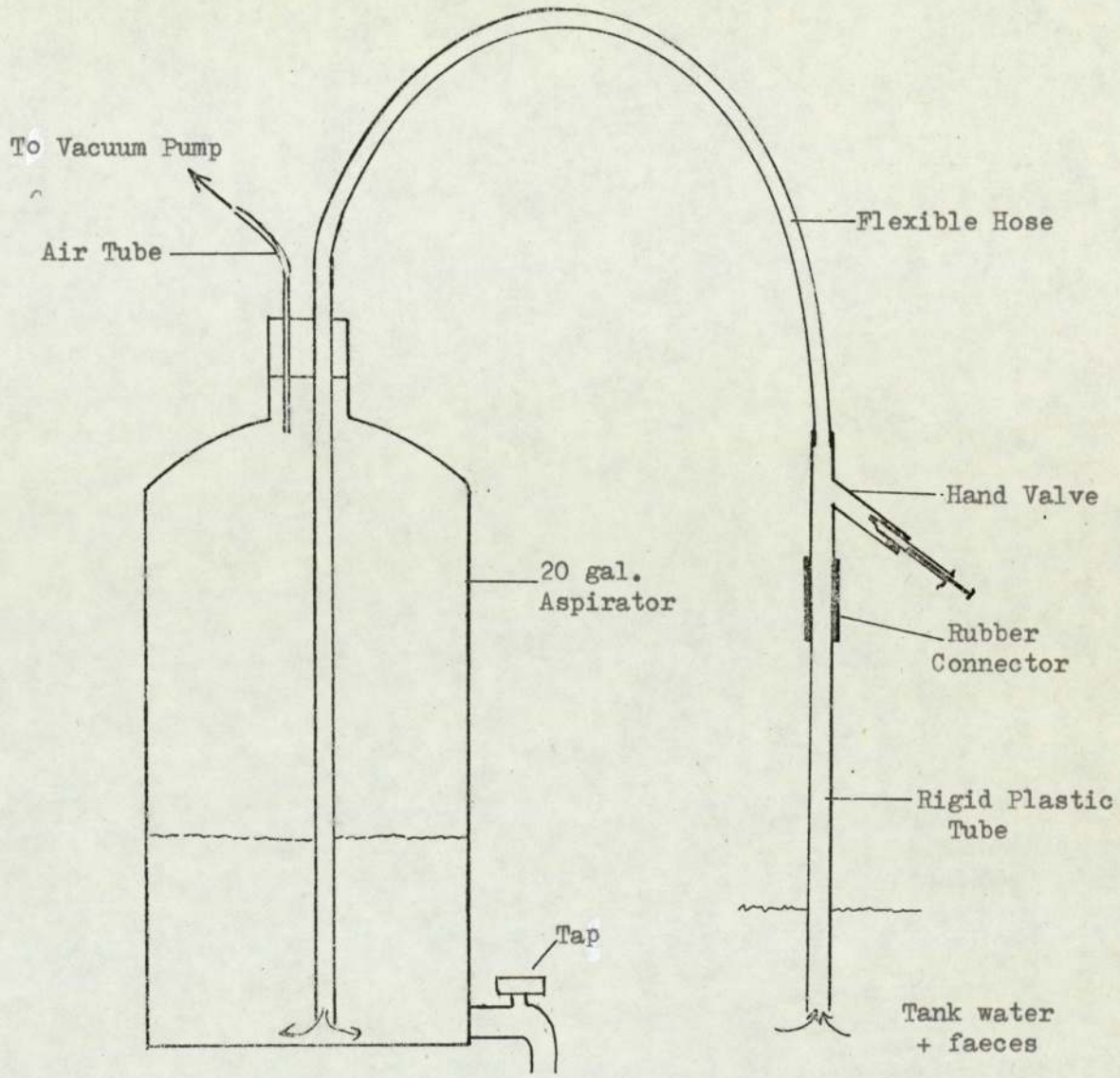
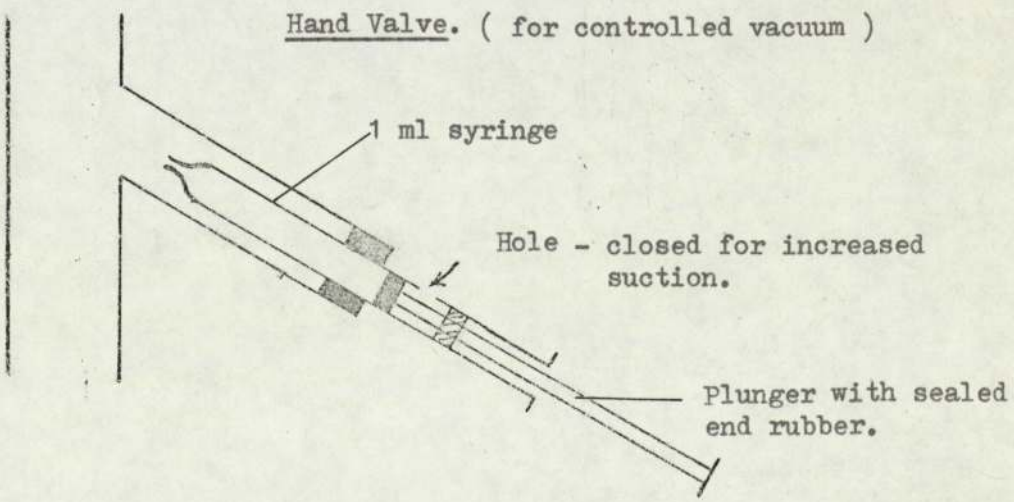


Fig. 11. Modified Tank System.

PLATE 6.

Fig. 12. Tank Vacuum Cleaner.





DIET PREPARATION

Introduction

The dry pelleting method used to produce the experimental feeds for the initial trial was not considered satisfactory. The pellets were softer, breaking down quickly in water by comparison to the commercially available control feed. In addition, as a result of the friable texture of the pellets, attempts to produce crumbles for smaller fish resulted only in the production of dust. There was little opportunity to produce feeds for fish much below 15g.

Whilst there was scope for improving the pellet hardness by the application of steam during the process, there was little opportunity to experiment with the operating conditions. Further, since 1-2 kilos of each feed would suffice for most of the planned experimental work, it was considered wasteful to produce 5 kilos of each mix in view of the limited availability of feed ingredients.

To overcome these difficulties, a method of processing feed on site on a routine basis was required. It was essential to develop a standardised method which was suitable for producing a range of water stable pellets which could be satisfactorily crumbled when necessary.

Ingredient Storage

All ingredients were received into the laboratory in minimum 10 kilo lots. Feed ingredients were checked for spoilage from insects on arrival. One bag of white fish meal was badly contaminated with a beetle, Ptinus tectus (Howe and Burgess, 1953) and was consequently discarded. When checked, the feedstuffs were rebagged in large strong plastic bags, tied off tightly to exclude air, and clearly labelled. These were then placed in a second bag, also tied off and labelled clearly. The

bags were stored in a cool dry place.

Mixing

Mixing was as previously described pg65. The vitamin and salt mixes were prepared separately and the ingredients were added to the main mix, the smallest quantities first.

A Kenwood Chef Major replaced the Hobart mixers. The mixing motion was identical, being planetary, and the stainless steel bowl was large enough to accommodate up to 1500g of feed.

1250g of each formulation was generally mixed at a time. This was then put through a Christie Norris Hammer Mill fitted with a 1mm screen. As a result, a fine homogenous powder was produced. This was found to have better pelleting qualities than a coarser, unground mix.

The quantity of the dry, ground mix required to produce 1 kilo of finished feed was weighed accurately back into the mixing bowl. The oil component of the feed was then weighed and added to 200mls of water in the liquidiser attachment of the Kenwood Major. A homogenous emulsion was obtained from the oil/water mixture. This was next transferred in a fine stream to the dry mix whilst the paddle was in motion. The greater volume obtained by mixing the oil with the water provided a more even distribution of oil throughout the feed mix. A further quantity of water was added until the mix had a suitable consistency for extrusion. The quantity involved was different for each formulation and was judged subjectively. However, there were obviously tolerance limits.

Pelleting

The Kenwood Major was fitted with a mincer attachment. This had two die plates drilled with $\frac{1}{8}$" and $\frac{1}{4}$" holes. The paste was extruded

through the $\frac{1}{8}$" die plate at a low speed with the maximum compaction pressure that could be physically applied. In this way, a relatively dense pellet was produced.

The extruded material was broken off at approximately 3-4" lengths and spread out onto plastic sheets. These were then transferred to a drying area for 24-36 hours. The material was dried at room temperature with the aid of an electric fan. Although this process was satisfactory, the room temperature and humidity fluctuated and the drying time and final moisture content of the pellets was slightly variable. The method would have been better standardised by using a thermostatically controlled drying oven fitted with an electrically operated fan.

The Kenwood Major is shown in Plate 7 fitted with the three attachments as used for mixing the dry ingredients, emulsifying the oil with water and extruding the final paste. The drying process is shown in Plate 8.

Crumbling

The dried 'worms' were then disintegrated in the liquidiser attachment in approximately 200g quantities and a range of granules were subsequently separated out using standard sieves as follows:

Granule Size		Sieve Size: Opening
$\frac{1}{8}$" pellet	to pass over	3·18 mm
Crumble 2	to pass through	3·18 mm
	to pass over	2411 microns
Crumble 1	to pass through	2411 microns
	to pass over	1200 microns
Dust fraction	to pass through	1200 microns

The $\frac{1}{8}$" pellets become more regular in size and length with successive crumbling periods. The two crumbs and the pellets were satisfact-

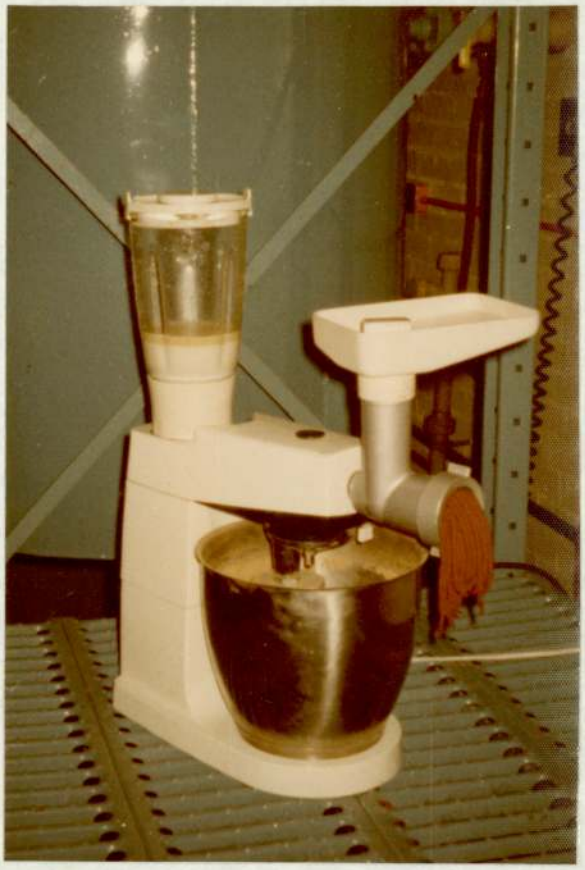


PLATE 7.

PLATE 8.

ory for feeding trials on a range of fish sizes from 5g-100g, and the dust was discarded.

Diet C 02 of the initial experiment was prepared in this way for comparison with pellets produced on the California Laboratory Pellet Mill and those produced commercially by Cooper Nutrition Products Ltd.

Pellet Quality

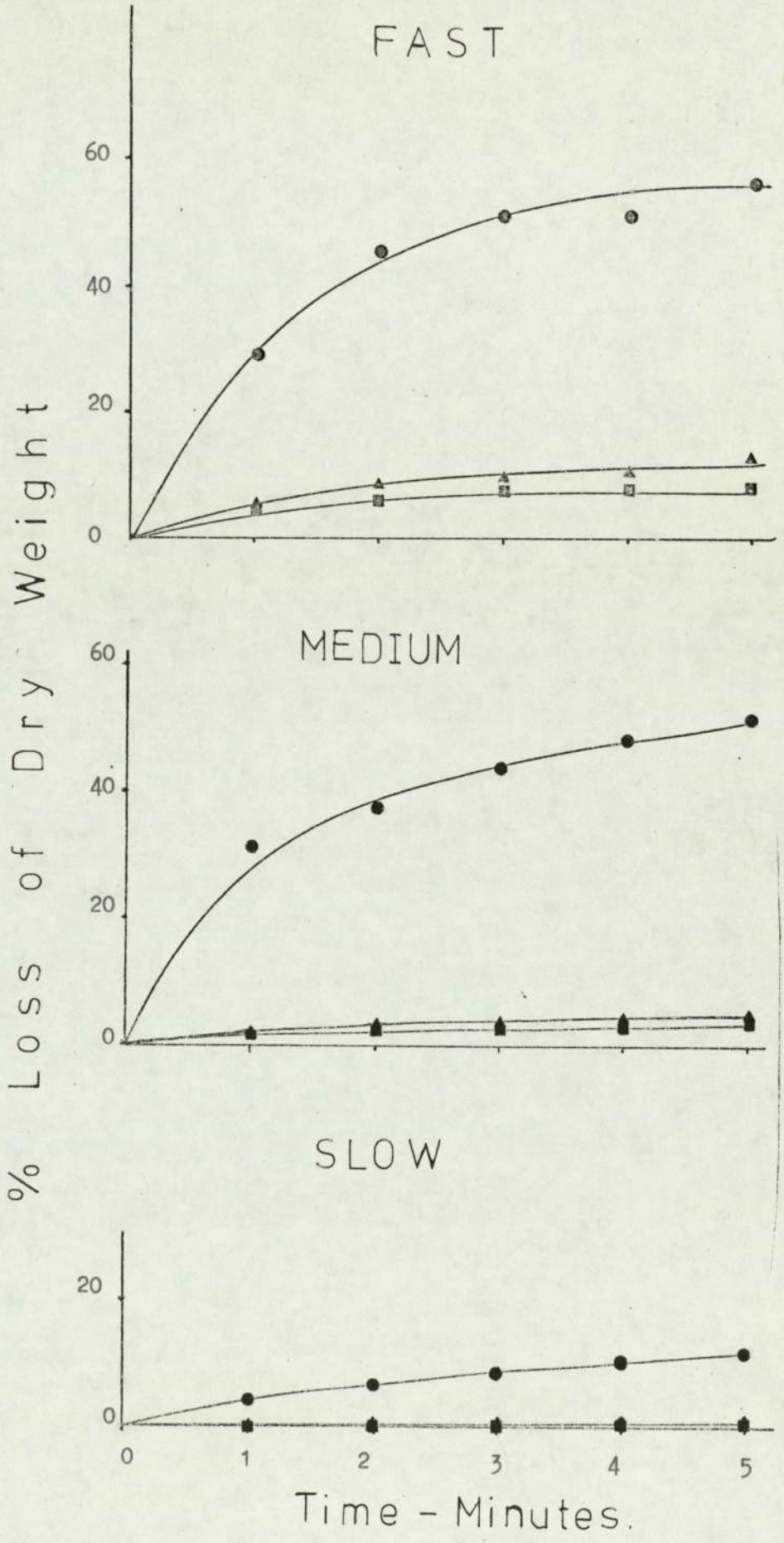
The pellets produced previously on the California Laboratory Pellet Mill were quite soft and friable and there was a considerable degree of dusting on handling as previously discussed. The pellets produced by the wet extrusion method, however, were very hard by comparison and there did not appear to be excessive dusting associated with handling. An attempt was made to quantify this difference.

The method simply involved clamping a Macartney bottle in one arm of an electrical shaker. The shaker could be set at various speeds which produced a range of vibratory movements from a gentle shaking action to an extremely vigorous vibration. Three arbitrary speeds, described as slow, medium and fast, were selected on the dial. At each speed 5g quantities of each of the three diets were tested in triplicate for five timed periods at minute intervals, removing the dust formed each time by gently shaking in a small sieve with a 1mm screen. The quantity of material retained was weighed. The mean results are tabulated in Table 32 and represented graphically in Fig 13 .

Clearly the pellets produced by the wet extrusion method were considerably better than those produced using the California Laboratory Pellet Mill. There was in fact, little difference between the hardness of these pellets and the expanded pellets produced commercially.

Water Stability

The importance of the water stability of pelleted fish feeds has al-


FAST
MEDIUM
SLOW
% Loss of Dry Weight
60
40
20
0
60
40
20
0
20
0
0
1
2
3
4
5
Time - Minutes.

ready been discussed and a technique for quantifying this has been described. However, further use of the apparatus (fig2), for assessing the quality of the pellets produced by the wet extrusion method showed that the technique was not entirely satisfactory. As the pellets disintegrated and lost weight, they began to rise in the tube and were eventually held against the top mesh by virtue of the direction of water flow. This prevented particles less than 1mm being carried out of the chamber. Attempts to carry out comparative assessment of pellet quality were abandoned using this technique.

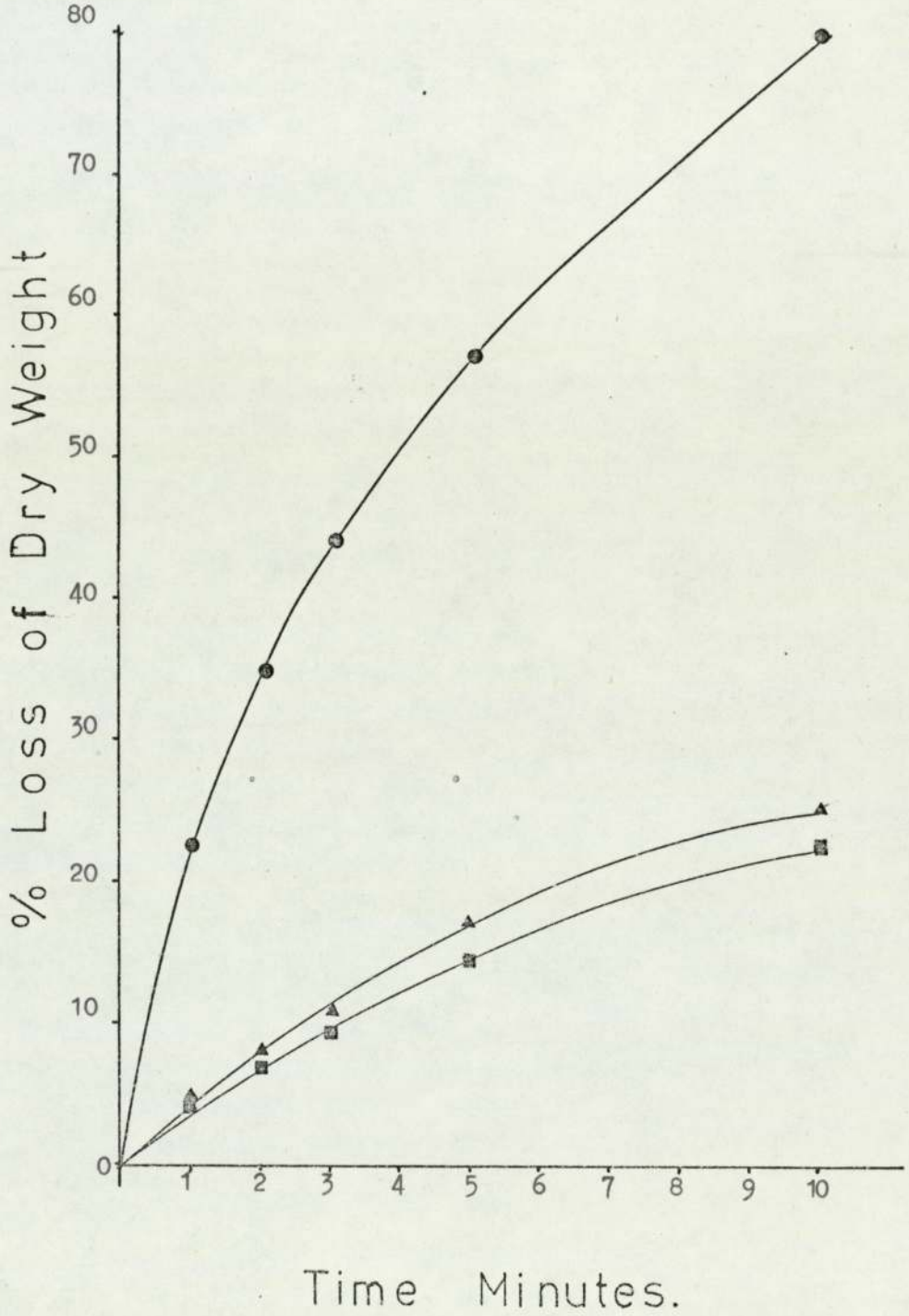
However, a simple method was developed using the shaker. The method was similar to that described by Hepher (1969). Small cylindrical cages, approximately 4" long and 1" diameter, were made from 1mm gauge, stainless steel mesh. The cages were clamped to the arm of the shaker, which was set to give a gentle shaking movement. 5g quantities of pellets were dropped into the cage which was then immersed in a 250ml glass beaker filled with water maintained at a constant temperature of 12^{o}C by a Churchill 'Circotherm'.

5g quantities of each of the three pelleted diets were tested in triplicate for 1, 2, 3, 5 and 10 minute periods. At the end of each period, the cage was removed, and the material remaining emptied onto a filter paper which was subsequently placed into a vacuum drying oven set at 95^{o}C and 100mms pressure. The material was dried overnight.

The loss of material was reported as the % loss of dry weight. The results are shown in Table33 and illustrated graphically in Fig14. The results were very similar to those for testing the pellet hardness, once again showing the marked improvement in pellet quality when produced by the wet extrusion method compared to those produced on the California Laboratory Pellet Mill. The water stability approached that of the commercial expanded pellets.

Conclusion

A method had been developed for the production of small quantities


% Loss of Dry Weight
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
Time Minutes.

of pelleted material for use in laboratory-scale comparative feeding trials. The method was not only convenient, but the product was of a high quality, comparable with commercially available compound feeds, being equally as hard and having similar water stability characteristics.

FEEDING METHODS

Introduction

'Ad libitum' feeding was evaluated as an alternative method to the controlled feeding used in the initial experiment.

As mentioned previously, ad libitum feeding cannot be achieved in the aquatic environment as conveniently as it can for terrestrial livestock. Demand feeding is as close as one might expect to get to true ad libitum feeding, where food is available at all times. Unfortunately, demand feeding can be carried out on a small-scale only with a sophisticated feeding mechanism and it was therefore necessary to evaluate the alternative procedure which involves feeding fish to satiation a number of times per day. Although this is not strictly an ad libitum feeding technique, it is a compromise which has frequently been used by fish biologists to represent 'ad libitum' feeding.

Method

The technique was evaluated in the 10 litre tanks used in Phase II of the initial experiment and subsequently modified as described. Up to twenty 50g rainbow trout could be maintained in these tanks on open flow provided that the oxygen supply was not limiting. The fish could be carefully observed during feeding. The technique was assessed using 4" fish fed diet CO_2 produced by the wet extrusion technique.

The method used involved administering feed twice daily, seven days/week and, at each feed, the fish were offered as much food as they would readily consume. The technique employed was relatively simple. Feed was held in individually weighed, capped, plastic containers which were placed each morning in loops of wire attached to the plastic sheeting adjacent to each tank. In the morning and late afternoon, each tank in

turn received approximately 1g lots of pellets from the appropriate container. By the time all tanks of fish had received their first portion, those fish fed first were ready to receive a second portion and so on. This procedure was repeated several times until there was evidence of some loss in feeding vigour. The fish were then fed until a few pellets remained unconsumed, showing the point of satiation. This was a small proportion of the total food offered and it was generally consumed within 10-15 minutes of the fish receiving their final food lot. Food consumption/day was calculated as the difference between the weight of the container and food at the beginning of the day and at the end of the day when the containers were removed.

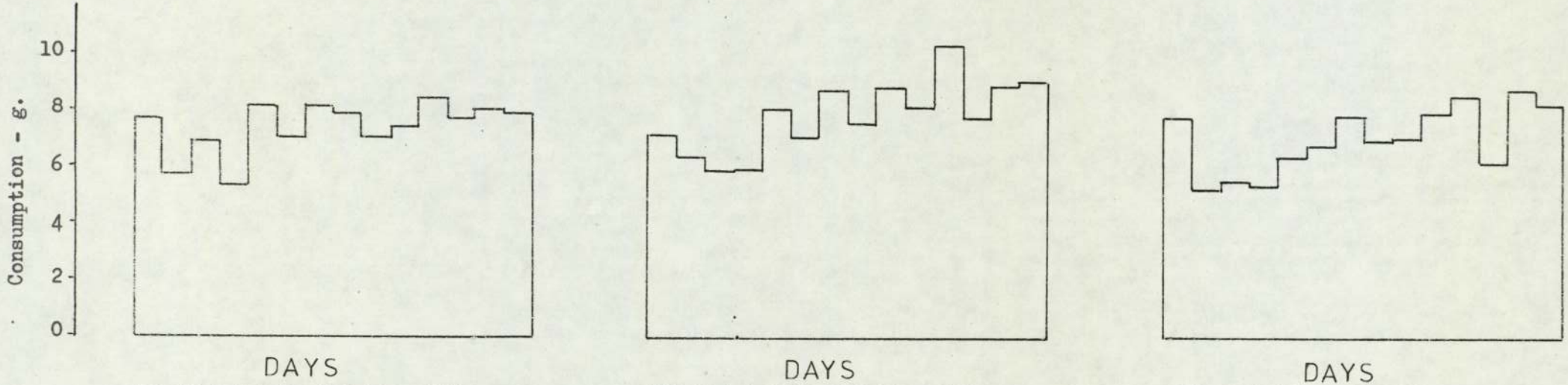
To avoid bias in the amount of feed offered to each tank, for example in favour of or against those first receiving food, the tank at which the sequence was commenced was changed regularly and the rotation was sometimes reversed, although strict numerical sequence was followed on all occasions to prevent confusion.

Results

For the most part, it was felt that feeding was achieved with a high degree of accuracy. Fig 15 shows the pattern of feeding values obtained with 3 similar populations of fish during a 13 day period. Daily food consumption was reasonably constant. The recommended level of feeding for that size of fish at 11°C is 2·3% body weight/day. Clearly the feeding level achieved by this technique was similar.

Occasionally, it was not possible to feed twice/day and consequently a low feeding level resulted. This is clearly shown in Fig 16. On such occasions consumption was consistently reduced in all tanks. It is interesting to note, however, that on the following day a high feeding value generally resulted, the fish obviously making up for the deficit of the previous day. Frequently there was an over compensation as shown for day 9. The feeding value for day 10 was low despite the fact that two feeds were offered on that day.

Fig.15 Satiation Feeding - Daily Consumption Pattern for Three Populations of Trout During a 14 Day Feeding Period



Mid-Point Body Weight = 331 g

% Feeding Rate = 2.2

Feed Conversion Ratio = 1.26

Mid-Point Body Weight = 324 g

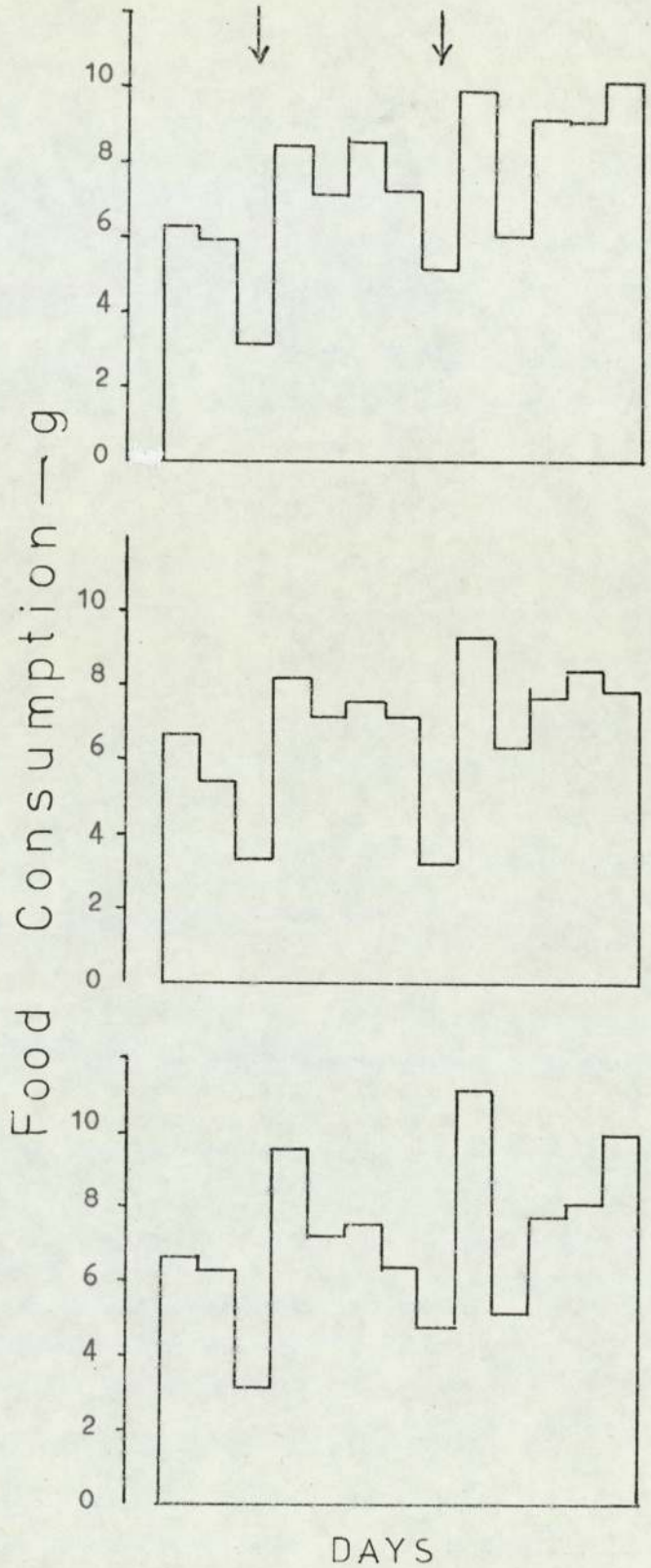
% Feeding Rate = 2.4

Feed Conversion Ratio = 1.51

Mid_Point Body Weight = 331 g

% Feeding Rate = 2.1

Feed Conversion Ratio = 1.25



↓ Indicates days on which feeding was restricted to one feed only.

Fig. 16. Satiation Feeding

Effect of occassional restriction of intake on daily consumption pattern.

Occasionally food consumption was reduced in one tank alone. Generally this was related to a reduction in water flow due to a pipe blockage and consequent lowering of the oxygen content of the water, a condition which was quickly corrected. On the odd occasion, there was also a loss of appetite in a particular tank for no apparent reason. The important point about this feeding technique, however, is that such occasions are recognised and food is not 'blindly administered'.

There was no substantial increase in consumption as a result of increasing the number of feeds / day to three and there seemed little advantage in doing so. However, this was based on a very limited observation period and no attempt was made to see if the food conversion ratio was affected.

Discussion

It is difficult to judge whether the feeding levels achieved using this technique truly reflect the appetite of the fish or whether they represent a restricted feed intake

If the feeding levels do reflect the appetite of the fish, it is not clear to what extent the fluctuations in daily consumption reflect slight variations in daily appetite caused by environmental influences. It is possible that the fluctuations represent an 'artifact' due to the nature of the technique. It was difficult to judge the point of satiation even on this scale. However, it is interesting to note that a low feed intake was compensated the following day by an abnormally high feed intake. This would seem to substantiate the theory that the level of consumption obtained does reflect the appetite of the fish and the technique is reasonably sound even in the event of the occurrence of anomalies in the daily pattern of consumption.

Using this technique no fish or group of fish are penalized as a

result of their having a proportionally higher metabolic demand since they have the opportunity to feed to satiation.

The feeding tables used previously for controlled feeding are designed for good quality feeds. However, diets of varying quality and composition, such as may be used in experiments involving the evaluation of different proteins, might not necessarily be consumed at the same rate. The 'ad libitum' technique takes account of such differences and could be used to determine appetite differences.

In addition, the appetite of fish may be greatly affected by changes in the environmental conditions, notably temperature, percentage of oxygen saturation and water quality and by disturbance. Failure to recognise and correct for changes in appetite which may occur in such circumstances, or indeed for no readily apparent reason, as occasionally happens, will again result in food wastage and therefore apparently poor conversion ratios. Such was the case in the initial feeding trial (Section 3). Although complete consumption of the ration offered was frequently achieved, there were occasions, particularly towards the end of the experiment when environmental conditions were by no means ideal, when food was incompletely consumed, resulting in poorer conversion ratios than had been previously experienced. 'Ad libitum' feeding takes account of such changes in appetite.

Conclusion

The 'ad libitum' technique would appear to be sound in principle. However, the absolute success of its application to feeding trials depends on the regularity of feeding and the degree of care exercised at each feeding. The method is tedious and requires a considerable degree of patience from the operator.

SECTION 5.

EVALUATION OF SINGLE-CELL PROTEIN IN COMPOUND FEEDS 2. THE EFFECT OF REPLACING FISH MEAL WITH A YEAST, BACTERIA, ALGA AND TWO FUNGI.

INTRODUCTION

In the previous experiment on compound feeds, only small quantities of three single-cell proteins had been available. Larger quantities of Candida lipolytica and Penicillium chrysogenum were obtained from B.P. Proteins Ltd, and Glaxo Laboratories respectively. A larger quantity of Aspergillus niger was produced in the Tate & Lyle Pilot Fermentation Plant within the department and was subsequently ring dried providing 10kg of dried mycellium. In addition two more sources of single-cell protein had been obtained. These were a bacterium, Pseudomonas spp., supplied by I.C.I. Ltd and an alga, Spirulina maxima, supplied by Sosa Texcoco S.A. of Mexico. The opportunity arose, therefore, to evaluate at least one single-cell protein from each of the main groups - yeasts, bacteria, algae and filamentous fungi.

As a result of the collapse of the Peruvian Anchoveta Industry, fish meal had become a scarce commodity as described in Section 1. It was therefore appropriate to evaluate the novel proteins as substitutes for fish meal in a conventional trout compound feed and to determine the main limiting factors. For comparison, three conventional oil seed meals, soya bean, cotton seed and groundnut, were included in the evaluation programme.

The experimental facilities had been modified to allow a longer term experiment to be carried out hopefully without the decline in environmental conditions experienced previously and diet preparation methods had been improved. The development of a method for producing crumbs provided an opportunity to work on smaller fish.

Previously, mortality, growth and conversion efficiency had been the only criteria on which the single-cell proteins had been assessed. On this occasion, however, a longer term trial was conducted in which the effects on appetite, digestibility, growth, conversion efficiency and

general pathology could be assessed. To improve the sensitivity of the trial, it was started at a time when temperatures were more favourable for rapid growth.

EXPERIMENTAL FEEDS

Ingredient Availability

The nine test proteins, including herring meal as the control, are listed in Table 34, together with the suppliers. Listed also in Table 34 are the conventional ingredients used to prepare the basal ration.

Analysis

The feed ingredients held in store were sub-sampled and prepared for analysis as described on pg 62. All analyses were carried out at least in duplicate.

1) Moisture

The moisture content was determined by drying a weighed quantity of the sample, approximately 2g, in a vacuum oven set at 95 - 100°C and evacuated to 100mm pressure (A.O.A.C. Methods of Analysis, 1970). The moisture content was reported as a percentage of the original sample.

2) Ash

Ashing was carried out on a weighed quantity of sample, approximately 2g, in nickel crucibles placed in a muffle furnace set at 550°C for 2 hours. Ash content was reported as a percentage of the original sample.

3) Crude Oil

Crude oil was determined by petroleum ether extraction (40-60 B.P.) in a soxlet extraction apparatus (Fertilizer and Feeding Stuffs Regul-

ations, 1968). The extracted oil was dried, weighed and reported as a percentage of the original sample.

4) Crude Protein

Crude protein was calculated from the nitrogen content reported as a percentage of the original sample, multiplied by 6·25. Nitrogen was determined using a slightly modified version of the microkjeldahl technique referred to on pg 62. In an attempt to overcome the problem of variability in the estimation, caused by the use of such a small sample size, the technique was scaled up by a factor of 4. Material containing approximately 4 mg of nitrogen was weighed accurately into 100ml kjeldahl flasks. 600mg of a catalyst mixture containing 32 parts potassium sulphate, 8 parts copper sulphate and 1 part selenium powder was added, together with 4ml concentrated sulphuric acid. The material was digested for at least 2 hours on an electric heating mantle. The digested sample was cooled and transferred quantitatively to a 100ml volumetric flask and diluted to 100ml. Distillation was carried out on duplicate 25ml aliquots of the diluted digest using a Markham distillation apparatus modified to accommodate large samples. The ammonia was removed by the addition of 4ml 40% sodium hydroxide and was subsequently collected with the distillate in 5ml saturated boric acid. The ammonia was determined by direct titration with 0·01N HCl, using Tshiro's Indicator.

5) Gross Caloric Content

This was determined directly using a Bomb Calorimeter.

Formulation

It was proposed that a feeding trial lasting 12 weeks should be conducted on nine diets, including a control diet, being a slightly modified McNenney formulation containing 40% herring meal, and eight experimental diets containing the eight test proteins each substituted for 50% of the herring meal of the control diet. The formulations of the diets,

CO9 to C17, are shown in Table 35.

It was further proposed that the level of inclusion of the five single-cell proteins should be raised to 40% of the diet by weight and that these five diets and the control diet should be fed for a further 10 weeks. Since three of the single-cell proteins had been substituted for the delactosed whey and distillers' solubles of the control diet in the previous experiment without causing any marked fall in growth rate or conversion efficiency, the further quantities of test proteins were substituted for these same ingredients. The 2% binder was also removed to make up the 20% required for the substitution. The formulations of the diets, C18 - C22 are compared to the control formulation CO9, as shown in Table 36.

Diet Preparation

All diets were prepared by the wet-extrusion method described in Section 4. Three feed sizes were prepared using the sieves listed and the three fractions were stored separately in plastic bags tied off to exclude air and kept in labelled air tight polythene containers until required for feeding.

Diet Analysis

The pellet hardness and water stability was compared as described in Section 4.

The water content of the pellets was determined as for the ingredients. Diet proximate analyses were then calculated from values for the ingredients and corrected for the change in water content, as measured.

GENERAL METHODS

Trial Schedule

During the first ten weeks, each of the nine experimental diets, CO9 - C17, were fed to three replicate groups of 20 rainbow trout. The diets were randomly allocated to the 27 tanks.

Between the 10th and the 12th week, an inert indicator was included in the 9 experimental diets for the purpose of determining the protein digestibility.

At the end of 12 weeks, 6 fish from every tank were removed for pathological examination as described below. 10 fish from each of the 3 tanks fed the control diet, CO9, and those diets containing the five single-cell proteins, C13 - C17, were then pooled for continued feeding of the control diet and the new formulations, C18 - C22, each containing 40% single-cell protein, for a further 10 weeks; i.e. 30 individuals were fed each S.C.P. for a total of 22 weeks, 12 weeks at a level of 20% and 10 weeks at a level of 40%. At the end of this period, 18 fish from each group were sacrificed for pathological examination.

Experimental Animals

560 rainbow trout, approximately 7cm in length, were selected from the stocks which had been transported from Vortex Ltd and maintained in the 200 litre stocking tanks, as described in Section 2. The fish were randomly sorted into the 27, 10 litre tanks modified as described in Section 4, in lots of 20 individuals and allowed to adapt to their new environment for 14 days prior to feeding on the experimental diets. During this adaptation period, the fish were fed a standard commercial crumble obtained from C.N.P. Ltd.

Environmental Parameters

The tanks used during the first 12 weeks were a fixed volume of

10 litres and the flow rate was adjusted to approximately 250ml per minute. The range of stocking densities and loading factors at the start of the experiment were 11 - 13 g l^{-1} and 440 - 520 g l^{-1} min respectively. These had increased to 43 - 61 g l^{-1} and 1720 - 2500 g l^{-1} min respectively by the end of the 12th week. However, an adequate supply of oxygen was ensured throughout by supplying a stream of air to the air lift tubes.

The tanks used from 12 - 22 weeks were a fixed volume of 30 litres and the water flow was adjusted to approximately 500 ml per minute. The stocking densities and loading factors at the start of this period varied from 22 - 31 g l^{-1} and 1324 - 1854 g l^{-1} min respectively and at the end between 35 - 53 g l^{-1} and 2108 - 3374 g l^{-1} min respectively. The tanks were not aerated during this time, oxygen being entirely supplied from the incoming water.

Throughout the experiment the temperature was ambient, being that of the mains water. The temperature of the water leaving the header tank and supplying all experimental systems was recorded daily and with the flow rates used, there was little variation between that temperature and the water temperature within the experimental tanks. The temperature has been recorded with the growth data relevant to the two phases of the experiment, Table 50 and 56.

Oxygen was measured at regular intervals during the experiment and was not considered to be limiting at any time when the flow rates were correctly adjusted. It was difficult to balance the flow rates and maintain them absolutely constant at all times, however, and on occasions flow to individual tanks was reduced or cut off due to blockages within the delivery pipes, and the fish were kept alive by the air lift mechanism but at a much reduced oxygen concentration. This situation was quickly corrected.

Total ammonia of the effluent was measured only once in randomly

selected tanks during the 10th week of the experiment. The levels were very low and were in no case considered to be limiting.

Feeding

Fish were initially fed on the smallest crumb but as they grew, the larger crumbs and finally the pellets were readily consumed. Feeding was by the 'ad libitum' technique discussed in Section 4. Feeding was generally carried out twice per day, on each occasion feeding to satiation. The food consumed by the fish in each tank was measured and recorded daily.

Tank Cleaning

The tanks were not entirely self-cleaning, particularly during the early stages when the fish were small. Faecal material which had not been carried away in the effluent was removed prior to the morning feed, using the tank 'vacuum cleaner' described in Section 4. This did not unduly disturb the fish and they fed voraciously shortly afterwards.

Once every two weeks, when the fish were removed for weighing, the tanks were thoroughly cleaned to remove the algal coat which gradually built up, particularly in the summer months.

Weighing

All fish were weighed individually every two weeks. They were lightly anaesthetised in a solution containing 50ppm MS222 (Sandoz), rolled gently on a cotton wool pad to remove excess water and weighed to 0·01g on a top loading electronic balance. After weighing, the fish were returned to well aerated fresh water and, after recovery, were moved back to the cleaned experimental tanks.

Individual results were recorded, the mean values computed and the growth rate and growth efficiencies determined.

Dying or dead fish were removed from the tanks as and when they occurred and the weight was measured and recorded for the computation of conversion ratios.

PROTEIN DIGESTIBILITY DETERMINATION

Methods of digestibility determination have been discussed by Maynard and Loosli (1969). The use of inert indicators, which pass undigested through the alimentary tract, have provided a convenient method of measuring digestibility in a number of animals without having to collect faecal material quantitatively. The method has been applied successfully to fish digestion studies using chromium III oxide as the indicator, as discussed by Nose (1967).

For the purpose of determining the apparent crude protein digestibility of the nine experimental feeds, CO9 - C17, 0·5% of chromium III oxide was added to each of the diets during the mixing process. The diets were otherwise prepared as discussed in Section 4.

The diets were green coloured but were well accepted by the fish. Each of the 27 lots of fish received the appropriate diet for 12 days during the 10th - 12th week of the experiment. The diets were fed in the normal way. On the 8th and 12th days, the fish were lightly anaesthetised and faecal material was collected by the gentle application of pressure to the abdomen in the region between the pelvic fins and the anus. The faecal material, collected in this way from each of 60 individuals from each of the diet treatments, was pooled and dried in a vacuum oven.

Analyses were carried out on the food and dried faeces. Nitrogen was estimated using the semi-microkjeldahl method previously des-

cribed. Chromium III oxide was determined by the wet ashing method of Furukawa (1966). The method briefly involved digestion of the samples containing chromium III oxide in concentrated nitric acid, followed by the oxidation of the chromium ions by perchloric acid. The resultant solution was diluted volumetrically and the absorbance determined using a Unicam SP 600 Spectrophotometer. The quantity of chromium III oxide contained in the original sample was then determined from a calibration curve prepared from absorbance values obtained from a series of standards containing known quantities of chromium III oxide added to a compound feed mix. The method is described in detail in the paper by Furukawa (1966).

Apparant digestibility of the protein was determined from the equation

$$\text{Digestibility \%} = 100 - \left(\frac{\text{\% indicator in feed}}{\text{\% indicator in faeces}} \times \frac{\text{\% N in faeces}}{\text{\% N in feed}} \times 100\right)$$

TERMINAL PATHOLOGY

At the end of the 12 weeks, 6 fish from each of the 27 tanks, making a total of 18 fish on each of the experimental diets C9 - C17, were randomly selected for examination. At the end of 22 weeks, a further 18 fish from each of the 6 lots of fish continued on diets C9 and C18-C22 were randomly selected for examination.

Condition Factor

The relationship between weight and length of fish may be expressed generally in the form $W = aL^n$ where W = weight, L = length, a is a constant and n is an exponent usually lying between 2·5 and 4·0 (Le Cren, 1951). For trout, the value of n has been taken as 3 and the factor a, called the condition factor (K) has been determined by the simplified equation

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

(Brown, 1957)

Brown (1957) has discussed the use of the value of K as an index of fatness or body shape. According to Lee and Putnam (1973) rapidly growing trout fed good diets normally have a larger condition factor than those fed marginal feeds. Therefore, in an attempt to compare the adequacy of the experimental feeds, the condition factor of the 18 randomly selected fish for each diet treatment was determined. However, since the use of the simplified equation, in which n is assumed to be 3, has been criticised (Hastings and Dickie, 1972), the relationship between length and weight for the sampled fish was determined for each diet using the general formula $W = aL^n$ which, taking logarithms, may be written

$$\log_e W = \log_e a + n\log_e L$$

The constant n will be given by the slope of the graph of $\log_e W$ against $\log_e L$. The mean condition factor can be determined from the graph or alternatively, the condition factor of the individual trout can be determined from $\frac{W}{L^n}$ using the value of n determined graphically.

External Appearance

The external appearance of each fish was examined, noting the general colouring, the condition of the scales, the amount of mucus covering the body and the condition of the fins, eyes and gills. Any abnormal conditions such as opaqueness of the lens, increased vascularisation in the iris and cornea, the presence of fluid in the eye socket or the abdomen, or the presence of body sores, was particularly noted.

Haematocrit

The most convenient tool for the fish nutritionist to assess clinical status is haematology (Halver, 1972). Methods of fish haematology have been reviewed by Hesser (1960). Of the methods discussed, haematocrit is the most convenient for the examination of large numbers of fish. The haematocrit technique has been discussed more specific-

ally by Snieszko (1960). He has shown that haematocrit values correlate with red cell count and haemoglobin values determined on the same fish. The microhaematocrit method is extremely reliable and can be easily and rapidly performed with fish blood. Commercial heparinized capillary tubes available for haematological studies are suitable, although, due to the rapid and unique way in which fish blood coagulates, values may be 7-18% higher than a true reading (Snieszko, 1960). In view of this, a modified method was suggested (Larsen and Snieszko, 1961), although Snieszko (1961) suggests that the errors caused by the use of commercial capillaries can be considered of little significance when haematocrit values recorded using the same techniques are compared with each other.

Methods of sampling trout blood have been considered by Steucke and Schoettger (1967). Methods involving caudal puncture and incision of the sinus venosus were found to produce higher haematocrit values than the method involving excision of the caudal fin used by Snieszko (1960). The differences were not significant, however.

The methods used in this experiment were therefore essentially as described by Snieszko (1960). The fish were lightly anaesthetised in 50ppm MS-222 (Sandoz). They were then gently rolled on a damp cotton wool pad to absorb excess water and prevent dilution of the collected blood sample. The caudal fin was removed using a sharp scalpel and blood was collected directly from the dorsel blood vessel into duplicate commercial heparinized capillary tubes. One end of each tube was sealed using a micro-burner and the tubes were centrifuged in a microhaematocrit centrifuge for five minutes. The packed cell volume was measured directly as a % of the total blood volume using a microhaematocrit reader.

Internal Appearance

The internal appearance was examined after making an incision

along the mid-line of the ventral surface. Of particular interest was the colour and appearance of the organs, particularly the liver, and the quantity of visceral fat.

The liver was quickly removed from each of the 18 fish examined on each diet treatment. Six livers from each group were cut into small pieces and fixed in 10% neutral buffered formalin. The remaining 12 livers were dried at 95°C in a vacuum (100mm Hg) for 12 hours and weighed.

Liver Histology

After fixation, pieces of each of the livers were prepared for histological examination. Processing was carried out by the Departmental Histologist, Mrs M. Varley, for which due acknowledgement is given. The material was embedded in paraffin wax, sectioned at 6μ, and stained with Haematoxylin and Eosin stains. The prepared slides were examined using both light and phase contrast microscopy.

RESULTS AND DISCUSSION

In view of the number of factors under consideration in the experiment, the results and discussions have been considered together for each separate heading.

Experimental Feeds

The results of the analysis of the ingredients used in the experimental diets are shown in Tables 37 and 38.

The water content and the computed proximate analyses of diets C9-C17, used in the first 12 weeks, and diets C9 and C18-C22, used in the second 12 weeks, are shown in Tables 39 and 40 respectively.

Differences in the composition of the experimental diets largely reflect differences in the composition of the 9 test proteins. Differences in the water content of the finished feeds have a small additional effect. No attempt was made to balance any of the nutrient components in the diets as shown by the range of values reported for each nutrient parameter.

The crude protein content varies between 32% for the fungi and 78% for the bacteria. The fungi and algae are generally lower than the yeast and bacteria, as discussed in Section 1. The values for B.P. yeast and ICI bacteria are close to those published but the value for the alga Spirulina is somewhat lower than the 62% crude protein content reported by Clément et al (1967). As discussed in Section 1, single-cell protein may contain substantial amounts of non-protein nitrogen and the crude protein values reported may be considerably higher than the true protein content. However, no attempt was made to measure the non-protein nitrogen components. N x 6·25 remains the method generally reported in connection with feedstuffs.

In addition, values reported as % ether extract will not be a rel-

iable estimate of true lipid. Mixed solvent systems used for lipid extraction generally yield higher values, particularly after acid hydrolysis. This method has been used to determine the lipid content of B.P. yeast (Shacklady, 1972) and the alga Spirulina (Hudson and Karis, 1974), in both cases giving higher values than reported here.

The estimate of total energy by combustion of the materials in a bomb calorimeter is, however, a measure which is generally reproducible, as is the estimate of ash.

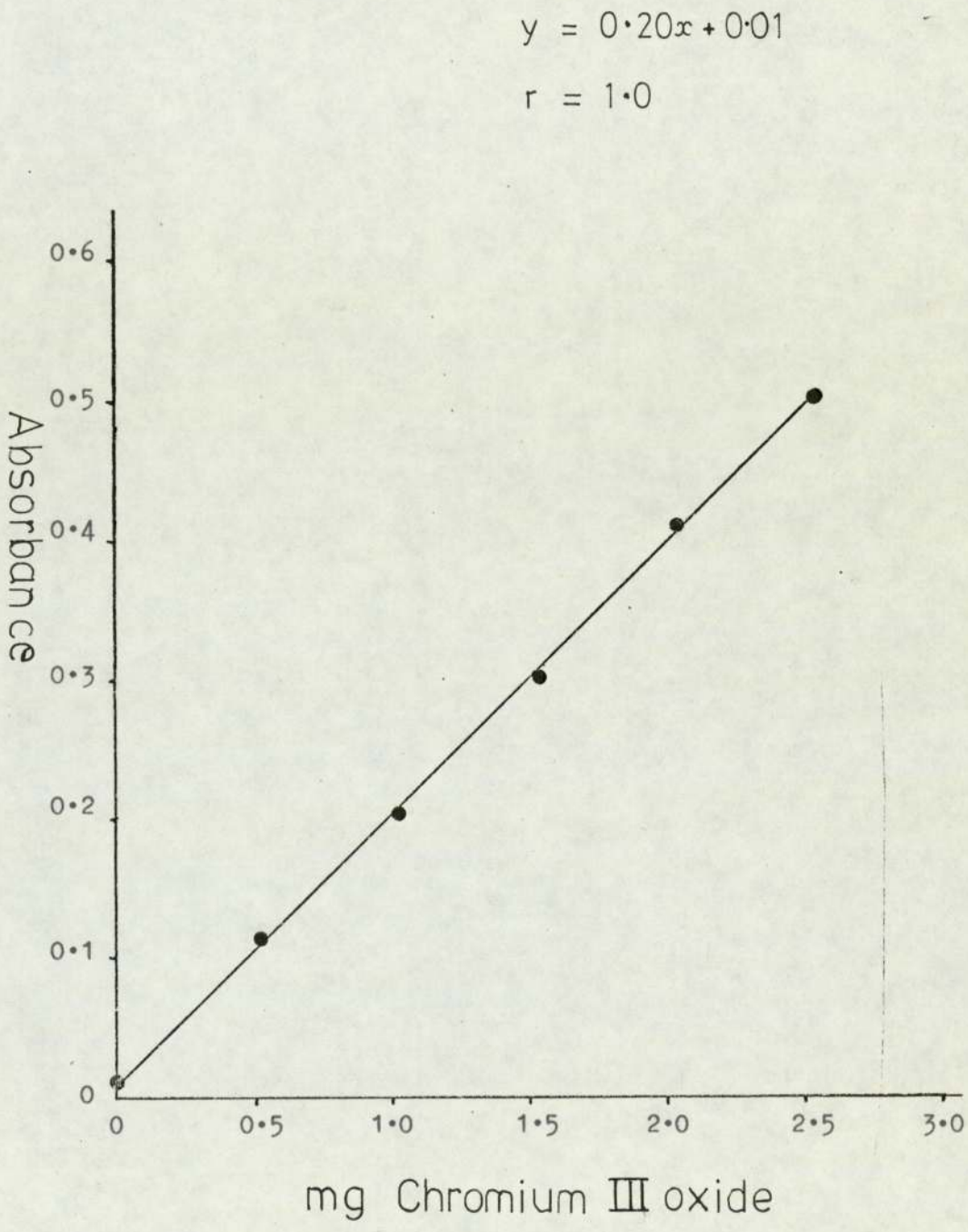
All formulations pelleted successfully using the wet extrusion method. The pellets were, without exception, hard and dust free and there were no marked differences between the pellet quality as determined using the shaker methods for testing hardness and water stability. Pellet quality was comparable to the commercial expanded product of C.N.P. Ltd.

Digestibility

The calibration curve used for determining chromium III oxide values in food and faecal samples is shown in Fig 17. The values for nitrogen and chromium III oxide content of the food and faecal samples are shown in Table 41 together with the calculated values for crude protein digestibility.

The apparent crude protein digestibility of the diet containing the yeast protein was higher than that of the fish meal control diet and the diets containing the groundnut meal and bacterial protein were somewhat similar to it. Diets containing cotton and soya bean meal were lower. The algal and fungal proteins were comparatively poorly digested. Metabolic faecal nitrogen was not determined. From the data of Nose (1966), however, it is unlikely that the differences between apparent and true protein digestibility would have been

Fig. 17. Calibration Curve for Chromium III oxide Estimation.



very marked at the levels of crude protein used in the diets. Assuming that the basal component is equally well digested in all diets, the observed differences will reflect differences in the crude protein digestibility of the test proteins. On the basis of this assumption, crude protein digestibility has been calculated for each test protein relative to that of fish meal at 100%, as shown in Table 41.

The protein of ground nut, yeast and cotton seed meal appears to have been more digestible than that of the fish meal, bacterial protein equally as well, and soya protein somewhat lower. Protein of Aspergillus niger appeared to be better digested than that of Penicillium chrysogenum which was comparatively poorly digested. The algal protein was a little better digested, although still quite low.

Digestibility values for several feedstuffs have been reported in the literature. Some of the data has been reviewed by Nose (1966) and Hastings (1969). Windell (1974) reports two values for protein digestibility of herring meal at 99·5% and 94·6%, whilst Nose (1966) reports a value of 77% for this meal. Values for white fish meal have varied between 74% and 94% (Hastings, 1969). Windell (1974) reports the protein digestibility of soya bean meal as 63·5%, Kitamikado et al (1964) reports the value as 71%, whilst Nose (1966) reports values of 81% and 87%. Hastings (1969) reports values of 72·7% and 84% for protein digestibility of two samples of cotton seed meal. Clearly, values for the conventional feed ingredients vary substantially.

In view of the relatively high values reported for herring meal, the results for the yeast and the bacterial single-cell proteins are encouraging, whilst the values for the alga and fungi, although somewhat lower, may be quite comparable with the digestibility of some of the plant seed proteins.

Appetite

Differences in food consumption rates were apparent as early as

day two, and were clearly established by day four of the experiment, as shown in Fig 18. Mean daily feed consumption for each two week period of the ten week trial are shown in Table 42. Relatively low values for bacterial protein are clearly noticeable.

Feed consumption rates are frequently reported in the literature as percentages of the body weight (Brown, 1946; Page and Andrews, 1973; Smith, 1974). Since the weight of fish on each treatment is changing with time, the rate of consumption expressed in this form would be given by

$$Q = \frac{F}{\int_0^T W dt} \times 100 \ \%$$

where Q is a quotient representing the level of feed intake and F is the total intake in g over the period $T-T_o$, where T is in days. $\int_0^T W dt$ is the weight of fish (given in g) after time T. During short periods of measurement, W is considered to be proportional to T, and Q is given by

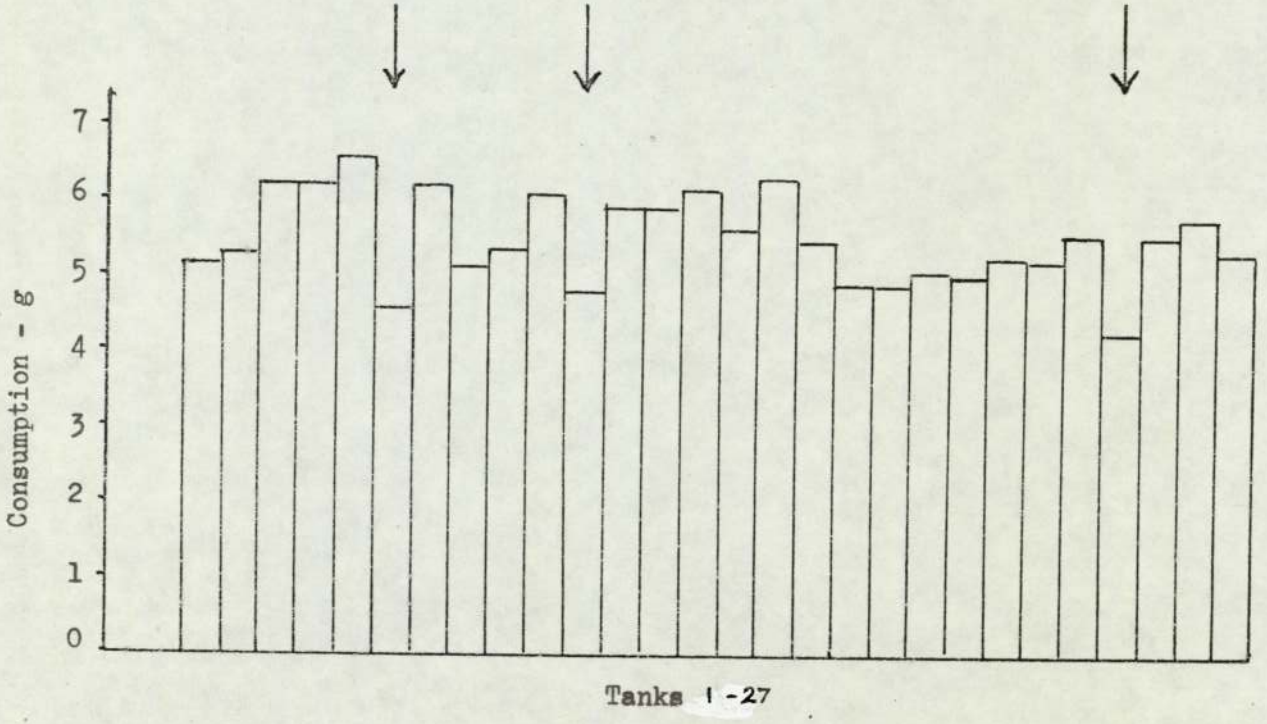
$$Q = \frac{F}{\left(\frac{W_T + W_{T_o}}{2}\right) T-T_o} \times 100 \ \%$$

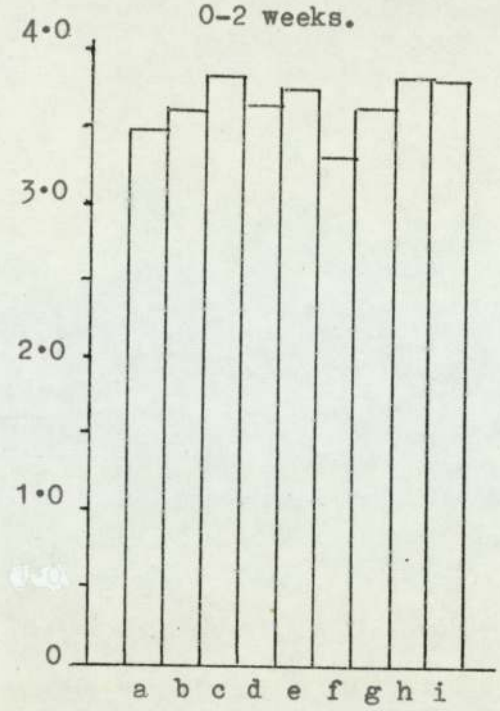
The data for each two week period is shown in Table 43 and Fig 19. Clearly the trend in feed consumption established during the initial two week feeding period was similar throughout the trial, there being generally lower consumption rates relative to body weight of fish on the feeds containing a comparatively higher energy content, notably the control diet containing herring meal and that containing bacterial protein (see Table 39).

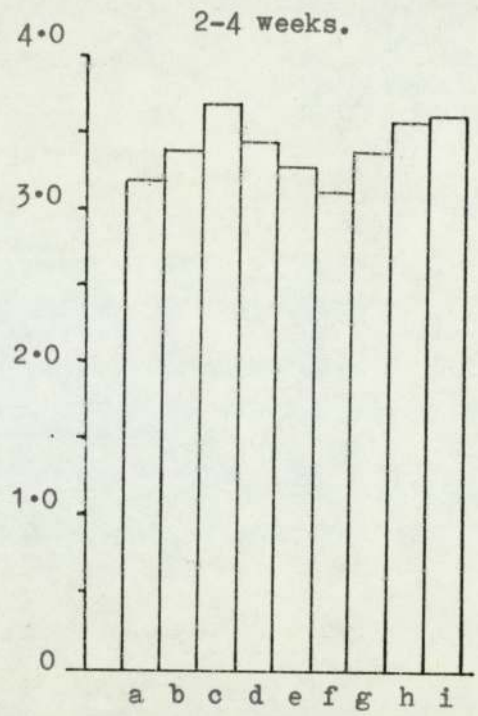
That food consumption rate in fish is related to the energy content of the feed has been shown by the observations of Rozin and Mayer (1961). In their experiments, goldfish adjusted their rate of feeding to a nearly constant caloric intake per day despite a two fold intake in the bulk ingested. The results of Lee and Putnam (1973) and Page and

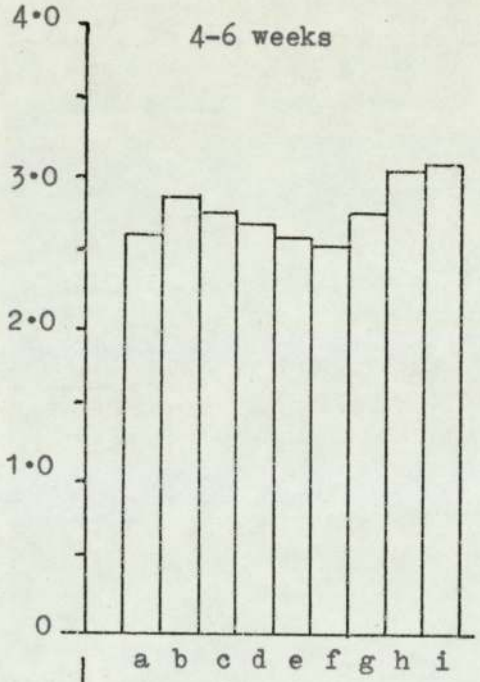
Fig.18 Food Consumption on the 4th Day of Trial 2 on Compound Feeds

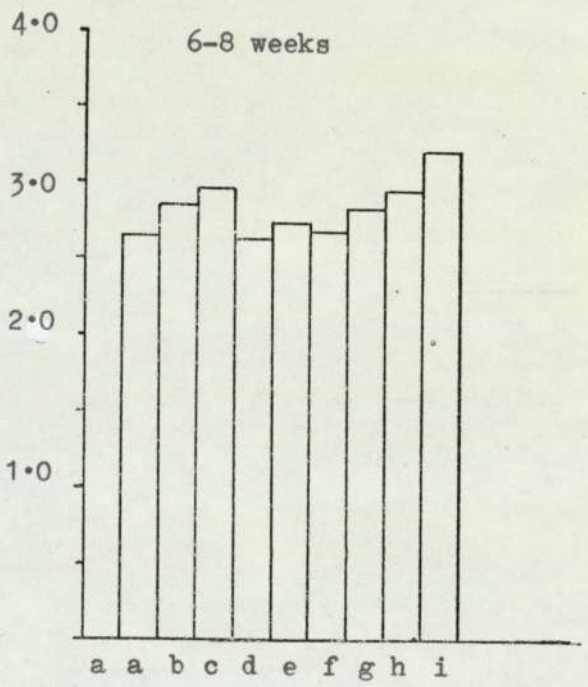
- Showing the Established Feeding Pattern

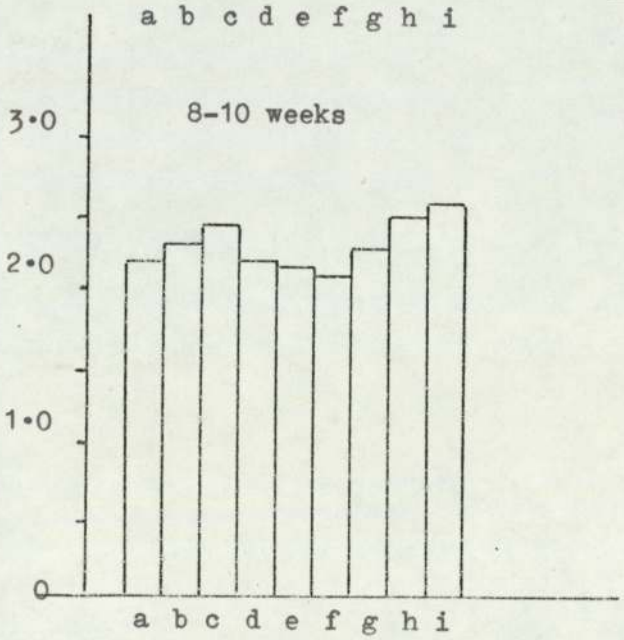



0-2 weeks.
4·0
3·0
2·0
1·0
0
a b c d e f g h i


2-4 weeks.
4·0
3·0
2·0
1·0
0
a b c d e f g h i


4-6 weeks
4·0
3·0
2·0
1·0
0
a b c d e f g h i


6-8 weeks
4·0
3·0
2·0
1·0
a a b c d e f g h i


8-10 weeks
3·0
2·0
1·0
0
a b c d e f g h i

Andrews (1973), working on the interactions of protein and energy ratios on feed consumption rates, among other things, of rainbow trout and channel catfish respectively, give further evidence that fish, like other animals, for example poultry (Hill and Dansky, 1954) and pigs (Cole, Duckworth and Holmes, 1967), eat to satisfy their energy requirement. A comparison of consumption data as a measure of appetite should therefore take into account differences in the energy content of the feeds.

Further, a useful comparison of food consumption rates reported as a percentage of the body weight could be made only if the food consumption rate is proportional to body weight. This is not the case, however. That appetite reduces with size of fish is widely known and it is for this reason that the suggested feeding levels in tables designed for trout (Phillips, 1970) reduce with increasing size of fish. Page and Andrews (1973), working on catfish, also showed clearly that, in constant environmental conditions, feed intake was greatly influenced by fish size. In this experiment, therefore, since the fish on each of the experimental diets were growing at different rates, such a comparison is useful only at the beginning of the trial when the fish weights were comparable, and only then might any observed differences in feed consumption reflect differences in the metabolisable energy content of the feeds.

Carpenter (1953) discusses the concept of an 'appetite quotient' for the interpretation of ad-libitum feeding experiments, which would take into account differences in both the metabolisable energy content of the feeds and differences in body weight. He suggests a terminology and a standard method of mathematical treatment to test whether or not rats in an ad libitum feeding trial have 'had the same level of food intake', a condition defined by Kleiber (1947) as being such that "the rate of intake of metabolisable energy is the same multiple of the standard metabolic rate" and taken by Carpenter (1953) as being that "the rate of intake of metabolisable energy is the same proportion

to (body weight)$^{0\cdot88}$".

The factor 0·88 was used by Carpenter since Hegsted and Haffenreffer (1949) had found that the nutrient intake (measured as metabolisable energy) of growing rats varied as their mean body weight raised to the power 0·88, and the results of Brody (1945) had also indicated that the basal metabolic rate of comparable rats varies with their body weight raised to a power not significantly different from 0·88.

Beamish and Dickie (1967), in a review of the relationship between body weight and metabolism in fish, state that 'it is generally agreed that the logarithm of oxygen consumed, T, is linearly related to the logarithm of body weight, W, i.e.

$$T = \alpha W^{\gamma}$$

$$\log T = \log \alpha + \gamma \log W$$

and that the parameters α and γ of this general equation, termed the T line, take various values depending on conditions of both fish and its environment and on the level of activity at which measurements are made. At standard and low routine oxygen consumption Winberg (1956) and Paloheimo and Dickie (1966a) conclude that a value of the slope of about 0·8 provides an adequate general description of the relation among most species.

Winberg (1956) further proposed writing the energy equation

Energy input = energy expenditure + energy accumulation

in the form

$$pR = T + \frac{\Delta W}{\Delta t}$$

where R is the ration size and p is a correction factor used to convert R to an assimilated ration, T is the total metabolic expenditure, and $\frac{\Delta W}{\Delta t}$ is the weight gain, all parameters being expressed in common energy units, and demonstrated that an index of 'routine' metabolism derived from the data of food-growth experiments as

$$T = pR - \frac{\Delta W}{\Delta t}$$

was essentially the same as had been well established in studies of

oxygen consumption under rather similar experimental conditions. This was confirmed by Paloheimo and Dickie (1965, 1966a) in an analysis of food-growth data published by a number of authors. From these studies, it appears that under normal non-stress living conditions in nature, the value of the slope γ is about 0·8 and that this value may be used to describe the relationship between metabolism and body weight.

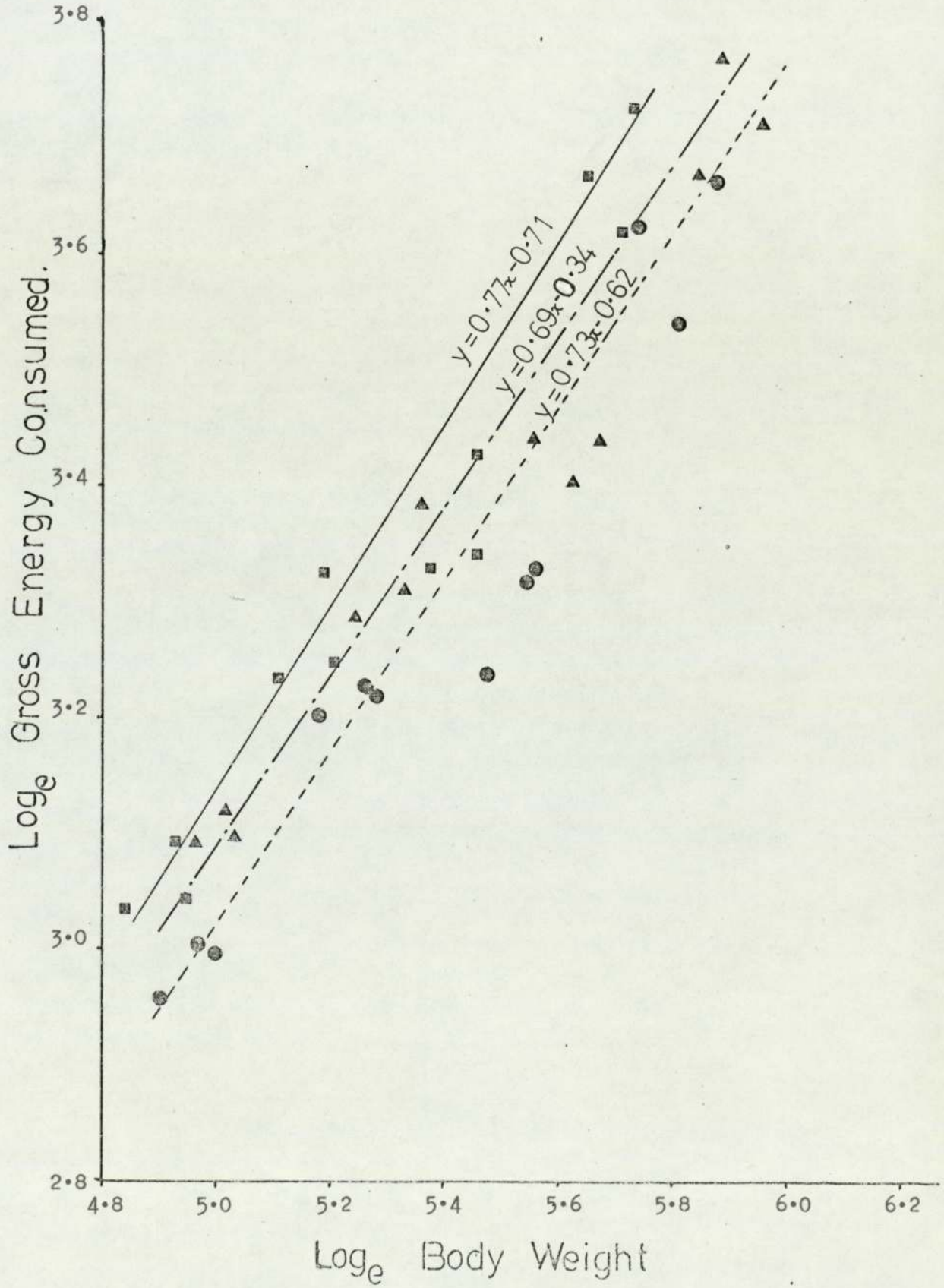
Paloheimo and Dickie (1966a) further showed that where fish were fed a maintenance ration, so that in the long term they neither lost nor gained weight, the level of metabolism appeared to correspond with that characterising the "routine" metabolic level in oxygen consumption studies. When fish were fed ad libitum, however, the value of γ appeared to remain unaffected but the value of α was raised by a factor of 4-5 times to a level approaching that known in oxygen consumption studies as "active" metabolic levels. Intermediate levels of feeding gave rise to intermediate levels of α. From such data they concluded that the level of food intake by fish is reflected in the total metabolism through its effect on metabolism α.

The criterion for ad libitum feeding was that there should always be a small amount of food in the tank in excess of the fish's consumption of food. A maintenance diet was one in which the feeding rate was adjusted by the experimenter in accordance with the response of the weight of the fish. Fish fed to satiation at a predetermined frequency, such as in the experiments of Kinne (1960, 1962) described by Paloheimo and Dickie (1966a), were considered to be on a restricted diet intake. While there was a different level of metabolism for the different feeding levels, these systems of adjusting feeding to fish demands resulted in a common relation of metabolism to body size, evident by an apparent parallelism of the slopes within a species for all feeding levels. Thus, according to Paloheimo and Dickie, given a constant availability of food of a particular type, it appeared that fish of different sizes feed at rates which result in the maintenance of

a value of γ characteristic of the species and a level of α characteristic of the level of food supply and the environmental conditions.

Following Paloheimo and Dickie's terminology, the fish in this experiment would be considered as being on a restricted feed intake rather than an ad libitum feeding regime. However, feeding to satiation twice per day is likely to give a feed intake which approaches the maximum ad libitum feeding rate. In addition, environmental conditions were relatively constant only during the first four weeks of the experiment, after which time there was a decline in temperature.

Food consumption rates for each two week period were calculated in gross energy units. The log e values were plotted against log e values of the mid-point body weight $\frac{W_T + W_{To}}{2}$ for each period (T-To) where (T-To) was 14 days.
The consumption rates for diets 1, 6 and 9 during four two week periods illustrate the trends and are shown in Table 46, and plotted in Fig 20. The data for period 5 (8 - 10 weeks) has been omitted. The temperatures had fallen substantially by this time and, due to uncontrollable circumstances, feeding was more restricted, fish being fed to satiation only once per day on five occasions during this period. Both a lowering of temperature and a lower feeding rate would reduce the level of metabolism, (Paloheimo and Dickie, 1966a). Ignoring the data for period three, there is a positive correlation between the points for periods 1, 2 and 4 for each diet which reveals three separate lines with a similar slope value. In this case it is possible that a fall in temperature over the range 16·5 - 14°C during the eight week period had only a small effect on the level of metabolism, and the low values for period three were caused by inadvertently restricting food intake. The method of feeding is difficult, particularly in bad weather conditions and restriction conceivably could have happened.


3·8
3·6
3·4
3·2
3·0
2·8
4·8
5·0
5·2
5·4
5·6
5·8
6·0
6·2
Log$_e$ Gross Energy Consumed.
Log$_e$ Body Weight
y=0·77x-0·71
y=0·69x-0·34
y=0·73x-0·62

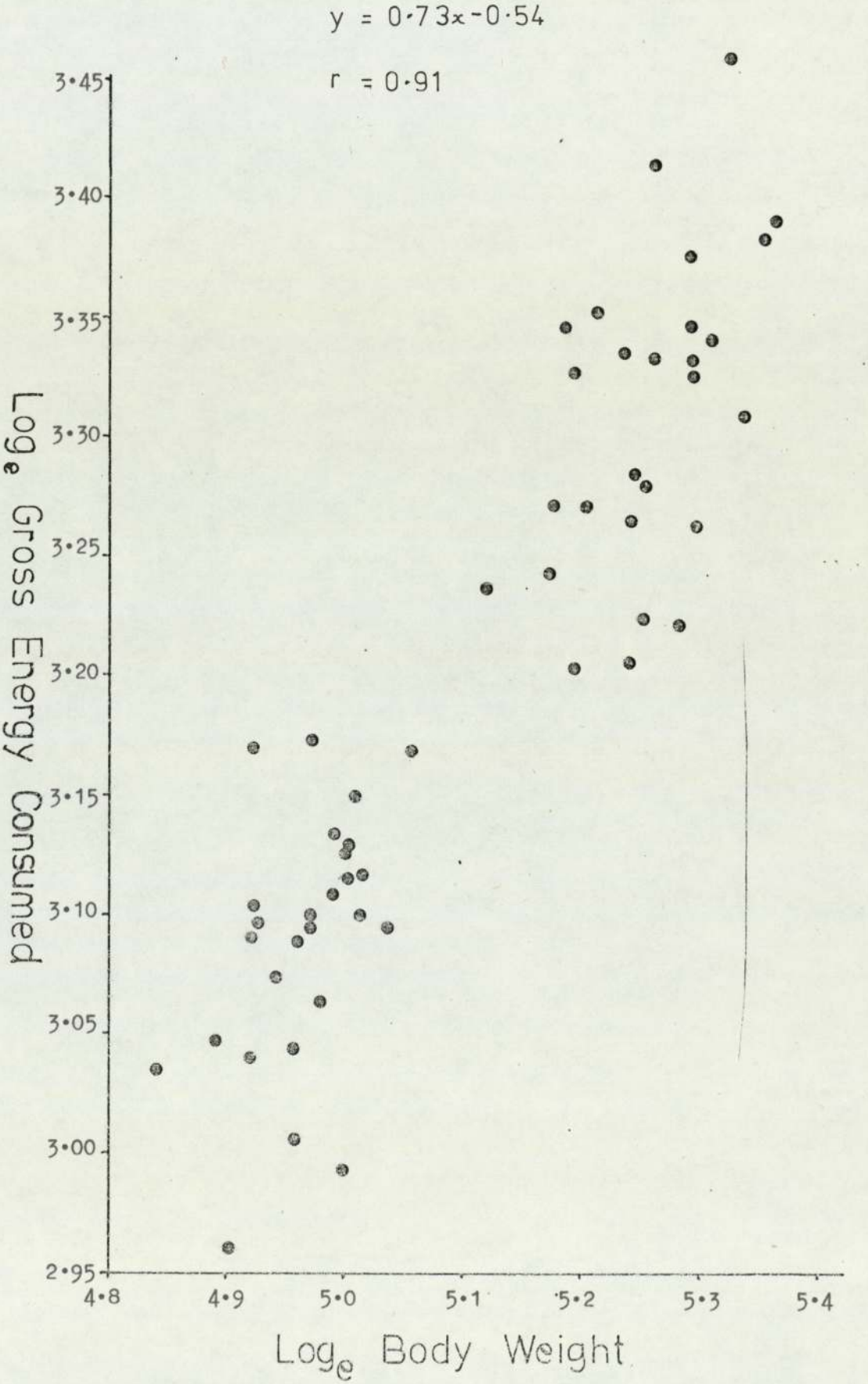
Whichever way the results of this experiment are interpreted, it is clear that the slope value is similar for the three diets. Strictly speaking the $\log_e$ of the metabolisable energy content of each feed should be plotted. The metabolisable energy content of each feed was not determined, however, and there is little point in assuming a value such as 0·8 considered by Winberg (1956) as being appropriate for the conversion of gross energy to assimilated energy values. It is likely that the value of the conversion factor is variable being dependent on digestibility of the major nutrient components-lipid, carbohydrate and protein, and the quality specifically of the protein component which is dependant on the amino acid profile. Such differences may account in part for differences in the intercept value of the three diets.

Despite such differences, it is possible to obtain a mean value which adequately describes the relationship between food intake, as gross energy, and body weight by plotting the data for each of the three replicate tanks on each of the nine diets during the two periods in which environmental conditions were relatively constant. It is clear from Fig 21 that there is a positive correlation between $\log_e$ gross energy consumed and $\log_e$ of the mid point body weight (p 0·001) and the slope value, equal to 0·73, is similar to the value of 0·8 determined from the relationship: $\log T = \log\alpha + \gamma \log W$. (Winberg, 1956; Paloheimo and Dickie, 1966). The scatter of results about the mean line may be explained by the differences in the metabolisable energy content and calorigenic action of the diets as mentioned above.

Thus one has a situation analogous to that for the rat where Hegsted and Hoffenreffer (1949) had found that the nutrient intake (measured as metabolisable energy) of growing rats varied as their mean body weight raised to the power 0·88, a factor not significantly different

Fig. 21. Correlation of Log_e Gross Energy Consumed and Log_e Body Weight.

- All populations during period 1 and period 2.



from that found for the relationship between basal metabolic rate and body weight described by Brody (1945). Thus it is possible to use Carpenter's concept of an appetite quotient (Carpenter, 1953).

According to Carpenter, at any time in the experiment, theoretically, the appetite quotient Q is defined by the equation

$$\frac{\Delta F}{\Delta t} \times Cx = Qx \times W^{0.88}$$

Rate of food consumption	Metabolisable calories per gram of food	Appetite quotient	Body Weight

Since food consumption is irregular from day to day the calculation is most accurately made over the whole period of the experiment (T) measured in days.

Total feed eaten ;

$$F \cdot Cx = Qx \int_0^T W^{0.88} dt$$

$$\text{or} \quad Qx = \frac{F \cdot Cx}{\int_0^T W^{0.88} dt}$$

Since in fish, metabolism and food consumption rate may be considered to be more nearly proportional to the body weight raised to the power 0·8, this value is substituted for the 0·88 used by Carpenter

for the rat. For convenience of calculation, again $W^{0\cdot 8}$ is considered to increase proportionately with time, an assumption which should not lead to gross errors if the measurements are made over short periods and repeated as was the case in this experiment. Thus we have

$$Q_x = \frac{F \cdot C_x}{\left(\frac{W_T^{0\cdot 8} + W_{T_o}^{0\cdot 8}}{2} \right)^{T-T_o}}$$

As mentioned previously, the metabolisable energy content of the feeds was not determined. One of the problems in estimating metabolisable energy content of feeds for fish is that of assigning a value to the available calories per g of protein consumed. Unlike the situation in mammals, fish excrete much of the nitrogen as gaseous ammonia, rather than urea. The caloric value of protein will depend on the relative quantities of ammonia and other metabolites excreted. Different workers have ascribed different values to dietary protein as discussed by Cowey and Sargent (1972). For example, Phillips (1970) suggests a value of 4·1 kcals/g, which assumes that a large proportion of the excreted nitrogen is in the form of urea, and Brett et al (1969) suggest a value of 5·7 kcals/g, which assumes that ammonia is the only excretory metabolite.

The assimilation coefficients shown in Table 44 refer to digestible energy content rather than metabolisable energy content. The digestible energy content of fat was assumed to be 8·0 kcals/g and that of carbohydrate was assumed to be 1·6 kcals/g. The carbohydrate content of each diet was determined by difference and the value used therefore includes the fibre component. The low digestibility value assumes that the carbohydrate is composed mainly of starchy materials with a digestibility of 40%. The digestible energy content of the protein was determined using the digestibility values reported earlier in Table 41 and assuming a caloric value of 5·65 kcals per g of digestible protein. In this respect, a further assumption made,

however, was that all of the digested protein was true protein. This is unlikely since many of the non-protein nitrogen components will be digested. The caloric values of such components would not be 5·65 kcals/g.

The assimilation coefficients have been used to determine the digestible energy consumed during each period. This value was used in place of F·Cx in the equation described above for the determination of appetite quotients for the three replicate groups of fish on each diet for each 2 week period of the experiment. The results are shown in Table 46. Analysis of variance showed a highly significant period effect ($p<0·001$) and a highly significant diet effect ($p<0·001$) as shown in Table 47. Interaction between diet and period was not significant at the 5% level. There was a significant variation between the replicates of each diet at the 5% level but not at the 1% level ($p>0·01$, $<0·05$).

The main effect appeared to be the period effect. Since fish are poikilothermous, one would expect the level of metabolism to decrease with decreasing temperature. The effect of temperature on metabolism has been discussed by Paloheimo and Dickie (1966a) Warren and Davis (1967), Beamish and Dickie (1967) and Hastings and Dickie (1972). Since metabolism decreases with temperature and food intake is related to metabolism, as discussed, one would expect a highly significant effect of temperature on the appetite quotients. In addition, however, as previously mentioned, the consumption was restricted to one feed only on 5 days during period 5 which may have reduced the appetite quotient. This could also have been so for period 3.

The differences between the values obtained for the 9 experimental feeds have been analysed using a sequential t test. The results are shown in Table 47. The mean values are ranked in ascending order. Values not significantly different at the 5% and 1% levels are indicated.

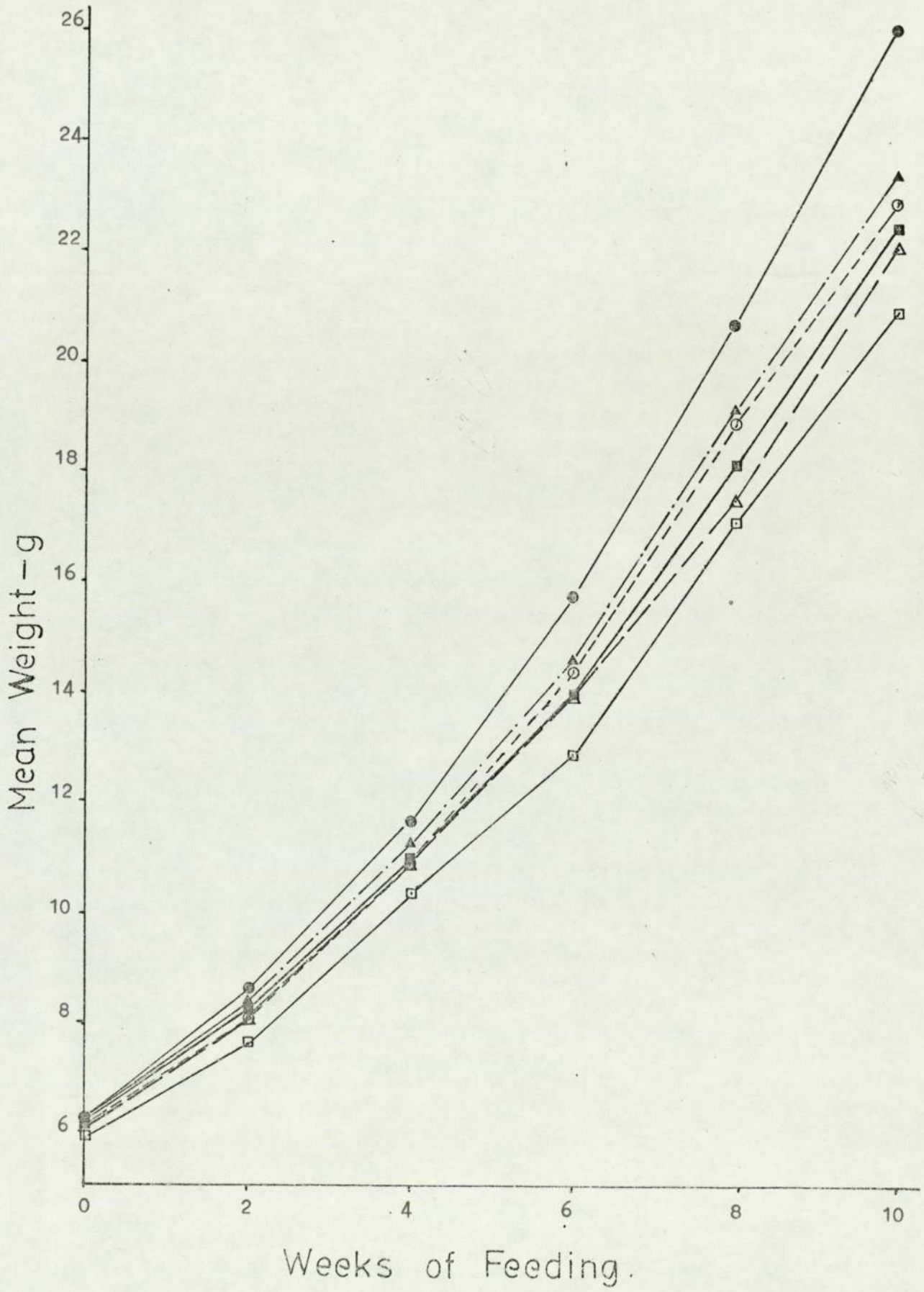
Reference to the apparently low consumption rate of bacterial protein was made earlier when differences were recognised as early as day 2 of the experiment. Much of this observed difference is probably related to the comparatively high energy content of the feed and is therefore lost when the data is calculated as appetite quotients. Nevertheless the appetite quotient for diet CO6 containing bacterial protein was the lowest. Appetite quotients for fish fed algal protein also appeared to be low. However, there was no significant difference at the 5% level between any of the diets containing S.C.P. or that containing ground nut. The soya bean meal was added to this list at the 1% level of significance. Diets containing fish meal and cotton seed meal appeared to give significantly higher appetite quotients than all other diets.

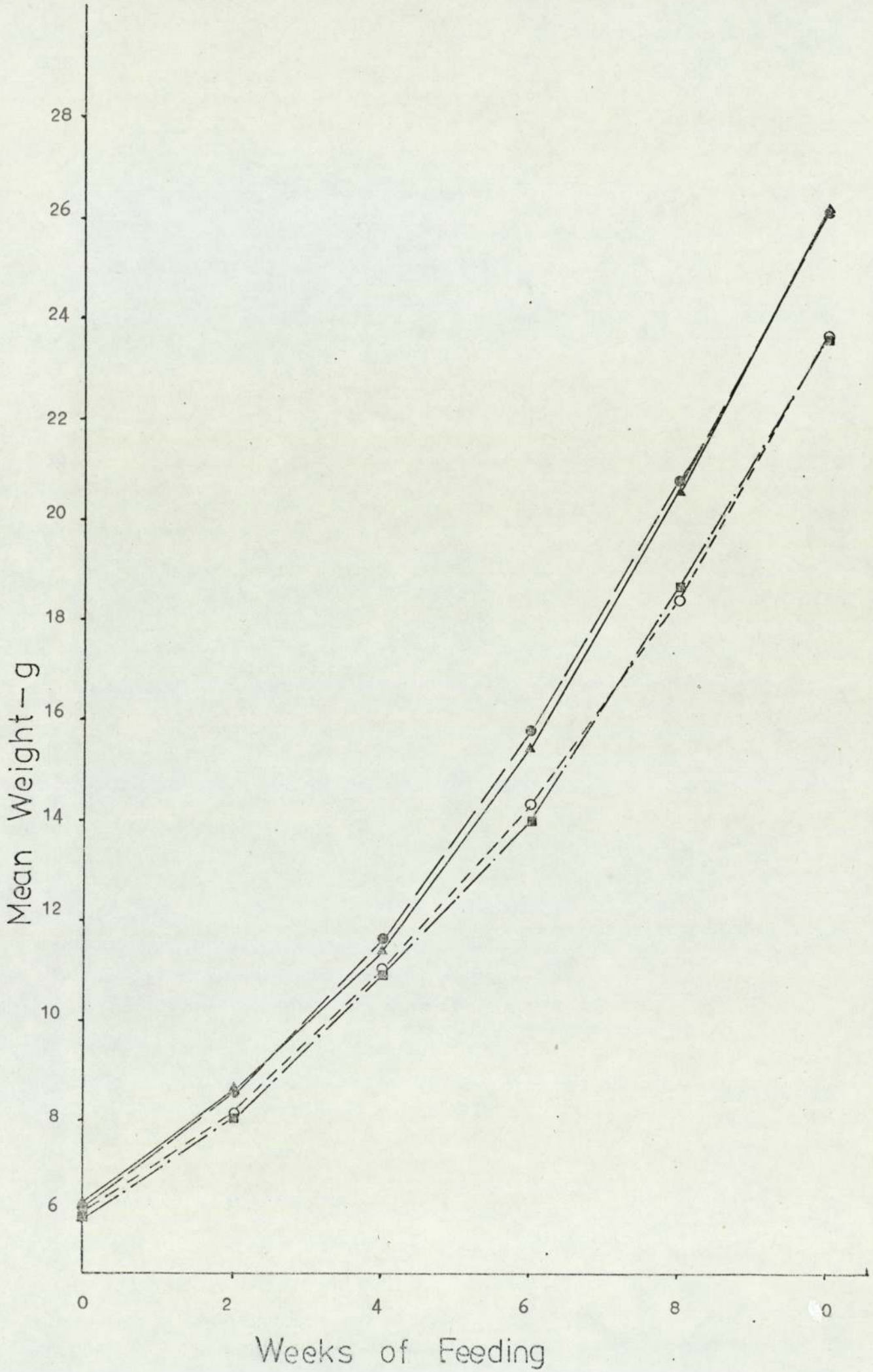
The grouping is interesting in that all of the diets containing S.C.P. are together. A number of assumptions are made in arriving at the values for appetite quotients. One possible explanation for the observed differences could be that the metabolisable energy content of the single-cell protein feeds has been underestimated. The measured lipid values, for example, may be low, as previously discussed. If lipid which was not extractable with petroleum ether is available to the fish, then higher assimilation coefficients should be used giving rise to higher appetite quotients.

Generally the observed differences in appetite quotients for the 9 experimental diets are small and palatability problems seem unlikely.

Growth and Food Conversion Efficiency

The mean fish weights for each of the replicate groups on the nine experimental feeds are shown in Table 48, as recorded at the end of each 14 day period during the first 10 weeks. The range of fish weights within each tank increased considerably during the trial. The mean results for each diet have been plotted in Figs 22 and 23. Log values


Mean Weight - g
26
24
22
20
18
16
14
12
10
8
6
0
2
4
6
8
10
Weeks of Feeding.


28
26
24
22
20
18
16
14
12
10
8
6
Mean Weight – g
0
2
4
6
8
0
Weeks of Feeding

have been calculated (Table 49) and curves of specific growth are shown in Figs 24 and 25. Mean specific growth rates for each period are shown in Table 50 and the result of an analysis of variance is shown in Table 51.

Food conversion efficiencies have been calculated for each diet, as shown in Table 52. Analysis of variance has been computed (Table 53).

By the end of 10 weeks the fish fed the control diet containing 40% herring meal and those fed diet C10 containing 20% soya bean meal, in place of an equal quantity of herring meal, had the highest mean weights. There was little difference between the final weights of fish fed diets containing cotton seed meal, groundnut and BP yeast. The weight of fish fed ICI bacterial protein and algal protein were a little lower, and those fish fed the moulds had the lowest recorded weights (Table 49 and Fig 23). Small differences in specific growth were evident particularly during the initial 2 weeks of the experiment (Table 50), the highest rate being recorded for the control diet and the lowest for the mould-containing diets, particularly for diet C17 containing Penicillium chrysogenum. The rates for the control diet were generally high throughout the experiment whilst those for fish fed the Penicillium chrysogenum were generally low throughout. However, taken over the whole 10 week period, there was no significant difference ($p > 0{\cdot}05$) between the mean specific growth rates of the fish on any of the diets. Specific growth rate reduced with time for all treatment groups and the period effect was highly significant ($p < 0{\cdot}001$).

The results for food conversion efficiency (Tables 52 and 53) also show a highly significant period effect. There were also significant differences ($p < 0{\cdot}001$) between the diet treatments. In addition there was a significant interaction ($p < 0{\cdot}05$) between the values for conversion efficiency and the period, indicating that the magnitude of the

Fig.24. Specific Growth Rates - Phase I.

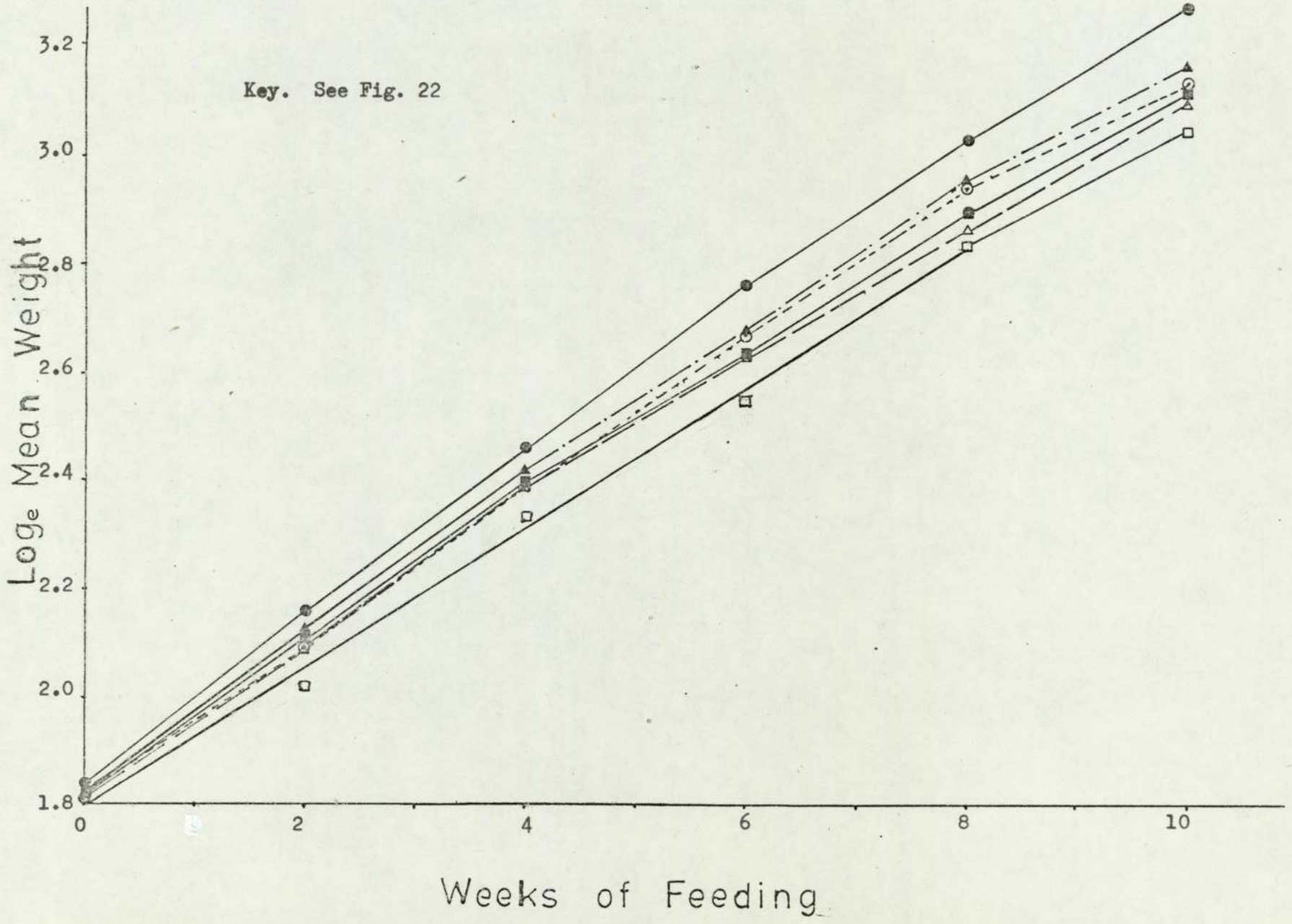
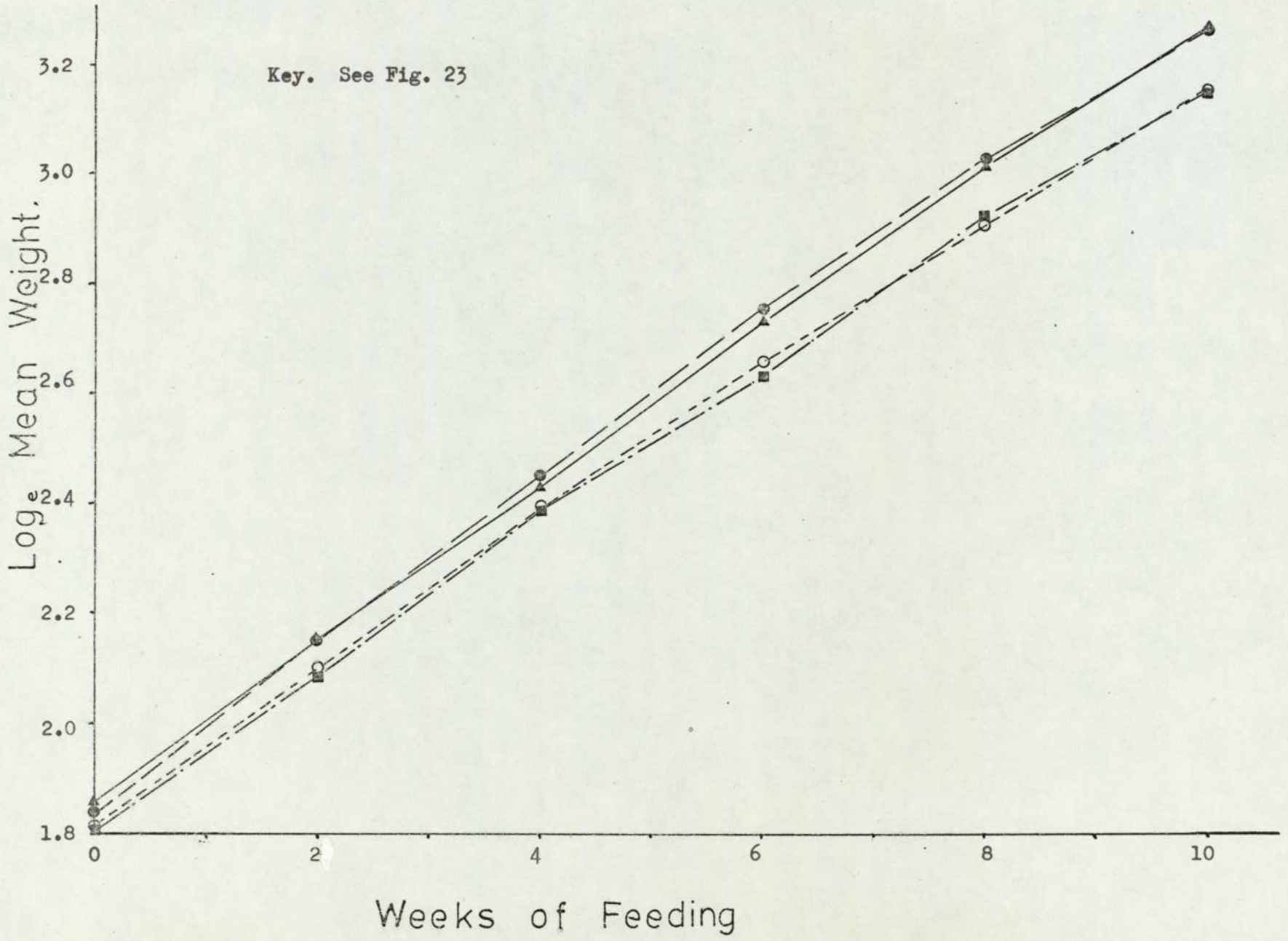


Fig. 25. Specific Growth Rates - Phase 1.



period effect was different for the different treatment groups. Finally, there was a small but significant variation ($p < 0{\cdot}05$) between the replicates within treatment groups.

The relationship between the food energy, growth and growth efficiency have been discussed in detail in reviews by Winberg (1956), Paloheimo and Dickie (1965, 1966 a and b), Beamish and Dickie (1967), Warren and Davis (1967) and Hastings and Dickie (1972).

According to Beamish and Dickie (1967), there are 2 major pathways of energy use:

1) invariably the larger, is the release and expenditure of energy through a series of chemical reactions collectively known as metabolism.
2) is the conversion and deposition or storage of energy in the animal body in a series of processes which collectively may be called growth.

Thus, the growth formula may be written as the basic energy equation of Winberg (1956)

$$\frac{\Delta W}{\Delta t} = pR - T$$

where W, the energy equivalent of growth during a period of time t, is the energy acquired in the assimilated ration, pR, minus the total energy expended in metabolism T.

Gross growth efficiency is the percentage of the ingested energy stored as body fabric (Needham, 1964) given as $K = \frac{\Delta W}{R\Delta t}$. The relationship between growth efficiency and ration size was investigated by Paloheimo and Dickie (1966b). By examining values for food consumption and growth of fish, as published in the literature, they concluded that the gross efficiency decreases from a maximum value at low feeding levels and that the change was independent of the body weight of the animal. However, Warren and Davies (1967) suggest that this statement is not restrictive enough. They state that unless

net efficiency (given as $\frac{\Delta W}{\Delta t(R-M)}$, where M is the maintenance ration) declines drastically with increases in ration from low levels, and this is not generally the case, gross efficiency will not decline with increasing ration except beyond some intermediate level of feeding. Brett (1969), working on the effect of ration size and temperature on growth and conversion efficiency, found that at all temperatures K reached an optimum as rations increased and suggested that the inference of Paloheimo and Dickie may have been based on cases in which the range of ration sizes considered lay above the optimum.

The effect of temperature on feed consumption rate, growth and gross conversion efficiency warrants further discussion. Brown (1957) has expressed the view that temperature was not of direct importance to growth efficiency, except through its general influence on metabolic rates and corresponding appetite. This contention appears to have been supported by the analysis of Paloheimo and Dickie (1966b).

In this experiment, temperature has already been shown to have affected 'appetite', food consumption rates generally reducing throughout the experiment as the temperature dropped. Clearly from Fig 24, 25 specific growth rate was not constant in the experiment but began to fall off with time. The most likely cause for this reduction is the general decrease in food consumption throughout the experiment. Feed conversion efficiency generally improved during the experiment, the highest value being recorded when the temperature had fallen to 12·5°C.

There have been several attempts to determine the optimum temperature for the growth of salmonids. Brown (1946) reported two optimum temperature ranges, 7-9°C and 16-19°C, for brown trout (Salmo trutta). However, this work was criticised by Swift (1961) who suggested that Brown's two temperatures resulted from considering an insufficient number of fish, and, using larger numbers of animals, he was able to show only one optimum temperature at 12°C. Baldwin (1956) suggested a temperature of 13°C as being the optimum

for the growth of brook trout (Salvelinus fontinalis) fed on minnows.

More recently Brett (1969) has determined the temperatures at which optimum growth and efficiency occurred in sockeye salmon (Onchoryncus nerka) fed a range of ration sizes. When fed an excess ration, optimum growth occurred at 14·5°C whilst maximum conversion efficiency occurred at 11·5°C. That optimum growth and optimum conversion efficiency occur at different temperatures has also been shown for the coho salmon (Onchoryncus kisuch) (Doudoroff, 1969) and catfish (Ictalurus punctatus) (Allen and Strawn, 1967).

Whilst there may be species differences in the salmonid group, it is possible that differences in the methods used, particularly with respect to food type and feeding levels, both of which have a marked effect on growth, could explain observed differences reported in the literature. Brett (1969) fed sockeye salmon high energy compound feeds at a number of different levels up to excess. The optimum temperatures for growth and food conversion varied with the level of feeding. Repeating Brett's work on rainbow trout, brown trout and brook trout may reveal optimum temperatures for growth and food conversion efficiency similar to those observed for sockeye salmon.

Apart from the general decrease in consumption rate due to the fall in temperature, food conversion may have been more restricted during periods 3 and 5, as previously discussed. This would tend to increase feed conversion efficiency since the feeding rate was likely to have been greater than the optimum.

Other factors may also have influenced food conversion. For example, food particle size may be of importance as discussed by Paloheimo and Dickie (1966). Hastings and Dickie (1972) point out that particle size has been recognised as one of practical importance in fish culture for many years but there appear to be no reports of experiments which have been deliberately designed to measure the

effect and provide an explanation of the role which food particle size plays in either fish culture or in nature. They concluded that 'at this junction in the state of the art of fish feeding, we can only conclude that characteristics of physical distribution and availability for grazing may have an influence on food value for growth, which are as important as substantial changes in the chemical or bulk characteristics'. In this experiment, the particle size was increased from crumb 1 to the $\frac{1}{8}$" pellet during the course of the experiment and this may also have contributed to the increased conversion efficiency by increasing 'grazing efficiency'.

Analysis of variance showed a highly significant effect of diet treatment on food conversion efficiency and the results were therefore further analysed using the sequential t test as shown in Table 53. The means are ranked in ascending order and values not significantly different are indicated.

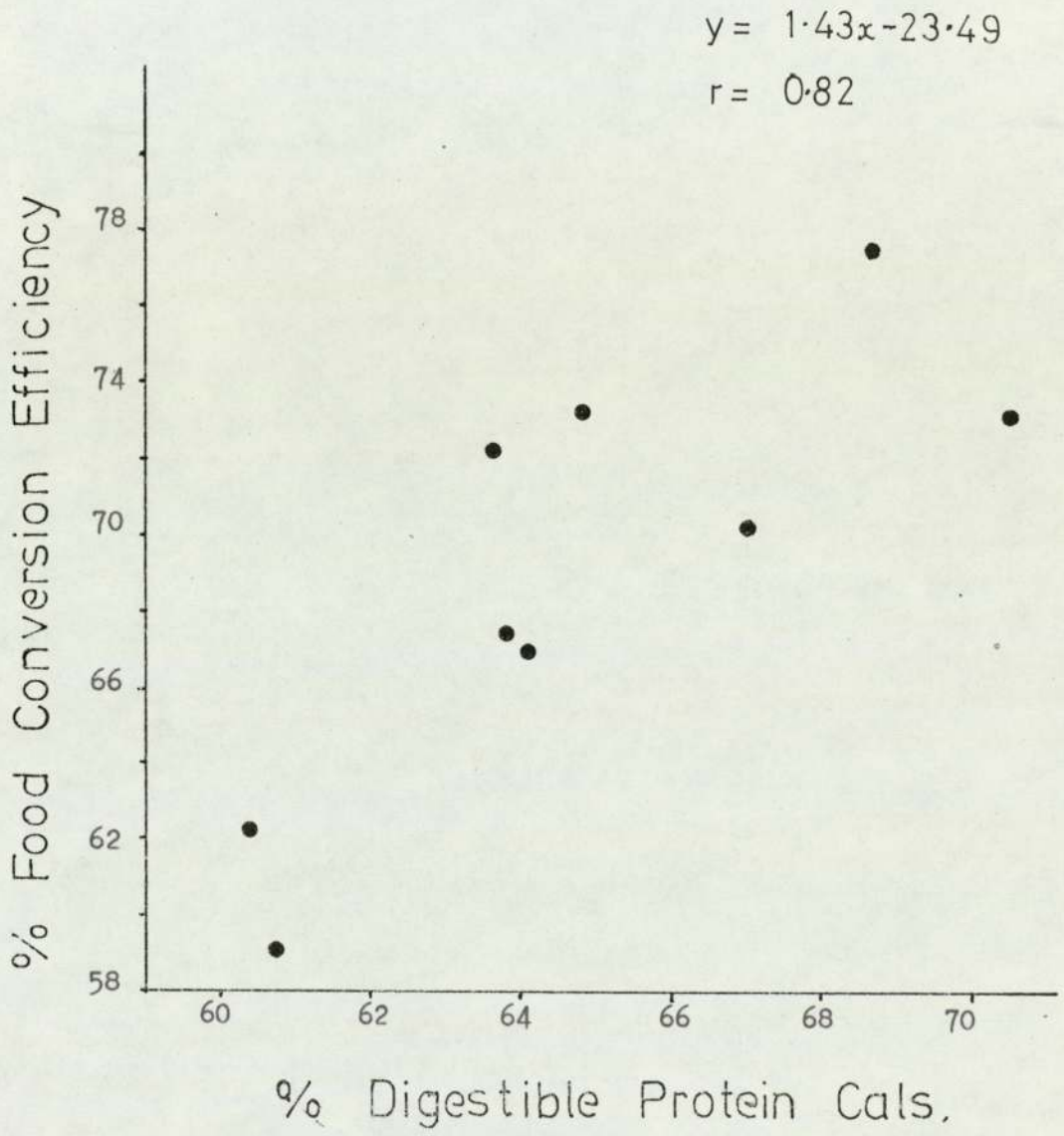
Food conversion efficiency was correlated with the digestible protein content and the digestible caloric content. The regression equations are:

Digestible Protein %	$y = 1{\cdot}57x + 16{\cdot}68$	$r = 0{\cdot}89$
Digestible Cals. %	$y = 1{\cdot}93x - 65{\cdot}14$	$r = 0{\cdot}92$

However, differences in caloric content were largely due to differences in the content of digestible protein. Conversion efficiency generally improved as the % digestible protein calories increased (Fig26).

The importance of large quantities of protein in salmonid diets has already been mentioned. The optimum protein content for chinook salmon and rainbow trout has been shown to be approximately 40% using semi-purified diets. Practical diets containing protein of lower quality are generally formulated to contain 40 - 45% protein, contributing some 55 - 60% of the total calories. The McNenney formulation, on which the control diet CO9 was based, is formulated

Fig. 26. Correlation of % Food Conversion Efficiency and % Digestible Protein Calories.



for optimum performance and in this instance contained 45·5% protein on analysis, contributing 59·8% of the total calories and 68·6% of the digestible calories. As the protein content reduced, one would therefore expect a fall-off in performance. The scatter of points about the mean regression line will be partly the result of experimental errors, particularly in the assumption that all the measured nitrogen was protein nitrogen, as previously discussed, and partly due to differences in the quality of the test proteins. The value of each protein used will be dependent on its amino acid profile and, in this instance, on its ability to compliment the amino acid profile of the basal ration. Protein quality is discussed further in Section 6.

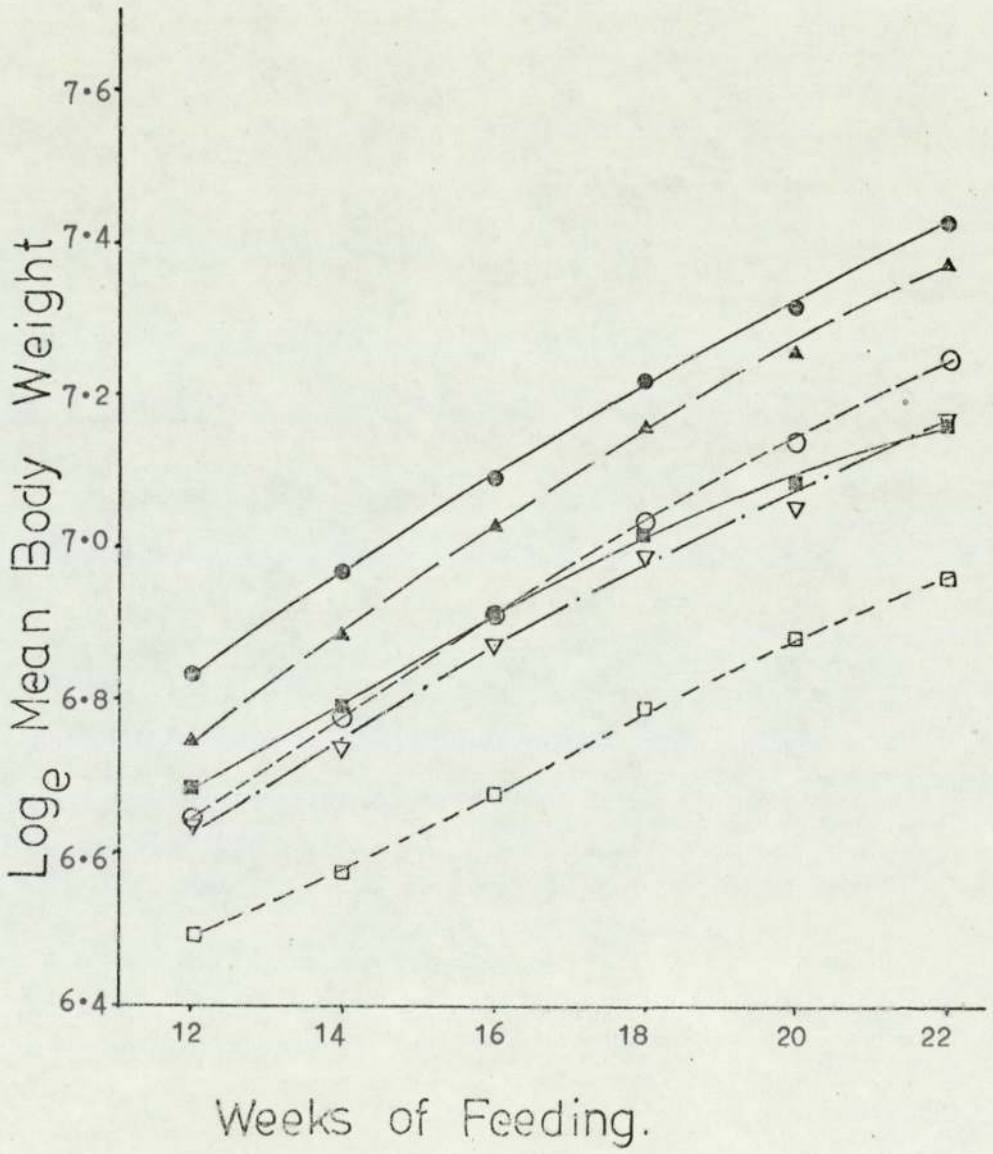
The data for the second phase of the experiment is shown in Tables 54, 55, 56 and 57. Food consumption values (Table 54) have been reported as:

$$\frac{\text{Food consumed}}{\frac{1}{2}\left(W_{To}^{0\cdot 8} + W_{T}^{0\cdot 8}\right) T\text{-}To}$$

where food consumption is given as gross energy (Kcals) and weight W is given in gs. The values differ from the appetite quotients reported earlier in that they have not been corrected using assimilation coefficients. Values for diet C19, containing the ICI bacterial protein, and diet C22, containing Penicillium chrysogenum, were comparatively low. It is unlikely that the differences are entirely due to differences in assimilation coefficients and appetite could be adversely affected for these single-cell proteins at this level of inclusion. Appetite does not appear to be affected by the yeast, alga or the mould, Aspergillus niger.

Growth data is shown in Table 55 and specific growth plots are shown in Fig 27. The specific growth rates and food conversion efficiency for each two week period have also been determined and are reported in Tables 56 and 57 respectively.

Conversion efficiency values for the control diet were very high


Log$_e$ Mean Body Weight
7·6
7·4
7·2
7·0
6·8
6·6
6·4
12
14
16
18
20
22
Weeks of Feeding.

despite the low temperatures recorded during this phase of the experiment. Warren and Davis (1967) reported that unpublished data of Brocksen suggest that cutthroat trout (Salmo clarki) may utilize food for growth under some conditions in late winter with higher gross efficiencies than in spring, at least over the narrow range of rations the fish will consume in the winter. According to Warren and Davis, the higher gross efficiencies in winter were primarily due to the lower maintenance costs, probably resulting from lower temperatures, and the data suggests that there are seasons and temperatures at which maintenance costs may be low but at which fish are still metabolically able to utilize food effectively for growth. Under these circumstances the gross efficiencies will be high. This appeared to be the case in this experiment.

There was little difference in food conversion efficiencies (F.C.E.) for the groups fed the control diet (C09) and that containing the yeast protein (C18). The specific growth rate of the latter was marginally better, probably reflecting the slightly increased appetite (Table 54). The F.C.E. of diet C19, containing the bacterial protein, was also very high, although the specific growth rate was lower than the control as a result of a generally reduced feed intake. Both diets (C18 and C19) had a higher protein and caloric content than the control diet and both single-cell proteins have already been shown to be well assimilated. It is likely that the optimum protein content has been exceeded in these diets, resulting in no further improvement in F.C.E. over the control diet.

The growth rate of fish fed the algal containing diet (C20) was also comparatively high although F.C.E. was generally somewhat lower than the control. This diet had a similar protein content and more calories than the control but the algal protein has a lower digestibility as indicated earlier. This probably accounts for the poorer F.C.E.

The growth rate of fish on the diet C21, containing Aspergillus niger, varied between periods, being higher than the control during two periods. The F.C.E. was, however, somewhat poorer. F.C.E. of fish fed diet C22, containing Penicillium chrysogenum, was also comparatively low and specific growth rate was poor as a result of this and a generally depressed 'appetite' as indicated previously. Both C21 and C22 had a lower protein content than the control. The protein digestibility of these components has also been shown to be lower, as indicated previously.

Terminal Pathology

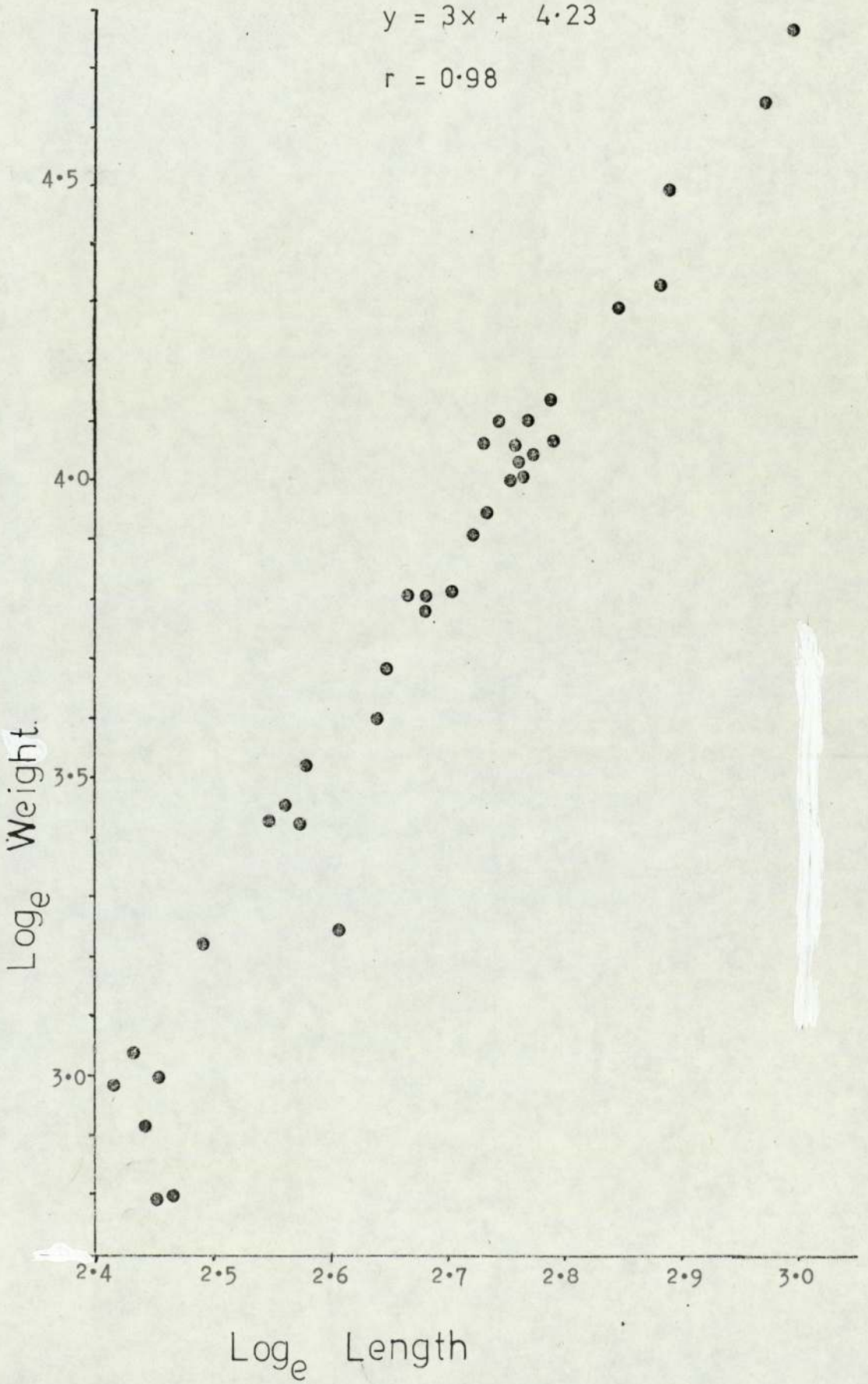
Condition

The general relationship between $\log_e$ weight and $\log_e$ length is shown for diet CO9 in Fig 28. There was a significant positive correlation between $\log_e$ weight and $\log_e$ length. However, the relationship appeared to break down for the smallest fish. The weight range of fish within each group was shown to increase substantially during the trial. However, within each group there was a small number of fish that grew very little during the trial. These fish were probably the lowest members of the hierarchy, generally stressed and therefore in poor condition having a comparatively low weight for their length. Omission of the data for such fish increased the correlation coefficient and decreased the slope value.

n was considered close enough to 3 to permit its use generally in determining the condition factor of the 18 fish from each diet at the end of each phase of the experiment. The mean results are shown in Table 58. The results have been computed for analysis of variance and the significance of differences between diets have been tested using sequential t tests. (TABLE 59)

There was no significant difference in the mean condition factor

Fig. 28. Correlation of Length and Weight Data - Control Diet.



for each treatment group at the end of Phase I. However, a positive correlation ($p<0.02$) was obtained between condition factor and the digestible caloric content of the diet (Fig29), condition factor generally increasing with increasing digestible calories in the diet.

During Phase II, there was an increase in the condition factor in all groups except those on the diet containing bacterial protein. However, the increase was not significant in some cases as shown in Table 58. The condition factor significantly increased in the group fed the control diet. This could be related to the temperature change. It is possible that there was a greater tendency for fat storage in the colder months when metabolic energy demand was at a low level. This may equally apply to the other groups, although the energy content of the diets generally increased during Phase II which may have accounted in part for the observed increase in condition factor.

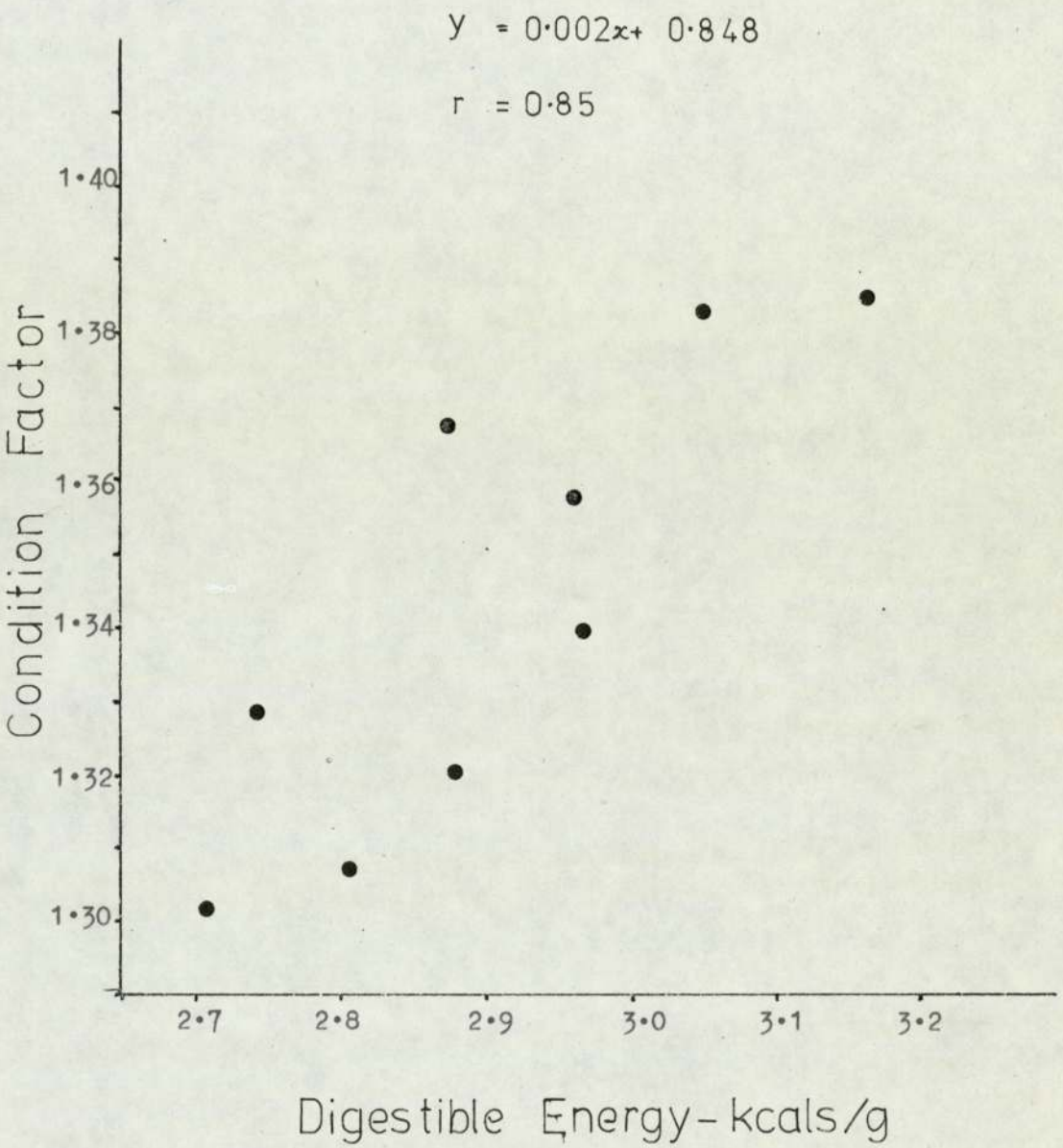
Differences between the groups were noted at the end of Phase II as shown by analysis of variance (Table59). This may reflect differences in fat storage but since body composition was not determined this could not be verified. The fish on the control diet CO9 and those on diet C18 containing BP yeast had significantly higher condition factors and these diets had the highest energy content. They had also generally performed better than other diets during the second phase of the experiment.

External appearance

There were no substantial differences in the external appearance of fish from any of the groups. At the end of Phase I, the smaller fish in each group were in poorer condition, having slightly increased fin erosion as a result of fin "nipping". This was not so apparent at the end of Phase II after 10 weeks in the colder environmental conditions.

Fish fed algal protein had slightly improved colouration probably

Fig. 29. Correlation of Condition Factor and Digestible Energy.



resulting from the higher quantities of caratenoid pigments found in algae.

Haematocrit

The mean results for the haematocrit tests are shown in Table 60, 61. There were significant differences between the diet groups ($p<0.001$). The means have been compared using the sequential t test. Values indicated are not significantly different. The fish fed the control diet had a significantly higher haematocrit than the other diets at the 5% level of significance, although this was not significantly different from diets containing algal protein, groundnut, yeast or bacteria at the 1% level of significance. Fish fed diets containing mould protein had significantly lower haematocrit values than all others.

Haematocrit values for the 6 groups at the end of Phase II are shown in Table 60. There was a significant difference between haematocrit values for Phase I and Phase II only for those fish fed diets containing Penicillium chrysogenum, as shown in Table 60. Haematocrit increased substantially in this case. Small increases were also shown for diets containing yeast protein, algal protein and the mould, Aspergillus niger, but the differences were not significant ($p>0.05$). There were small decreases in the groups fed the control diet and the diet containing bacterial protein. The protein content of all test diets increased and thus may have accounted for the small improvements in most cases. The marked improvement for the diet containing Penicillium chrysogenum, however, could not be explained.

At the end of Phase II only the diet containing the mould Aspergillus niger had a significantly lower haematocrit value at the 1% level of significance. (TABLE 62).

The first general sign of abnormal haematology is impaired haemopoiesis which is characterised mainly by depressed values for

erythrocytes and haemoglobin. There are two basic causes: 1) the lack of elements permitting fish to produce blood and 2) nutritional diseases which affect haematopoetic organs (Snieszko, 1972). Snieszko suggests that some of the essential vitamins, minerals, and possibly also amino acids, belong to the first group, and fatty degeneration of the liver, visceral granuloma, hepatoma in terminal stages, and possibly others, belong to the second group. In addition to the many nutritional diseases in which anaemia is listed as a symptom (Snieszko, 1972; Ashley, 1972) most communicable diseases in their acute form may also result in anaemia.

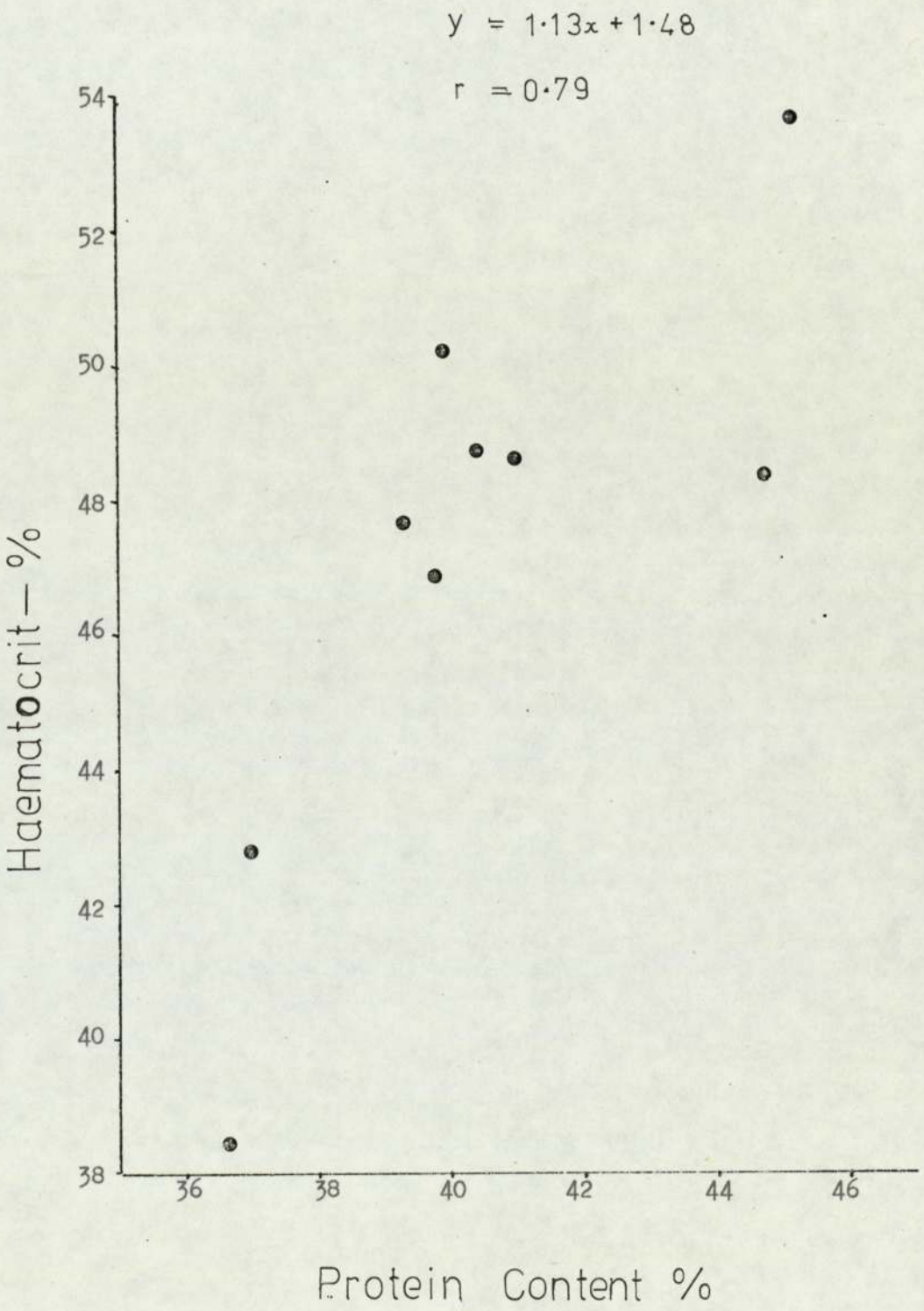
There is a wide range of 'normal' haematocrit values for rainbow trout. Snieszko (1961) reports 44·7% as the mean result of 84 rainbow trout aged between 3 - 10 months. In a further analysis he showed a seasonal variation, values varying from 43·8% in May to 53·0% in January. Values for groups of fish on the mould proteins were just outside this range at the end of Phase I. However, diet composition will have an effect on haematocrit. Thus, there was a positive correlation between haematocrit and protein content as shown in Fig 30. There was a marked resemblance between the plots of haematocrit against protein content and that of F.C.E. against protein content. Values for the bacterial protein were somewhat lower than might be expected in both cases.

The lower mean values for groups of fish fed diets containing the mould proteins are probably related to diet composition rather than to pathological disturbances listed in group 2) above. Protein content, or perhaps amino acid balance specifically, appear to have the same effect on haematocrit as it has on growth and food conversion.

Internal appearance

Internal examination revealed no obvious differences between the groups after 12 or 22 weeks. The amount of visceral fat was variable

Fig. 30. Correlation of Haematocrit and Protein Content.



and some livers were pale and in a few cases had a 'blotchy' appearance, but there did not appear to be any dietary related differences.

With the exception of diets C12 and C15, there were no significant differences in mean liver weight at the end of Phase I (Table 63). Values for C12 were significantly lower, and C15 significantly higher than the remaining diets at the 5% level of significance. There were no significant differences at the end of Phase II. There was a significant difference between Phase I and Phase II results only for fish in the groups fed the mould, Aspergillus niger. There were no obvious correlations between these results and any of the measured diet parameters.

The variation between observations within a treatment group were significantly different i.e. non-homogeneity of variances was shown by Bartlett's chi-square test. The variances were high in the case of the group on the diets containing algal protein and low in the group containing groundnut at the end of Phase I and these are the groups in which significant differences were noted. This finding could not be explained. The interpretation of the results must be treated with some caution.

None of the groups were considered to have abnormally large livers and there was no evidence of tumours. This was confirmed by histological examination. All livers were generally highly vacuolated but there were no obvious differences between treatment groups . Livers of this appearance are typical for hatchery trout reared on artificial compound feeds. Such vacuoles are generally considered to result from storage of lipid. The degree of vacuolation was variable but in no case was there a complete breakdown of liver tissue organisation indicating liver lipoid degeneration.

CONCLUSIONS

The performance data has been summarised in Tables 65 and 66. Differences in growth and conversion were largely related to differences in composition, particularly of the protein content.

All five single-cell proteins were well tolerated at a 20% inclusion rate, but at the 40% level there was some reduction in feed intake of the groups of fish fed diets containing Pseudomonas and Penicillium chrysogenum. Since there were many variables it is difficult to ascertain the cause of this. Amino acid imbalance is one possibility.

Pathological examination revealed no abnormalities in any of the fish fed single-cell protein for 22 weeks and mortalities were very low throughout the experiment. In this respect the results were most encouraging.

SECTION 6.

DESIGN OF EXPERIMENTAL FACILITIES FOR THE EVALUATION OF PROTEIN QUALITY AND FURTHER ASSESSEMENT OF FEEDING TECHNIQUES.

DESIGN OF EXPERIMENTAL FACILITIES

Introduction

The initial trials carried out in the systems outlined previously, showed certain inadequacies in the design of the experimental facilities, as discussed. It was clear that more space and water were required for a satisfactory feed evaluation programme and that it was necessary to improve the design of the experimental facilities.

The Fish Laboratory

For further work, increased space and water was made available for the development of a laboratory specifically for fish studies. The laboratory was a small two-storey building. The upper floor provided general office and laboratory facilities and the ground floor, the floor of which was concrete and sloped to a 6" drainage grid, was fitted with the tank systems. In addition, there was a small room adjacent to the general laboratory on the upper level. This also had a concrete floor and provided an excellent site for placing a 250 gallon header tank. This was served by a 1" inlet from the mains, and fitted with a ball cock control valve. The laboratory facilities were to be shared and it was necessary to design systems for the evaluation studies within the confines of the limited space and with the water available. It was also considered desirable to further develop the design criteria for the nitrogen balance system previously built and evaluated and to use as many materials as possible from that system.

At the time the facilities were designed, it was proposed to evaluate the test proteins by a number of methods, including tests for protein efficiency ratio, net protein utilization and biological value. In order to make it possible to compare such values directly and to establish possible correlations, it was considered that a desirable feature of the

facilities was the pairing of two systems where control of environmental parameters in each could be achieved in an attempt to maintain similarity of such factors as light and water temperature. The facilities therefore included a redesigned 16-tank nitrogen balance system for the determination of Biological Value (B.V.) and an additional 16 circular tanks for the determination of nutritional parameters other than B.V.

A large 'Bartangle' framework was built to accommodate both tank systems as shown in Plate 9 .

Experimental Tank System 1

The nitrogen balance system, previously described, was modified in an attempt to overcome the problems encountered in the initial experiments. The same two 8 tank units were retained, but were built adjacent on the same level, as opposed to one above the other (Plate 9). The enclosures were rebuilt completely to give a larger working space 9' x 3' x 2'. The front face was made of a considerably more sturdy timber frame which was bolted to the 'Bartangle'; 2' x 2' framed doors provided easier access. The light fittings described previously were retained. The whole of the inside of the enclosure was lined with polystyrene tiles providing a completely light-proof box with reduced noise disturbance and, it was hoped, a generally more stable environment (Plate 10).

The circotherm was dispensed with on the basis that it was inadequate to cope with the volume of water required to be cooled/heated and it was considered better to record the ambient water temperatures rather than create unwanted temperature gradients. On the basis that two 16 tank systems were to be run together (experimental tank systems 1 and 2), providing a similar environment for correlation studies, the plumbing system was arranged as shown in Fig 31.

The balance systems were supplied independently from the header

PLATE 9

PLATE 10.

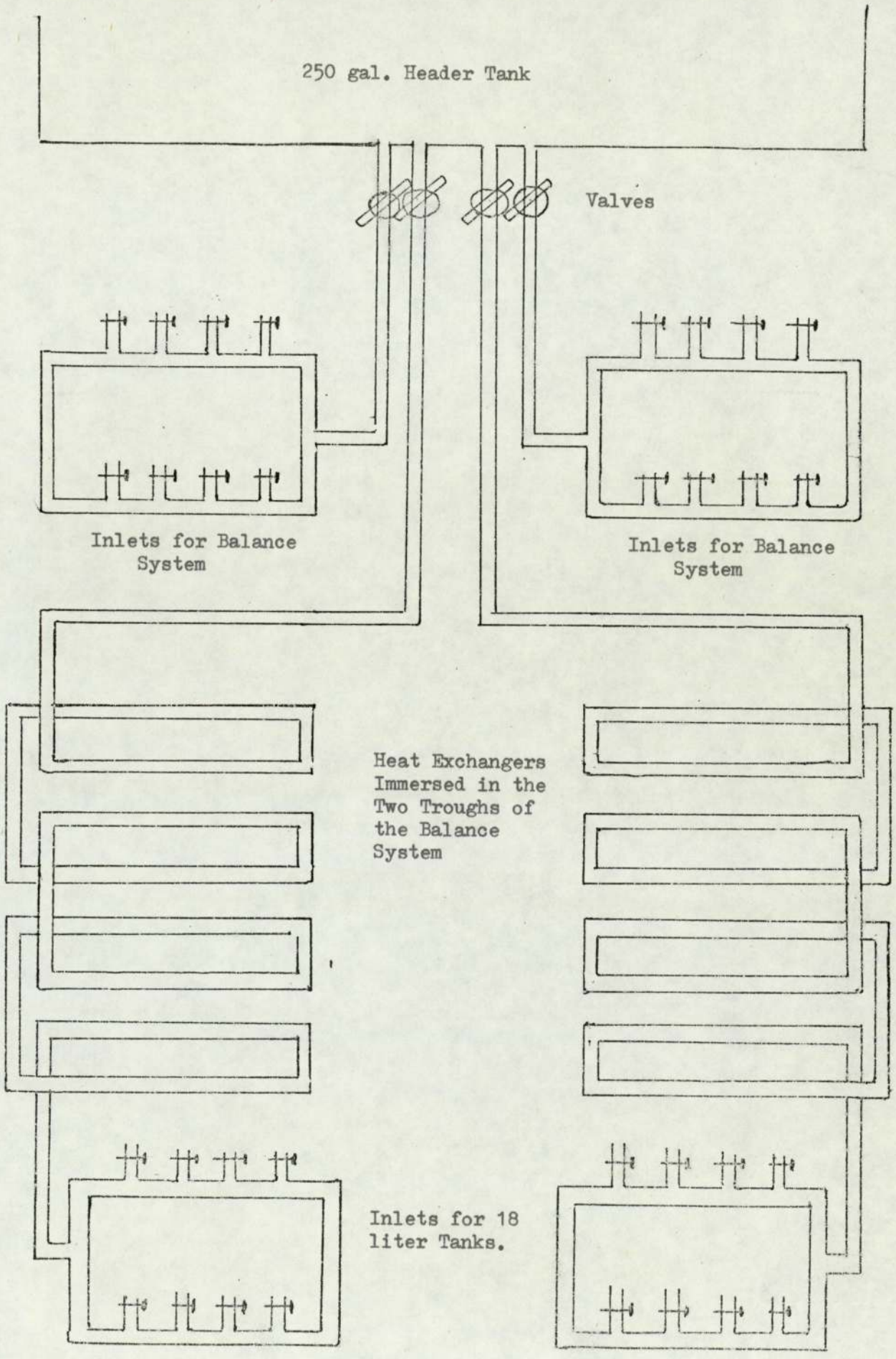


Fig. 31. Inlet Plumbing for Laboratory Tank Systems.

tank, each individual tank being served from a ring main as before. On open flow, the same temperature was maintained in all 32 tanks within the laboratory, since they were supplied from a common header and it had been found that fluctuating air temperatures had no observable effect on the tank water temperature with the flow rates generally used. However, so that the same temperature was maintained throughout a N-balance collection period (24 hrs.), when the balance tanks were not supplied with incoming water, the two water lines to the medium tank system were diverted through the 40' heat exchangers immersed as before in the troughs. Once equilibriated, the water temperature within the balance system should be maintained equal to that in the medium tank system (i.e. ambient mains water temperature).

Considerable modifications, as shown in Fig 32 and Plate 9, were made to the outlet and drainage system to overcome the problem of faecal/fungal build up in the traps.

The small traps were replaced by 12 fl oz disposable glass bottles from which the bottom had been cut. 53mm rubber bungs were used instead of corks, providing an adequate seal without additional use of sealing compounds. The trap could therefore be stripped down for cleaning purposes. It was not positioned directly beneath the tanks but moved to the front of the system, being more accessible in that position. A simple valve was made from 5 ml disposable plastic syringes and incorporated in each outlet line (Fig 32). Thus the trap could be closed off during feeding, when uneaten food could be removed by the side arm, and during faecal collection to prevent the water in the tank rushing in to the trap causing upwelling as described previously. The outlet from the trap was via a swing arm providing the water levelling device previously described. The waste discharge, however, was to an open 4" gutter which could not block and which could be cleaned. Rubber connectors were inserted in the outlet lines before and after the trap, so that the trap could be bypassed completely by a simple disconnection and reconnection of the tubing, as shown in Fig 32.

The neck of the trap had a $\frac{1}{2}$" open end from which faecal material could be drained very much more easily. A simple valve was again made from 5ml disposable plastic syringes (Fig32) and inserted into each bottle neck. The valve could be opened easily using one hand and very little force, leaving the other hand free to hold a collection vessel. The traps drained into a filter funnel for direct collection of faeces if desired. In this case water from the trap was discharged directly to the lower drainage system of experimental tank system 2 by connecting a hose to the filter funnel. The tank could be completely emptied to the drains in this way when desired.

Finally the air lift system was changed slightly. 6 mm. tubing replaced the $\frac{1}{4}$" previously used and air tube was passed into this via a T-piece, as shown in fig32. The water flow into the tank from the air-lift was very much smoother because of the larger bore.

The nylon covers were replaced by $\frac{1}{8}$" diamond netlon clipped around the circumference of the tank as shown in Plate 10.

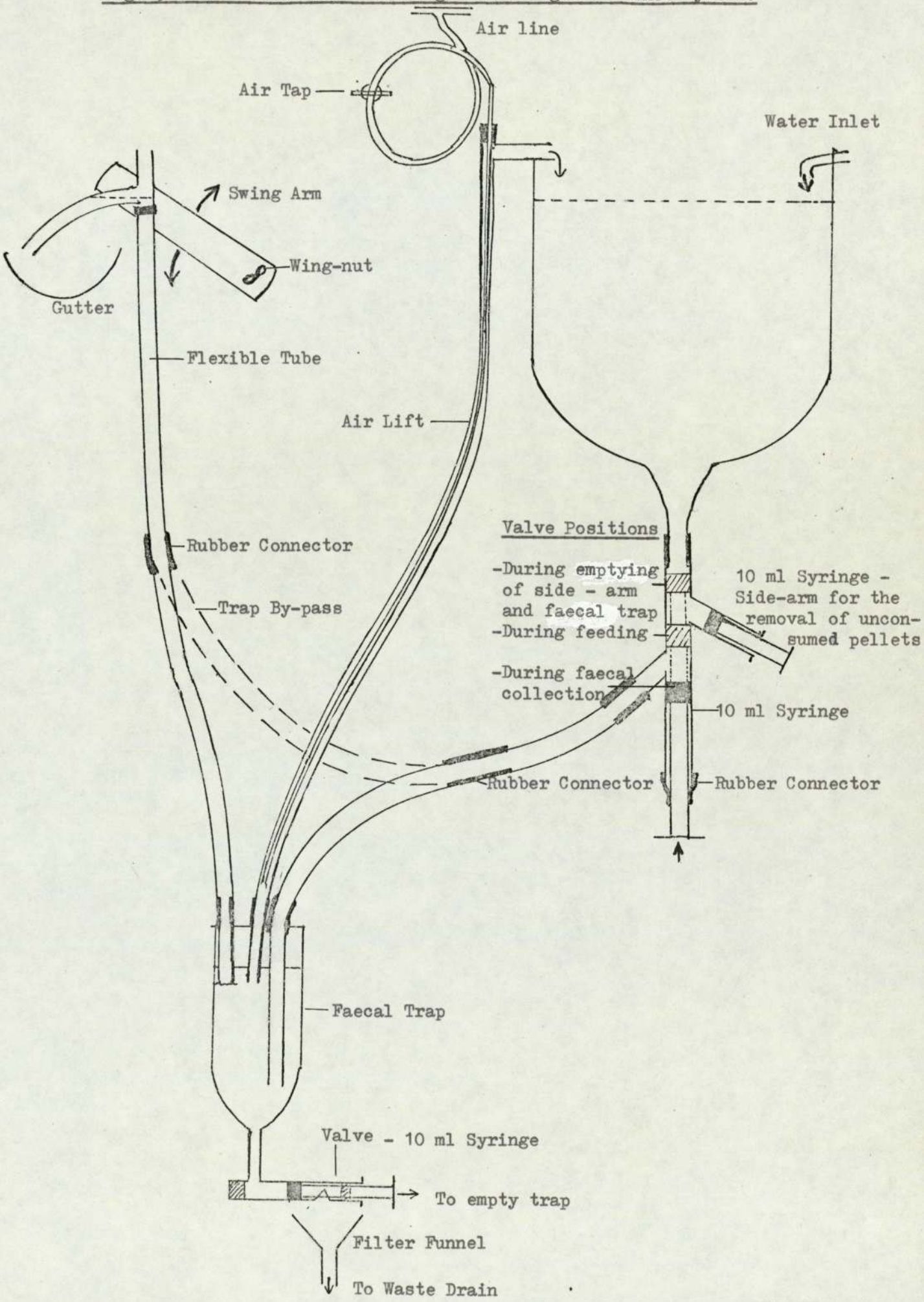
The whole system was very much improved providing greater ease of operation and increased working efficiency.

Experimental Tank System 2

The second system installed in the laboratory was of a very much simpler design, incorporating 18" diameter circular tanks with a capacity of 18 litres. The saucer-shaped tanks were positioned on the 'Bartangle' framework below the balance system, as shown in plate 11. The tanks were modified hatchery trays made of fibreglass, with a smooth inside, shelving to a 6" diameter, shallow 'sump' with a 1" central drain hole into which a length of P.V.C. pipe was fitted and sealed. A netlon 'plug' was inserted into the hole to prevent fish escaping via the outlet.

The drainage mechanism was a simple 'overflow swing-arm'. This

Fig. 32. Modified Oulet Plumbing for Nitrogen Banlance System.



consisted of a rubber hose looped from the tank's central drain to a P.V.C. T-piece which was bolted to one end of a length of metal strip. The other end of the strip was attached to the main framework of the system with a bolt and wingnut. The whole assembly thus acted as a swinging arm, enabling the water level in the tank to be set at any desired volume (plate 12). Water drained from the T-piece to 4" open gutters positioned beneath the tank system.

The inlet system was via the heat-exchangers immersed in the troughs as described earlier (fig 31). The individual tanks were supplied from a 'ring main' via $\frac{1}{4}$" inlet tubes terminated by a bend positioned in the tank so as to give a circular flow pattern, in an attempt to encourage an even distribution of fish within the tank and also to provide a self-cleaning system. The flow rate to each tank could be adjusted.

Light was provided to each tank from individual 24 volt, 12 watt bulbs supplied from a transformer as for the balance system. As a safety precaution, the bulb holders were mounted on timber enclosures as shown in fig 33. Each enclosure was mounted directly over the central drain hole of a tank, fastened to the hardboard 'shelf' between experimental tank systems 1 and 2. The bulb holders were assembled as shown in fig 33. The insertion of a rubber washer effectively sealed in the electrical contacts preventing damp 'shorting' the circuit.

At the light fitting, P.V.C. discs were used for the attachment of the tank covers which were cut in the shape of a cone from $\frac{1}{8}$" diamond netlon and attached to the tanks around the circumference using nylon line. An overlap was allowed at the front to allow easy access to the tank.

The system was relatively easily maintained. The tanks were self-cleaning. The rubber overflow tubes were squeezed daily to loosen accumulating faecal/fungal clumps and the swing arms lowered to carry the solids to the open gutters.

Fig. 33. Assembly of Light Fitting.

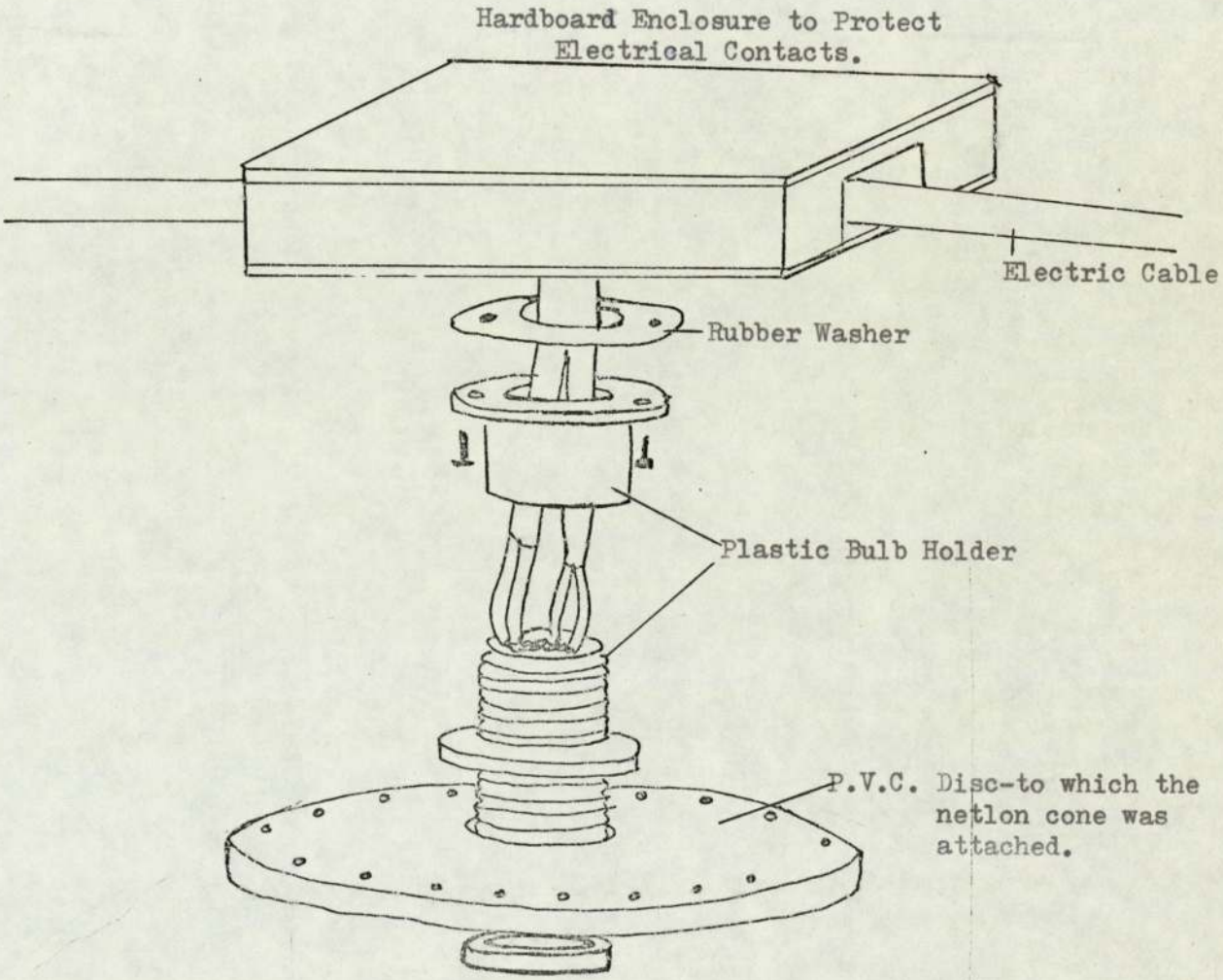


PLATE 11.

PLATE 12.

DEVELOPMENT OF MECHANISED FEEDING SYSTEMS

Introduction

Feeding techniques commonly used in feed evaluation trials were discussed in Section 3 and both controlled feeding and 'ad libitum' techniques have subsequently been used in separate trials as described in Sections 3 and 5 respectively. One problem common to both techniques used was that they were time consuming if food wastage was to be avoided and they required personal presence at least twice per day, seven days per week. The incorporation of a mechanised feeding system, complete with an automatic facility, into the fish laboratory was therefore considered to be desirable.

A number of criteria considered to be necessary for a feeding system designed for use in small-scale experimental feed trials were defined.

It should -

(1) be capable of dispensing a range of food sizes up to $\frac{1}{8}$" pellets without jamming.
(2) be capable of dispensing variable amounts of pellets, the smaller the number at the lower limit the better.
(3) be capable of dispensing pellets without causing excessive dusting.
(4) have a self-filling mechanism from a hopper holding sufficient quantity of feed for a minimum of two days at the maximum feeding rate envisaged.
(5) be capable of dispensing pellets automatically at any chosen interval up to six hours.
(6) have a manual overide to facilitate 'ad libitum' feeding.
(7) be easy to make with the minimum skill, using readily available, inexpensive materials.

Design of System

An air-powered feeding system, consisting of feed dispenser units

and control mechanisms, was developed.

Feed dispenser

The design of the dispenser unit was developed from an idea in which pellets were simply pushed out of a tube by an advancing plunger. When it retracted, pellets flowed from a hopper by gravity feed replacing those dispensed. The details of the design are shown in Fig 34 and a complete unit is shown in Plate 13. The unit was modified slightly for use outside as shown in Fig 35 and Plate 14. The method of construction is described in detail in Appendix 2.

Bottled compressed air, at approximately 25p.s.i., acting on the rubber seal of the power piston (Fig 34) supplied sufficient force to overcome the frictional resistance offered by the seal, the elastic force of the spring and the small weight of pellets in the body of the dispenser tube. The piston was consequently pushed outwards moving the plunger with it, thus dispensing the pellets from the open end. When the power was released the potential energy stored in the extension spring was sufficient to return the piston against the frictional resistance of the seal. As the plunger retracted, pellets flowed by gravity from the hopper into the main body of the dispenser tube. The quantity of feed dispensed during each operation was adjusted by altering the traverse of the plunger.

Automatic, controlled feeding

For automatic operation, the air supply was connected to the feeder units via solenoid operated valves, designed as shown in Fig 36. Construction and operation details are described in Appendix 2.

The important feature of the design is that when a valve is open, the air passes into a closed system, where one can fully utilize the power (25 p.s.i.) with very little loss of air volume from the bottle, and that

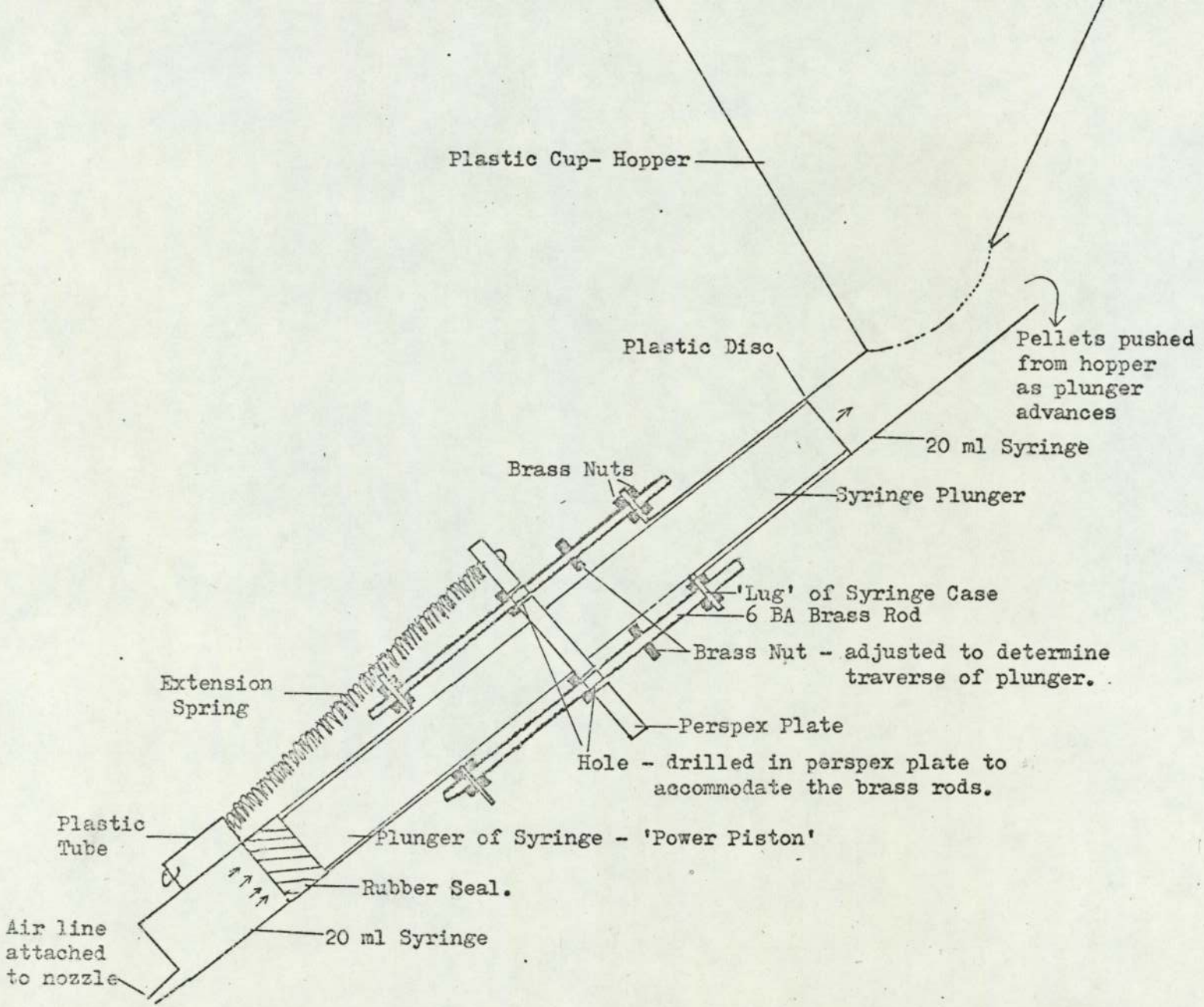
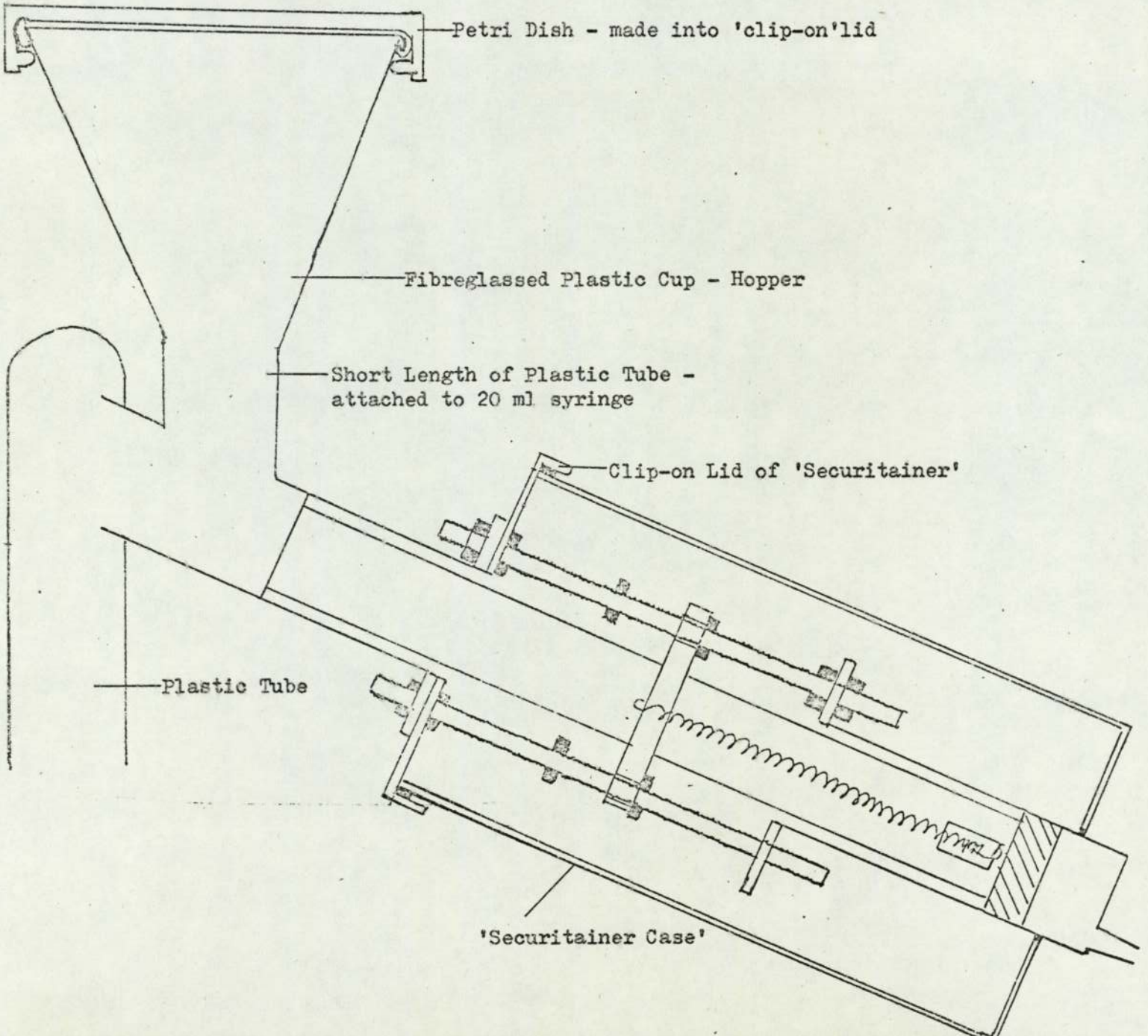


Fig 34. Feed Dispeser Unit.

Fig. 33. Feed dispenser unit - modified for use outside



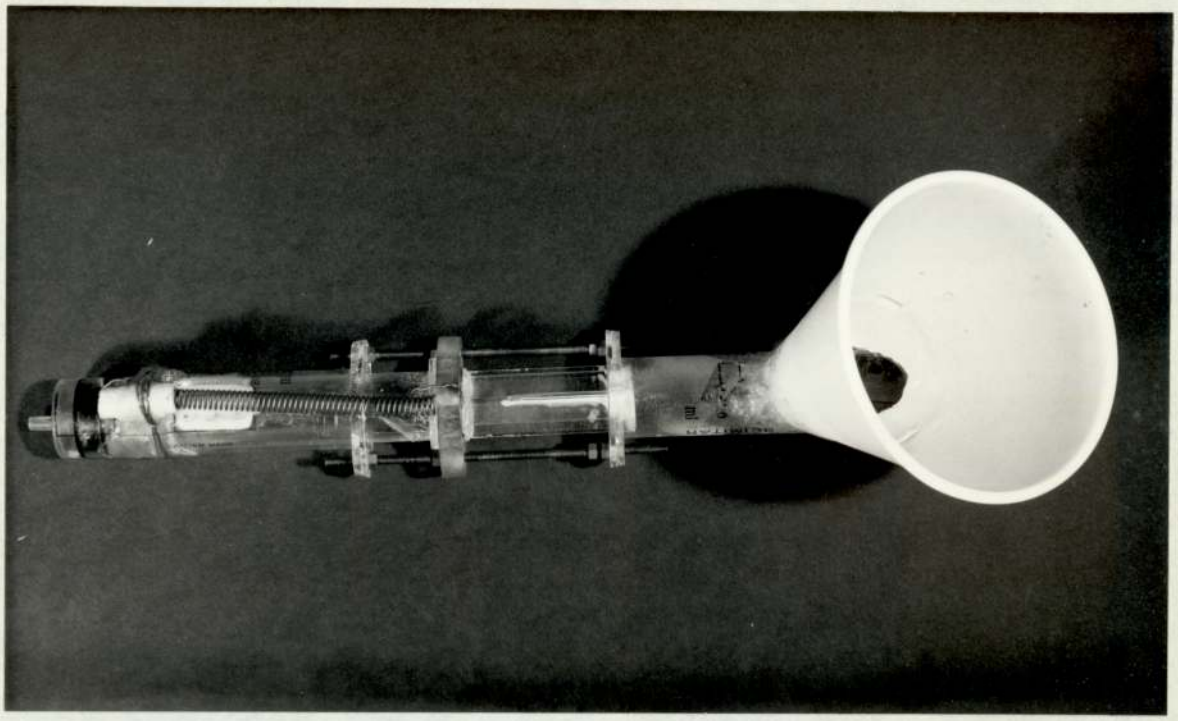


PLATE 13.

PLATE 14.

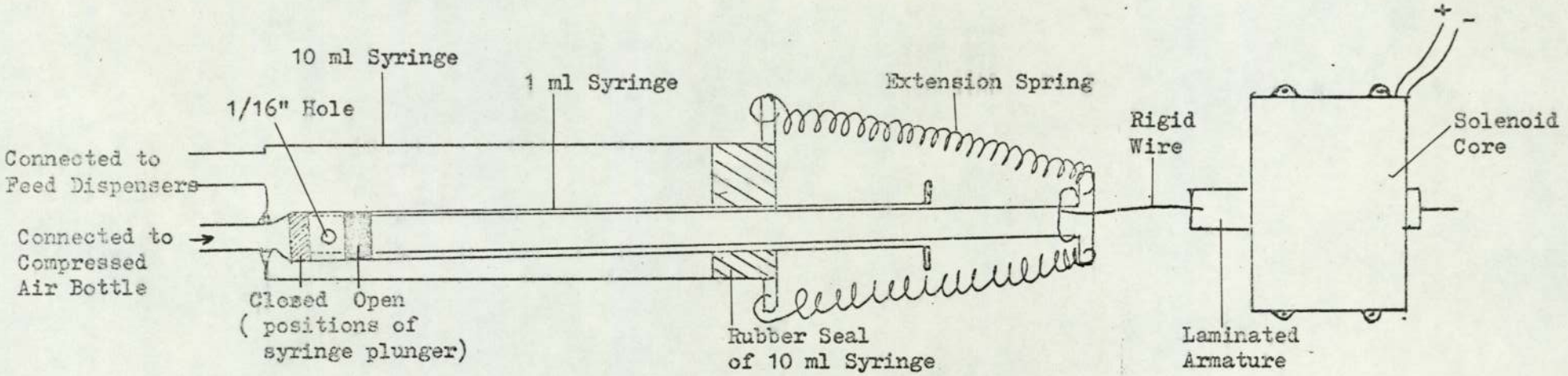


Fig. 36. Solenoid Operated Feeder Control Valve.

in its closed position, the valve allows the system to exhaust, when the extended spring on the dispenser could retract, allowing the feeder tube to refill with pellets.

Four valves were installed in a control box sited between the two parts of the balance system as shown in Plate 9 . The exterior and interior of the control box is shown in Plates 15 and 16 respectively. In the latter, the four solenoid valves can be clearly seen. Any number of feed dispensers could be connected into an air ring main supplied from each valve. All dispensers connected into a common ring main would discharge food simultaneously. Four valves were installed in order to give a versatile system with optional timed sequences.

Fixed timing sequences were obtained from a cam-timer rotating once per hour, whilst variable timing was achieved by means of an electromechanical timer. These two timers operated only during daylight hours, being controlled by a 24 hour Venner timer which supplied power to both the feeding and lighting systems for 12 hours only. A detailed account of the timing mechanisms complete with circuit diagrams is given in Appendix 2. All electrical components were wired in to the control box which was isolated as far as possible from water.

Tank System 2 in the laboratory was fitted with 16 dispensers, one for each tank. The dispensers were light enough to be clipped to a piece of metal strip bolted to the main 'Bartangle' frame of the system. The strip could be swivelled in a horizontal plane whilst the clip could be turned so that the feeder could be rotated vertically about the axis of the clip. In this way the position of the feeder unit could be adjusted with respect to the tank it served. The open end of the dispenser was normally pushed through a hole cut from the netlon cone some 9-12 inches from the water surface as shown in Plate 17. All of the 16 feeders could be connected into a single air ring main served from any one of the four solenoid operated valves. Alternatively, the feeders could be split into four groups, each group being connected to a different valve

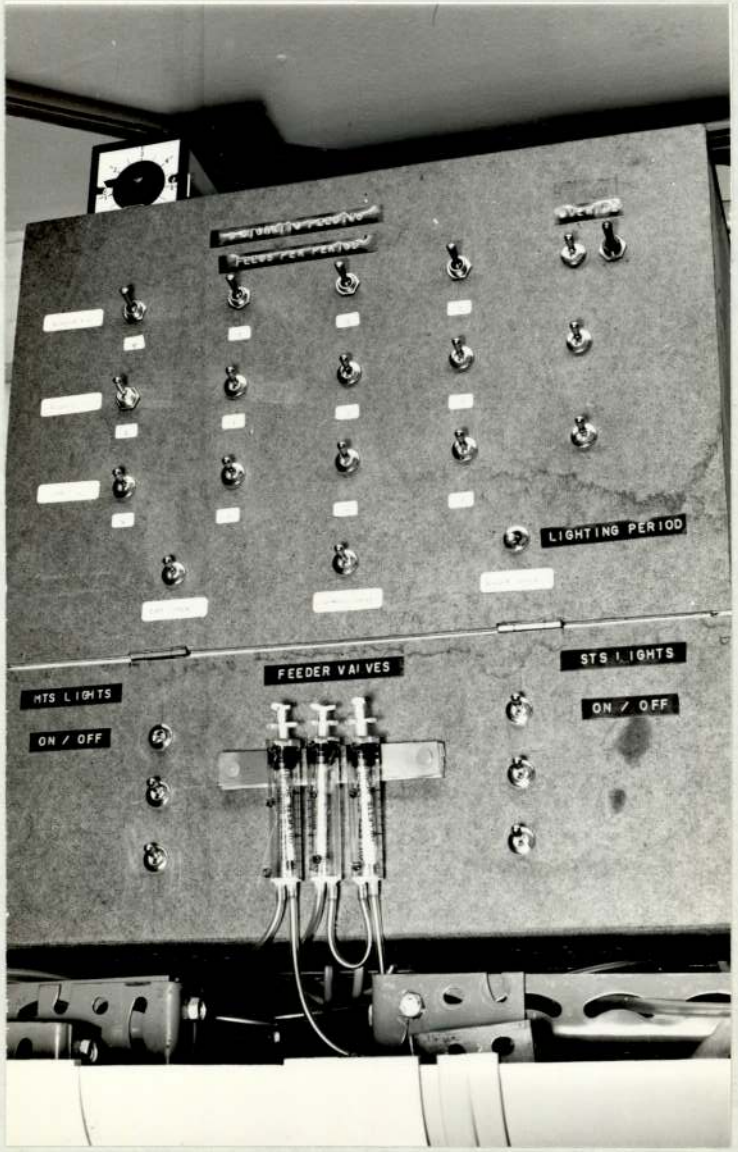


PLATE 15.

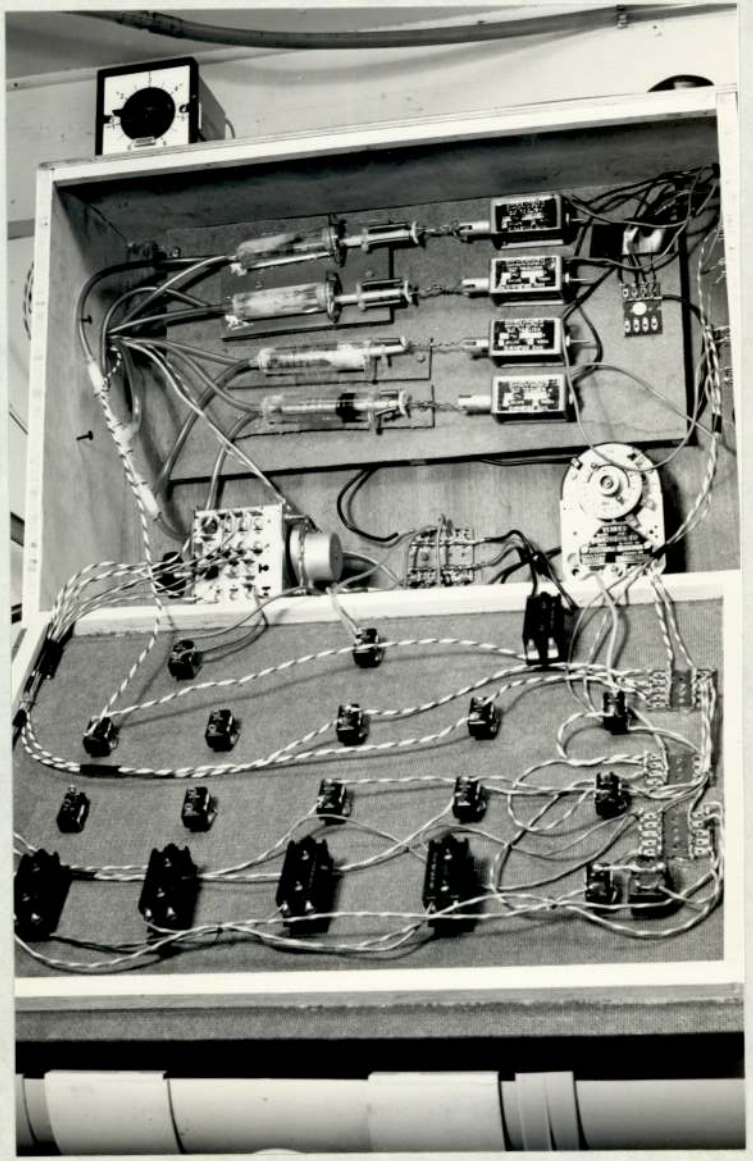


PLATE 16.

PLATE 17.

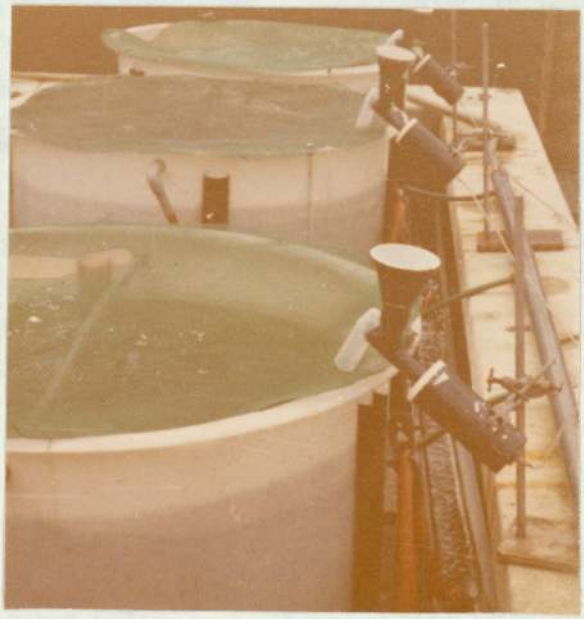


PLATE 18.

operated independently, providing different feeding regimes. The system was therefore well equipped for controlled, automatic feeding.

Since there were no electrical parts on the dispenser units, they could be positioned on tanks situated outside the laboratory. Furthermore, they could be positioned some distance from the laboratory as long as it was practicable to run an air line from the control box in the laboratory to the feed dispenser units. Thus the stock tanks situated in the open, some 20 yards from the laboratory, were fitted with automatic feeders, as shown in Plate 18. The feed dispensers used in this case were those modified to protect the mechanism and the feed from rain and dirt, etc.

Mechanised 'ad libitum' feeding

Whilst the feeding system installed in the laboratory was ideal for experiments in which feeding was controlled, or indeed during the pre-experimental period when fish were being adapted to the experimental environment, there was no facility for operating each feeder independently as was required for the 'ad libitum' feeding technique. This was rectified, however, by incorporating a hand-operated valve into the air supply line between the ring main supply and each feed dispenser. When air pressure was allowed into the ring main, any feeder could be operated manually, simply by opening the appropriate valve. Each valve was located by the tank it served, so that the fish could be observed whilst feed was being dispensed from the feeder. One such valve is shown in Plate 19 and Fig 37. The beads prevented the plungers from being blown out of the valves when the air pressure was on.

In order to allow air into the ring main, it was necessary to operate the appropriate solenoid valve, using, for example, the manual overide switches. However, to protect the solenoids from excessive use during 'ad libitum' feeding, a set of three manually operated master control valves were incorporated into the design and positioned on the front of

PLATE 19.

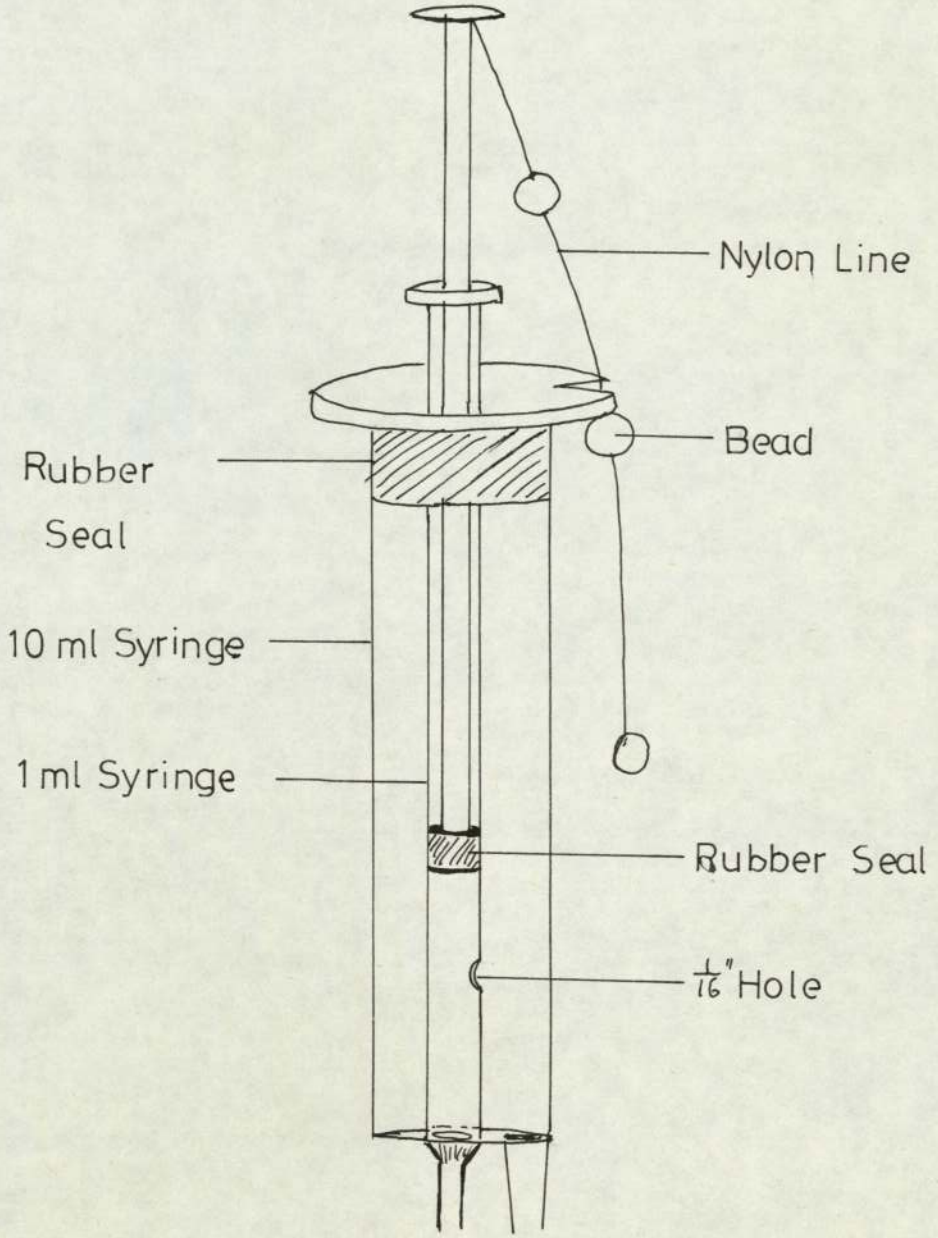


Fig.37. Hand Valve.

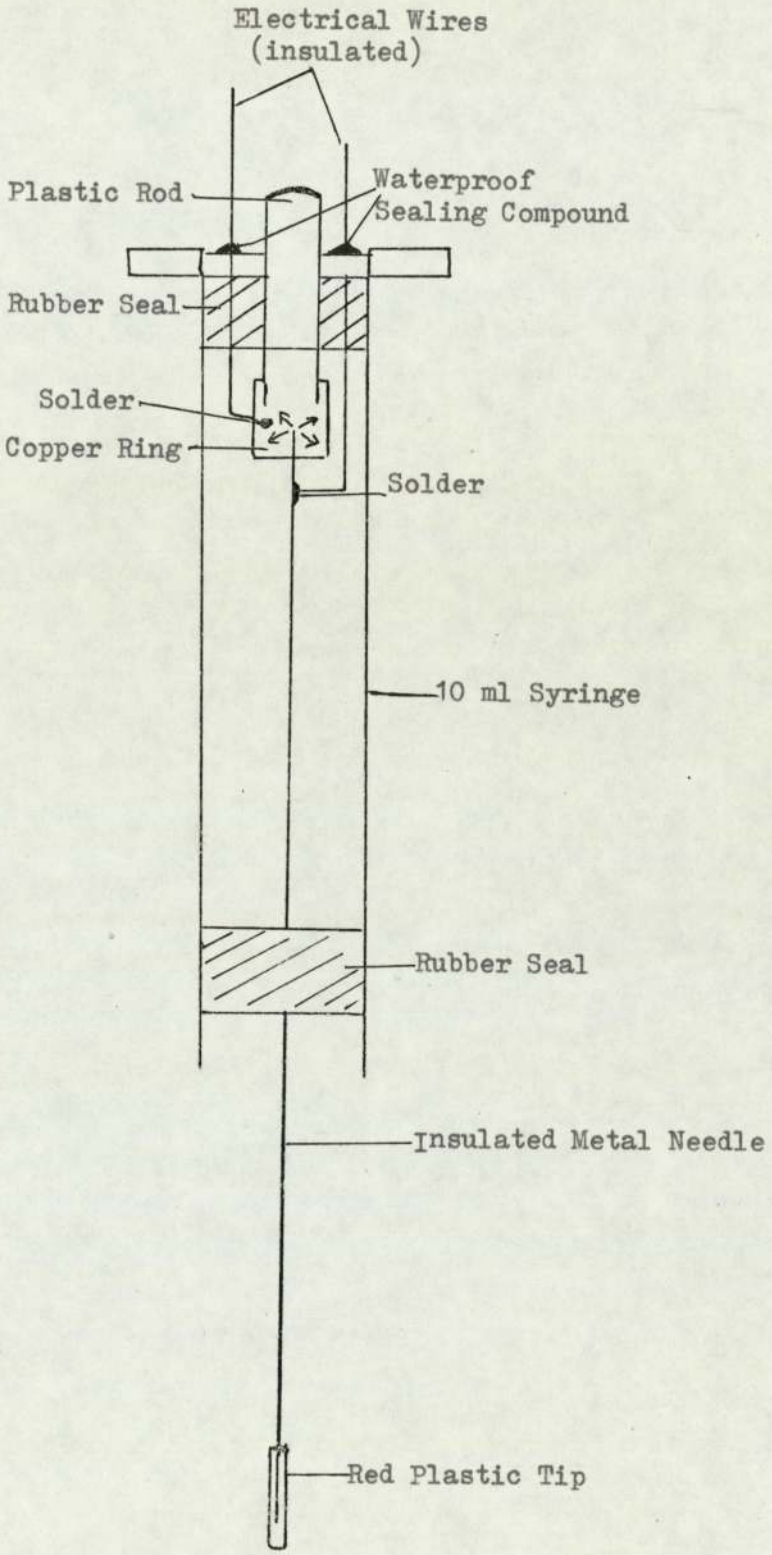
the control box as shown in Plate 15. The valves allowed the solenoid valves to be by-passed. The detailed function of the three valves is described in Appendix 2 along with the sequence of operation. The advantage of using the system for 'ad libitum' feeding compared with hand feeding using the techniques described previously, was that the time taken to feed a number of tanks could be substantially reduced. Generally fish in all tanks take some food. Thus with all the feeder valves open, food could be dispensed to all tanks at one time using the manual overide on the box. This could be repeated several times. When necessary, the air supply to the feeder supplying any particular tank, e.g. when fish were reaching the point of satiation, could be shut-off by closing the appropriate feeder valve. Fish in all tanks would progressively reach this point. Thus, with all individual feeder valves closed, the control valves were changed over, air therefore by-passing the solenoid-operated valves and passing directly into the ring main. Each feeder valve was then operated in turn as many times as was required to complete the feeding.

Demand feeding

Demand feeding has been described by Adron et al. (1973). He had used the principle to examine various aspects of the feeding behaviour of rainbow trout. He had shown that rainbow trout could learn to make contact with a switch positioned in the water, in order to obtain food.

The air-powered feeding system was modified to incorporate the demand feeding principle. The feed dispenser unit was retained. Each feeder required an independent solenoid-operated valve for demand feeding but the valve design was unchanged. A simple switch was designed as shown in Fig 38 The details of construction are given in Appendix 2. The electrical contacts were isolated in a sealed tube allowing the switch to be used safely outside the laboratory. When contact was made, a relay was actuated. This operated a counter in addition to the solenoid valve which was connected to the feed dispenser

Fig. 38. Demand Feeder Tank Switch.



unit in the normal way. A monostable multi-vibrator circuit was incorporated into the circuit design in order to open the air valve for a 1 second time interval to ensure operation of the feed dispenser. Details of the electrical circuit are described in Appendix 2. The circuitry was developed in this instance for two demand feeders. The electrical components, including the solenoid operated valves, were incorporated into a control box. An air operated pen recording system was also incorporated into the design. Details are described in Appendix 2. The chart recording system and control box are shown in Plate 20.

The demand feeding system was operated successfully in the three foot diameter stocking tanks using Cooper Nutrition Products 'Beta' Floating Trout Pellets (Size No. 4) during the summer of 1973 when the water temperatures were relatively high $14^{o}C$-$17 \cdot 5^{o}C$. During this period, the feeding rates were comparatively high and fish fed voraciously. Seventy rainbow trout (15+cm) were given an opportunity to adapt to the feeding regime. The behaviour and learning pattern of the fish confronted with such a system has been fully described by Adron et al. (1973).

The approach of the fish to the red trigger tip changed with time from a vigorous 'attack' to a gentle push. A trained fish about to operate the trigger is shown in Plate 21.

The 'ad libitum' feeding technique used previously could be carried out more conveniently using the mechanised feeding system, but the method was still time consuming and required personal presence. Furthermore, one could not be sure that feed intake was as much as it would have been if fish had been allowed to feed at all times. Demand feeding, however, allowed fish to feed continuously and may be considered to represent true ad libitum feeding. The feeder design had been successfully modified for use as a demand feeding system and it was therefore appropriate to evaluate the technique and to determine the feasibility of using it routinely in small-scale feeding trials.

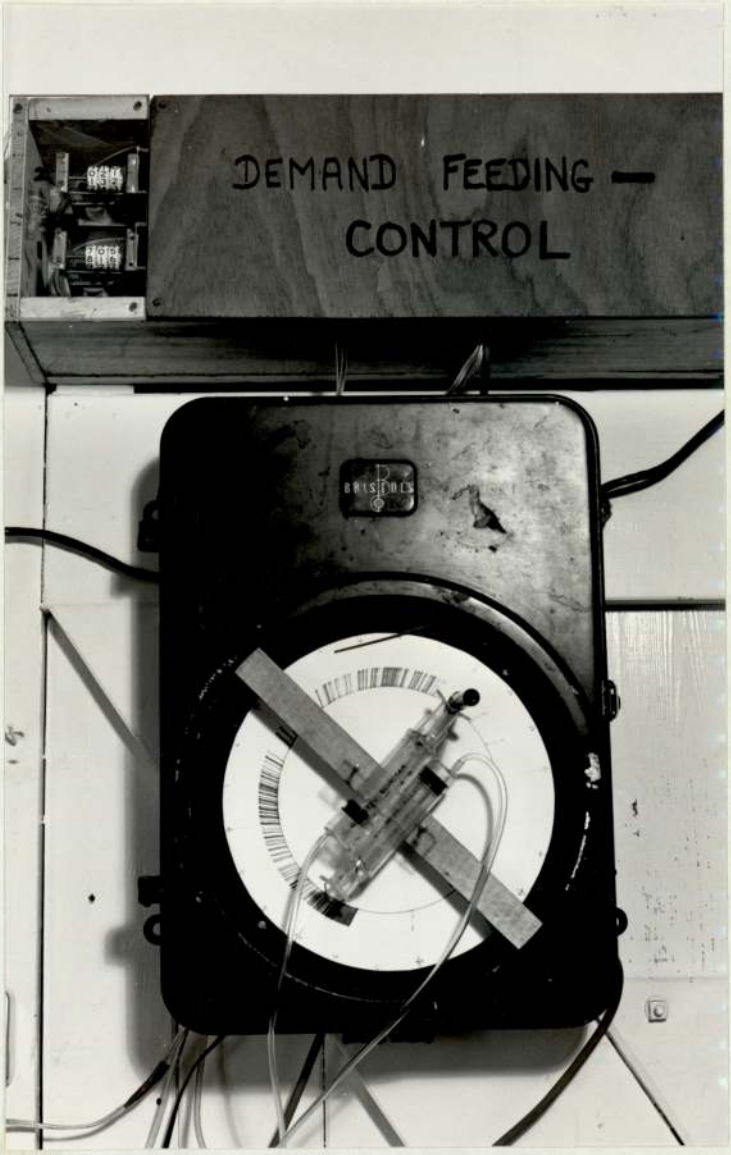


PLATE 20.

PLATE 21.

Observations With Demand Feeding

1) Effect of light periodicity on feeding rhythm

Introduction

Adron (1973 et al.) had shown that trained populations of rainbow trout maintained in tanks supplied with artificial light between the hours 08.00 and 20.00 fed rapidly during the first hour after the presentation of the demand trigger at 09.00hrs. Feeding rate then declined until the late afternoon when there was a small but consistent increase in feeding rate prior to removing the demand feeder at 16.00hrs. When the fish were subsequently allowed to demand feed in continuous light, a fairly regular pattern of feed activity was established after about ten days, when peaks of feeding activity occurred at about 8 hour intervals. Adron suggested that the self-feeding rhythm, with bursts of activity at approximately 8 hour intervals, may be related to the overall rate of gastric evacuation. He therefore suggested that the choice of a 7 hour feeding period in the controlled 12 hour lighting experiment was not ideal. Although there was a consistent increase in feeding activity towards the end of the period, this may have been more marked if the fish were allowed a longer feeding period.

It seemed appropriate to repeat Adron's experiment using a longer feeding period in the controlled artificial lighting conditions of the laboratory and to compare any established rhythm in this circumstance with that established for a similar population of fish maintained in similar conditions outside the laboratory in normal daylight.

Method

70 'trained' fish were divided into two populations of approximately equal weight. One population of 35 fish was placed in a 3' diameter tank within the laboratory. The tank was supplied with light from an

individual 12 watt light bulb situated approximately 2'6" above the water surface. Extraneous light was kept from the tank by a black plastic curtain surrounding it. The lighting was of 12 hours duration: 08.00 to 20.00hrs. The laboratory was in complete darkness during the hours 20.00 to 08.00hrs.

The second population of 35 fish was placed in an identical tank outside in natural daylight. During the experimental period, dusk was approximately 22.00hrs. and dawn approximately 03.30hrs.

Both tanks were supplied with tap water at the rate of approximately 4litre/minute. The tanks were fitted with a demand feeder and the trigger in each tank was placed in the same position relative to the water inlet position. The trigger was placed 6" in front of the feeder relative to the fish which lined up against the direction of flow. The feeders were filled daily with Cooper Nutrition Products' 'Beta" Floating Trout Pellets, size no. 4.

At the end of a preliminary period of seven days in which the fish became adapted to the environmental conditions, both populations were weighed. The temperature was recorded and the feeding rhythms were recorded during the following 12 days using the air-powered recording system.

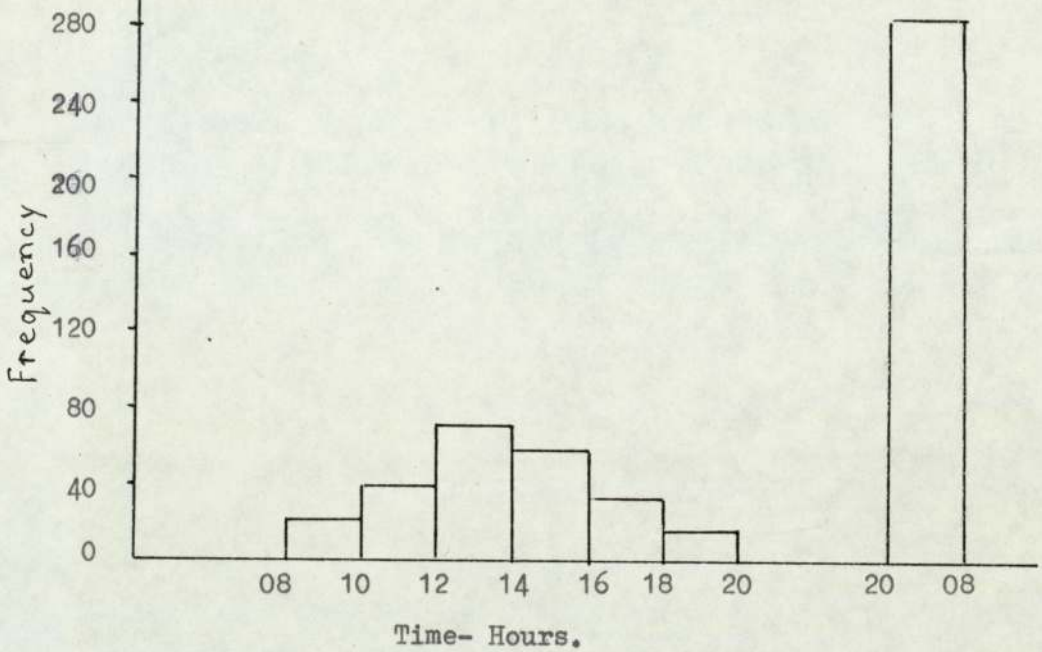
Results

Weight of fish in laboratory (lighting - 12 hrs. on/off) = 2580g
Weight of fish outside (lighting - natural daylight) = 2590g
Temperature = 15°C

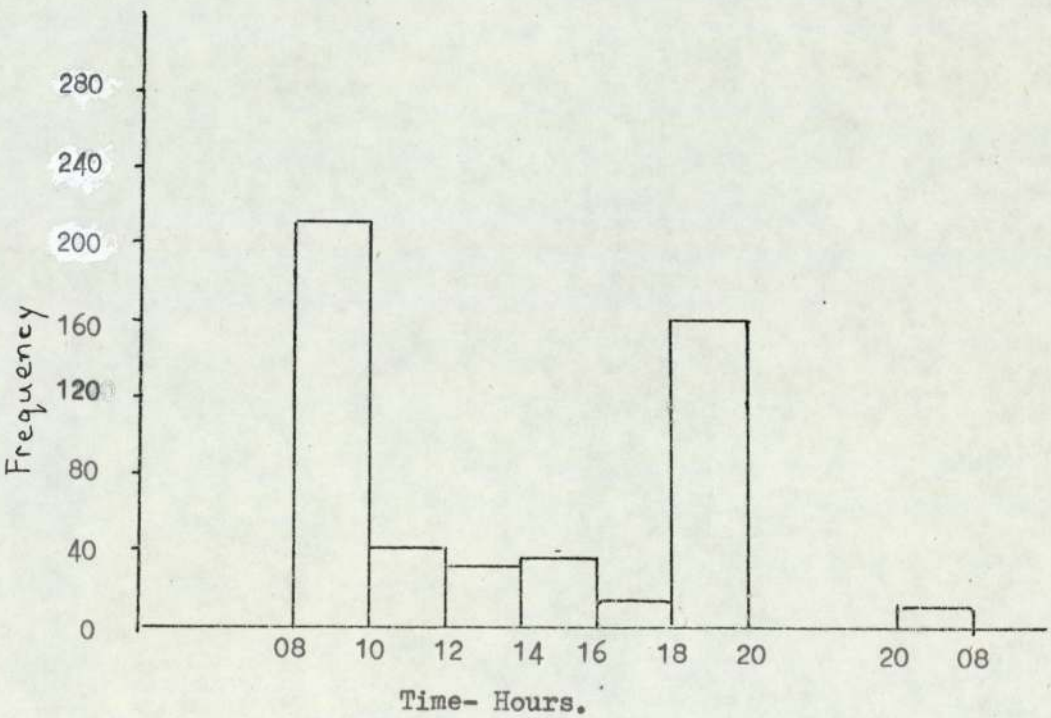
Typical feeding rhythms for the two lighting conditions are shown in Fig 39. The frequency of contacts during a 12 hour period, 08.00hrs. to 20.00hrs., recording at two hour intervals, is shown in Fig 40 along with the total number of contacts for the remaining 12 hours.

Fig. 40. Demand Feeding Experiment.

Effect of Light Periodicity on Feeding Frequency.



NATURAL LIGHT



CONTROLLED LIGHT

In the natural daylight conditions, feeding was continuous throughout the daylight hours although the heaviest feeding appeared to occur at dusk and dawn. The dawn peak was most noticeable.

In the laboratory, feeding was also continuous during the hours of light but two very noticeable peaks occurred between the hours 08.00 - 10.00hrs. and between the hours of 18.00 and 20.00hrs., that is during the first and last two hours of light.

The number of contacts and the weight of food dispensed was similar for the two populations in the different lighting regimes. The rate of food consumption was about 2·4% body weight/day. There was no observable food wastage. The fish appeared to consume the food immediately after it was dispensed.

Discussion

It was interesting to note that in intensive conditions where food is not limiting, feeding activity increased at dawn and dusk in natural daylight. This is in agreement with observations on wild populations of trout in Scottish Lochs where the activity of trout has been shown to increase substantially during the twilight periods, possibly indicating increased movement associated with searching for natural food organisms. (Holliday, personal communication). Even more interesting was the fact that this same pattern developed in the artificial laboratory light conditions, where dawn and dusk were not artificially simulated, the light coming on to full intensity at 08.00hrs. and switching off to complete darkness at 20.00hrs.

In the natural light conditions the dusk and dawn peaks occurred at approximately 7-8 hour intervals which, at first sight, would appear to confirm Adron's belief that feeding activity is associated with gastric evacuation time. However, although there was a general increase in the number of contacts between 10.00hrs, and 04.00hrs., there was no

clear evidence of a marked peak of activity about midday equal in magnitude to that of the dawn and dusk peaks. The feeding activity may be related to an inherent biological rhythm controlled by light and dark phases of the day, feeding activity being maximal during the first and last hours of light. If this is so, the 7-8 hour interval between the dawn and dusk peaks in the natural light conditions could be coincidental.

The results in the controlled light conditions clearly confirm Adron's observations that very active feeding occurs in the first two hours after presenting fish with feeding opportunity. In this instance, the feeders were not removed but remained in position the whole 24 hours. It was, however, unlikely that the fish could see the triggers during the hours of darkness and, although there were occasional discharges during this time, these would be the result of accidental contact. Feeding became possible as soon as the lights came on. It is unlikely that this increased activity is associated with external influences.

The second peak in feeding activity did not occur 8 hours after the commencement of the first as Adron had predicted based on gastric evacuation time, but in this case occurred some 10 hours after the first peak. Whilst sufficient time had elapsed for gastric evacuation, this observation possibly confirms the existence of an inherent biological rhythm, in which feeding activity is maximal during the first and last two hours of light, and that the fish in the controlled lighting conditions have adapted their feeding habits accordingly.

It was further interesting to note that the fish population in the laboratory appeared to consume as much food in the 12 hours light as the paired population outside were able to in the 19 hours natural light. The intensity of feeding in the first and last hours of light was such that they seemed to make up for any deficit.

Such observations are not only of academic interest but, if reproducible, could be of tremendous practical importance to the fish farming situation where optimum feeding regimes and rhythms are not well established. The experiments were not continued for long enough and quantitative measurements of feed consumption were made on too few occasions to place any significance on the results. It would be interesting to determine the effect on consumption of reducing the lighting period progressively from the 20hrs. to 4hrs., or indeed of having split light/dark periods.

2) Demand Feeding as a Measure of Appetite

Introduction

A major disadvantage of controlled feeding was that it took no account of differences in palatability between diets of varying composition. Adron et al (1973) had shown that rainbow trout showed a highly selective preference for a particular taste or odour of their diets. Only small differences in appetite were detected in the experiment in which 9 compound feeds were evaluated, as described in Section 5, and these may not have been due to difference in palatability. Nevertheless, since the demand feeding system had been used by rainbow trout without any evidence of food wastage, it seemed possible to use the system to measure palatability and to detect if changes in diet composition affected appetite.

Method

Two populations each of 40 individual rainbow trout, mean weight 47·8g and 47·9g respectively were trained in separate 100 litre circular polypropylene tanks each fitted with a demand feeder placed in a similar position and diametrically opposite to the inlet pipe. In each case the trigger was placed 6" in front of the feed dispenser relative to the fish, assuming that they line up against the direction of flow. The flow rate

in each case was adjusted to 3litre/min. The tanks were situated outside in natural light conditions. Being December, the daylight period was short, approximately 8 hours, and the ambient water temperature was $7^{o}C$.

The feeder triggers were lowered into the tanks at 09.30hours and lifted at 16.30hours daily, feeding opportunity being restricted therefore to daylight hours only. The food was Cooper Nutrition Products' 'Beta' floating trout pellets.

After 14 days feeding on the floating pellets, the fish were weighed. No access to food was permitted on the weigh day, being the 15th day of the experiment. On the 16th day, the food hoppers were filled with pellets of formula C09 (pg103) prepared by the wet method described in Section 4. This was a sinking food, the pellets being approximately $\frac{1}{8}''$ diameter and approximately $\frac{1}{8}''$ long. The experiment was continued for 6 days on this feed, after which time it was hoped that diets of varying composition could be tested.

Results

The feeding response was immediate and there was no indication of a progressive increase in the frequency of trigger contact. It was not clear at what stage the fish 'learned' to correlate pressing the trigger with the food reward, if indeed they did. Excessive food was present in the tanks on most occasions as little as one hour after lowering the trigger into the water. Unconsumed food generally floated on the surface for some time. The results for each population of fish are expressed in Table 67. The frequency of trigger contact and food dispensed during the 7 hour daylight period is shown for each of the first 2 weeks, together with the mean quantity dispensed for each trigger contact.

The quantity of food dispensed daily was reasonably consistent and

there was little difference between the results for the two populations of fish. The mean quantity of food dispensed daily for the two populations of fish represented 2·28% and 2·51% of the body weight at the beginning of the experiment and the food conversion ratios for the two populations for the first 14 days were 5·06 and 5·86 respectively. The frequency of trigger contact and the quantity of food dispensed daily increased when the food type was changed from Cooper Nutrition Products' 'Beta' floating type to a sinking pellet produced in the laboratory. This probably reflected the difference in the density of the two feeds. Again there was a considerable excess of food which remained unconsumed.

The capacity of the tanks used in this experiment were only half that of those used the previous summer during the experiment on light periodicity and feeding rhythm. However, it was unlikely that this was responsible for the excess of food dispensed since the food wastage continued when the fish were moved into the 200 litre tanks fitted with demand feeders, as used previously.

Discussion

In these conditions, in which the quantity of food dispensed was obviously excessive to that consumed, it was not possible to continue the experiment to determine if the demand feeding principle could be used to show differences in the palatability of diets of varying composition.

It was interesting to note the feed quantity dispensed bore no relation to that consumed, a situation which differed from that in the summer months, where food consumption was complete. According to the feed tables, the consumption of fish at 7^{o}C would be expected to be approximately 1·2% body weight/day which is considerably less than that required during the warm summer period. However, feed was dispensed at approximately twice that level. This would account for the excess quantities of food which remained unconsumed and the poor food con-

version ratios obtained during the first 14 day period.

The smaller 100 litre tanks used in this experiment and the comparatively higher stocking densitites could not be implicated as the cause for the food wastage since, at the end of the experiment, the fish were transferred to 200 litre tanks where food wastage was also apparent.

General Conclusions

A feeding system which met all the design criteria listed on page had been developed.

The feed dispensers were easily constructed from readily available, cheap materials. They were light but relatively sturdy. The basic design was simple enough to allow modification of dimensions to suit individual requirements. In this case the units were successfully used for all granule sizes of feed and for $\frac{1}{8}$" pellets. Larger units can be made to hold larger quantities of food or to dispense larger pellet sizes. The quantity of food dispensed could be finely adjusted, allowing very small quantities of granular feeds, or small numbers of pellets, to be dispensed at the lower limit and up to several grams of feed at the upper limit. Furthermore, there was no evidence of excessive dusting of pellets during its operation.

Any number of feeders could be operated automatically from one simple electrical circuit, requiring only one solenoid, therefore keeping costs to a minimum. Further, the feeders could be operated remotely from the control box allowing for the complete isolation of the electrical components. The system was therefore safe to use in damp conditions. An automatic system of this design was used successfully in the laboratory for 18 months with minimal maintenance.

In addition to the automatic feeding facility, the use of air provided

an opportunity for mechanisation of feeding in times of power failure or when using an 'ad libitum' technique, in which fish were being fed to satiation several times a day.

Conversion of the automatic feeding system to a demand feeding system had also been shown to be feasible. Unfortunately, however, each demand feeder required an independant solenoid incorporated into a monostable multivibrator circuit. One of the main advantages of an air operated feeding system was that one solenoid and electrical circuit could operate any number of feeder units. This advantage was lost with the demand feeders and consequently the cost to fit any one of the 16 tank experimental systems would be considerably increased.

Although populations of rainbow trout could clearly obtain food using the system, the quantity of food dispensed during the colder periods was excessive to that actually consumed. In such conditions the system could not be used for comparative feeding trials. Temperature records for the previous two years had shown that the temperature of the mains water remained below $12^{o}C$ for 8 months of the year. If the food wastage was related to temperature, demand feeding would be useful only for limited periods of the year unless temperature control was possible.

Furthermore, a high percentage of food wastage was also observed in small tanks, 10-20 litres, stocked with 20 individual rainbow trout, and fitted with the demand feeding system, even in optimum temperature conditions. It would not be practical, therefore, to use this system on any of the tank systems designed for comparative feeding trials.

The most appropriate feeding method for the conditions within the laboratory and for the type of nutritional work conducted, appeared to be the mechanised 'ad libitum' technique. The automatic feeding facility was convenient for feeding stock fish and fish adapting to the experimental tank systems prior to experiments.

SECTION 7.

METHODS OF EVALUATING PROTEIN QUALITY.

INTRODUCTION

The nutritive value of a protein is determined by its ability to supply the amino acids essential for the metabolism and growth of the animal under study. Several biological methods are used to determine protein quality but most are tedious and time consuming, and chemical methods are therefore frequently used for routine estimations. Chemical and biological procedures in common use, and their relative merits, have been discussed fully in reviews by Block and Mitchell (1946), Albanese (1959), Rice and Beuk (1953), Carpenter (1963), Morrison (1964), Hegsted and Chang (1965), Morrison and Rao (1966), Woodham (1968), McLaughlan and Campbell (1969), and the Agricultural Research Council (1970), among others.

CHEMICAL METHODS

The concept of a chemical score based on the quantity of essential amino acids present in a protein was introduced by Block and Mitchell (1946). The method involves the quantitative determination of the essential amino acids present in the protein under study, the value being expressed in g per 16g N. The quantity of each amino acid is then compared with that present in whole egg protein which is known to be very efficiently utilized by several animals. The amino acid in greatest deficit is taken as the first limiting amino acid and 100 - % deficit of that amino acid is the chemical score.

An alternative method of estimating protein quality from the chemically determined amino acid values was proposed by Oser (1959). The method is based on the contribution of all the essential amino acids rather than the one in greatest deficit. The value, called 'the essential amino acid index', is defined as the geometric mean of the ratios of the essential amino acids in a protein relative to their respective amounts in whole egg protein.

Hastings (1969) lists values for chemical score and essential amino acid index of several feedstuffs used in fish diets and in some commercially available fish feeds. The range of values varied substantially, but tryptophan was reported as first limiting amino acid in most cases.

Typical amino acid values for the nine proteins used in the feeding trial described in Section 5 were similarly analysed. The values reported in Table 68 are the percentage deviations of each amino acid from the corresponding value in whole egg protein. The sulphur amino acids, methionine and cystine, were first limiting in all proteins except herring meal in which isoleucine was first limiting. Hastings (1969) reported tryptophan as first limiting for herring meal and cotton seed meal. This clearly was not the case for the values used in this comparison.

Quantitative amino acid requirements have been reported in detail for only one species of fish, namely the chinook salmon. The requirements are discussed by Mertz (1972). Amino acid values reported for the nine proteins have been compared to the requirements reported for the chinook salmon as shown in Table 69.

The actual methionine requirement is 1·25 - 1·5 g/16g N in the presence of 2·5g cystine (Halver et al, 1959). The methionine requirement would therefore be met by the herring meal, cotton seed meal, BP yeast, ICI bacteria and Penicillium chrysogenum, if sufficient cystine was present. This would not appear to be the case, however, for any of these proteins and therefore the methionine requirement has been assumed to be higher. Assuming that cystine spares an equivalent quantity of methionine, the methionine requirement would be 4·0 g/16g N in the absence of cystine. The combined quantities of methionine and cystine would not appear to satisfy the requirement in any diet and these amino acids are likely therefore to be limiting. They are first limiting in all proteins except herring

meal, where there is a slightly greater deficit of arginine. Arginine is second limiting amino acid in yeast, bacterial and one of the mould proteins. Lysine is second limiting amino acid in groundnut and the algal protein.

Another important consideration is the relative quantities of leucine and isoleucine. Excess leucine can have an antagonistic effect on the isoleucine requirements by inhibiting growth. Similarly, high isoleucine can have a detrimental effect on growth when leucine levels are comparatively low. However, the ratio of the two amino acids does not appear to be too unfavourable in any of the proteins listed. High levels of valine, over 3·0% of the ration equivalent to 7·5% of the protein, also results in lower dietary efficiency. High levels of valine in Penicillium chrysogenum resulted in the highest EAA index value recorded, yet this quantity may be detrimental to performance.

The accuracy of comparisons based on amino acid values depend on the reliability of the chemical methods used for breaking down the protein into its constituent amino acids and their subsequent quantitative determination. The problems involved in this respect have been discussed by Block and Mitchell (1946) and Carpenter and Bjarnason (1970).

The methods now commonly used for amino acid determinations generally involve hydrolysis of the protein with strong acids or alkalis followed by colorimetric estimation of the amino acids following separation by column chromatography. Automated procedures for the colorimetric estimations are now routinely used. Although such procedures are more convenient and less time consuming, the basic problems remain. For example, during acid hydrolysis of whole foods, methionine and particularly cystine are liable to destruction and oxidation to various degrees. This would account for the absence of cystine values in many reports of amino

acid profiles and perhaps accounts for low values in others. The comparison and conclusions made above are therefore based on values which may not be accurate. Furthermore, variation in amino acid values determined by different laboratories on sub-samples of the same material are reported. Add to this the variation that can exist between different batches of feedstuffs and it is not difficult to see how different conclusions as to which amino acid is actually limiting in a feedstuff can arise. These points emphasize the caution that must be exercised when using such techniques to estimate protein quality.

Comparisons based on the salmonid requirements must also be treated with caution for the same reasons, but in addition it should be emphasized that the amino acid requirements reported thus far are specific for the set of conditions prevalent during the experiments in which the quantitative requirements were determined. A purified diet was used containing casein and gelatin supplemented with amino acids to give an overall amino acid profile similar to that for whole egg protein and the quantities of each amino acid determined are thus specific for the situation in which the remaining amino acids are balanced in that way. The balance of amino acids in the protein of practical feedstuffs may be such as to change the requirement for any one specific amino acid.

A further problem is that the method does not take into account differences in availability of the amino acids. The true value of a protein will be determined by the quantity of the amino acids available to the animal, which will be somewhat less than that determined after chemical hydrolysis. Nevertheless, Block and Mitchell (1946) showed a good correlation between chemical score values and protein quality determined by several biological assay techniques. They found a strong correlation between chemical score and both protein efficiency ratios and biological values of 23 feedstuffs as determined in the rat ($r = 0 \cdot 889$ and $0 \cdot 861$ respectively). There have been no such thorough

investigations in fish although Nose (1963) reported a positive correlation ($r = 0 \cdot 69$) between essential amino acid index and biological values for a series of proteins including casein, gluten and zein in rainbow trout.

BIOLOGICAL METHODS

Biological methods for estimating protein quality generally fall into 2 groups:

1) Those in which growth is measured as an index of protein quality e.g. the protein efficiency ratio (P.E.R.) method, originally proposed by Osborne and co-workers (1919), in which protein quality is determined by the ratio of the gain in weight to protein consumed, and the slope ratio assay method based on the regression of gain in weight on nitrogen intake (Hegsted and Chang, 1965).

2) Those in which nitrogen retention is measured as the index of protein quality e.g. the determination of the biological value (B.V.) using nitrogen balance techniques commonly described as the Thomas-Mitchell method (Thomas, 1909 and Mitchell, 1924) and the determination of net protein utilization (N.P.U.) by direct measurement of nitrogen retention by carcass analysis (Bender and Miller, 1953).

The P.E.R. technique is relatively simple, convenient and has been widely used. However, P.E.R. is affected by the level of protein inclusion in the diet, age, sex and strain of the experimental animals, length of assay, level and source of dietary calories and the method of feeding, as discussed by Morrison (1964). The method has been further criticised on the basis that the gain in body weight may not be of constant composition and that the method assumes all of the

protein consumed is used for growth. The most serious criticisms appear to be the latter two since standardisation of the assay conditions would reduce the variation caused by other factors. The technique commonly used involves a 4 week test in which rats are fed ad libitum with diets containing protein at a single level of 10%. The short duration minimizes the effect of changing body composition and the 10% protein level has been found to yield the most sensitive and valid results.

Methods involving measurement of nitrogen retention take into account changes in composition and the requirement of nitrogen for processes other than growth. The determination of the biological value of a protein may be determined from the equation:

$$B.V. = \frac{N_i - ((N_f - MFN) + (N_u - ENE))}{N_i - (N_f - MFN)}$$

where N_i = nitrogen intake

N_f = faecal nitrogen

MFN = metabolic faecal nitrogen (obligatory loss on a nitrogen-free diet)

N_u = excreted nitrogen

ENE = endogenous nitrogen excreted (obligatory loss on a nitrogen-free diet)

The procedures must be carried out under closely controlled conditions since values are influenced by the same factors as for the P.E.R. determinations. In view of this and the more tedious and time consuming nature of the procedures involved, the method is not generally applicable to the routine evaluation of foods.

An alternative method for determining protein quality by nitrogen retention was the N.P.U. method described by Bender and Miller (1953) and Miller and Bender (1955). The method involved the direct determination of nitrogen retention by carcass analysis and is somewhat simpler. N.P.U. is given as:

$$N.P.U. = \frac{N_b - N_{bo}}{N_i}$$

where N_b = body nitrogen content of animal fed test protein

N_{bo} = body nitrogen content of animals fed non-protein nitrogen diet

N_i = nitrogen intake

Bender and Doell (1957) reported that, under their experimental conditions, N.P.U. values were much more precise than P.E.R. values but this finding has not been confirmed by a number of other workers, as discussed by Morrison (1964).

APPLICATION OF BIOLOGICAL METHODS TO FISH STUDIES

There have been very few attempts to apply standardised methods to the determination of protein quality in fish. Nitrogen balance studies are complicated because of the necessity of collecting faeces in an aqueous environment. One problem is that soluble nitrogen leaches from the faeces in the collection vessel and contaminates the excreted nitrogen. Several methods have been described for estimating values for faecal nitrogen and excreted nitrogen (Tunison et al, 1942; Ogino & Chen, 1973; Smith, 1974). Methods for the estimation of metabolic faecal nitrogen and endogenous nitrogen excretion have been described by Gerking (1955), Nose (1967), Ogino & Chen (1973).

N.P.U. of a number of proteins has been determined in fish by carcass analysis by Nose (1961) and Cowey et al (1974). The method has fewer imponderables but is not without problems e.g. some species of fish do not readily consume a protein-free diet. Cowey et al (1974) used a low protein diet rather than a protein-free diet for the determination of N.P.U. values in plaice. N.P.U. was given by:

$N.P.U. = B - (B_k - I_k)/I$ where B and B_k are the total body nitrogen and I and I_k the nitrogen intakes of the fish on the test and low-protein diets respectively.

The relationship between N.P.U. and protein level has been described by Cowey and Sargent (1972). In plaice fed varying levels of freeze-dried cod, and carp fed varying levels of casein, N.P.U. is highest at dietary protein levels near the maintenance requirement and falls with increase in dietary protein. Cowey (1975) has further discussed results for B.V. and N.P.U. of a number of feedstuffs as determined in carp and plaice respectively. In these species of fish, differences in B.V. and N.P.U. between feeds of varying quality appear to exist even at high dietary protein levels. This result is in contrast with that found in mammals and Cowey suggests that it may be an expression of the high essential amino acid requirement of the fish.

Growth methods have been used to estimate protein quality in fish. Hastings (1969) has reported P.E.R. values obtained for a number of feedstuffs as determined by Shanks in chinook salmon. P.E.R. values for liver was the highest, followed by fish meal, casein, cotton seed meal and brewer's yeast. The values were 3·04, 2·65, 2·38, 0·91 and 0·85 respectively. He further reported values for casein at four different protein levels and three different levels of dextrin. P.E.R. varied with both protein content and energy level as shown for the rat. Optimum values for casein occurred at progressively lower protein levels as the energy content increased. Dextrin was likely to have 'spared' protein for growth, therefore accounting for the higher optimum P.E.R. value obtained for casein in the diets containing dextrin at the highest level.

Cowey and Sargent (1972) have also discussed the effect of protein level on P.E.R. Working with plaice, Cowey and co-workers found that P.E.R. varied with protein content in a manner not dissimilar to that obtained with mammals, although the optimum values were obtained at a much higher protein level. They have also discussed the results reported by Ogino and Saito (1970) for carp fed casein. In this case the pattern was different, P.E.R. decreasing

linearly as the protein level increased. Cowey and Sargent suggest that this may indicate a very low maintenance requirement for protein in carp, possibly due to the activity of micro-organisms in the alimentary tract.

CONCLUSION

There is a considerable need for confirmation that the amino acids indicated as limiting on the basis of chemical methods, are actually limiting in practical fish feeds. Determination of the protein quality of feeds using bioassay techniques on fish is necessary. Values obtained could then be correlated with chemically determined amino acids in an attempt to identify the limiting amino acids. A prerequisite to such comparative trials, however, is the establishment of clearly defined methods for routine estimations on a laboratory scale.

The facilities discussed in Section 5 were designed to evaluate protein quality using both nitrogen balance techniques and growth techniques simultaneously. However, standardization of the nitrogen balance technique proved difficult. Although the system could be worked much more efficiently than previously, in some of the tanks fish did not adapt too well to the system. There appeared to be an excessive amount of 'fin nipping' causing high mortalities and general stress. In some cases there was a diminished response to feeding resulting in loss of weight. Furthermore, fish did not readily consume a protein-free diet. In view of the difficulties and the fact that the methods were extremely time consuming, growth methods, involving measurement of feed intake and weight gain only, were considered to be more convenient, at least for initial screening experiments.

SECTION 8.

EVALUATION OF BACTERIA, ALGAE AND YEAST

IN SEMI-PURIFIED DIETS.

INTRODUCTION

There has yet been little attempt to establish a standardised method for the determination of protein efficiency ratio in fish. The 28 day experiment on diets containing 10% dietary protein routinely used to estimate protein quality in rats is unlikely to be applicable to fish in view of their higher dietary protein requirement. The effect of protein content on PER was therefore determined for bacterial, algal and yeast proteins in separate experiments conducted on rainbow trout. The apparent crude protein digestibility of these protein sources was further determined and finally a comparative assessement of the growth, gross conversion efficiency and protein conversion efficiency was attempted on trout fed diets containing fish meal, soybean meal, bacteria, algae and yeast.

(1.) EFFECT OF PROTEIN CONTENT ON GROWTH, GROSS CONVERSION EFFICIENCY AND PROTEIN CONVERSION EFFICIENCY.

Bacteria, yeast and algae were included in diet formulations as the sole protein component. Basal ingredients common to all diets were a salt mix (U.S.P. XVIII), a vitamin mix (Halver, 1969) and a lignosulphate binder (Trade-name Durabond). The single-cell proteins had been analysed previously, Table 37. The weight of each required to give approximately 20%, 25%, 30%, and 35% crude protein in the diet was added to the basal ingredients. Glucose and starch were then added to 100% in the proportions shown in Tables 70, 71, and 72.

In each series of diets the SCP replaced the glucose on a weight for weight basis. The diets were considered to be approximately isocaloric, assuming assuming assimilation coefficients of 0·8 for the SCP and 1·0 for the glucose.

Diet Preparation.

Each series of four diets was prepared at one time during the week preceeding the commencement of each separate feeding trial. The wet extrusion method was used as described in Section 4 of this thesis. The quantity of water added in each case was that which gave a suitable consistency for extrusion. The diets containing ICI and BP proteins were extremely pale and to facilitate ease of feeding during the experiments a red dye, permecol red, was added to the diet at a rate of 0·1%. This was added to the water prior tomixing with the dietingredients. The dye was not necessary with the diets containing algal protein, but it was included. All diets were air dried at room temperature.

Diet Analysis.

The moisture and nitrogen content of each diet were determined using the analytical procedures described in Section 5 (pg102). Gross caloric content of each feed was estimated from the values for the ingredients as determined by bomb calorimetry and reported at the foot of each table.

GENERAL METHODS.

A separate feeding trial was carried out on each set of diets.

Experimental Facilities.

The sixteen 18 litre tanks installed in the fish laboratory were used for all 3 trials. The system has been fully described in Section 6.

Experimental Animals.

For each trial 320 rainbow trout fingerlings, in the range 4-5",

were selected from the stocking tanks. All fish had been maintained on the standard commercial feed. Fish were selected as near as possible for uniformity of size and were randomly distributed into the tanks in lots of 20. The fish were allowed to acclimatize to the experimental facility for 14 days prior to feeding on the experimental diets.

3 tanks were allocated to each of the four experimental diet treatments and the remaining 4 allocated to the standard commercial pellet (CNP No. 4 Beta Floating Trout Pellets). The diet allocations were selected randomly. During the 3 days immediately preceeding the commencement of the experiment, the 12 tanks which had been allocated the experimental diets were fed on diet C09 (Table 35) to accustom them to a sinking pellet. They changed readily to this diet and they were easily able to take food from the bottom of the tank.

Environmental Parameters.

The water was supplied directly from the header tank. The flow rates were fixed as near as possible to 500ml/min. The water leaving the header tank was 100% saturated with oxygen and no additional aeration was needed. Oxygen was not considered to be limiting at any time during the 3 experiments.

The water temperature was ambient and recorded daily in the laboratory. The mean weekly temperatures for each experiment were as follows:

EXPT. WEEK.	1	2	3	4
PER 01) Bacteria experiment	12·0	13·0	14·5	15·0
PER 02) Algae experiment	15·5	16·5	17·5	16·5
PER 03) Yeast experiment	15·5	14·5	14·0	13·0

For each experiment the stocking and loading parameters at the beginning and end of the experiment were in the following range:

Experiment	Stocking Density gl^{-1} Start	End	Loading gl^{-1} min. Start	End
PER 01) Bacteria	15·1-16·3	17·2-25·3	542-588	618- 910
PER 02) Algae	19·7-22·6	13·7-43·3	708-816	494-1560
PER 03) Yeast	15·0-16·4	19·5-28·3	540-592	702-1018

At no time was ammonia likely to have been limiting. The effect of tank size and stocking density on growth could not be assessed.

Weighing.

Each lot of 20 fish was weighed in total on a 10kg Sauter electronic balance using a wet weighing method. A bucket containing approximately 5kg of water was 'tared'. Fish were netted from the experimental tanks, drained for a standard 12 second period and added to the 'tared' bucket of water. The weight was read off directly from the scale to the nearest gram.

Feeding.

Fish were fed to satiation twice daily, using the feeding system described in Section 6. A record of food consumption was kept. All diets had good water stability properties and in all cases the diet offered was consumed completely.

RESULTS.

The formulations and the relevant analytical data is shown in Tables 70, 71 and 72 as previously indicated.

The performance data for the trial in which the bacterial protein

was tested has been tabulated in Table 74. There were no mortalities during the 28 day trial.

The food consumption rate was low throughout the trial when compared to the commercial feed (Fig 41). The appetite has been quantified as the $\frac{\text{Metabolizable Energy Intake (kcals)}}{\frac{1}{2}\left[(\text{Weight}_{T_0}) + (\text{Weight}_{T_{28}})\right] \cdot 28}$

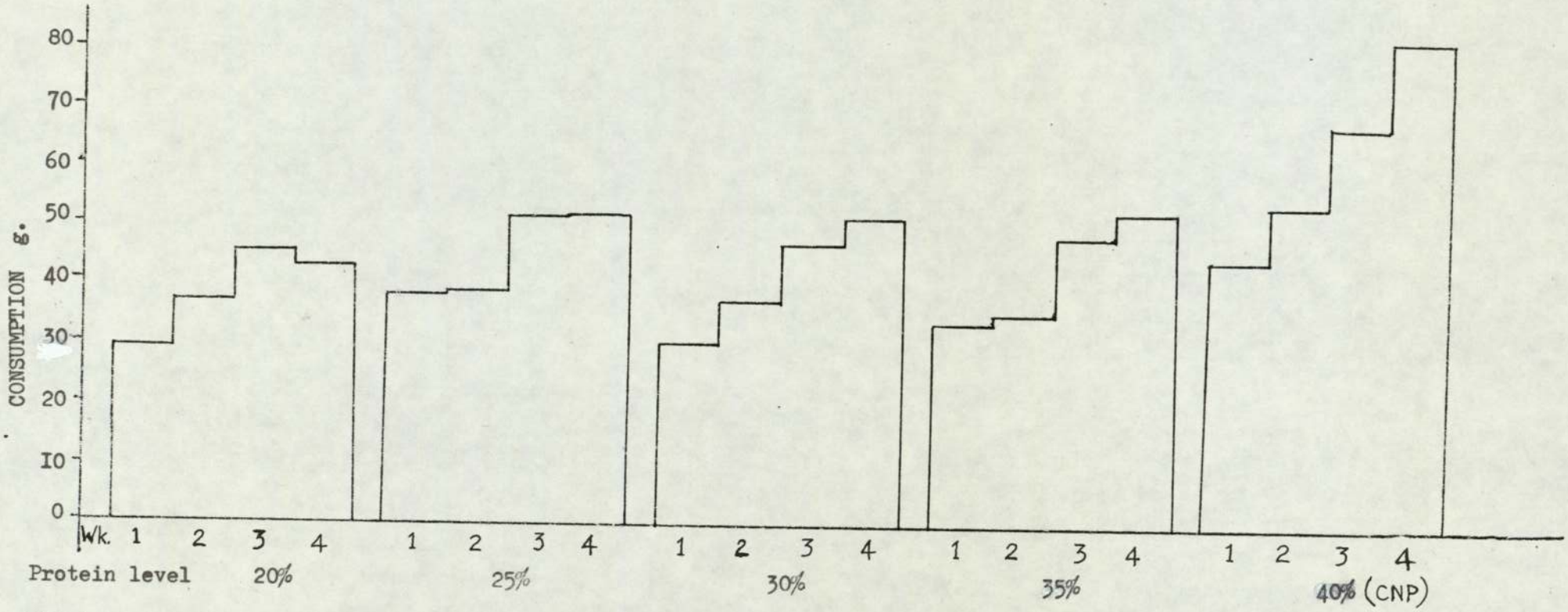
The values for the 28 day period were 0·157, 0·17, 0·159 and 0·165 for the 4 diets containing 20, 25, 30, and 35% bacterial protein. respectively, compared to 0·225 for the commercial diet.

The mean values for % weight gain, food conversion efficiency (FCE %) and protein efficiency ratio (PER) at each protein level shown in Table 74,are graphically represented in Fig. **42**, and the results have been further analysed for statistical significance as shown in Table 75.

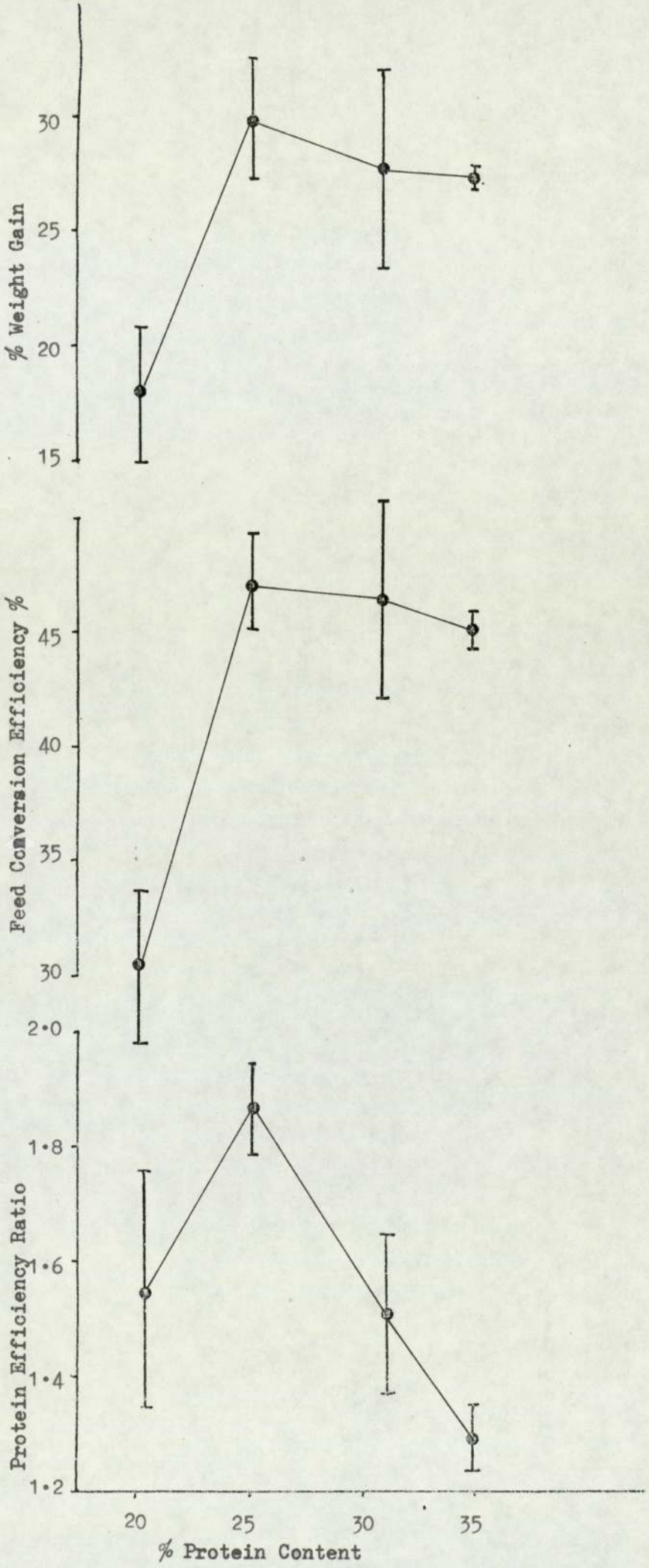
Under the conditions of the experiment, the maximum growth and FCE occurred at the 25% level of protein inclusion (Fig. **42**). An increase in protein content from 20% to 25% significantly increased the growth rate and FCE ($p<0·001$), but further increases in protein content caused a slight, but not significant, depression of the growth rate and FCE. (Table 75). The best of the diets containing bacterial protein had a significantly lower FCE than the commercial feed ($p<0·001$), amounting to 71% of it, and a significantly lower growth rate ($p<0·001$) amounting to only 50%.

The maximum PER was also achieved at the 25% level of protein inclusion. The lowest PER recorded was for the 35% protein diet. The variation between the replicate groups was quite marked and the dietary effect was not as significant (Table 75). Although the highest PER value, achieved at 25% protein inclusion, was significantly higher than the lowest at 35% protein inclusion ($p<0·05$), there was no significant difference between either of

Fig. 4I. Food Consumption Data - Bacterial Protein.



Protein level	20%	25%	30%	35%	40% (CNP)
Total food consumed. g.	156·4	181·7	168·0	173·4	252·3
M.E. kcals	422·28	490·59	453·60	468·18	706·44
Appetite quotient	0·157	0·170	0·159	0·165	0·225


% Weight Gain
30
25
20
15
Feed Conversion Efficiency %
45
40
35
30
Protein Efficiency Ratio
2·0
1·8
1·6
1·4
1·2
20
25
30
35
% Protein Content

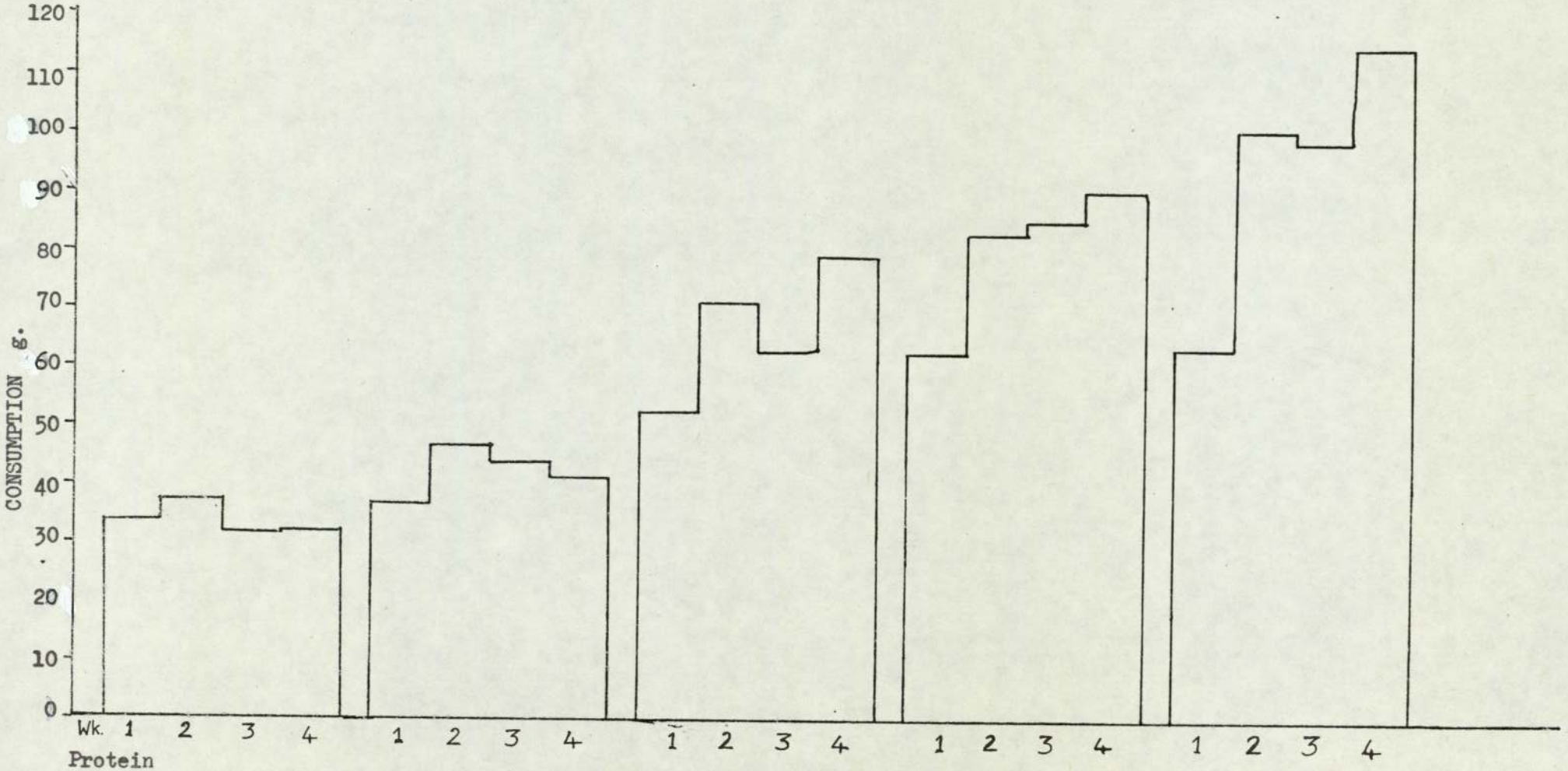
these values and those at 20% and 30% protein inclusion or indeed the commercial feed.

The performance data for the trial in which algal protein was tested has been tabulated in Table 76. On this occasion there were mortalities as shown. The mortalities did not appear to be dietary related. During the pre-experimental adaptation period, heavy losses were incurred on the standard commercial feed. These fish were replaced prior to the commencement of the trial. Some fish were apparently stressed in the small tanks at the higher temperature experienced during this experiment, although oxygen was not limiting. The fish were generally more aggressive and 'fin nipping' was severe in some cases. The experiment was abandoned in one group of fish fed diet P06 following high mortalities in the first week and a generally diminished feeding response. Heavy mortalities also occurred in one of the groups fed on diet P08, but the feeding response remained satisfactory throughout the trial period. Dead or dying fish were removed and their weights were recorded for the computation of the results.

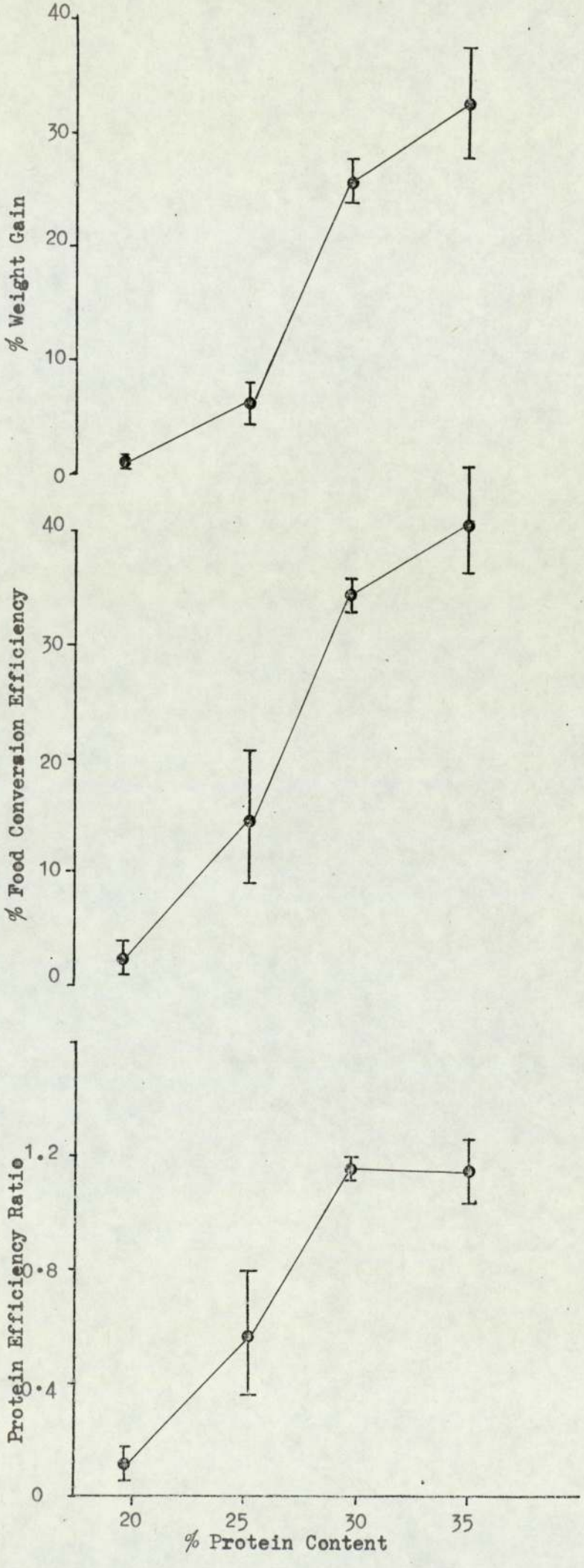
The food consumption data for each 7 day period is shown in Fig 43. Food consumption was slightly lower during week 3 when the temperatures exceeded 17^{o}C. Food consumption by groups of fish fed diets containing 20% and 25% algal protein was low. The appetite data, quantified as previously, was 0·134, 0·166, 0·247 and 0·285 for the diets containing 20, 25, 30 and 35% algal protein respectively compared to 0 280 for the commercial diet. Only the groups of fish on the 35% test diets had an appetite comparable with those on the commercial feed, although the appetite of fish on the 30% diet was acceptably high. Intake was low on the lower protein test diets.

The mean values for weight gain %, FCE and PER shown in Table 76 are graphically represented in Fig 44 and the results have been further analysed for statistical significance as shown in Table 72. Growth rate

Fig. 43. Food Consumption Data - Algal Protein.



Protein level	20%	25%	30%	35%	40% (CNP)
Total food consumed g.	134·0	167·5	263·8	319·9	376·6
M.E. kcals.	460·53	527·62	830·97	1007·7	1054·5
Appetite quotient	0·134	0·166	0·247	0·285	0·280


% Weight Gain
40
30
20
10
0
% Food Conversion Efficiency
40
30
20
10
0
Protein Efficiency Ratio
1.2
0.8
0.4
0
20
25
30
35
% Protein Content

and FCE increased with each 5% increase in protein content. There was no significant difference between the values for the 20 and 25% test diets or the 30 and 35% test diets ($p>0{\cdot}05$). The increased efficiency from 25 to 30% protein inclusion was however highly significant ($p<0{\cdot}001$). The highest growth rate and FCE, achieved on the 35% test diet, was only 38% and 48% of that achieved on the commercial diet.

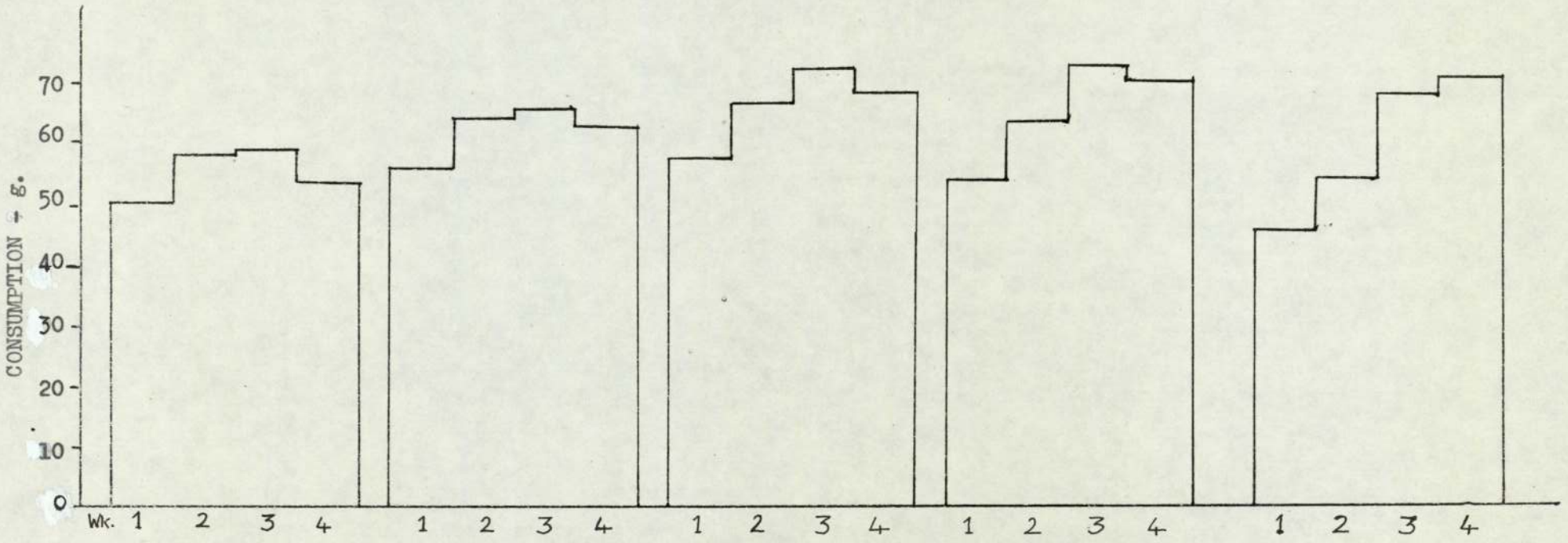
PER increased with each 5% protein increment to 30% but there was no further increase with a further 5% increase to 35% protein inclusion. The differences between the test diets containing 20 and 25% protein and 30 and 35% protein were again not significant ($p>0{\cdot}05$). The increased efficiency from 25% to 30% protein inclusion was highly significant, however, as was the difference between the 35% test protein diet and the commercial feed ($p<0{\cdot}001$).

The performance data for the final trial of this series, on diets containing yeast protein, has been tabulated in Table 78. There were no mortalities during the 28 day trial, and food consumption was high, being comparable with the commercial feed on all test diets, as shown in Fig 45. The appetite data, quantified as previously, was 0·229, 0·245, 0·249, 0·246 for the test diets containing 20, 25, 30 and 35% yeast protein repsectively compared to 0·218 for the commercial control.

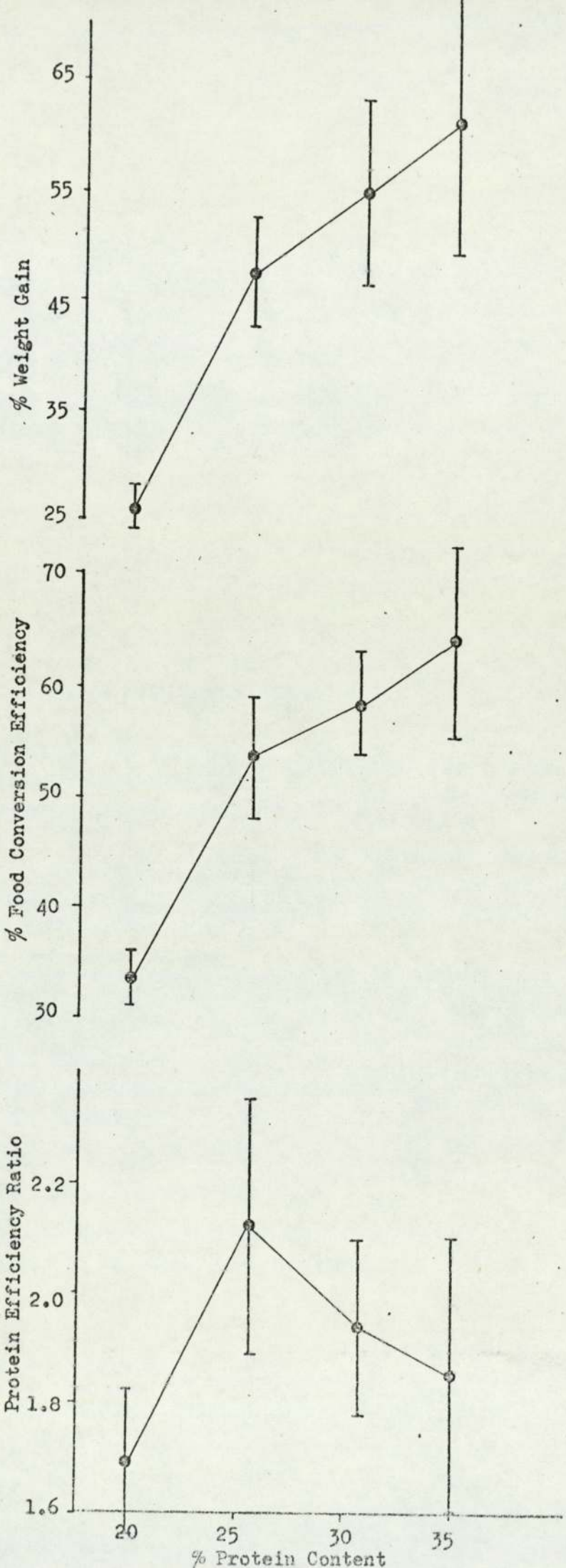
The mean values for % weight gain, FCE and PER shown in Table 78 are represented graphically in Fig 46 and the results have been further analysed for statistical significance as shown in Table 79.

Weight gain and FCE increased with each 5% increment in yeast protein. The increase from 20 to 25% was significant ($p<0{\cdot}05$) but the further increases achieved at 30 and 35% were not significant. The FCE for the 35% yeast diet was slightly lower than that for the commercial feed, although the difference was not significant. The

Fig. 45. Food Consumption Data - Yeast Protein.



Protein level	20%	25%	30%	35%	40% (CNP)
Total food Consumed-g	218·8	248·1	264·1	260·4	233·7
M.E. kcals	647·65	734·38	781·74	770·78	691·75
Appetite quotient	0·229	0·245	0·249	0·246	0·218


% Weight Gain
65
55
45
35
25
% Food Conversion Efficiency
70
60
50
40
30
Protein Efficiency Ratio
2.2
2.0
1.8
1.6
20
25
30
35
% Protein Content

growth rate on the 35% yeast diet, however, was higher than the commercial diet by virtue of the increased feed intake. The difference was again not significant.

PER appeared to be optimum at 25% protein, although the variation within treatment groups was high and analysis of variance failed to show any significant differences at the 5% level of significance between any of the groups including those fish on the commercial feed.

DISCUSSION.

In each series of diets the glucose was replaced on a weight for weight basis with the test material in an attempt to maintain the level of metabolisable energy, assuming that 80% of the gross energy of the test material and 100% of the gross energy of the glucose, is assimilated. The latter assumption is based on the experimental evidence of Singh & Nose (1967) showing that more than 99% of glucose was digested by rainbow trout at all levels of inclusion between 20 and 60%. The use of an energy assimilation coefficient of 0·8 for the test material, however, is not based on experimental evidence, although it is likely that the values for the dry feedstuffs will have been of that order. More precise values could not be used in the absence of accurate values for the true protein, lipid and carbohydrate and their corresponding digestibility coefficients.

In Section 5, the interpretation of food consumption data using Carpenter's Appetite Quotient (Carpenter, 1953) was discussed in some detail. It appeared likely that trout, like birds and mammals consume food in proportion to their demand for metabolisable energy. On this basis, if the diets in any one series contained the same quantity of metabolisable energy, as indicated, one would expect that the consumption of energy in each case would be in the same

proportion to the metabolic demand, i.e. fish weight$^{0\cdot8}$. Clearly in the case of the yeast, this appeared to be so. There was little difference in the calculated appetite quotients for each of the 4 test diets. The difference between the commercial feed and the test diets could be the result of either an overestimate of the metabolisable energy of the test protein or an underestimate of the metabolisable energy of the commercial feed. However, the result indicates an equal or better appetite of diets containing up to 58% by weight of yeast supplying the entire protein content of the diet at a level of up to 35%, when compared to a commercial feed.

Appetite quotients for the diets containing bacterial protein were also of the same order of magnitude but in this case were considerably lower than that for the commercial diet. It is unlikely that errors in the estimation of metabolisable energy would account for this difference. It will be recalled that the bacterial protein appeared to cause a lowering of the consumption rate in compound diets containing bacterial protein at 20% and particularly at the 40% level. Clearly, the same effect has been observed in this experiment. The response was noticeable on the first day of feeding with a reduced intake consistently lower than the commercial feed by 40-50%, indicating a palatability difference. However, feed intake did not appear to reduce as the level of bacteria increased to the maximum of 45%.

In view of the results for the proximate analyses and apparent digestibility of the crude protein of the alga, reported earlier, it is possible that the estimated assimilation coefficient of 0·8 for this material is in error, in view of it having a higher carbohydrate content and lower protein digestibility than the yeast and bacteria. On this basis, it is likely that the metabolisable energy content of the diets containing algal protein actually decreased as the quantity of the test material increased in replacement of glucose. This will

have contributed in part for the observed differences in the calculated appetite quotients. However, the large differences in appetite quotients for the 20 and 25% protein diets and the 30 and 35% protein diets will almost certainly be the result of other factors including an inadequate supply of essential amino acids from this protein at such low levels of inclusion. Additionally, the lower protein diets containing algal protein had the highest glucose levels in any series. Phillips et al (1948) have shown that glucose absorbed into the bloodstream of brook trout is not rapidly taken up by the tissues or excreted and consequently causes a pronounced and prolonged hyperglycaemia. He further reported that when diets containing large quantities of digestible carbohydrate were fed to trout, the fish accumulated large quantities of glycogen in their livers which led to growth retardation and increased mortality. On the basis of this evidence, it was suggested that the maximum limit of digestible carbohydrate should be 9% of the diet on a wet weight basis, equivalent to 33% on a dry weight basis. Buhler and Halver (1961) were unable to show deleterious effects of feeding dextrin up to 48% in the diet of chinook salmon. They further showed that 20% glucose promoted the highest growth rate and best protein efficiency ratio in a series of diets containing several carbohydrates at a level of 20%, showing that carbohydrate was effectively used as an energy source by chinook salmon. They further suggested that the observed effects in the experiments reported by Phillips (1948) were the result of using inadequately balanced diets. However, assuming a digestibility of 50% for the dextrin at this level of inclusion (Singh & Nose, 1967), the digestible carbohydrate of their diet would be 24% which is within the maximum limit suggested by Phillips (1948). Furthermore, there may be species differences. Thus, Palmer and Ryman (1972) have also reported pronounced and prolonged hyperglycaemia and increased liver glycogen in rainbow trout following oral administration of glucose. In view of this, it is possible that the level of digestible carbohydrate in this experiment caused some

problems including a diminished feed intake.

Of the three test materials, the yeast protein was the best utilized for growth and gave generally higher FCE values when compared to the commercial diet. The optimum protein content for growth and FCE was not exceeded in this case and is therefore likely to be in the range found by Satia (1974) for trout fed diets containing fish meal and generally accepted as the optimum for this species i.e. 40-50%.

The growth rate and FCE reached an optimum at a level of 25% protein, however, for diets containing bacterial protein possibly indicating nutritional imbalance. The growth rate was considerably poorer than that of fish on the commercial diet in view of the poorer conversion and reduced feed intake.

The diets containing algal protein produced the poorest growth rates and FCE. Diets containing 20% and 25% algal protein did not appear to be adequate to promote growth, possibly indicating limiting essential amino acids at that level of protein intake or possibly dietary disturbance caused by excessive carbohydrate as discussed. The optimum protein content for growth and FCE was not exceeded.

The maximum protein efficiency ratio occurred at the 25% level of inclusion in diets containing yeast and bacteria and 30% in the case of the algae. The optimum PER in the rat occurs for most proteins at alower level of protein inclusion as shown, for example, by Hegsted and Chang (1965) but the shape of the plot of PER against the protein content is generally as for the rat.

Hastings (1969) reports data of Shanks in which casein was tested in the diets of chinook salmon at 4 levels of protein - 27%, 32·5%, 40% and 47·5% each in three diets containing 10%, 22% and 34% dextrin. The highest PER was achieved in the diet containing

32·5% protein and 34% dextrin. Luquet (1970) reported PER data for diets containing 30, 40, 50 and 60% protein derived from herring meal and fed to rainbow trout. The best PER was at 30%. Cowey and Sargent (1972) report data for plaice fed freeze-dried cod and casein in which the maximum PER was achieved at even higher protein contents, 40% and 50% respectively. That optimum PER is achieved in the carnivorous fish species at a higher protein content than mammals and birds is a reflection of their higher protein requirement. PER in carp, however, an omnivorous species of fish, was reported to decrease linearly as protein increased from very low levels of inclusion (Ogino and Saito, 1970).

The maximum PER achieved for the bacteria, algae and yeast was 1·88, 1·17 and 2·12 respectively. The values expressed as a % of the values for the commercial diet in each case were 113·3%, 55·2% and 119·1% respectively. However, until such time as a suitable standardised method is established, some caution must be exercised in comparing values produced from different experiments, particularly when such factors as temperature is variable. Furthermore, although an attempt was made to produce diets in each series with apparently similar metabolisable energy contents, no attempt was made to use the same proportions of the carbohydrates, starch and glucose, in all three experiments. Examination of the data of Buhler and Halver (1961) and that of Shanks, reported by Hastings (1969) shows that PER varies with the level and source of energy as for the rat and comparative trials should therefore be conducted on diets containing carefully controlled proportions of the energy yielding components.

(2.) DIGESTIBILITY OF DIETS CONTAINING BACTERIA, ALGAE AND YEAST AS SOLE PROTEIN COMPONENTS IN SEMI-PURIFIED DIETS.

METHODS.

Diets P03 and P04 containing bacteria (Table 70), P07 and P08

containing algae (Table 71) and P11 and P12 containing yeast, with 1·0% CrIII oxide in replacement of starch in each case, were prepared by the wet extrusion method as previously described. It was originally proposed that nitrogen balance techniques should be used to assess the biological value of the proteins but difficulties were experienced with adapting the fish to the nitrogen balance system as described in the previous section. The diets were therefore fed to duplicate populations of 20 fish in the 18 litre tanks described in Section 6, for a period of 12 days, during which time the water temperature was in the range 15-16°C. Faeces were collected directly from anaesthetised fish on the 9th and 12th day as described in Section 5 pg 108. The faecal samples from the duplicate populations of fish were pooled and dried. The apparent crude protein digestibility was determined using the methods of Furukawa (1966) as described in Section 5 pg 108. Estimations were carried out on duplicate samples of the diets and dried faecal material. Unfortunately, insufficient faecal material remained for bomb calorimetry and the estimation of the digestible energy.

RESULTS.

The relevant analytical data and the computed crude protein digestibility is shown in Table 80. The mean digestibility of the crude protein of the three single-cell proteins was 86·0%, 71·0% and 87·7% for the bacteria, alga and yeast respectively.

DISCUSSION.

The results for the crude protein digestibility of bacteria, alga and yeast in semi-purified diets in which the SCP supplied the sole protein component, were in accordance with the results shown previously in Section 5. Both the bacterial and yeast protein was highly digestible, giving values similar to those reported for fish meals, whilst the algal protein was less well digested, but nevertheless comparable with many of the plant seed proteins.

(3.) COMPARATIVE ASSESSMENT OF PROTEIN QUALITY IN DIETS CONTAINING FISH MEAL, SOYABEAN MEAL, YEAST, BACTERIA AND ALGAE.

EXPERIMENTAL DIETS.

Diets containing the 5 test feeds were formulated to contain 27·5g of crude protein per 100g dry matter. The feeds were samples from the same batches evaluated in the compound feeds, Section 5. The relevant analytical data was reported in Table 37. Each protein source was added to a basal diet containing 5·0g of vitamin mix (Table 73), 4·0g of 'Durabond' binder and 3·0g of cod liver oil.

Since the Soxlet oil extraction procedure, using petroleum ether (pg 102), was not considered suitable for the complete extraction of all the lipid in the dry samples, as discussed previously, a method of lipid extraction by mixed solvents, following acid hydrolysis, was used to estimate total lipid content of the 5 test materials. The materials were hydrolysed with 3N HCl and the lipids extracted with industrial methylated spirits and a mixture of diethyl ether and petroleum ether (40-60 BP) as described in A.O.A.C. (1965). The following values were obtained: herring meal, 8·7%; soyabean meal, 1·2%; BP yeast, 9·4%; ICI bacteria, 6·8% and the alga, Spirulina maxima, 7·3%. Thelipid contribution from each of the test materials was calculated and the total lipid in each diet was then made up to 10·0g/100g dry matter by adding an appropriate quantity of soyabean oil.

The total caloric content of the test materials and starch has been reported previously and the value for the added oil was taken as 9·4kcals/g. The energy contribution to each diet from the test material and oils was then calculated and sufficient potato starch was added to raise the gross energy content to 400kcals/100g dry matter.

The ash content of the mixture was then calculated and a sufficient quantity of the dry mineral mix was then added to bring the total estimated ash content to 10g/100g dry matter.

Cellulose (Solka Floc) was finally added to bring the dry matter content to 100g.

The composition of the balanced diets and the theoretical specifications are shown in Table 81.

The % moisture and % nitrogen were determined by the techniques described in Section 5, pg 102. The % moisture for the 5 experimental diets was 8·9, 9·2, 9·1, 10·0 and 11·2, for diets P13, 14, 15, 16 and 17 respectively. The corresponding % crude protein for each diet was 25·6, 25·4, 25·2, 25·0 and 24·8 respectively.

The digestible energy was estimated from the calculated proximate analyses, assuming the following calorific equivalents: 8·0 kcals/g for lipids, 1·6 kcals/g for the carbohydrate, calculated by difference and 5·65 kcals/g for the digestible protein. Values used for the digestible protein content were those from Table 80 for yeast, bacteria and the alga. Values for herring meal and soyabean meal were calculated from the values reported in Table 41 corrected by multiplying each value by the ratio of the yeast values reported in Table 80 and Table 41.

It was apparent that the diet containing fish meal was very poorly bound when dried. A large percentage of the diet was reduced to dust in the crumbling process and the remaining pellets had a very poor water stability, disintegrating rapidly in water. In view of this, the diet containing fish meal was omitted from the trial. The remaining diets were well bound and had an acceptable water stability. The coarse texture of the fish meal was responsible for the poor binding capacity.

GENERAL METHODS.

The methods were essentially as used previously for experiments PER 01, 02 and 03. However, in view of the fact that the fish meal diet was omitted from the trial, 4 tanks were randomly allocated to each of the four remaining test diets. Each diet was therefore assessed on 4 replicate groups of 20 individual rainbow trout in the size range 5-6".

The mean temperature for each week was:

WEEK	1	2	3	4
TEMP. ^{o}C	7·5	7·5	6·5	7·5

The stocking densities and loading factors at the beginning and end of the trial were in the following ranges:

DIET.	Stocking Density gl^{-1}	Loading Factor gl^{-1} min.
P14	36·8 - 33·5	1324 - 1204
P15	36·8 - 47·0	1324 - 1692
P16	36·9 - 38·5	1323 - 1386
P17	36·9 - 44·2	1329 - 1591

Oxygen was not considered to be limiting at any time during the 28 day trial.

Feeding and weighing was as reported previously.

RESULTS.

The results have been tabulated in Table 82. There were no mortalities during the trial. The results for % weight gain, FCE and PER have been further analysed for statistical significance in Table 83.

The first striking feature is the apparently low consumption of

diet P14 containing soyabean meal. This diet was not consumed at all at any time during the trial, the quantity shown representing only the small amount of feed offered daily. Fish frequently showed interest in the feed offered and would pick up pellets but they would not swallow them. The three diets containing SCP were consumed, although at different rates, as shown. The corresponding appetite quotients for the yeast, bacteria and algae were 0·145, 0·065, 0·110 respectively.

As a result of the low consumption rates, the % weight gain was also generally low. Fish on diet P14 containing soyabean meal lost weight through starvation. There was considerable variation in the group fed bacterial protein, one group actually losing weight. The mean % weight gain for the bacterial protein diet was only 4·4%. This was significantly lower than the mean weight gain of 19·7% for groups on the algal protein diet ($p<0·001$). The highest mean growth rate, equal to 27·9%, was achieved on the yeast protein diet, although this result was not found to be significantly different from that for the algal protein diet.

The FCE for the diets containing bacteria, algae and yeast were 19·7%, 52·0% and 57·7%. Again the difference between the result for the diet containing the bacteria and algae was significant ($p<0·001$) but the difference between the result for the diets containing algae and yeast was not significant.

PER results followed the same pattern, the lowest result being 0·79 for the diet containing the bacterial protein, which was significantly lower than the 2·09 obtained for the diet containing algal protein. The highest value, 2·29, obtained for the diet containing yeast protein, was not significantly different from the result obtained for the diet containing algal protein.

DISCUSSION.

The relatively low consumption rates found in this experiment were related to the low temperatures throughout. There was, however, obviously a definite palatability problem with the feed containing soyabean meal. The result for the bacterial protein also indicated a palatability problem as found in previous experiments.

The low food consumption rate in the case of 2 of the groups on the bacterial protein diet possibly prevented the fish from obtaining sufficient protein for growth. The other 2 groups of fish, however, consumed a little more and some growth occurred. The results were generally poorer than those obtained previously for this material at 25% protein inclusion. The results on this occasion for % weight gain, FCE and PER were 4·4, 19·7 and 0·79 compared to 30·6, 47·3 and 1·88 reported previously.

The results for the algal protein were a marked improvement on those obtained previously for this material at 25% inclusion. The results for % weight gain, FCE and PER were 19·7, 52·0 and 2·09 on this occasion, compared to 6·0, 14·9 and 0·59 respectively, as reported previously. The improved result is probably a reflection of the lower demand for energy at this temperature, allowing more effective use of the protein. However, a more favourably balanced diet may have contributed to the improvement. Two dietary factors were changed from the previous experiment; notably the glucose was omitted and the corn oil was substituted by a mixture of cod liver oil and soyabean oil. The possible harmful effects of high levels of digestible carbohydrate have already been indicated. In this experiment a greater proportion of the metabolisable energy was supplied by oil. Furthermore, the use of cod liver oil and soyabean oil may have been of additional benefit supplying linolenic acid, which has been reported as being essential for the growth of rainbow trout (Lee et al, 1967).

The results for the yeast were 27·9, 57·7 and 2·29 for % weight gain, FCE and PER repsectively compared to 47·5, 53·5 and 2·12 reported previously. The lower growth rate was the result of a reduced feed intake which was related to the lower temperature as mentioned previously. The results for FCE and PER were comparable.

CONCLUSIONS.

The results shown in this series of experiments emphasised the importance of determining a standardised PER method for evaluating protein quality in fish diets. One factor which affects PER in trout is the level of dietary protein. The protein content yielding the highest PER appeared to be 25-30% in the conditions of these experiments. However, the level and source of dietary calories will exert an additional effect. Further experiments are required on several feedstuffs of varying protein quality used in diets with varying caloric sources and levels. An added complication in fish research is the effect of environmental conditions, particularly temperature. Since the level of metabolism, hence the energy requirement, and the dietary protein requirement are temperature dependant functions, they will clearly have an effect on PER in experiments carried out at the variable temperatures. This probably accounted in part for the observed differences. Further experimental work is required to determine the extent of such effects. The method of feeding will also have an effect. In these experiments satiation feeding was carried out. Palatability effects were observed in at least two feeds tested, and therefore the level of intake of dietary calories and protein was dissimilar. Experimentation is required to evaluate the effect of controlled feeding, as opposed to ad-libitum feeding, in an attempt to determine the extent of the effect of feed intake on PER. Finally, experimentation is required to determine the effects of the length of assay on PER values in fish.

Clearly it will be some time before a standardised method applicable to fish studies can be proposed.

The results for the yeast protein were most encouraging. The feed intake was higher than that achieved on a commercial feed, indicating that the yeast would be very acceptable as a major protein source. Furthermore it was highly digestible and the growth rate, FCE and PER values were consisitently high.

The second experiment in which algal protein was evaluated also produced most encouraging results, particularly in view of the lower protein digestibility of this material. It is acceptable to trout and could conceivably be used as an important component in well balanced diets. The bacterial protein has a high digestibility and the results of the first trial indicated that the protein could be reasonably efficiently utilized for growth. However, the appetite restriction, which has been shown repeatedly for this material, could be a serious limiting factor in its use.

It is unfortunate that the diets containing the two conventional feedstuffs, fish meal and soyabean meal, could not be evaluated for comparison.

GENERAL CONCLUSIONS.

The urgent necessity of evaluating novel proteins as potential ingredients in trout diets was stressed in Section 1.

Two large U.K. companies have developed processes for the industrial production of SCP. B.P. have developed a process for the production of yeast on n-paraffins, whilst I.C.I. have developed a process for the production of bacteria on methanol. Both companies are at a fairly advanced stage in evaluating their products for all classes of livestock. It was therefore appropriate to evaluate these materials in trout diets.

Tate & Lyle have developed fermentation processes for the production of filamentous fungi on carbohydrate wastes. Although their concern appears to be the development of processes applicable to the developing countries, similar processes are at present being applied to the treatment of industrial wastes containing low levels of carbohydrate which can be utilized by fungi and such materials could conceivably become available in small quantities in the future. Industrial production of penicillin produces large quantities of waste mycelia which could also find application in feedstuffs. It was therefore appropriate to evaluate the Tate & Lyle and Glaxo products.

Although the environmental conditions in the U.K. are unsuitable for industrial production of algal protein, a product produced in Mexico was evaluated to complete the range of single-cell proteins tested.

Facilities for conducting feeding trials under controlled laboratory conditions were designed. A major constraint, however, was the limited space and water availability. Improvements were made as the work progressed and factors such as oxygen and ammonia

were shown not to be limiting during the experiments. However, the effect of tank size could not be assessed and there were occas ions, particularly when the water temperatures were at the highest, when the more aggressive individuals in a population caused a considerable degree of damage by fin nipping. This was most noticeable during an experiment on algal protein at a time when the water temperature peaked at 17°C. Mortalities were high during this period. Some of the fish removed had severe damage to their fins and tails, as shown in Plate 22. In a few cases the fish at the top of the hierarchy occupied most of the tank space, the other members of the population being 'herded' into the centre or into a tight pack at the edge of the tank, as shown in Plates 23 and 24. Fish in this situation were obviously stressed.

Where possible, for sound nutritional work on this species of fish, more extensive facilities allowing the use of larger tanks and a greater number of fish in each replicate group, would be beneficial. Furthermore, constant environmental conditions would be a great advantage.

There is considerable scope for further evaluation of methodology of protein evaluation and the establishment of a convenient technique for the routine assessement of protein quality in practical feedstuffs. In the meantime, further evidence of the basic nutritional requirements of this species of fish should be sought so that constraints in feed formulation can be used with more confidence.

Despite the problems encountered in conducting nutritional research in such limited facilities, some interesting results were obtained with the five single-cell proteins. All of the materials were incorporated into compound feeds for rainbow trout at a level of 20% without any marked effect on feed intake and there was no sign of deleterious effects after 12 weeks feeding. Feeding for a further period of 10 weeks at a level of 40% in the diets did not produce

PLATE 22.

PLATE 23.

PLATE 24.

any noticeable pathological disturbance but feed intake of fish fed the diets containing the bacteria and the mould, Penicillium chrysogenum, was low, indicating a palatability problem of these feeds at that level.

The performance of the diets appeared to be largely related to the protein content of the feed and in this respect the two moulds have a limited value as a major ingredient of trout diets since their protein content (30 - 32 %) is below the optimum for this species. Their effectiveness as a protein supplement would be further limited in view of the lower crude protein digestibility observed for these materials. They may find wider application in the diets of such species as carp, the major cultured species in many developing countries where the production of moulds on agricultural wastes appears to offer some promise. Certainly research on this species would be valuable.

The algal protein had a higher protein content (49%) and a crude protein digestibility comparable to many of the plantseed meals. It could find some use, therefore, as a supplementary protein source in diets of rainbow trout, in the same way as soyabean meal or cottonseed meal. One experiment conducted on a diet containing 25% protein solely from algae, produced a very acceptable food conversion efficiency (52%) and a relatively high protein efficiency ratio (2·1). Algal proteins may not be widely available, however, being restricted to countries with a suitable climate and they may be used more effectively in compound feeds for herbivorous or omnivorous species of fish in the country of production.

Of the single-cell proteins tested, the yeast and bacteria would seem to have the widest application in trout compound feeds. They will be produced industrially in large quantities in several European countries, including Britain, and they could be used by the highly organised feed industry. They have a high protein content, of the

order of 50 - 80 %, and appear to have a favourable balance of amino acids, with the possible exception of low S-amino acids. They could therefore be used as a major protein component of compound trout feeds. The apparently poor palatability of the bacterial protein, however, may impose an upper limit on the levels used. Further research may be required to identify the cause of this problem, if indeed it is a consistent feature of the material and not merely contamination of the particular batch evaluated here.

The best results have been obtained with B.P. yeast. The material has been successfully utilized by several classes of livestock and claims of a very high biological value have been made, as discussed in Section1. Certainly the material was very acceptable to rainbow trout in compound feeds and as the sole source of dietary protein in semi-purified diets. In the latter case, relatively high growth rates have been obtained and feed conversion efficiency of 58% and a protein efficiency ratio of 2·3 was achieved on diets containing only 25% protein. These results were most encouraging and indicated a considerable potential for this material. It may be used to replace all or part of the fish meal in compound trout diets carefully formulated to contain an optimum balance of nutrients. Amino acid supplementation is an area which warrants consideration. Other factors which will be important in the production of balanced feeds containing yeast protein will be the lipid levels and particularly the supply of $\omega 3$ fatty acids, the vitamin levels and the mineral balance. Further work on such aspects could lead to the successful use of this material routinely in compound trout feeds. Unfortunately, however, it is unlikely to significantly reduce the cost of trout feeds since it will find a place in the feed commodity market at a price applicable to its performance as measured against such conventional proteins as fish meal and soyabean meal.

TABLE 1.

PROTEIN CONTENT OF SOME MICRO-ORGANISMS.

ORGANISM	CRUDE PROTEIN %	NOTES BELOW	REFERENCE
Bacteria			
Pseudomonas spp (I.C.I.)	83.0	1	I.C.I. Information Booklet
Pseudomonas spp.(C.P.C.)	78.5	2	Ko and Yu. (1968)
Unidentified (Esso-Nestle)	62.73	3	Pillai et al (1972).
Escherichia coli	82.0	3	Bunker (1968)
Alcaligenes viscosus	84.0	3	" "
Lactobacillus fermentans	87.0	3	" "
Bacillus subtilis	63.1	1	Miller (1968)
Yeasts			
Saccharomyces cerevisiae	53.1	2	Peppler (1968)
Candida utilis	58.5	2	" "
Candida fragilis	58.1	2	" "
Saccharomyces cerevisiae	47.9	2	" "
Candida spp (B.P.)	60-62	1	Shacklady (1972)
Candida spp (B.P.)	68-70	1	" "
Endomycopsis lipolytica (R.R.L.)	59.1	2	Pillai et al (1972)
Candida spp (Kyowa Hakko Kogyo)	61.8	1	Hoshai (1972)
Candida spp (Kanegafuchi Chem. Ind).	59.2	1	" "

Notes.

1. Reported on dry weight basis
2. Reported for dried material as received
3. Moisture not stated

( ) Information in parentheses indicates Industry involved - See Table

TABLE 1 (Cont).

ORGANISM	CRUDE PROTEIN %	NOTES BELOW	REFERENCE
Fungi			
Fusarium semitectum	65.0	3	Worgan (1974)
Fusarium moniliforme	54.9-58.2	1	Christias (1975)
Fusarium oxysporum	54.9-55.5	1	" "
Fusarium lini	37.0	1	Litchfield (1968)
Rhizopus oligosporius	44.5	3	Worgan (1974)
Rhizopus stolonifer	34.8	3	" "
Rhizopus nigricans	36.0	1	Litchfield (1968)
Aspergillus niger	39·6-41.6	1	Christias (1975)
Aspergillus oryzae	38.0	1	Litchfield (1968)
Penicillium notatum	38.4	1	" "
Algae (Green)			
Chlorella 7-11-05	59.7	2	Lubitz (1961)
Chlorella pyrenoidosa	61.9	1	Leveille et al (1962)
Spongiococcum eccentricum	31.6	1	" "
Uronemagigas	50.0	3	Gordon (1969)
Stigeoclonium spp	48.0	3	" "
Hormidium spp	46.0	3	" "
Ulothrix spp	42.0	3	" "
Scenedesmus quadricauda + Chlorella spp	43.8	2	Cook (1962)
Scenedesmus dimorphus + Chlorella spp	43.0	2	Erchul & Isenberg 1968)
Algae (Blue-green)			
Spirulina Maxima	61.8	1	Clement et al (1967)
Plectonema boryanum	34.0	3	Gordon (1969)
Mastigocladus laminosus	34.0	3	" "
Gloeocapsa alpicola	36.0	3	" "
Fremyella diplosiphon	38.0	3	" "

Notes

1. Reported on dry weight basis
2. Reported for dried material as received
3. Moisture not stated.

TABLE 2.

ESSENTIAL AMINO ACID COMPOSITION OF SOME YEASTS. (g/16g N)

ORGANISM.	Isoleucine	Leucine	Phenylalanine	Tyrosine	Tryptophan	Valine	Arginine	Histidine	Lysine	Methionine	Cystine	Threonine	REFERENCES
Endomycopsis lipolytica (R.R.L.)	7.2	7.4	3.9	-	1.0	7.8	6.4	2.0	8.9	1.7	-	4.9	Pillai et al (1972)
Candida lipolytica (BP-G)	5.0	7.4	4.3	3.6	1.4	5.8	5.1	2.1	7.4	1.8	1.1	4.9	Shacklady (1972)
Candida lipolytica BP-L)	5.3	7.8	4.8	4.0	1.3	5.8	5.0	2.1	7.8	1.6	0.9	5.4	" "
Candida spp (Kyowa Hakko Kogyo)	4.5	7.0	4.3	3.4	1.4.	5.4	5.0	2.0	7.3	1.8	1.1	4.5	Hoshai (1972)
Candida spp (Kanegafuchi Chem.Ind.)	4.8	6.9	4.3	3.2	1.2	5.1	4.8	2.3	6.9	1.4	1.2	5.1	" "
Saccharomyces cerevisiae	5.5	7.9	4.5	-	1.2	5.5	5.0	4.0	8.2	2.5	-	4.8	Peppler (1968)
Candida utilis	5.3	7.0	4.3	-	1.2	6.3	5.4	1.9	6.7	1.2	-	5.5	" "
Candida fragilis	5.5	9.9	3.9	-	1.5	6.6	4.9	2.5	8.8	1.5	-	4.4	" "
Saccharomyces cerevisiae	5.7	6.3	4.4	-	1.1	5.2	4.7	1.5	7.3	1.2	-	4.8	" "

TABLE 3.

ESSENTIAL AMINO ACID COMPOSITION OF SOME BACTERIA.(g/16g N)

ORGANISM	Isoleucine	Leucine	Phenylalaline	Tyrosine	Tryptophan	Valine	Arginine	Histidine	Lysine	Meth ionine	Cystine	Threonine	REFERENCE.
Pseudomonas spp (I.C.I)	4.4	6.8	7.6		1.0	5.3	4.5	2.0	6.3	2.5	0.6	4.6	Tolan (1974)
Pseudomonas spp (C.P.C)	3.8	6.6	3.3	-	1.2	3.5	5.0	1.2	4.3	1.3	-	4.1	Ko & Yu (1968)
Pseudomonas spp (C.P.C)	4.3	6.7	3.4	-	0.9	5.1	4.7	1.9	9.1	0.8	-	3.8	" " " "
Mixed species of methane utilizing bacteria (Shell)	4.3	8.1	8.4		1.6	6.5	6.2	2.2	5.7	2.7	1.2	4.6	Tolan (1974)
Methane utilizing bacteria	5.4	0.0	5.4	4.2	1.3	7.1	5.7	2.3	6.0	2.4	0.8	6.0	D'Mello and Whitte more (1973)
Methane utilizing bacteria	5.0	8.4	5.2	-	3.0	6.8	-	-	6.4	2.4	-	5.0	Norris (1968)
H-C utilizing bacteria (Esso-Nestle)	3.6	5.6	2.9	-	0.9	4.5	-	-	6.5	2.0	0.6	4.0	Enebo (1970)
Bacillus megaterium	4.4	1.0	5.4	5.0	-	6.5	6.7	2.3	6.3	3.1	-	4.8	Tannenbaum et al (1966)

TABLE 4.

ESSENTIAL AMINO ACID COMPOSITION OF SOME FUNGI. g/16g N

ORGANISM.	Isoleucine	Leucine	Phenylalanine	Tyrosine	Tryptophan	Valine	Arginine	Histidine	Lysine	Methionine	Cystine	Threonine	REFERENCE
Fusarium spp (R.H.H)	4.2	7.1	2.8	2.8	1.4	4.2	-	-	4.2	2.2	2.0	1.8	Spicer (1973)
Aspergillus niger (T & L)	4.3	7.6	6.9		2.1	5.3	-	-	5.8	2.5	1.0	5.1	Tol an (1974)
Graphium spp	4.2	9.9	3.3	4.0	1.3	4.9	5.0	3.7	6.1	1.2	0.5	4.9	Volesky et al (1975)
Penicillium notatum	3.2	5.5	2.8	-	1.3	3.9	3.7	4.4	4.0	1.0	-	-	Litchfield (1968)
Tricholoma nudum	3.2	6.7	3.7	-	3.5	3.9	4.6	3.0	6.7	1.7	-	-	"
Aspergillus niger (wild type)	-	-	-	-	-	-	-	-	6.5	1.4	-	-	Christias et al (1975) [1]
Aspergillus niger (mutant 70)	-	-	-	-	-	-	-	-	6.7	1.1	-	-	"
Fusarium oxysporum	-	-	-	-	-	-	-	-	6.8	1.3	-	-	"
Fusarium moniliforme									6.6	1.3	-	-	"

[1] Calculated from values given in μ moles/g.

TABLE 5.

ESSENTIAL AMINO ACID COMPOSITION OF SOME AIGAE (g/16gN)

ORGANISM.	Isoleucine	Leucine	Phenylalanine	Tyrosine	Tryptophan	Valine	Arginine	Histidine	Lysine	Methionine	Cystine	Threonine	REFERENCE.
Spirulina maxima	6.0	8.0	5.0	4.0	1.4	6.5	-	-	4.6	1.4	0.4	4.6	Clément et al (1967)
Anabaena cylindrica	5.8	9.7	4.4	-	-	6.8	-	-	5.3	2.1	0.8	5.8	Gordon (1969)
Gloeocapsa alpicola	6.2	10.9	2.2	-	-	6.6	-	-	4.9	2.7	1.2	6.3	"
Chlorella vulgaris	3.5	6.1	2.8	2.8	2.1	5.5	-	-	10.2	1.4	0.2	2.9	Fowden (1954)
Chlorella pyrenoidosa	3.6	4.1	4.8	2.9	1.5	5.8	5.4	1.5	7.8	2.0	-	3.4	Lubitz (1961)
Scenedesmus spp + Chlorella spp	3.7	8.2	6.9	3.0	1.8	5.1	5.7	1.5	5.9	3.0	-	4.0	Leveille et al (1962)

TABLE 6.

COMPOSITION OF SOME CONVENTIONAL FEEDSTUFFS.

(Essential Amino Acid Content - g/16g N)

FEEDSTUFF	Isoleucine	Leucine	Phenyl-alanine	Tyrosine	Tryptophan	Valine	Arginine	Histidine	Lysine	Methionine	Cystine	Threonine	REFERENCE
Whole Egg	8.0	9.2	6.3	4.5	1.5	7.3	6.4	2.1	7.2	4.1	2.4	4.9	Block & Mitchell (1946)
Herring Meal	4.5	7.5	3.9	3.1	1.2	5.4	5.8	2.4	7.7	2.9	1.0	4.3	IA.F.M.M. (1970)
White Fish Meal	3.7	6.5	3.3	2.6	0.9	4.5	6.4	2.0	6.9	2.6	0.9	3.9	"
Soyabean Meal	5.4	7.4	4.8	3.1	1.3	5.2	7.0	2.4	6.3	1.3	1.5	3.7	U.S.National Research Council (1973)
Cottonseed Meal	3.9	6.1	5.7	1.7	1.6	5.0	10.4	2.7	4.2	1.6	2.1	3.5	" "
Groundnut Meal	4.2	7.8	5.7	3.8	1.0	5.9	12.4	2.5	4.9	0.8	1.3	3.2	" "

NUTRITIONAL VALUE OF SOME YEASTS.

T A B L E 7 . (from Bressani, 1968)

Sample	Digestibility	B.V.	PER.
Bakers' Yeast (Saccharomyces cerevisiae)	81	59	1.4
Brewers' Yeast (Saccharomyces cerevisiae)	80-90	58-69	1.7
Torula Yeast (Candida utilis)	85-88	32-48	0.9 - 1.4
Torula Yeast + 0.5% D.L. Methionine.	90	88	2.0 -2.3

T A B L E 8 . (From Mitsuda et al, 1967).

Sample	Digestibility	B.V.	N.P.U.	PER
Torula Yeast (Candida utilis)	65	37	24	1.1
Extracted protein of Torula Yeast	96	47	45	1.6
Extracted protein + methionine	95	61	58	2.2
Casein	98	68	66	2.3

NUTRITIONAL VALUE OF SOME YEASTS

T A B L E 9 . (from Shacklady, 1970).

Sample	Digestibility	B.V.	N.P.U.
B.P. yeast (n-para)	96	61	59
" + 0.3% DL-Met.	96	91	88
B.P. yeast (gas-oil)	94	54	50
" + 0.3% DL-Met)	95	96	91
Soya protein isolate	100	42	42
" + 0.3% DL-Met	99	65	64
Dried whole egg	100	90	90
" + 0.3% DL-Met	100	97	97

T A B L E 1 0 . (from Palmer & Smith, 1971).

Sample No.	2	3	4	5
Digestibility	94	93	91	79
N.P.U.	56	58	50	44
N.P.U.+ 0.3% L-Met	58	62	69	50
N.P.U.+ 0.6% L-Met	68	69	73	54
N.P.U. + 0.9% L-Met	74	78	75	60
B.V.	60	62	54	56
B.V.+ 0.9% L-Met	79	84	82	76

NUTRITIONAL VALUE OF SOME BACTERIA.

TABLE 11. (from Tannenbaum, 1968).

Sample	Digestibility	B.V.	N.P.U.
Bacillus Megaterium			
Whole cells	76	60	46
Extr cell contents	94	65	61
Whole cells	55.6	62.3	34.5
Broken cells	67.3	70.0	47.1
Casein + 2.5% Methionine	88.4	88.7	78.3

TABLE 12. (from La Chance, 1968).

Sample	Digestibility	B.V.	N.P.U.
Hydrogenomonas eutropha (boiled)	93.0	77.1	72.0
Hydrogenomonas eutropha (sonified)	93.6	76.0	71.0
Casein	98.9	77.3	76.0

TABLE 13. (from Palmer & Smith, 1971).

	Digestibility	B.V.	N.P.U.
Sample 6	90	42	38
" + 0.3% L-Met	-	-	49
" + 0.6% L-Met	-	-	54
" + 0.9% L-Met	-	70	63
Sample 7	91	51	46
" + 0.6% L-Met	-	-	57
" + 0.9% L-Met	-	75	68

NUTRITIONAL VALUE OF AIGAE

TABLE 14. (from Cook, 1962)

Sample	Digestibility	B.V.	N.P.U.
Dried algae	65.4	54.3	35.5
Autoclaved algae	65.5	54.5	35.6
Algae cooked for 30 minutes	73.0	56.0	40.9
Algae cooked for 2 hours	69.8	48.7	44.0

TABLE 15. (from Erchul and Isenberg, 1968).

Sample	PER	Digesti-bility	B.V.	N.P.U.
1. 30% A + 30% B + 25% C + 10% D	-	67	71	47
2. 75% C + 18% B	1.71	65	73	48
3. 95% D	0.92	51	60	31
4. 95% B	0.72	-	-	-
5. 95% E	1.15	-	-	-
6. 75% B + 20% F + 4% G.	1.98	75	6	57
7. 50% B + 30% F	1.46	-	-	-
Casein control	(1) 2.63	(3) 97	(3)77	(3)75
	(2) 2.73	(4) 98	(4)81	(4)79

Species A. Closterium, B. Chlorella. C. Scenedesmus dimorphus
D. S. quadricauda. E. S. intermedius F. Scenedesmus (unspecified).

Notes 1. Control for samples 1 - 6.
2. Control for sample 7.
3 and 4. Control values determined approximately 6 months apart.

T A B L E 1 6 . (from Clément et al, 1967).

Sample	Digestibility	B.V.	N.P.U.
Raw Spirulina	75.5 - 76.7	60 - 65	45.6 - 49.8
Stewed Spirulina	74.3	48 - 54	36.0 - 40.0

TABLE 17.

POTENTIAL WORLD-WIDE PRODUCTION AND PILOT FACILITIES FOR S.C.P. BY 1980. (WELLS, 1975).

	Organism	Substrate	Estimated 1975-80 capacity (t/a)
CZECHOSLOVAKIA			
Slovnaft Kojetin	Yeast	Ethanol	60,000
Slovnaft Kojetin	Yeast	n-Paraffins	100,000
FINLAND			
United Paper Mills	Fungi	Sulphite liquor	10,000
FRANCE			
British Petroleum, Lavera	Yeast	Gas Oil	100, 000.
Group Francais des Proteines	Yeast	n-Paraffins	40,000
GERMANY			
Biological Carbon Research	Algae	Carbon dioxide	Pilot
ITALY			
Italproteine, Sardinia	Yeast	n-Paraffins	100,000
Liquichimica, Calabria	Yeast	n-Paraffins	200,000
MEXICO			
Sosa Texcoco	Algae	Carbon dioxide	Pilot
ROMANIA			
Realprot	Yeast	n-Paraffins	60,000
REPUBLIC OF CHINA	Yeast	n-Paraffins	1,000
SWITZERLAND			
Nestle	Bacteria	Ethanol	1,000
TAIWAN			
Chinese Petroleum	Yeast	n-Paraffins	100,000
UNITED KINGDOM			
British Petroleum	Yeast	n-Paraffins	100,000
I.C.I.	Bacteria	Methanol	100,000
Shell Chemical	Bacteria	Methanol	1,000
R.H.M. Foods	Fungi	Carbohydrates	10,000
Tate and Lyle	Fungi	Carbohydrates	4,000
U.S.S.R.	Yeast	n-Paraffins	200,000
UNITED STATES			
Amoco Foods Co.	Yeast	Ethanol	5,000
LSU/Bechtol	Bacteria	Cellulose	10,000
US Army	Bacteria	Cellulose	10,000
JAPAN			
Mitsubishi Gas	Yeast	Methanol	100,000
Sumimoto	Bacteria	Methanol	100,000
Kyowa Hakko	Yeast	n-Paraffins	100,000

TABLE 18.

COMPOSITION OF FEED INGREDIENTS (1)

Ingredient	Water Content %	Crude Protein %	Pet.Ether Extr. %	Ash %
White fish meal	9.0	64.7	4.8	15.2
Wheat middlings	13.2	15.0	3.6	3.9
Soyabean meal	9.0	45.5	1.1	5.7
Delactosed whey	4.1	26.0	1.7	23.5
Distillers' solubles	5.8	25.9	0.3	24.9
Corn gluten meal	6.1	61.3	2.0	2.1
Brewers' yeast	8.1	45.4	0.9	13.0
Dehydrated grass meal	8.8	13.2	2.0	12.0
Candida lipolytica	5.1	58.9	2.8	10.6
Penicillium chrysogenum	6.5	32.5	0.5	12.9
Aspergillus niger	3.6	30.1	2.1	7.3

(1) Proximate analyses - all analyses reported as % of the original sample 'as received'.

TABLE 19

FORMUIATIONS OF EXPERIMENTAL DIETS.

Ingredient	% in diet						
	CO 2	CO 3	CO4	CO 5	CO6	CO 7	CO 8
White fish meal	35	35	35	35	17	17	-
Delactosed whey	10	-	-	-	10	10	10
Distillers' solubles	8	-	-	-	8	8	8
Penicillium chrysogenum	-	18	-	-	18	-	-
Candida lipolytica	-	-	18	-	-	18	35
Aspergillus niger	-	-	-	18	-	-	-
Wheat middlings	13	13	13	13	13	13	13
Soya bean meal	10	10	10	10	10	10	10
Corn gluten meal	6	6	6	6	6	6	6
Brewers' yeast	5	5	5	5	5	5	5
Dehydrated grass meal	3	3	3	3	3	3	3
Trace mineral salts (1)	4	4	4	4	4	4	4
Vitamin mix. (2)	4	4	4	4	4	4	4
Soya bean oil	4	4	4	4	4	4	4

(1) Trace Mineral salts shown in Table 20.

(2) Vitamin mix shown in Table 21.

TABLE 20

MINERAL MIX (U.S.P. XVIII).

Ingredient	Quantity (g)
NaCl	139.3
K I	0.79
KH_2PO_4	389.0
Mg SO_4	57.3
$CaCo_3$	381.4
$FeSO_4 7H_2O$	27.0
$MnSO_4 H_2O$	4.01
$ZnSO_4 7H_2O$	0.548
$CuSO_4 5H_2O$	0.477
$CoCl_2 6H_2O$	0.023

TABLE 21

VITAMIN PREMIX (1)

Vitamin	mg vitamins/lb premix (2)
D-Ca Pantothenate	600
Pyridoxine	5
Riboflavin	600
Niacin	6,250
Folic acid	100
Thiamin	500
Biotin	5
B 12	0.25
Menadione sodium bisulphate	125
B.H.T.	250
Vitmin E	2,000 I.U. *
Ditamin D_3	15,000 I.U. *
Vitamin A	75,000 I.U. *
Chloine chloride (3)	4,000
Ascorbic Acid (3)	5,000

(1) Vitamin premix used in McNenney diet (Orme, 1970).

(2) mg/lb of premix being made up of pure vitamins premixed in wheat middlings, except * given in international units.

(3) Vitamins added to the diet at the time of mixing the main ingredients

TABLE 22

PELLET QUALITY.

Diet Number	% diet remaining as pellets after sieving.	Water stability % loss in 5 minutes.
C01	Not included	3.2
C02	77.2	28.9
C03	67.2	39.5
C04	67.6	36.3
C05	81.2	97.7
C06	67.6	28.9
C07	66.8	28.8
C08	60.6	34.4

TABLE 23.

Composition of Experimental Diets.

Diet Number	Water Content[1] %	Crude Protein[2] %	Pet.Ether Extr.[2] %	Ash[2] %
CO 1	8.8	40.6[1]	4.8[1]	12.2[1]
CO 2	9.6	40.1	6.8	13.8
CO 3	8.5	42.0	6.7	12.0
CO 4	8.0	46.8	7.1	11.6
CO 5	9.4	40.9	7.0	10.9
CO 6	10.9	33.8	6.0	13.2
CO 7	10.3	38.5	6.4	12.9
CO 8	9.9	37.5	6.1	12.1

(1) As measured.

(2) As calculated from ingredient analyses and corrected to measured moisture content.

TABLE 24.

GROWTH DATA - PHASE I.

No. of days of trial	0	14	28	61	% increase in mean weight 0 - 61 days.
Mean temperature (1)	11	10	9	9	
Feeding rate (2)	2.3	2.1	1.9	1.8	
Diet Number	Mean weight of fish - g (3)				
CO 1	17.0	20.4	22.9	30.6	80.0
CO 2	16.6	19.3	22.5	30.3	82.5
CO 3	16.6	20.0	22.1	29.7	78.9
CO 4	16.9	20.1	23.8	31.0	83.4
CO 5	17.0	20.3	22.9	30.3	78.2
CO 6	16.7	19.5	21.5	26.5	58.7
CO 7	16.8	19.2	22.9	29.2	73.8
CO 8	16.6	19.2	21.6	26.6	60.2

(1) Ambient mains water temperature to nearest degree.

(2) Feeding rate suggested in tables given as % body weight/day.

(3) Mean weight of fish from duplicate groups on each diet treatment.

TABLE 25.

CUMULATIVE MORTALITY, STOCKING AND LOADING DENSITIES.

(End of Phase I).

Tank Number	Diet Number	Cumulative Mortality	Total Weight g	Stocking Density $g\,l^{-1}$	Loading[1] $g\,l^{-1}$ min.
1	CO 1	0	873	87.3	3492
2	CO 2	4	712	71.2	2848
3	CO 3	3	742	74.2	2968
4	CO 4	28 (2)	-	-	-
5	CO 5	0	847	84.7	3388
6	CO 6	2	671	67.1	2684
7	CO 7	1	783	78.3	3132
8	CO 8	2	718	71.8	2872
9	CO 8	0	714	71.4	2856
10	CO 7	11	499	49.9	1996
11	CO 6	1	731	73.1	2924
12	CO 5	5	706	70.6	2824
13	CO 4	0	871	87.1	3484
14	CO 3	19 (0)	186	18.6	744
15	CO 2	3	774	77.4	3096
16	CO 1	1	810	81.0	3240

(1) Approximate values only, assuming a constant flow rate of 250 ml/min

( ) Numbers in parentheses show the mortality prior to the systems problems described in the text.

TABLE 26.

ENVIRONMENTAL CONDITIONS (60 DAYS).

Tank Number	Temp. °C	pH	Ammonia p.p.m.	Unionised Ammonia $\mu g l^{-1}$
1	9.0	6.22	0.6	0.1716
2	9.0	6.08	0.9	0.1864
3	9.0	6.11	1.0	0.2220
4	9.0	-	-	-
5	9.0	6.06	0.96	0.1899
6	9.0	6.07	0.56	0.1134
7	9.0	6.09	1.15	0.2413
8	9.1	6.05	0.98	0.1910
9	9.3	6.19	0.85	0.2323
10	9.4	6.10	0.80	0.1791
11	9.5	6.15	0.80	0.2025
12	9.5	6.08	1.98	0.4267
13	9.5	6.17	1.25	0.3314
14	9.5	6.19	0.48	0.1308
15	9.5	6.12	1.25	0.2954
16	9.5	6.11	1.15	0.2655

TABLE 27

STOCKING DENSITY AND LOADING FACTOR (START OF PHASE 2.)

Tank Number	Diet Number	Total Weight g	Stocking Density $g\ l^{-1}$	Loading Factor $g\ l^{-1}$ min.
1	CO 1	632	63.2	2528
2	CO 2	644	64.4	2576
3	CO 3	613	61.3	2452
4	CO 4	409	40.9	1636
5	CO 5	617	61.7	2468
6	CO 6	536	53.6	2144
7	CO 7	602	60.2	2408
8	CO 8	586	58.6	2344
9	CO 8	574	57.4	2296
10	CO 7	614	61.4	2456
11	CO 6	550	55.0	2200
12	CO 5	631	63.1	2524
13	CO 4	462	46.2	1848
14	CO 3	360	36.0	1440
15	CO 2	627	62.7	2508
16	CO 1	666	66.6	2664

TABLE 28.

MEAN FISH WEIGHTS - PHASE II

Number of days of trial	61	78	92	110
Mean Temperature °C (1)	9	8	7	7
Feeding (in range) (2)	1.8-1.7	1.7-1.6	1.6-1.5	1.5-1.4
Diet (3) Number	Mean Weight of Fish - g. ± S.E.(4)			
CO 2 (2)	32.2 ± 1.33	39.5 ± 1.60	46.6 ± 1.97	53.1 ± 2.42
(15)	31.4 ± 1.38	38.3 ± 1.71	44.8 ± 1.73	56.1 ± 2.28
CO 3 (3)	30.6 ± 1.34	37.6 ± 1.72	44.0 ± 2.09	53.7 ± 2.67
(14)	25.7 ± 2.05	31.2 ± 2.62	36.8 ± 2.97	46.0 ± 3.77
CO 4 (4)	29.2 ± 1.14	35.7 ± 1.63	42.8 ± 1.98	55.1 ± 2.60
(13)	33.0 ± 1.92	42.0 ± 2.37	51.1 ± 2.93	63.6 ± 3.10
CO 5 (5)	30.8 ± 1.22	37.8 ± 1.47	44.9 ± 1.75	55.7 ± 2.14
(12)	31.6 ± 1.56	39.2 ± 1.93	45.4 ± 2.15	53.5 ± 2.76
CO 6 (6)	28.6 ± 0.89	30.4 ± 0.84	34.2 ± 1.28	40.6 ± 1.58
(11)	27.5 ± 1.51	31.5 ± 1.6	36.2 ± 1.85	40.5 ± 2.15
CO 7 (7)	30.1 ± 1.43	36.2 ± 1.79	42.2 ± 2.03	52.2 ± 2.42
(10)	29.3 ± 1.24	35.1 ± 1.45	41.7 ± 1.65	48.4 ± 2.03
CO 8 (8)	29.3 ± 0.90	35.0 ± 1.07	40.8 ± 1.26	49.2 ± 1.62
(9)	28.7 ± 0.99	33.6 ± 1.2	38.3 ± 1.36	46.4 ± 1.68

(1) Mean ambient mains water temperature.

(2) Feeding rate % Body Weight/Day.

(3) Diet number with tank number in parentheses.

(4) Values are means for each of the duplicate populations on each diet.

TABLE 29

CONVERSION EFFICIENCY DATA - PHASE II. (1)

Period Days / Diet Number (2)	61 - 78	79 - 92	93 - 110	61 - 92 Mean result.
CO 2 (2)	88.5	80.0	48.8	82.7
(15)	87.0	75.2	87.7	
CO 3 (3)	89.3	79.4	76.9	83.0
(14)	83.3	80.0	87.0	
CO 4 (4)	87.0	88.5	100.0	93.2
(13)	100.4	97.1	85.5	
CO 5 (5)	87.7	80.0	84.0	83.0
(12)	93.5	70.9	62.1	
CO 6 (6)	52.4	56.5	64.5	58.1
(11)	56.8	66.7	54.9	
CO 7 (7)	78.1	75.2	82.0	79.7
(10)	81.3	84.0	55.9	
CO 8 (8)	76.3	74.1	71.4	70.0
(9)	67.1	62.5	73.0	
Temp(Range)°C	9 - 8	8 - 7	7	

(1) % Conversion efficiency $\frac{\text{Weight gain-g}}{\text{Weight food consumed-g}} \times 100$ (for duplicate groups of fish on each diet.)

(2) Tank number in parentheses.

TABLE 30

ENVIRONMENTAL CONDITIONS (110 DAYS).

Tank	Diet No.	Oxygen saturation %	pH	Total Ammonia p.p.m.	Stocking Density g l^{-1}	Loading (1) g l^{-1} min.
1	CO 1	71	6.5	0.25	73.2	2927
2	CO 2	36	6.3	0.34	106.3	4248
3	CO 3	51	6.5	0.44	107.5	4296
4	CO 4	47	6.4	0.65	77.2	3087
5	CO 5	45	6.5	0.52	105.9	4236
6	CO 6	51	6.3	0.45	81.3	3251
7	CO 7	59	6.4	0.31	104.3	4172
8	CO 8	55	6.4	0.26	98.4	3936
9	CO 8	65	6.5	0.21	92.9	3714
10	CO 7	45	6.4	0.34	101.6	4064
11 (2)	CO 6	83	-	-	-	-
12	CO 5	64	6.5	0.43	107.0	4278
13	CO 4	54	6.5	0.30	89.1	3564
14	CO 3	62	6.5	0.34	64.4	2575
15	CO 2	62	6.4	0.28	112.3	4492
16	CO 1	61	6.5	0.33	108.9	4356

Temperature in all tanks = 7.0 °C.

(1) Loading - approximate values only.

(2) 5 fish only.

TABLE 31.

MEAN WEIGHT OF FISH (LOGE VALUES) AND SPECIFIC GROWTH RATE.

NUMBER OF DAYS / DIET NUMBER	61	78	92	110	61 - 92
	Log_e mean weight ——				Mean Sp. Gr. %
CO 2	3.46	3.66	3.82	4.00	1.16
CO 3	3.34	3.54	3.70	3.91	1.16
CO 4	3.44	3.66	3.85	4.08	1.32
CO 5	3.44	3.65	3.81	4.00	1.19
CO 6	3.30	3.43	3.56	3.72	0.84
CO 7	3.39	3.57	3.74	3.90	1.12
CO 8	3.37	3.54	3.68	3.87	1.00

TABLE 32.

PELLET HARDNESS.

Diet	Speed (1)	(2) %Loss of weight in time (mins). 1	2	3	4	5
C.N.P. (expanded)	Slow	0	0	0.2	0.2	0.2
	Medium	1.8	2.4	3.6	4.4	5.0
	Fast	5.2	6.4	7.6	8.0	8.4
CO 2 (Dry Method)	Slow	4.0	6.2	8.2	9.8	11.4
	Medium	30.8	37.4	44.2	48.4	51.8
	Fast	29.4	45.4	51.0	51.4	56.6
CO 2 (Wet Method)	Slow	0	0.2	0.4	0.4	0.6
	Medium	1.6	2.4	3.0	3.4	3.6
	Fast	5.2	8.2	10.2	10.8	13.0

(1) Arbitrary selection on dial of shaker.

(2) Values represent mean of 3 samples.

TABLE 33.

WATER STABILITY OF PELLETS

Pellet Type	% loss in dry weight in time (mins)				
	1	2	3	5	10
C.N.P. (expanded)	4.0	7.0	9.5	14.5	22.5
CO 2 (dry pelleting)	23.0	35.0	44.0	57.0	80.5
CO 2 (wet pelleting)	5.0	8.0	11.5	17.0	25.0

TABLE 34.

FOOD INGREDIENTS USED IN THE COMPOUND FEEDS.

TEST PROTEIN	SUPPLIER
Herring meal	Herring By-Products Ltd.
Soyabean oil Meal	Rowett Research Institute
Cotton seed meal	" " "
Groundnut meal	" " "
Yeast Protein	B.P. Proteins Ltd.
Bacterial Protein	I.C.I. Ltd.
Algal Protein	Sosa Texcoco, S.A. Mexico.
Mould Protein	Glaxo Laboratories
Mould Protein	Tate & Lyle Ltd.

BASAL INGREDIENTS	
Corn gluten meal (Globe Prairie)	C.P.C. Ltd.
Delactosed whey	Cooper Nutrition Products Ltd.
Distillers' solubles	" " " "
Brewers' yeast	" " " "
Wheat feed	Spillers Ltd.
Durabond	Reeson Bubsby & Co. Ltd.
Pure Vitamins	Cooper Nutrition Products Ltd.
Salt Mixture	(Laboratory Chemicals)

TABLE 35.

FORMULATION OF EXPERIMENTAL DIETS - PHASE I.

Basal Ration (1)	%
Herring meal	20
Corn gluten meal	10
Brewers' yeast	5
Delactosed whey	10
Distillers' solubles	8
Wheat feed	15
Vitamin premix (3)	4
Mineral mix (4)	2
Durabond binder	2
Soyabean oil	4
Test Protein (2)	20

(1) All diets contain basal ingredients.

(2)

Diet Number	Test Protein
CO 9	Herring meal
C 10	Soyabean oil meal
C 11	Cotton seed meal
C 12	Groundnut meal
C 13	Yeast Protein (B.P.)
C 14	Bacterial protein (I.C.I.)
C 15	Algal protein (Sosa Texcoco)
C 16	Mould protein (Tate & Lyle)
C 17	Mould protein (Glaxo Laboratories.

(3) Vitamin mix as shown in Table 21.

(4) Mineral mix as shown in Table 20.

TABLE 36.

FORMULATION OF EXPERIMENTAL DIETS - PHASE II.

Basal Ration[1]	%
Herring meal	20
Corn gluten meal	10
Brewers' Yeast	5
Wheat feed	15
Vitamin mix[3]	4
Mineral mix[4]	2
Soya bean oil	4
Test Protein[2]	40

(1) All diets contain basal ingredients.

(2)

Diet Number	Test Protein
C 18	Yeast Protein (B.P.)
C 19	Bacterial protein (I.C.I.)
C 20	Algal Protein (Sosa Texcoco)
C 21	Mould protein (Tate & Lyle)
C 22	Mould protein (Glaxo Laboratories).

(3) Vitamin mix as shown in Table 21.

(4) Mineral mix as shown in Table 20.

TABLE 37.

COMPOSITION OF TEST INGREDIENTS

(Proximate Analysis).

Ingredient	Water Content %	Crude Protein %	Pet Ether Extr. %	Ash. %	Total Energy kcals/10 g
Herring Meal	9.3	72.5	5.8	12.2	46.41
Soybean Oil Meal	7.8	45.9	1.4	6.1	42.50
Cottonseed Meal	7.0	44.6	1.8	6.9	42.22
Groundnut Meal	8.1	49.0	2.3	5.4	42.64
Yeast Protein (B.P. Ltd)	5.3	58.2	2.9	13.3	48.57
Bacterial Protein (I.C.I. Ltd)	3.4	77.7	1.6	16.3	49.70
Algal Protein (Sosa Texcoco SA)	7.5	49.8	2.3	10.3	47.20
Fungal Protein (Tate & Lyle Ltd)	8.5	32.7	2.1	3.0	43.72
Fungal Protein (Glaxo Laboratories)	6.7	32.1	0.5	11.0	39.33

TABLE 38

COMPOSITION OF INGREDIENTS OF BASAL RATION
(Proximate Analysis).

Ingredient	Water Content %	Crude Protein %	Pet Ether Extr. %	Ash %
Corn Gluten meal	8.0	60.0	2.5	3.8
Delactosed whey	9.9	27.9	1.7	21.6
Distillers solubles	5.6	25.3	10.5	13.9
Brewers' yeast	7.1	45.0	1.0	5.6
Wheat feed	13.2	15.5	3.4	3.9
Durabond	9.6	Nil	1.0	11.0

TABLE 39.

COMPOSITION OF TEST DIETS.

(Proximate Analysis)

TEST DIET		Water %	Crude Protein %	Pet. Ether Extr. %	Ash %	Gross Energy Kcals/100 g.
C 09	Herring Meal	8.3	45.51	7.50	14.05	429.93
C10	Soybean Oil Meal	8.7	39.82	6.61	12.72	418.82
C11	Cottonseed Meal	9.2	39.28	6.64	12.79	415.25
C12	Groundnut Meal.	8.9	40.38	6.78	12.56	418.44
C13	Yeast Protein (B.P. Ltd.,)	11.0	41.00	6.70	13.74	417.81
C14	Bacterial Protein (I.C.I.Ltd)	10.3	44.95	6.47	14.37	421.56
C15	Algal Protein (Sosa Texcoco SA)	10.2	39.91	6.67	13.33	420.90
C16	Fungal Protein (Tate & Lyle)	10.1	36.67	6.65	16.93	415.43
C17	Fungal Protein (Glaxo Laboratories)	8.7	36.97	6.41	13.67	411.48

TABLE 40

COMPOSITION OF TEST DIETS

(Proximate Analysis)

TEST DIET		Water %	Crude Protein %	Pet. Ether Extn %	Ash %	Gross Energy Kcals/100 g.
C09	Herring meal	7.0	46.1	7.7	14.3	436.0
C18	Yeast Protein	7.6	49.1	7.3	13.4	458.1
C19	Bacterial Protein	8.4	56.0	6.6	14.3	454.9
C20	Algal Protein	8.5	45.8	7.0	12.1	452.5
C21	Fungal Protein (Tate & Lyle)	8.0	39.3	7.0	9.3	442.9
C22	Fungal Protein 'Glaxo)	7.1	39.2	6.4	12.6	426.1

TABLE 41.

APPARENT PROTEIN DIGESTIBILITY(1)

Diet No.	% Cr.III Oxide		% Nitrogen		% Apparent Digestibility	Relative % Digestibility of Test Protein (2)
	Feed	Faeces	Feed	Faeces		
C09	0.475	1.466	7.47	3.57	84.4	100.0
C10	0.493	1.332	6.56	3.29	81.4	95.9
C11	0.487	1.256	6.53	2.88	82.9	102.7
C12	0.495	1.426	6.78	3.09	84.2	106.3
C13	0.484	1.411	6.88	2.95	85.3	106.9
C14	0.465	1.494	7.23	3.58	84.6	99.4
C15	0.475	1.403	6.78	4.02	79.9	88.6
C16	0.473	1.210	6.14	3.14	80.0	93.7
C17	0.459	1.093	6.14	3.12	78.7	86.1

(1) Calculated from:

$$\text{Digestibility \%} = 100 - \frac{\text{\% indicator in feed}}{\text{\% indicator in faeces}} \times \frac{\text{\% N in faeces}}{\text{\% N in feed.}} \times 100$$

(2) Digestibility of test protein component relative to herring meal at 100%, assuming the protein of the basal ration is digested equally in all diets.

TABLE 42

MEAN DAILY FOOD CONSUMPTION - 9

TIME WEEKS / DIET	0 - 2 g	± SE	2 - 4 g	± SE	4 - 6 g	± SE	6 - 8 g	± SE	8 - 10 g	± SE
C09 HERRING MEAL	5.11	0.37	6.22	0.26	7.35	0.57	9.19	0.75	10.00	1.14
	5.15	0.42	6.87	0.22	7.26	0.70	9.53	0.89	10.15	1.21
	5.26	0.40	6.37	0.28	7.00	0.64	10.09	1.02	10.12	1.20
SOYABEAN OIL MEAL	5.69	0.39	7.10	0.33	8.54	0.62	11.38	0.98	11.28	1.43
	5.11	0.39	6.38	0.26	7.50	0.57	9.88	0.81	10.72	1.44
	5.57	0.38	7.00	0.30	7.09	0.59	9.68	0.76	10.00	1.18
C11 COTTONSEED MEAL	5.74	0.45	7.33	0.37	6.62	0.56	10.11	0.83	11.23	1.55
	5.49	0.46	7.66	0.32	7.65	0.73	10.25	0.91	10.42	1.24
	5.04	0.41	6.17	0.22	6.45	0.60	8.76	0.84	9.18	1.28
C12 GROUNDNUT MEAL	5.03	0.35	6.80	0.23	6.73	0.50	7.96	0.63	8.75	1.01
	5.31	0.42	6.24	0.36	6.94	0.56	9.30	0.91	9.53	1.33
	5.36	0.45	6.80	0.26	6.89	0.73	8.82	0.75	9.49	1.13
C13 YEAST PROTEIN	5.72	0.40	6.72	0.29	7.16	0.61	9.27	0.78	9.24	1.08
	5.32	0.35	5.92	0.31	6.08	0.53	8.56	0.72	8.41	1.13
	5.41	0.44	6.67	0.39	6.90	0.74	9.36	0.87	9.38	1.13
C14 BACTERIAL PROTEIN	4.58	0.34	5.84	0.12	6.05	0.57	8.90	0.80	8.91	1.05
	4.80	0.37	5.98	0.20	6.59	0.54	9.27	0.75	8.85	1.03
	4.73	0.35	5.94	0.19	6.60	0.53	8.17	0.85	8.14	0.99
C15 ALGAL PROTEIN	5.14	0.40	6.69	0.27	6.41	0.60	8.72	0.84	9.14	1.07
	5.26	0.41	6.22	0.27	7.23	0.57	9.30	0.71	9.72	1.20
	5.44	0.43	6.72	0.33	7.12	0.70	8.75	0.75	8.87	1.03
C16 FUNGAL PROTEIN (Tate & Lyle)	5.38	0.37	6.90	0.24	7.75	0.62	8.55	0.83	9.12	1.17
	5.30	0.40	6.25	0.25	6.34	0.66	8.73	0.72	9.18	1.16
	5.54	0.47	6.76	0.30	7.82	0.66	9.58	0.84	9.90	1.17
C17 FUNGAL PROTEIN (GLAXO)	5.38	0.38	6.78	0.19	7.52	0.56	10.13	0.84	10.46	1.16
	5.06	0.40	6.19	0.17	6.81	0.63	9.55	0.79	9.26	1.12
	5.10	0.43	6.28	0.32	6.89	0.66	9.06	0.84	9.31	1.19

TABLE 43.

SUMMARY OF FOOD CONSUMPTION DATA. (g/100g fish)

(Mean result for each diet expressed as % Mid-point Body Weight per Day for each period).

TEST DIET \ TIME WEEKS	0 - 2	2 - 4	4 - 6	6 - 8	8 - 10
C09 FISH MEAL	3.48	3.20	2.63	2.66	2.19
C10 SOYBEAN MEAL	3.63	3.40	2.87	2.86	2.30
C11 COTTONSEED MEAL	3.85	3.69	2.76	2.97	2.43
C12 GROUNDNUT MEAL	3.66	3.45	2.69	2.64	2.20
C13 YEAST PROTEIN	3.77	3.29	2.61	2.74	2.16
C14 BACTERIAL PROTEIN	3.32	3.13	2.55	2.67	2.10
C15 ALGAL PROTEIN	3.65	3.40	2.77	2.78	2.27
C16 FUNGAL PROTEIN (T & L)	3.85	3.60	3.05	2.95	2.48
C17 FUNGAL PROTEIN (GIAXO)	3.83	3.64	3.09	3.22	2.58
MEAN TEMPERATURE °C	16.5	16.75	16.0	14.5	12.5

TABLE 44.

RELATIONSHIP BETWEEN BODY WEIGHT(1) AND GROSS ENERGY CONSUMPTION.(2)

PERIOD-WEEKS	DIET 1 (3)		DIET 6 (3)		DIET 9 (3)	
	Log_e BWt	Log_e G.E.	Log_e BWt	Log_e G.E.	Log_e BWt.	Log_e G.E.
0 - 2	4.957	3.090	4.902	2.961	4.924	3.097
	5.035	3.097	4.959	3.007	4.843	3.036
	5.015	3.118	5.001	2.993	4.954	3.044
2 - 4	5.241	3.286	5.193	3.204	5.197	3.329
	5.354	3.386	5.256	3.227	5.112	3.238
	5.335	3.310	5.282	3.220	5.203	3.252
4 - 6	5.546	3.453	5.476	3.239	5.463	3.432
	5.670	3.441	5.551	3.324	5.377	3.333
	5.622	3.404	5.557	3.326	5.455	3.345
6 - 8	5.840	3.677	5.741	3.625	5.732	3.730
	5.950	3.713	5.836	3.666	5.645	3.671
	5.879	3.770	5.811	3.539	5.709	3.618

(1) Body weight calculated as $\frac{Wt_T + Wt_{To}}{2}$ where T - To = 14 days.

(2) Gross energy consumption calculated from food consumption (g) for each period from Table 41 multiplied by the gross energy content of the diets (I cal/g) from Table 38.

(3) Values given are for each of 3 groups of fish on each diet.

TABLE 45.

ASSIMILATION COEFFICIENTS.

Diet	Assimilation Coefficient(1)
CO 9	0.74
C10	0.69
C11	0.69
C12	0.71
C13	0.71
C14	0.72
C15	0.67
C16	0.66
C17	0.66

(1) Assumes following digestible energy values:-

Fat	=	8.0 Kcals/g intake
Carbohydrate	=	1·6 Kcals/g intake
Protein	=	5.65 Kcals/g digested.

TABLE 46.

APPETITE QUOTIENT (1)

DIET \ PERIOD WEEKS	0 - 2	2 - 4	4 - 6	6 - 8	8 - 10	Diet Mean
C09	0.309	0.299	0.277	0.274	0.242	
	0.292	0.302	0.248	0.260	0.229	0.273
	0.303	0.284	0.248	0.291	0.243	
C10	0.289	0.281	0.263	0.272	0.218	
	0.275	0.276	0.257	0.269	0.238	0.263
	0.293	0.293	0.239	0.264	0.224	
C11	0.321	0.312	0.229	0.282	0.251	
	0.288	0.311	0.245	0.264	0.219	0.268
	0.281	0.283	0.242	0.266	0.228	
C12	0.298	0.319	0.248	0.244	0.223	
	0.286	0.268	0.241	0.258	0.214	0.259
	0.295	0.293	0.237	0.249	0.218	
C13	0.319	0.290	0.244	0.256	0.214	
	0.296	0.266	0.226	0.268	0.219	0.258
	0.293	0.286	0.236	0.254	0.210	
C14	0.275	0.278	0.230	0.274	0.228	
	0.276	0.271	0.235	0.264	0.210	0.249
	0.263	0.264	0.235	0.238	0.200	
C15	0.277	0.287	0.224	0.249	0.217	
	0.279	0.265	0.248	0.257	0.218	0.250
	0.279	0.272	0.236	0.241	0.204	
C16	0.288	0.292	0.260	0.237	0.222	
	0.283	0.273	0.232	0.268	0.232	0.258
	0.280	0.275	0.258	0.255	0.215	
C17	0.284	0.249	0.258	0.281	0.242	
	0.286	0.282	0.251	0.284	0.227	0.259
	0.263	0.265	0.238	0.255	0.218	
PERIOD MEAN	0.288	0.283	0.244	0.262	0.223	

(1) Calculated for each of the three replicate groups on each diet for each two-week period.

TABLE 47

ANALYSIS OF APPETITE DATA.

ANALYSIS OF VARIANCE

Source	Degrees of Freedom	Sum of Squares	Mean Squares	F. Ratio	Probability
Period (P)	4	0.07887	0.01972	164.33	< 0.001
Diet (D)	8	0.00710	0.00089	7.42	< 0.001
P x D	32	0.00563	0.00018	1.50	> 0.05 (N.S)
Replicates in D	18	0.00401	0.00022	1.83	0.01 - 0.05
Residual	72	0.00870	0.00012		
TOTAL	134	0.10432			

FURTHER ANALYSIS OF DIET EFFECT (using sequential form of the Q method based on the Tables of the Studentized Range).

Diet	Mean	5% Level (1)	1% Level (2)
C14 Bact	0.2494	a	a
C15 Alg	0.2502	a b	a
C16 A.nig.	0.2580	a b c	a b
C13 Yeast	0.2585	a b c	a b
C 17 P.Chr.	0.2589	a b c	a b
C12 G.N.	0.2594	a b c	a b
C10 Soya	0.2634	b c	a b c
C11 Cot.	0.2681	c d	b c
C09 H.M.	0.2734	d	c

(1) Diet means with same letter are not significantly different.

(2) Diet means with same letter are not significantly different.

WEIGHT DATA - PHASE I.

DIET \ TIME WEEKS	0		2		4		6		8		10	
	g.	± S.E.	g.	± S.E.	g.	± S.E.	g.	± S.E.	g.	± S.E.	g.	± S.E.
C09	5.99	0.31	8.22	0.52	10.67	0.74	14.95	1.44	20.44	2.04	26.43	2.75
	6.53	0.37	8.84	0.60	12.30	1.06	16.70	1.73	21.67	2.22	27.06	2.83
	6.28	0.26	8.79	0.40	11.95	0.61	15.70	0.95	20.06	1.13	24.95	1.43
C10	6.52	0.31	9.12	0.44	12.22	0.68	16.97	1.07	23.09	1.50	29.18	1.97
	6.27	0.28	8.26	0.38	10.85	0.54	14.63	0.90	19.41	1.23	24.61	1.72
	6.32	0.26	8.63	0.43	11.24	0.68	14.76	1.05	19.28	1.49	24.20	1.81
C11	5.80	0.36	8.14	0.65	11.14	1.03	13.87	1.50	18.88	2.13	24.16	2.81
	6.32	0.28	8.51	0.42	11.94	0.69	15.61	1.06	20.48	1.44	26.02	1.95
	6.12	0.22	7.64	0.33	9.93	0.50	12.67	0.86	16.76	1.34	20.93	1.82
C12	5.81	0.22	7.53	0.26	10.35	0.45	13.81	0.68	16.68	0.89	21.58	1.30
	6.35	0.37	8.67	0.58	11.27	0.87	14.81	1.37	19.67	1.89	25.18	2.54
	6.35	0.36	8.31	0.50	11.57	0.72	14.79	1.04	19.07	1.37	24.54	1.80
C13	5.99	0.36	8.38	0.63	11.44	1.03	15.17	1.60	20.39	2.28	24.98	2.80
	6.18	0.24	8.22	0.30	10.65	0.42	13.15	0.58	16.49	0.78	20.82	1.01
	6.40	0.22	8.47	0.30	11.48	0.47	15.04	0.78	20.29	1.17	24.54	1.60
C14	5.72	0.34	7.74	0.50	10.26	0.78	13.62	1.37	18.42	2.11	22.73	2.88
	6.17	0.33	8.08	0.55	11.10	0.93	14.65	1.53	19.59	2.41	23.63	2.89
	6.45	0.17	8.40	0.34	11.27	0.61	14.64	1.01	18.77	1.36	22.42	1.66
C15	6.00	0.33	8.04	0.47	10.69	0.71	13.52	1.09	17.56	1.65	21.64	2.10
	6.24	0.28	8.13	0.44	10.74	0.61	14.07	0.92	18.46	1.34	23.57	1.82
	6.36	0.30	8.61	0.45	11.51	0.67	14.32	0.99	18.32	1.31	22.40	1.71
C16	5.88	0.30	7.83	0.40	11.07	0.63	14.79	0.98	18.00	1.17	21.86	1.59
	6.00	0.22	7.73	0.31	9.89	0.44	12.77	0.69	16.13	0.91	20.83	1.26
	6.29	0.28	8.42	0.43	10.88	0.64	14.21	0.97	18.64	1.34	23.59	1.79
C17	5.97	0.34	7.79	0.50	10.29	0.75	13.28	1.15	17.57	1.68	22.11	2.09
	5.54	0.22	7.14	0.30	9.46	0.45	12.17	0.65	16.12	0.89	19.86	1.11
	6.28	0.22	7.89	0.34	10.30	0.59	13.10	0.99	17.07	1.52	20.90	1.73

TABLE 49

MEAN GROWTH DATA - PHASE I.

DIET.	TIME WEEKS	0	2	4	6	8	10
C09	Weight g	6.27	8.62	11.64	15.78	20.72	26.15
	Log_e wt	1.836	2.154	2.454	2.759	3.031	3.264
C10	Weight g	6.37	8.67	11.44	15.45	20.59	26.19
	Log_e wt	1.852	2.160	2.437	2.738	3.025	3.265
C11	Weight g	6.08	8.10	11.00	14.05	18.71	23.70
	Log_e wt	1.805	2.092	2.398	2.64	2.93	3.16
C12	Weight g	6.17	8.17	11.06	14.37	18.47	23.77
	Log_e wt	1.820	2.100	2.403	2.665	2.916	3.168
C13	Weight g	6.19	8.36	11.19	14.53	19.06	23.45
	Log_e wt	1.823	2.123	2.415	2.676	2.948	3.155
C14	Weight g	6.11	8.07	10.88	14.40	18.91	22.93
	Log_e wt	1.810	2.088	2.387	2.67	2.940	3.13
C16	Weight g	6.06	8.05	10.89	13.92	17.49	22.09
	Log_e wt	1.802	2.086	2.388	2.637	2.862	3.095
C15	Weight g	6.20	8.26	10.98	13.97	18.11	22.54
	Log_e wt	1.825	2.111	2.396	2.637	2.896	3.115
C17	Weight g	5.93	7.61	10.35	12.85	17.07	20.96
	Log_e wt	1.78	2.029	2.337	2.550	2.837	3.043

TABLE 50

MEAN SPECIFIC GROWTH RATES - PHASE I

PERIOD WEEKS / DIET	0 - 2	2 - 4	4 - 6	6 - 8	8 - 10	Diet Mean
C09	2.26 2.16 2.40	1.86 2.36 2.19	2.41 2.18 1.95	2.24 1.86 1.75	1.84 1.59 1.56	2.04
C10	2.40 1.97 2.00	2.09 1.95 1.89	2.35 2.13 1.95	2.20 2.02 1.91	1.67 1.70 1.62	1.99
C11	2.42 2.13 1.59	2.24 2.42 1.87	1.57 1.91 1.74	2.20 1.94 2.00	1.76 1.71 1.59	1.94
C12	1.85 2.22 1.92	2.27 1.87 2.36	2.06 1.95 1.75	1.35 2.03 1.81	1.84 1.76 1.80	1.92
C13	2.40 2.04 2.00	2.22 1.85 2.17	2.02 1.50 1.93	2.11 1.62 2.14	1.45 1.67 1.36	1.90
C14	2.16 1.93 1.89	2.01 2.27 2.10	2.02 1.98 1.87	2.16 2.07 1.77	1.50 1.34 1.27	1.89
C16	2.09 1.89 2.16	2.03 1.99 2.07	1.68 1.93 1.56	1.87 1.94 1.76	1.49 1.75 1.44	1.84
C15	2.05 1.81 2.08	2.47 1.76 1.84	2.07 1.83 1.91	1.40 1.67 1.94	1.39 1.83 1.68	1.85
C17	1.90 1.81 1.63	1.99 2.01 1.91	1.82 1.80 1.72	2.00 2.01 1.89	1.64 1.49 1.45	1.80
PERIOD MEAN	2.04	2.08	1.91	1.91	1.60	
TEMP °C	16.5	16.75	16.0	14.5	12.5	

TABLE 51.

ANALYSIS OF SPECIFIC GROWTH RATE DATA

ANALYSIS OF VARIANCE

Source	Degrees of Freedom	Sum of Squares	Mean Squares	F Ratio	Probability
Pericd (P)	4	3.82496	0.95624	19.64	<0.001
Diet (1)	8	0.66501	0.08313	1.71	>0.05 (n.s.)
P x D	32	1.40653	0.04395	0.90	>0.05 (n.s.)
Replication in D	18	0.96926	0.05385	1.11	>0.05 (n.s.)
Residual	72	3.50567	0.04868		
Total	134	9.37143			

TABLE 52

FOOD CONVERSION EFFICIENCY

DIET PERIOD WEEKS	0 - 2	2 - 4	4 - 6	6 - 8	8-10	Diet Mean
C09	67.1 68.9 73.5	60.6 77.5 76.3	89.3 93.5 82.6	82.0 80.0 66.7	87.7 82.0 74.1	77.5
C10	70.4 59.9 63.7	67.1 62.5 57.5	85.5 77.5 76.3	82.6 74.6 71.9	83.3 74.6 75.8	72.2
C11	68.5 61.3 46.5	62.9 67.6 57.1	63.3 74.1 65.4	76.3 73.0 71.9	72.5 82.0 69.9	67.5
C12	52.6 67.1 56.2	63.7 64.1 73.5	79.4 78.7 71.9	74.6 80.6 74.6	86.2 89.3 88.5	73.4
C13	64.1 59.2 58.8	69.9 63.3 69.4	80.6 63.3 79.4	78.7 59.9 86.2	72.5 79.4 69.9	70.3
C14	67.6 61.3 63.3	66.2 77.5 74.1	85.5 82.6 78.7	74.1 82.0 77.5	70.9 70.4 69.0	73.4
C15	60.9 55.2 63.7	61.0 64.5 66.2	68.0 70.9 60.6	71.4 72.5 70.4	68.5 80.6 70.9	67.0
C16	55.9 50.3 59.2	64.9 52.9 56.2	70.4 61.0 65.4	54.6 56.2 70.9	63.3 74.6 76.9	62.2
C17	52.1 48.5 48.5	56.8 57.8 59.2	61.0 61.3 62.5	64.9 63.7 67.6	56.8 62.1 63.3	59.1
PERIOD MEAN	60.2	64.8	73.7	72.6	74.6	
TEMP °C	16.5	16.75	16.0	14.5	12.5	

TABLE 53

ANALYSIS OF CONVERSION EFFICIENCY DATA.

ANALYSIS OF VARIANCE

Source	Degrees of Freedom	Sum of Squares	Mean Squares	F.Ratio	Probability.
Period (P)	4	4361.97674	1090.49419	40.61	< 0.001
Diet (D)	8	4095.65037	511.95630	19.06	< 0.001
P x D	32	1498.55926	46.82998	1.74	0.01 - 0.05
Replicates in D	18	977.36666	54.29815	2.02	0.01 - 0.05
Error	72	1933.58000	26.85528		
TOTAL	134	12867.13304			

FURTHER ANALYSIS OF DIET EFFECT (using sequential form of the Q method based on the tables of the Studentized Range).

DIET	MEAN	5% LEVEL(1)	1% LEVEL (2)
C17 P.chr.	59.07	a	a
C16 A.nig.	62.18	a b	a
C15 Alg.	67.02	b c	a b
C11 Cotton	67.48	b c	a b
C13 Yeast	70.31	c d	b c
C10 Soya.	72.21	c d	b c
C16 Bact.	73.38	c d	b c
C14 G.N.	73.40	c d	b c
C09 H.M.	77.45	d	c

(1) Diet means with same letter are not significantly different.

(2) Diet means with same letter are not significantly different.

TABLE 54

FOOD CONSUMPTION[1] PHASE II (12-22 weeks)

PERIOD WEEKS / DIET	12 - 14	14 - 16	16 - 18	18 - 20[2]	20 - 22
C09	0.190 (141)	0.200 (166)	0.191 (180)	0.143 (143)	0.173 (188)
C18	0.212 (141)	0.224 (167)	0.216 (179)	0.156 (142)	0.187 (185)
C19	0.160 (100)	0.192 (132)	0.168 (126)	0.103 (83)	0.139 (119)
C20	0.226 (140)	0.242 (166)	0.220 (167)	0.156 (130)	0.195 (177)
C21	0.217 (134)	0.241 (164)	0.228 (172)	0.150 (122)	0.200 (174)
C22	0.180 (102)	0.194 (119)	0.189 (126)	0.165 (119)	0.169 (131)
Temperature °C	9.5	8.5	7.5	7.5	7.0

(1) Food consumption as Gross Energy Consumed (Kcals)

$$\left(\frac{Wt_{T_0}^{0.8} + Wt_{T_1}^{0.8}}{2} \right) T_1 - T_0 \qquad \text{(where } T_1 - T_0 = 14 \text{ days)}$$

Values in parentheses are the actual quantities consumed in each period in g.

(2) Feeding automatic during this period, and feed intake therefore controlled and restricted.

TABLE 55.

GROWTH DATA - PHASE II.

DIET	TIME WEEKS	12	14	16	18	20	22
C09	Tot. Wt.g.	927	1060	1207	1362	1504	1687
	Log_e Wt.	6.832	6.966	7.096	7.217	7.316	7.431
C18	Tot. Wt.g.	852	979	1132	1288	1421	1597
	Log_e Wt.	6.748	6.887	7.032	7.161	7.259	7.376
C19	Tot. Wt.g.	802	892	1006	1121	1195	1297
	Log_e Wt.	6.687	6.793	6.914	7.022	7.086	7.168
C20	Tot. Wt.g.	770	877	1006	1137	1262	1405
	Log_e wt.	6.646	6.777	6.914	7.036	7.140	7.248
C21	Tot. Wt.g.	762	842	965	1090	1163	1290
	Log_e wt.	6.636	6.736	6.872	6.994	7.059	7.163
C22	Totl. Wt.g.	662	717	793	891	972	1054
	Log_e wt.	6.495	6.575	6.676	6.793	6.880	6.960

TABLE 56

MEAN SPECIFIC GROWTH RATES.

PERIOD WEEKS / DIET	12 - 14	14 - 16	16 - 18	18 - 20	20 - 22.
C09	0.957	0.928	0.863	0.707	0.821
C18	0.993	1.038	0.921	0.702	0.834
C19	0.758	0.860	0.773	0.457	0.586
C20	0.931	0.980	0.875	0.743	0.768
C21	0.714	0.974	0.870	0.464	0.740
C22	0.571	0.720	0.832	0.622	0.577
TEMP. °C	9.5	8.5	7.5	7.5	7.0

TABLE 57.

FOOD CONVERSION EFFICIENCY (%) - PHASE II.

PERIOD WEEKS / DIET	12 - 14	14 - 16	16 - 18	18 - 20	20 - 22
C09	94.3	89.3	86.2	99.0	97.1
C18	90.1	91.7	87.0	94.3	95.2
C19	89.3	87.0	90.9	89.3	85.5
C20	76.3	77.5	78.1	96.2	80.6
C21	59.5	75.2	73.0	59.9	73.0
C22	53.8	63.7	78.1	68.0	62.9
Temp °C	9.5	8.5	7.5	7.5	7.0

TABLE 58

CONDITION FACTOR - PHASE I AND II DATA.

TEST PROTEIN IN DIET.	END PHASE I (12 WEEKS)				END PHASE II (22 WEEKS)				Comparison Phase I & II STUDENTS 't' PROBABILITY.
	DIET NO.	NO. OBS.	MEAN C.F.	± S.E	DIET NO.	NO. OBS.	MEAN C.F.	± S.E.	
HERRING	C09	18	1.384	0.017	C09	18	1.454	0.020	< 0.02
SOYABEAN	C10	18	1.367	0.026					
COTTONSEED	C11	18	1.320	0.017					
GROUNDNUT	C12	18	1.357	0.030					
YEAST	C13	18	1.338	0.022	C18	18	1.414	0.017	< 0.02
BACTERIA	C14	18	1.382	0.024	C19	18	1.351	0.014	> 0.05 (n.s.)
ALGAE	C15	18	1.306	0.017	C20	18	1.332	0.020	> 0.05 (n.s.)
MOULD (A.nig:)	C16	18	1.329	0.017	C21	18	1.380	0.017	> 0.0 (n.s.)
MOULD (P.chr)	C17	18	1.301	0.017	C22	18	1.350	0.014	< 0.05

TABLE 59

ANALYSIS OF CONDITION FACTOR DATA.

ANALYSIS OF VARIANCE - PHASE I DATA.

SOURCE	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARES	F RATIO	PROBABILITY.
Between Groups (1)	8	0.14121	0.01765	1.92	> 0.05 (n.s.)
Within Groups	153	1.40485	0.00918		
TOTAL	161	1.54606			

(1) Diet effect not significant.

ANALYSIS OF VARIANCE - PHASE II DATA.

SOURCE	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARES	F RATIO	Probability.
Between Groups (1)	5	0.19414	0.03883	6.53	< 0.001
Within Groups	102	0.60700	0.00595		
TOTAL	107	0.80114			

(1) Diet effect highly significant.

FURTHER ANALYSIS OF DIET EFFECT PHASE II - (using sequential form of the Q method based on the tables of the Studentized Range).

DIET	MEAN C.F.	5% LEVEL(1)	1% LEVEL(2)
C09 H.M.	1.4556	a	a
C18 YEAST	1.4139	a b	a b
C21 A.Nig.	1.3806	b c	a b
C19 BACT.	1.3511	b c	b
C22 P.CHR.	1.3506	b c	b
C20 ALG.	1.3328	c	b

(1) Diet means with same letter are not significantly different at 5% level.
(2) Diet means with same letter are not significantly different at 1% level.

TABLE 60

% HAEMATOCRIT VALUES - PHASE I AND II DATA.

TEST PROTEIN IN DIET.	END PHASE I (12 WEEKS)				END PHASE II (22 WEEKS)				COMPARISON PHASE I & II STUDENTS 't' PROBABILITY
	DIET NO.	NO. OBS.	MEAN HAEM.	± S.E.	DIET NO.	NO. OBS.	MEAN HAEM.	± S.E.	
HERRING	C09	18	53.61	1.37	C09	18	51.33	1.50	> 0.05 (n.s.)
SOYABEAN	C	17	46.94	1.21					
COTTONSEED	C11	18	47.67	1.27					
GROUNDNUT	C12	18	48.67	1.09					
YEAST	C13	18	48.61	0.80	C18	18	50.00	1.38	> 0.05 (n.s)
BACTERIA	C14	18	48.33	1.45	C19	18	46.94	1.35	> 0.05 (n.s.)
ALGAE	C15	18	50.17	0.85	C20	18.	52.83	1.71	> 0.05 (n.s.)
MOULD (A. nig.)	C16	18	38.39	1.24	C21	18	40.50	0.75	> 0.05 (n.s.)
MOULD (P. chr.)	C17	13	42.77	1.79	C22	18	49.78	1.33	< 0.01

TABLE 61.

ANALYSIS OF HAEMATOCRIT DATA - PHASE I

ANALYSIS OF VARIANCE

SOURCE	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARES	F RATIO	PROBA-BILITY
BETWEEN GROUPS (1)	8	2648.34087	331.04261	12.61	< 0.001
WITHIN GROUPS	147	3858.58220	26.24886		
TOTAL	155	6506.92308			

(1) Diet effect highly significant.

FURTHER ANALYSIS OF DIET EFFECT (using sequential form of the Q method based on the tables of the Studentized Range).

DIET	MEAN HAEM %	5% LEVEL(1)	1% LEVEL(2)
C09 HERRING	53.61		a
C15 ALGAE	50.17	a	a b
C12 GROUNDNUT	48.67	a	a b
C13 YEAST	48.61	a	a b
C14 BACTERIA	48.33	a	a b
C11 COTTONSEED	47.67	a	b c
C10 SOYABEAN	46.94	a	b c
C17 MOULD (P. chr.)	42.77		c d
C16 MOULD (A. nig.)	38.39		d

(1) Diet means with same letter are not significantly different at 5% level.

(2) Diet means with same letter are not significantly different at 1% level.

TABLE 62.

ANALYSIS OF HAEMATOCRIT DATA - PHASE II.

ANALYSIS OF VARIANCE.

SOURCE	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARES	F. RATIO	PROBAB-ILITY.
Between Groups (1)	5	1747.49074	349.49815	10.38	< 0.001
Within Groups	102	3433.05556	33.65741		
TOTAL	107	5180.54630			

(1) Diet effect highly significant.

FURTHER ANALYSIS OF DIET EFFECT (using sequential form of the Q method based on the tables of the Studentized Range).

DIET	MEAN HAEM %	5% LEVEL(1)	1% LEVEL (2)
C20 ALGAE	52.83	a	a
C09 HERRING	51.33	a b	a
C18 YEAST	50.00	a b	a
C22 MOULD (P. chr.)	49.78	a b	a
C19 BACTERIA	46.94	b	a
C21 MOULD (A. nig.)	40.50		

(1) Diet means with same letter are not significantly different at 5% level.

(2) Diet means with same letter are not significantly different at 1% level.

TABLE 63.

LIVER WEIGHTS (DRY WEIGHT/WET WEIGHT FISH %)

TEST PROTEIN IN DIET.	END PHASE I (12 WEEKS)				END PHASE II (22 WEEKS)				COMPARISON PHASE I & II STUDENTS 't' PROBABILITY.
	DIET NO.	NO. OBS.	MEAN W %	± S.E.	DIET NO.	NO. OBS.	MEAN W%	± S.E.	
HERRING	C09	12	0.408	0.017	C09	12	0.425	0.024	> 0.05 (n.s.)
SOYABEAN	C10	12	0.387	0.020					
COTTONSEED	C11	12	0.368	0.010					
GROUNDNUT	C12	12	0.295	0.017					
YEAST	C13	12	0.420	0.014	C18	12	0.420	0.014	> 0.05 (n.s.)
BACTERIA	C14	12	0.454	0.026	C19	12	0.464	0.020	> 0.05 (n.s.)
ALGAE	C15	12	0.501	0.033	C20	12	0.432	0.028	> 0.05 (n.s.)
MOULD (A.)	C16	11	0.446	0.022	C21	12	0.400	0.010	< 0.01
MOULD (P.)	C17	12	0.438	0.015	C22	12	0.446	0.010	> 0.05 (n.s.)

TABLE 64.

ANALYSIS OF LIVER WEIGHTS

ANALYSIS OF VARIANCE - PHASE I DATA.

SOURCE	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARES	F RATIO	PROBABILITY
Between Groups(1)	8	0.33376	0.04172	7.66	<0.001
Within Groups	98	0.53456	0.00545		
TOTAL	106	0.86831			

(1) Diet effect highly significant.

FURTHER ANALYSIS OF DIET EFFECT (using sequential form of the Q method based on the tables of the Studentized Range).

DIET	MEAN WEIGHT %	5% LEVEL(1)	1% LEVEL(2)
C12 GROUNDNUT	0.295		a
C11 COTTONSEED	0.368	a	a b
C10 SOYABEAN	0.387	a	a b
C09 HERRING	0.408	a	b
C13 YEAST	0.420	a b	b c
C17 MOULD(P. chr.)	0.438	a b	b c
C16 MOUID (A.nig.)	0.446	a b	b c
C14 BACTERIA	0.454	a b	b c
C15 AIGAE	0.501	b	c

(1) Diet means with same letter are not significantly different at 5% level.

(2) Diet means with same letter are not significantly different at 1% level.

ANALYSIS OF VARIANCE - PHASE II.

SOURCE	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARES	F RATIO	PROBABILITY
BETWEEN GROUPS(1)	5	0.02936	0.00587	1.17	>0.05 (n.s.)
WITHIN GROUPS	66	0.33167	0.00503		
TOTAL	71	0.36103			

(1) Diet effect not significant.

TABLE 65.

SUMMARY OF PERFORMANCE DATA - PHASE I.

DIET NO.	C09	C10	C11	C12	C13	C14	C15	C16	C17
DESCRIPTION	HERRING	SOY	COTTON	GROUNDNUT	YEAST	BACTERIA	ALGAE	MOULD (A.nig)	MOULD (P.chr.)
No. of fish at start	60	60	60	60	60	60	60	60	60
Mortality	1	0	0	0	1	1	0	3	1
Mean wt. starting-g.	6.27	6.37	6.08	6.17	6.19	6.11	6.20	6.06	5.93
Mean wt. 10 weeks.-g.	26.15	26.19	23.70	23.77	23.45	22.93	22.54	22.09	20.96
Total gain - g	1175	1178	1062	1056	1021	994	980	931	888
Total food consumed g.	1503	1598	1535	1403	1431	1344	1439	1470	1479
Food conversion Ratio (1)	1.28	1.36	1.45	1.33	1.40	1.35	1.47	1.58	1.67
Protein consumed g	684.0	636.3	602.9	566.5	586.7	604.1	574.3	539.0	546.8
Efficiency of protein utiliz'n (2)	1.72	1.85	1.76	1.86	1.74	1.65	1.71	1.73	1.62
Energy consumed Kcals	6462	6693	6374	5871	5979	5666	6057	107	6086
Kcals to produce 1 Kg fish	5499	5681	6002	5559	5856	5700	6180	6559	6853

(1) Food Conversion Ratio = $\frac{\text{Wt. Food consumed (g.'dry' food).}}{\text{Wt. gain of fish (g. wet weight).}}$

(2) Efficiency of Protein Utilization = $\frac{\text{Wt. gain of fish (g. wet weight).}}{\text{Wt. protein consumed (g).}}$

TABLE 66

SUMMARY OF PERFORMANCE DATA - PHASE II.

DIET NO. DESCRIPTION	C09 HERRING	C18 YEAST	C19 BACTERIA	C20 ALGAE	C21 MOULD (A.nig.)	C22 MOULD (P.chr.)
No.fish at start	30	30	30	30	30	30
Mortality	0	0	0	0	0	0
Mean body wt. starting	30.9	28.4	26.7	25.7	25.4	22.1
Mean weight 10 wks - g	56.2	53.2	43.2	46.8	43.0	35.1
Total gain g	760	745	495	635	528	392
Total food consumed - g	818	814	560	780	766	597
Food Conv.Rat-io	1.08	1.09	1.13	1.23	1.45	1.52
Protein consumed - g	377.1	399.7	313.6	357.2	301.0	234.0
Efficiency of Protein utili-zation	2.02	1.86	1.58	1.78	1.75	1.66
Energy consum-ed kcals.	3567	3729	2547	3530	3393	2544
Kcals to prod-uce 1 Kg. of fish.	4693	5005	5145	5559	6426	6490

TABLE 67

Week 1. C.N.P. Floating Pellets.

Mean Results for 7 days	No. of contacts per day ± S.E.	Weight dispensed per day. g ± S.E.	Weight dispensed per contact - g ± S.E.
FEEDER A	179.0 ± 17.3	52.14 ± 2.74 (1)	0.240 ± 0.014
FEEDER B	226.3 ± 12.0	48.87 ± 2.26(1)	0.214 ± 0

(1) Difference not significant ($p > 0.05$)

Week 2. C.N.P. Floating Pellets

Mean Results for 7 days	No. of contacts per day ± S.E.	Weight dispersed per day g ± S.E.	Weight dispensed per contact g ± S.E.
FEEDER A	195.6 ± 12.8	45.00 ± 3.91 (1) (2)	0.232 ± 0.01
FEEDER B	243.9 ± 24.0	47.00 ± 3.41 (1) (3)	0.196 ± 0

(1) Difference not significant ($p. > 0.05$).

Week 3. Diet C09 Sinking Pellets

Mean Results for 7 days	No. of Contacts per day ± S.E.	Weight dispersed per day g ± S.E.	Weight dispensed per contact g. ± S.E.
FEEDER A	336.3 ± 37.9	55.14 ± 5.35 (1) (2)	0.165 ± 0
FEEDER B	326.9 ± 30.0	52.38 ± 4.33(1) (3)	0.161 ± 0

(1) Difference not significant ($p > 0.05$)

(2) Increase in week 3 not significant ($p > 0.05$)

(3) " " " " " " " "

AMINO ACIDS OF PROTEINS COMPARED TO WHOLE EGG

(% Credit (+) or Deficit (-))

	Whole Egg g/100g Protein	Herring Meal	Soybean Oil Meal (1)	Cotton seed Meal (1)	Ground nut Meal (1)	B.P. Yeast (1)	I.C.I. Bacteria (1)	Spirulina Maxima (1)	A. niger (2)	P. chrysogenum (2)
Arginine	6.4	- 9	+ 9	+ 62	+ 94	-20	- 30	+ 2	+ 3	- 9
Histidine	2.1	+15	+14	+ 29	+ 19	0	NR	-14	+129	+114
Lysine	7.2	+ 7	-14	- 42	- 32	+ 4	- 18	-36	- 17	- 24
Tyrosine	4.5	-31	-31	- 62	- 16	-20	- 31	-11	- 29	- 34
Phenylalanine	6.3	-38	-24	- 10	- 10	-32	- 46	-21	- 13	- 14
Ph + tyr.	10.8	-35	-27	- 31	- 12	-27	- 40	-17	- 19	- 22
Cystine	2.4	-58	-37	- 12	- 46	-54	- 75	-83	-100	- 50
Methionine	4.1	-30	-68	- 61	- 80	-56	- 41	-66	- 66	- 61
Cyst + Meth.	6.5	-40	-57	- 43	- 68	-55	- 54	-72	- 78	- 57
Threonine	4.9	-12	-24	- 29	- 35	0	0	- 6	- 33	- 10
Leucine	9.2	-18	-20	- 34	- 15	-20	- 26	-13	- 43	- 39
Isoleucine	8.0	-44	-32	- 51	- 47	-37	- 46	-25	- 61	- 56
Valine	7.3	-26	-29	- 32	- 19	-21	- 23	-11	- 42	+ 44
Tryptophan	1.5	-23	-13	+ 7	- 33	- 7	- 40	- 7	NR	NR
Chemical Score		56	32(43)	39(49)	20(32)	44(45)	46	28	22	39(43)
Essential Amino Acid Index		79.5	78.2	77.6	76.7	79.52	67.5	76.2	67.8	82.3
1st Limiting amino acid		Isoleucine	Met (+Cyst)	Methionine	Met (+Cyst)	Met (+ Cyst)	Met + Cyst	Met + Cyst	Met + Cyst	Met (+Cyst)
2nd Limiting amino acid		Met + Cyst	Isoleucine	Isoleucine	Isoleucine	Isoleucine	Isoleucine	Lysine	Isoleucine	Isoleucine

(1) Calculated from values shown in Tables 2 - 6.

(2) Calculated from unpublished values supplied by Tate & Lyle Ltd.

TABLE 69.

AMINO ACIDS OF PROTEINS COMPARED TO SALMONID REQUIREMENTS

(% Credit (+) or Deficit (-))

	Salmonid Requirement g/100g Protein	Herring Meal	Soybean Oil Meal	Cottonseed Meal	Groundnut Meal	B.P. Yeast	I.C.I. Bacteria	Spirulina Maxima	A. Niger	P. Chrysogenum
Arginine	6.0	- 3	+ 17	+ 73	+107	- 15	- 25	+ 8	+ 10	- 3
Histidine	1.7	+41	+ 41	+ 59	+ 47	+ 24	NR	+ 6	+182	+165
Lysine	5.0	+54	+ 26	- 16	- 2	+ 50	+ 18	- 8	+ 20	+ 10
Phenylalanine	5.1	-24	- 6	+ 12	+ 12	- 16	- 33	- 2	+ 8	+ 6
Ph + tyr	(5.1)	+37	+ 55	+ 45	+ 86	+ 55	+ 27	+ 76	+ 71	+ 65
Cystine	(2.5)	-60	-40	- 16	- 48	- 56	- 76	- 84	-100	- 52
Methionine	1.5	+91	-13	+ 7	- 47	- 27	- 25	- 55	- 65	- 30
Cyst + Meth	(4.0)	- 2	- 30	- 7	- 47	- 27	- 25	- 55	- 65	- 30
Threonine	2.2	+95	+ 68	+ 59	+ 45	+123	+109	+109	+ 50	+100
Leucine	3.9	+92	+ 90	+ 56	+100	+ 90	+ 74	+105	+ 33	+ 44
Isoleucine	2.2	+105	+145	+ 77	+ 91	+127	+ 95	+173	+ 41	+ 59
Valine	3.2	+69	+ 63	+ 56	+ 84	+ 81	+ 75	+103	+ 31	+228
Tryptophan	0.5	+130	+160	+220	+100	+180	+ 80	+180	NR	NR
Limiting amino acids (and % deficit)	1	Arginine (3)	Cyst+Met (30)	Lysine (16)	Cyst+Met (47)	Cyst+Met (27)	Cyst+Met (25)	Cyst+Met (55)	Cyst+Met (65)	Cyst+Met (30)
	2	Cyst+Met (2)	-	Cyst+Met (7)	Lysine (2)	Arginine (15)	Arginine (25)	Lysine (8)	-	Arginine (3)

(1) In the absence of tyrosine.
(2) Assuming cystine can spare an equal proportion of methionine.

TABLE 70

EXPERIMENT PER 01 - COMPOSITION OF DIETS CONTAINING BACTERIA.

COMPOSITION

Ingredient %	P 0 1	P 0 2	P 0 3	P 0 4
Corn oil	6	6	6	6
Durabond	5	5	5	5
Salt Mix (1)	4	4	4	4
Vitamin Mix (2)	5	5	5	5
Bacteria	26	32	39	45
Glucose	19	13	6	0
Potato Starch	35	35	35	35
TOTAL	100	100	100	100
Water (3)	50	60	70	80

ANALYSIS

Moisture %	11.1	9.8	9.3	8.7
Protein %	20.3	25.2	30.9	35.0
Gross Energy (4) Kcals/100g.	372	381	388	394

(1) Salt Mix shown in Table 20

(2) Vitamin mix shown in Table 73

(3) Water - g added to produce suitable consistency + 0.1g. permacal red.

(4) Gross energy estimated from following values:-

Corn oil	9.40 Kcals/g
Starch	3.47 Kcals/g (As measured by bomb
Bacteria	4.86 Kcals/g. calorimetry)
Glucose	4.00 Kcals/g.

Assimilated energy approximately 270 Kcals/100g diet.

TABLE 71.

EXPERIMENT PER 02 - COMPOSITION OF DIETS CONTAINING ALGAE

COMPOSITION

Ingredient %	P 0 5	P 0 6	P 0 7	P 0 8
Corn oil	6	6	6	6
Durabond	5	5	5	5
Salt Mix (1)	4	4	4	4
Vitamin Mix (2)	5	5	5	5
Algae	38	48	58	68
Glucose	30	20	10	0
Potato Starch	12	12	12	12
TOTAL	100	100	100	100
Water (3)	34	39	50	70

ANALYSIS

Moisture	11.3	10.5	10.4	9.5
Protein	19.8	25.2	29.9	35.1
Gross Energy (4) Kcals/g.	386	393	400	411

(1) Salt Mix shown in Table 20

(2) Vitamin mix shown in Table 73

(3) Water-g added to produce suitable consistency + 0.1g per mecol red

(4) Gross Energy estimated from following values:-

Corn oil	9.40 Kcals/g.
Starch	3.47 Kcals/g.
Algae	4.72 Kcals/g.
Glucose	4.00 Kcals/g.

Assimilated energy approximately 315 Kcals/100g. diet.

TABLE 72.

EXPERIMENT PER 03 - COMPOSITION OF DIETS CONTAINING YEAST.

COMPOSITION

Ingredient %	P 0 9	P 10	P 11	P 12
Corn Oil	6	6	6	6
Durabond	5	5	5	5
Salt Mix (1)	4	4	4	4
Vitamin Mix (2)	5	5	5	5
Yeast	34	42	50	58
Glucose	24	16	8	0
Potato Starch	22	22	22	22
TOTAL	100	100	100	100
Water (3)	50	60	70	80

ANALYSIS

Moisture %	11.1	10.4	9.4	8.5
Crude Protein %	19.9	25.3	30.4	34.8
Gross Energy (4) Kcals/100 g.	380	388	398	407

(1) Salt Mix shown in Table 20

(2) Vitamin Mix shown in Table 73

(3) Water - g added to produce suitable consistency + 0.1g per mecol red

(4) Gross energy estimated from following values:-

Corn oil	9.40 Kcals/g	
Starch	3.47 Kcals/g	(As measured by bomb
Yeast	4.86 Kcals/g	calorimetry)
Glucose	4.00 Kcals/g	

Assimilated energy approximately 296 Kcals/100g diet.

TABLE 73

VITAMIN MIX.

Vitamin (pure)	mg per 5g premix (1)
Thiamin	5
Riboflavin	20
Pyridoxine	5
Nicotinic acid	75
Ca-pantothenate	50
Inositol	200
Biotin	0.5
Folic acid	1.5
B12	0.01
Menadione	4
∝ tocopherol acetate	40
Choline chloride	500 (2)
Ascorbic acid	100 (2)

(1) 5g vitamin premix added per 100g diet giving the vitamin levels suggested by Halver (1969). Vitamins werepre-mixed in cellulose. In addition to the vitamins suggested, 1.6mg A and D (containing 500 I.U./mg. of vitamin A and 100 I.U./mg of Vitamin D3) and 2.75 mg. B.H.T. were added.

(2) Added to the diet at the time the main ingredients were mixed.

TABLE 74.

EXPERIMENT PER 01 - PERFORMANCE DATA FOR DIETS CONTAINING BACTERIAL PROTEIN.

Diet	Repl.	Food Consumed g	Protein Consumed g	Initial Weight g	Weight Gain g	Weight Gain %	Food Conversion Efficiency %	Protein Efficiency Ratio
PO 1 (20% Prot)	1	139.6	28.3	271	38	14.0	27.2	1.34
	2	162.8	33.0	278	44	15.8	27.0	1.33
	3	166.7	33.8	277	67	24.2	37.0	1.98
	$\bar{x} \pm$ SE	156.4 ± 8.5	31.7 ± 1.7	275.3	49.7 ± 8.8	18.0 ± 3.1	30.4 ± 3.3	1.55 ± 0.21
PO 2 (25% Prot)	1	160.4	40.4	279	69	24.7	43.1	1.71
	2	191.1	48.2	289	94	32.5	49.3	1.95
	3	193.7	48.8	292	96	32.9	49.5	1.97
	$\bar{x} \pm$ SE	181.7 ± 10.7	45.8 ± 2.7	286.7	86.3 ± 8.7	30.0 ± 2.7	47.3 ± 2.1	1.88 ± 0.08
PO 3 (30% Prot)	1	156.6	48.4	275	70	25.5	44.6	1.45
	2	157.9	48.8	294	64	21.8	40.5	1.31
	3	189.6	58.6	288	104	36.1	54.9	1.78
	$\bar{x} \pm$ SE	168.0 ±10.8	51.9 ± 3.3	285.7	79.3 ± 12.5	27.8 ± 4.3	46.7 ± 4.3	1.51 ± 0.14
PO 4 (35% Prot)	1	168.3	58.9	276	74	26.8	44.1	1.26
	2	166.7	58.4	280	78	27.9	46.7	1.34
	3	185.1	64.8	293	83	28.3	44.8	1.28
	$\bar{x} \pm$SE	173.4 ± 5.9	60.7 ± 2.1	283.0	78.3 ± 2.6	27.7 ± 0.5	45.2 ± 0.8	1.29 ± 0.02
Commercial Feed (40% Prot.)	1	258.6	103.4	283	168	59.4	65.0	1.62
	2	249.3	99.7	291	152	52.2	61.0	1.52
	3	265.2	106.1	275	180	65.5	67.9	1.70
	4	236.1	94.4	279	171	61.3	72.4	1.81
	$\bar{x} \pm$SE	252.3 ± 6.3	100.9 ± 2.5	282.0	167.8 ± 5.8	59.6 ± 2.8	66.6 ± 2.4	166 ± 0.06

TABLE 75.

EXPERIMENT PER 01 - ANALYSIS OF DATA.

% Weight Gain (1)

SOURCE	Df	Sum of Squares	Mean Squares	F Ratio	Probability
Diet	4	3670.80	917.70	33.00	< 0.001
Residual	11	305.91	27.81		
Total	15	3976.71			

Comparison of Diet Means (2)

Diet	P01	P04	P03	P02	C.N.P.
Mean	18.00	27.67	27.80	30.00	59.80

Food Conversion Efficiency % (1)

SOURCE	Df	Sum of Squares	Mean Squares	F Ratio	Probability
Diet	4	2336.66	584.17	23.38	< 0.001
Residual	11	274.79	24.98		
Total	15	2611.46			

Comparison of Diet Means (2)

Diet	P01	P04	P03	P02	C.N.P.
Mean	30.40	45.20	46.67	47.30	66.58

Protein Efficiency Ratio (1)

SOURCE	Df	Sum of Squares	Mean Squares	F.Ratio	Probability.
Diet	3	0.554	0.138	3.14	>0.01<0.05
Residual	11	0.484	0.044		
Total	15	1.038			

Comparison of Diet Means (2)

Diet	P04	P03	P01	C.N.P.	P02
Mean	1.293	1.513	1.550	1.663	1.877

(1) Analysis of variance.

(2) Comparison of Means using sequential form of the Q method based on the tables of the Studentized Range. Values underlined are not significantly different ($p > 0.05$)

EXPERIMENT PER 02 - PERFORMANCE DATA FOR DIETS CONTAINING AIGAL PROTEIN

DIET	REPL.	MORTALITY	FOOD CONSUMED g	PROTEIN CONSUMED g	INITIAL WEIGHT g	WEIGHT GAIN g	WEIGHT GAIN %	FOOD CONVER-SION EFFICIENCY %	PROTEIN EFFICI-ENCY RATIO
PO 5 (20% Prot)	1	0	168.3	33.3	375	(-18)	0	0	0
	2	0	144.9	28.7	390	7	1.8	4.8	0.24
	3	1	125.3	24.8	353	4	1.1	3.2	0.16
	$\bar{x}$ ± S.E		146.2±12.4	28.9 ± 2.5	372.7	3.7 ±2.0	1.0 ± 0.5	2.7±1.4	0.13±0.07
P06 (25% Prot)	1	5	-	-	-	-	-	-	-
	2	0	174.0	43.9	3 70	16	4.3	9.2	0.36
	3	3	161.0	40.6	3 72	33	7.8	20.5	0.81
	$\bar{x}$ ± S.E		167.5±6.5	42.3± 1.7	371.0	24.5± 8.5	6.0 ±1.8	14.9±5.7	0.59±0.22
PO 7 (30%Prot)	1	2	251.1	74.6	366	79	23.0	31.5	1.06
	2	1	247.4	73.5	384	90	26.6	36.4	1.23
	3	2	292.9	87.0	390	106	29.1	36.2	1.22
	$\bar{x}$ ± S.E		263.8±14.6	78.4±4.3	380.0	91.7±7.8	26.2±1.8	34.7±1.6	1.17±0.05
PO 8 (35%Prot)	1	0	361.3	126.8	380	164	43.2	45.4	1.29
	2	0	329.6	115.7	406	104	25.6	31.6	0.90
	3	5	268.7	94.3	393	124	31.3	46.2	1.31
	$\bar{x}$ ± S.E		319.9±27.2	112.3±9.5	393.0	130.7±17.6	33.4 ±5.2	41.1±4.7	1.17± 0.13
Commer-cial Feed (40%Prot)	1	0	422.1	168.8	397	383	96.5	90.7	2.27
	2	0	379.3	151.7	355	330	93.0	87.0	2.18
	3	2	343.9	137.6	399	265	75.4	77.1	1.93
	4	2	360.9	144.4	371	304	89.3	84.2	2.11
	$\bar{x}$ ± S.E		376.6±16.8	150.6±6.7	380.5	320.5±24.7	88.6±4.6	84.8±2.9	2.12±0.07

TABLE 77

EXPERIMENT PER 02 - ANALYSIS OF DATA.

% Weight Gain. (1)

SOURCE	Df	Sum of Squares	Mean Squares	F, Ratio	Probability
Diet	4	17443.8	4361.0	180.9	< 0.001
Residual	9	217.0	24.1		
Total	13	17660.8			

Comparison of Diet Means. (2)

Diet	P05	P06	P07	P08	C.N.P.
Mean	1,00	6.05	26.23	33.37	93.55

Food Conversion Efficiency %. (1)

SOURCE	Df	Sum of Squares	Mean Squares	F.Ratio	Probability
Diet	4	11816.7	2954.2	84.5	< 0.001
Residual	9	314.5	35.0		
Total	13	12131.2			

Comparison of Diet Means (2)

Diet	P05	P06	P07	P08	C.N.P.
Mean	2.67	14.85	34.70	41.07	84.75

Protein Efficiency Ratio (1)

SOURCE	Df	Sum of Squares	Mean Squares	F.Ratio	Probability
Diet	4	6.161	1.540	47.5	<0.001
Residual	9	0.292	0.032		
Total	13	6.453			

Comparison of Diet Means (2)

Diet	P05	P06	P07	P08	C.N.P.
Mean	0.133	0.585	1.170	1.167	2.123

(1) Analysis of variance

(2) Comparison of means using sequential form of the Q method based on the tables of the Studentized Range. Values underlined are not significantly different. ($p > 0.05$).

TABLE 78

EXPERIMENT PER 03 - PERFORMANCE DATA FOR DIETS CONTAINING YEAST PROTEIN.

DIET	REPL.	FOOD CONSUMED g	PROTEIN CONSUMED g	INITIAL WEIGHT g	WEIGHT GAIN g	WEIGHT GAIN %	FOOD CONVERSION EFFICIENCY %	PROTEIN EFFICIENCY RATIO
P09 (20% Prot.)	1	209.4	41.7	270	81.	30.0	38.8	1.94
	2	216.0	43.0	290	66	22.8	30.6	1.53
	3	231.0	46.0	291	74	25.4	32.0	1.61
	$\bar{x} \pm$ SE	218.8 ± 6.4	43.6 ± 1.3	283.7	73.7 ± 4.3	26.1 ± 2.1	33.8 ± 2.5	1.69 ± 0.13
P10 (25% Prot.)	1	237.4	60.1	277	126	45.5	53.1	2.10
	2	256.0	64.8	284	163	57.4	63.7	2.52
	3	251.0	63.5	278	110	39.6	43.8	1.73
	$\bar{x} \pm$ SE	248.1 ± 5.6	62.8 ± 1.4	279.7	133.0 ± 15.7	47.5 ± 5.2	53.5 ± 5.7	2.12± 0.23
P11 (30% Prot.)	1	260.7	79.3	288	145	50.3	55.6	1.83
	2	230.4	70.0	282	120	42.6	52.2	1.71
	3	301.1	91.5	296	205	69.3	68.1	2.24
	$\bar{x} \pm$ SE	264.1± 20.5	80.3 ± 6.2	288.7	156.7 ± 25.2	54.1 ± 7.9	58.6 ± 4.8	1.93 ±0.16
P12 (35% Prot.)	1	256.9	89.4	272	169	62.1	65.8	1.89
	2	234.1	81.5	288	116	40.3	49.6	1.42
	3	290.2	101.0	279	230	82.4	79.3	2.17
	$\bar{x} \pm$SE	260.4 ±16.3	90.6 ±5.7	279.7	171.7 ±32.9	61.6± 12.2	64.9 ±8.6	1.86 ± 0.25
Commercial feed (40% Prot.)	1	243.9	97.6	290	182	62.8	74.6	1.86
	2	255.8	102.3	288	178	61.8	69.6	1.74
	3	229.7	91.9	292	173	59.2	75.3	1.88
	4	205.5	82.2	283	135	47.7	65.7	1.64
	$\bar{x} \pm$ SE	233.7 ± 10.8	93.5 ± 4.3	288.3	167.0 ± 10.8	57.9 ± 3.5	71.3 ± 2.3	1.78 ± 0.06

TABLE 79

EXPERIMENT PER 03 - ANALYSIS OF DATA

% Weight Gain (1)

SOURCE	Df	Sum of Squares	Mean Squares	F. Ratio	Probability
Diet	4	2438.48	609.62	4.19	$> 0.01 < 0.05$
Residual	11	1600.26	145.48		
Total	15	4038.74			

Comparison of Diet Means (2)

Diet	P09	P10	P11	C.N.P.	P.12
Mean	26.07	47.50	54.07	57.88	61.60

Food Conversion Efficiency (1)

SOURCE	Df	Sum of Squares	Mean Squares	F. Ratio	Probability
Diet	4	2661.86	665.47	8.31	< 0.001
Residual	11	880.37	80.03		
Total	15	3542.24			

Comparison of Diet Means (2)

Diet	P09	P.10	P11	P12	C.N.P.
Mean	33.8	53.53	58.63	64.90	71.3

Protein Efficiency Ratio (1)

SOURCE	Df	Sum of Squares	Mean Squares	F. Ratio	Probability
Diet	4	0.318	0.080	0.91	> 0.05 (ns)
Residual	11	0.962	0.087		
Total	15	1.280			

(1) Analysis of variance

(2) Comparison of means using sequential form of the Q method based on the tables of the Studentized Range. Values underlined are not significantly different ($p > 0.05$)

TABLE 80

APPARENT NITROGEN DIGESTIBILITY (1)

Test Protein	Diet No.	% Cr III oxide (2) Feed	% Cr III oxide (2) Faeces	% Nitrogen(2) Feed	% Nitrogen(2) Faeces	Apparent N Digestibility %.
BACTERIA	P03	0.92	2.25	4.85	1.50	87.4
	P04	0.85	1.64	5.63	1.68	84.5
ALGAE	P07	1.13	2.81	4.79	3.69	69.0
	P08	0.82	2.36	5.66	4.40	73.0
YEAST	P11	0.93	2.11	4.96	1.23	89.1
	P12	0.88	1.55	5.55	1.35	86.2

(1) Calculated from:

$$\text{Digestibility \%} = 100 - \left\{\frac{\%\text{ indicator in feed}}{\%\text{ indicator in faeces}} \times \frac{\%\text{ N in feed}}{\%\text{ N in faeces}} \times 100\right\}$$

(2) Values are the means of duplicate estimations.

TABLE 81.

EXPERIMENT PER 04 - COMPOSITION OF EXPERIMENTAL DIETS.

DIET NO. / INGRED-IENT	P13	P14	P15	P16	P17
Herring Meal	37.9				
Soybean Meal		59.9			
Yeast			47.3		
Bacteria				35.4	
Algae					55.2
Vitamin Mix (1)	5.0	5.0	5.0	5.0	5.0
Binder (2)	4.0	4.0	4.0	4.0	4.0
Cod liver oil	3.0	3.0	3.0	3.0	3.0
Soybean oil	3.5	6.0	3.0	4.5	3.0
Mineral Mix (3)	5.0	6.1	3.3	3.9	3.9
Potato starch	47.2	17.9	33.1	44.5	24.2
Cellulose (4)	5.0	6.5	9.5	7.9	10.4

Theoretical Specifications % Dry Matter

Dry matter	100	100	100	100	100
Crude Protein	27.5	27.5	27.5	27.5	27.5
Ash	10.0	10.0	10.0	10.0	10.0
Oil	10.0	10.0	10.0	10.0	10.0
Total Energy (5) Kcals/100g.D.M.	400.0	400.0	400.0	400.0	400.0
Digestible Energy (6) Kcals/100g.D.M.	282.9	268.5	280.0	284.8	269.7

(1) Vitamin Mix - Table 73 (2) Mineral Mix - Table 20 (3) Durabond
(4) Solka Floc.
(5) Total energy calculated from values for the test proteins and starch determined by bomb calorimetry and assuming a value of 9.4 Kcals/g for the added oil. The energy content of Durabond and Solka Floc is not included in the estimate.
(6) Digestible energy calculated, assuming values of 8.0 Kcals/g of oil, 1.6 Kcals/g of carbohydrate and 5.65 Kcals/g of digestible protein. The % digestible protein content was taken as: H.M. = 82.0, S.B. = 78.7, Y = 87.7, B = 86.0 , A = 71.0

EXPERIMENT PER 04 - PERFORMANCE DATA FOR DIETS CONTAINING YEAST, BACTERIA & AIGAE

DIET	PROTEIN %	REPL.	FOOD CONSUMED g	PROTEIN CONSUMED g	INITIAL WEIGHT g	WEIGHT GAIN g	WEIGHT GAIN %	FEED CONVER-SION EFF.%	PROTEIN EFF. RATIO
P.14 SOYBEAN MEAL	25.4	1	30.4	7.7	660	(-58)	-	-	-
		2	34.6	8.8	667	(-67)	-	-	-
		3	33.0	8.4	660	(-57)	-	-	-
		4	34.4	8.7	661	(-56)	-	-	-
		$\bar{x} \pm$ SE.	33.1 ± 1.0	8.4	662.0				
P15 YEAST	25.2	1	291.7	73.5	658	143	21.7	49.0	1.95
		2	318.2	80.2	668	184	27.5	57.8	2.29
		3	311.5	78.5	660	196	29.7	62.9	2.50
		4	352.6	88.9	663	215	32.4	61.0	2.42
		$\bar{x} \pm$ S.E.	318.5 ± 12.7	80.3 ± 3.2	662.2	184 ± 15	27.9 ± 2.3	57.7 ± 3.1	2.29 ± 0.12
P 16 BACTERIA	25.0	1	159.6	39.9	668	60	9.0	37.6	1.50
		2	145.5	36.4	657	45	6.9	31.0	1.24
		3	109.2	27.3	663	(-3)	0	0	0
		4	109.9	27.5	667	11	1.7	10.0	0.40
		$\bar{x} \pm$ S.E.	131.1 ± 12.7	32.8 ± 3.2	663.8	29 ± 14	4.4 ± 2.2	19.7 ± 8.3	0.79 ± 0.35
P17 AIGAE	24.8	1	278.9	69.2	666	150	22.5	53.8	2.16
		2	267.4	66.3	661	140	21.2	52.4	1.11
		3	278.0	68.9	670	145	21.6	52.1	2.11
		4	180.1	44.7	661	89	13.5	49.5	2.10
		$x \pm$ S.E.	251.1 ± 23.8	62.3 ± 5.9	664.5	131 ± 14	19.7 ± 2.1	52.0 ± 0.9	2.09 ± 0.03

TABLE 83.

EXPERIMENT PER 04 - ANALYSIS OF DATA.

% Weight Gain (1)

SOURCE	Df	Sum of Squares	Mean Squares	F. Ratio	Probability
Diet	2	835.7	417.9	23.3	< 0.001
Residual	8	143.2	17.9		
Total	10	978.9			

Comparison of Diet Means (2)

Diet	P16	P17	P15
Mean	4.4	19.7	27.9

Food Conversion Efficiency % (1)

SOURCE	Df	Sum of Squares	Mean Squares	F. Ratio	Probability
Diet	2	1851.8	925.9	13.75	$< 0,01$
Residual	8	538.7	67.3		
Total	10	2390.5			

Comparison of Diet Means (2)

Diet	P16	P17	P15
Mean	19.7	52.0	57.7

Protein Efficiency Ratio (1)

SOURCE	Df	Sum of Squares	Mean Squares	F. Ratio	Probability
Diet	2	2.932	1.466	13.75	< 0.01
Residual	8	0.853	0.107		
Total	10	3.785			

Comparison of Diet Means (2)

Diet	P16	P17	P15
Mean	0.79	2.09	2.29

(1) Analysis of variance.
(2) Comparison of means using sequential form of the Q method based on the tables of the Stud entized Range. Values underlined are not significantly different ($p>0.05$).

APPENDIX 1.

Meat-meal mixtures that have been successfully used as trout diets

(from Phillips, 1970)

	Percent in mixture number						
	6	6h	6i	6j	6k	1-66	1-67
Dried skim milk	12	--	--	--	--	--	--
Whitefish meal	12	12	12	--	--	--	--
Cottonseed meal	12	--	--	--	--	--	--
Wheat flour middlings	12	12	--	12	--	13	13
Distiller's solubles	--	12	12	12	12	13	5
Dehulled soy bean meal	--	12	12	12	12	--	--
Corn gluten meal	--	--	--	--	--	--	8
Red dog flour	--	--	12	--	12	--	--
Menhaden fish meal 1/	--	--	--	12	12	19	19
A-D feeding oil	--	--	--	3	3	3	3
Dried brewer's yeast	--	--	--	5	--	--	--
Mineralized salt	2	2	2	2	2	2	2
Pork spleen	30	--	--	--	--	--	--
Beef spleen	--	30	30	42	27	25	25
Beef liver	20	20	20	--	20	25	25

1/ Any good grade of fish meal may be substituted for this product such as redfish meal, or Peruvian fish meal.

Composition and Ingredient Specifications, Oregon Pellet, June 1972 (from Fish Commission of Oregon, 1972)

Ingredient	% of Diet	Specifications
MEAL MIX		
Herring meal	28.0	Canadian or domestic; minimum 70% protein; "full meal" (containing the solubles); maximum 3% NaCl.
Cottonseed meal	15.0	Minimum 50% protein; maximum 0.04% free gossypol; prime quality.
Dried whey product	5.0	Partially delactosed; minimum 15% protein.
Wheat germ meal	4.0	Minimum 25% protein and 7% fat.
Shrimp or crab meal	4.0	Maximum 3% NaCl, minimum 25% protein for crab meal.
Corn distiller's dried solubles	4.0	
VITAMIN PREMIX	1.5	See following page.
WET MIX		
Wet fish	30.0	Tuna viscera, herring, turbot, salmon viscera, dogfish, or hake; two or more, provided none shall exceed 15% of the diet and 1/32- and 3/64-inch pellets shall contain at least 7.5% tuna viscera; herring, salmon viscera, and hake must be pasteurized (see Page 9); nonvisceral products must be whole fish; livers included but no heads or gills in tuna or salmon viscera; note changes in oil level with use of hake or dogfish.
Kelp meal	2.0	Norwegian.
Herring or soy oil	6.0	Stabilized with 0.3% BHA-BHT (1:1); soy oil to be fully refined; herring oil to contain less than 5 ppm DDT or its analogs, less than 2% free fatty acids, and not to be alkaline reprocessed; add 0.5 parts oil for every 10 parts hake and delete 0.3 parts oil for every 10 parts dogfish in total diet.
Choline chloride	0.5	Liquid, 70% product.

Oregon Specifications - cont.

Oregon Vitamin Premix, Guaranteed Minimum Analysis and Vitamin Source Limitations, June 1972

Vitamin	Guaranteed Minimum Analysis per Pound of Premix	Source Limitation
Ascorbic acid	27.0 grams	
Biotin	18.0 milligrams	
B_{12}	1.8 milligrams	
E	15,200.0 International Units	Water dispersible, alpha tocopheryl acetate
Folic acid	215.0 milligrams	Not zinc folate
Inositol	17.0 grams	Not phytate
Menadione	180.0 milligrams 1/	Menadione sodium bisulfite complex or menadione dimethyl-pyrimidinol bisulfite
Niacin	5.7 grams	
d-Pantothenic acid	3.2 grams	Calcium pantothenate or choline pantothenate
Pyridoxine	535.0 milligrams	
Riboflavin	1.6 grams	
Thiamine	715.0 milligrams	

1/ *The biological activity of 180 mgs of menadione is required.*

McNenney Dry Diet - Specifications (from Orme, 1970)

FORMULATION SPECIFICATIONS PR6-25 (73)
(For No. 3 and No. 4 Granules and all pellet sizes)

1. Fish food mixture shall be composed of the following items in the proportionate quantities per hundred pounds. Final product to carry the following guaranteed analysis:

   Crude Protein, not less than 40%
   Fish Meal Protein, not less than 24.5%
   Crude Fat, not less than 7% or greater than 8%
   Crude Fiber, not more than 5%
   Moisture, not more than 10.5% at sack-off

		%
2.	Canadian or Alaskan Herring Meal, minimum protein 70%, maximum fat 10.5%, maximum salt (NaCl) 2%, maximum moisture 10%, stored at manufacturer's plant no longer than 6 months as indicated by Bill of Lading. Pepsin digestibility not less than 92.5%. (Stabilized meal preferred).	33-35 *
3.	Wheat Standard Middlings, minimum protein 15%, maximum fiber 9.5%	15-13 *
4.	Soybean Oil Meal, solvent extracted and dehulled, minimum protein 48.5%, maximum fiber 3%.	10
5.	Delactosed Whey, minimum protein 16%, maximum lactose 50%, Partially Delactosed Whey, Milk Nutrients Concentrated or equal.	10
6.	Corn Fermentation Extractives, minimum protein 25%, minimum fat 2%, maximum fiber 8%. Clinton CFS concentrate or equal..	8
7.	Corn Gluten Meal, minimum protein 60%, minimum fat 1%, maximum fiber 3%.	6
8.	Brewer's Dried Yeast, minimum protein 50%, minimum fat 0.7%, maximum fiber 3%.	5.
9.	Dehydrated Alfalfa Meal, reground pellets, minimum protein 17%, maximum fiber 27%.	3
10.	Trace Mineralized Salt, to contain FE, Cu, Co, I, maximum Zn .005%, maximum Mn .2%.	2
11.	Vitamin Premix No. 25 (See following page.)	4
12.	Soybean Oil, Fully Refined, (National Soybean Processors Association) with 0.01% BHA or BHT added.	4 *

* Fish Meal may be varied between 33-35% depending on protein content but must provide not less than 24.5% fish protein per hundred pounds. Quantity of added oil may be adjusted so that the finished feed shall not contain less than 7% or greater than 8% crude fat. Wheat Middlings are to be adjusted to compensate for the above variations.

McNenney Specifications - cont.

SPECIFICATIONS FOR VITAMIN PREMIX NO. 25

VITAMIN	GUARANTEED POTENCY PER POUND OF PREMIX (mg)
D Calcium Pantothenate	600.0
Pyridoxine	500.0
Riboflavin	600.0
Niacin	6,250.0
Folic Acid	100.0
Thiamine	500.0
Biotin	5.0
B-12	0.25
Menadione Sodium Bisulfite	125.0
B.H.T.	250.0
Vitamin E, from d or dl alpha tocopherol acetate	2,000.0 i.u.
Vitamin D3 Activity	15,000.0 i.u.
Vitamin A Activity (Vitamin A palmitate)	75,000.0 USP
Choline Chloride *	4,000.0
Ascorbic Acid *	5,000.0

* Choline Chloride and Ascorbic Acid will not be part of the packaged vitamin premix but shall be blended into the final mixture separate from each other and the vitamin premix. Choline Chloride, Ascorbic Acid, and Vitamin premix No. 25 are to be stored separately and never preblended before adding to the feed.

Certified vitamin premix to be supplied by recognized manufacturer and must show date of preparation. The vitamin premix to be used is not to be held in storage longer than 4 months after date of preparation.

Formula for the Abernathy Dry Diet (from Fowler, 1971)

Ingredient	Percent	Type
Fish Carcass Meal1)	44•5	Salmon, dogfish, hake, herring or turbot.
Dried Whey Product	17•0	Not less than 15% protein.
Wheat Germ Meal	16•5	Not less than 25% protein and 8% lipid.
Cottonseed Meal	15•0	Not less than 50% protein.
Soybean Oil	6•0	Fully refined with 0•01% BHA and 0•01 BHT added.
Vitamin Supplement	1•0	As for McNenney Diet - see previous page.

1) To have protein content of more than 70%, lipid less than 12%, water less than 7% and a T.B.A. value of less than 40.

APPENDIX 2

FEEDING SYSTEMS - DESIGN DETAILS

Feed Dispenser

The feed dispenser was shown in Fig 34 and Plate 13 in the main text. The unit was constructed as far as possible from components and with tools which are readily available in most laboratories. The hopper was a disposable plastic cup. The type used in this instance was shaped with steeply sloping walls to a 1" diameter base. This was considered an ideal size and shape, although the top part of some plastic bottles have been found adequate. The bottom of the cup was cut to a shape such that it could sit directly on the tubular portion of the unit, at an appropriate angle. The tube was a 20ml. disposable plastic syringe, from which the 'closed' end had been cut. A hole was burned in the syringe near the cut end using a $\frac{1}{2}$" heated cork borer. It was trimmed to a suitable size using a knife and file. The cup was then sellotaped in position over the hole. Final trimming inside was carried out with a heated scalpel to ensure that there was no break in the continuity between the cup and tubular portion of the dispenser. This is particularly important to avoid pellet blockages. The cup can be permanently fixed in position by brushing over the sellotape with a suitable hardener e.g. fibre glass resin. This keeps the feeder in tact in the dampest conditions.

The plunger was the normal syringe plunger cut to an appropriate length, $2\frac{1}{2}$" in this instance. A plastic disc, marginally smaller in diameter than the tube, was fixed onto the end of the plunger to prevent pellets escaping. Movement of the plunger was powered by a second syringe fixed rigidly to the first by two 6BA threaded brass rods inserted into holes drilled into the 'lugs' of each syringe tube and held by brass nuts fixed on each side. The syringe plunger of the 'power unit', hereafter called the piston, was left in tact with the rubber seal

providing a closed air pressure system essential for the operation of the feeder. The dispenser plunger and piston were glued to a shaped piece of $\frac{1}{8}$" perspex, which accommodated one end of a 2", 2lb extension spring.

Brass nuts were threaded onto the brass rods connecting the two syringe tubes and passing through the shaped piece of perspex joining the plunger to the piston. Nuts inserted on the piston side allowed for slight alteration of tension in the extension spring whilst nuts inserted on the plunger side acted as stops and could be adjusted to give a variable traverse of the plunger/piston unit.

Some problems with the dispensers had been experienced where the feeders were located outside and subjected to wind, rain and dirt. To overcome such problems the dispensers were slightly modified. The hopper was set back from the end of the dispenser tube allowing a small delivery tube to be placed on the end to prevent feed at the open end of the dispenser unit from becoming wet and consequently blocking the exit. To fully accommodate the delivery tube, the cup of the hopper was raised a little by the insertion of a short length of plastic tubing of approximately the same dimensions as the dispenser syringe tube. The whole hopper section was then fibreglassed. Clip-on lids were made from disposable petri-dishes, with two small protrusions fixed to the inside providing a snap-on facility, over the lip of the plastic cup. Finally, the power syringe and adjustment mechanism was enclosed in a plastic case made from a sodium hydroxide container (securitainer). The complete feeder is shown in Fig 34 and Plate 13 in the main text.

Solenoid Operated Valve

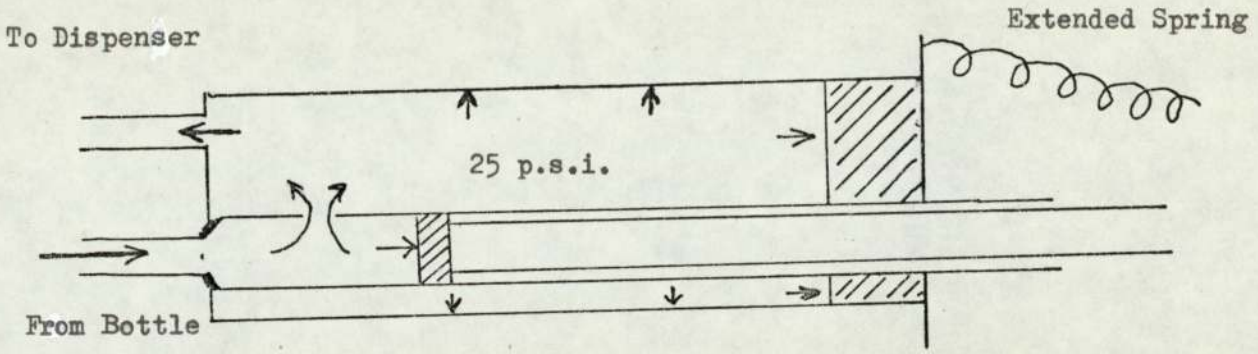
A valve was designed which was easy to make again with relatively inexpensive materials. The valve, shown in Fig 36 in the main text, was made from a 1ml and a 10ml syringe. A small hole was drilled in

the 1ml syringe about $\frac{1}{2}$" from the end. A hole, large enough to accommodate the nipple of the 1ml syringe, was then drilled in the flat end of the 10ml syringe. A hole was also burned in the solid rubber seal of the plunger of the 10ml syringe. The 1ml syringe was then inserted through the rubber seal and placed inside the 10ml syringe, with the nipple inserted into the drilled hole at the end. The rubber seal was forced into the 10ml syringe, forming a very tight seal and holding the 1ml syringe in place. The latter was glued to the 10ml syringe at the point where the nipple protruded from the drilled hole. Finally the plunger from the 1ml syringe was pushed to a position where its rubber seal was below the small hole drilled in its side.

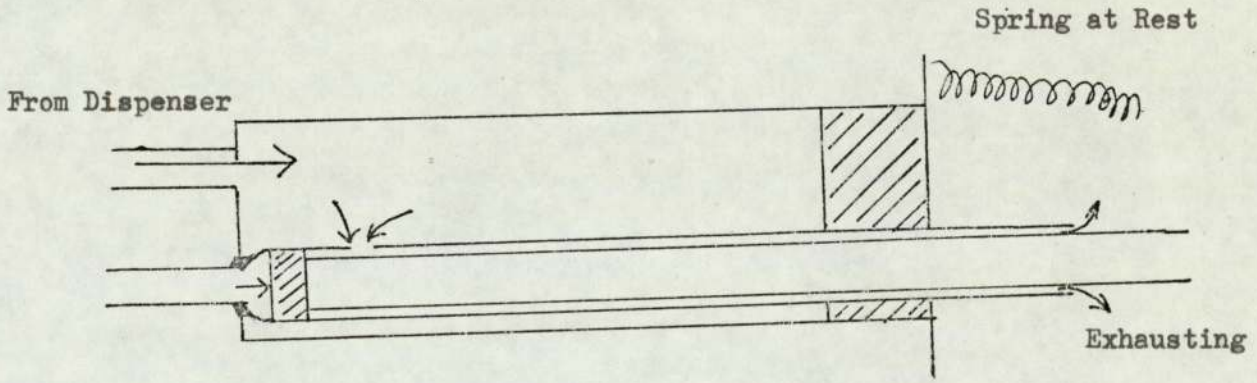
An air tube, supplying air at 25p.s.i. from a compressed air bottle, was connected to the nipple of the 1ml syringe. A second air tube was then connected between the nipple of the 10ml syringe and that of the power syringe on the feed dispenser unit. The operation was simple, Fig 4.7 (a and b). Air could not go any further than the 1ml syringe whilst its piston was positioned such that the rubber seal was below the hole. When retracted, however, air passed through the hole into the 10ml syringe and out to the feeder unit. Whilst the piston was in this position, the system was a closed pressure system. The pressure acting on the feeder piston therefore caused it to advance and feed to be dispensed. When the valve piston was returned to its position below the hole, Fig 4.7b, however, the pressure in the closed system was equalised to the atmosphere, the air exhausting through the 1ml syringe.

The valve piston was connected to the armature of an A.C. solenoid with a 3lb pull and a maximum 1" travel. These can frequently be purchased very cheaply from suppliers of surplus G.P.O. equipment etc. Extension springs were connected to the valve piston at one end and the body of the 10ml syringe at the other, and the position of the solenoid was adjusted so that the solenoid armature travelled $\frac{1}{2}$" - $\frac{3}{4}$",

Fig. 47. Operation of Solenoid Valve.



a) Valve Open



b) Valve Closed.

and in so doing, pulled the valve piston from its closed to its open position (either side of the hole). It was required that the extension springs be powerful enough to return the valve piston to its closed position against the frictional resistance of the seal and the force acting upon it by virtue of the 25p.s.i. air supply. It was consequently necessary to use a powerful enough solenoid to overcome the elastic force of the extension spring. The force required was, of course, less when the air was supplied than when it was turned off!

Automatic Control Facility

A versatile control system was installed in the laboratory to supply an automatic feeding facility for both experimental and stocking tanks. The lighting control system was also incorporated in the control box which was shown in Plates 15 and 16 in the main text.

The feeding duration was restricted to the hours of light by using a 24hr Venner timer to supply both the lighting and feeding systems (Fig 48). The Venner motor was generally left running continuously, although a toggle switch was connected in series with it, on the input side, so that it could be switched off if necessary. Two on/off periods could be set on the dial of the timer, although for the purpose of the experiments conducted within the laboratory, a single timed period, 12hrs on/12 hrs off, was selected. Further, the Venner was provided with an overide-switch on the output side, so that the output could be switched on or off during the timed periods, without disturbing the input, i.e. the motor could be left running. Two transformers and two further timers were connected to the output from the Venner. Each could be switched on or off independently. The transformers provided a low voltage source output for each of the lighting systems described previously, and the two timers provided a versatile timing system for the automation of the feeders.

The first of these was an ex-washing machine programmer which

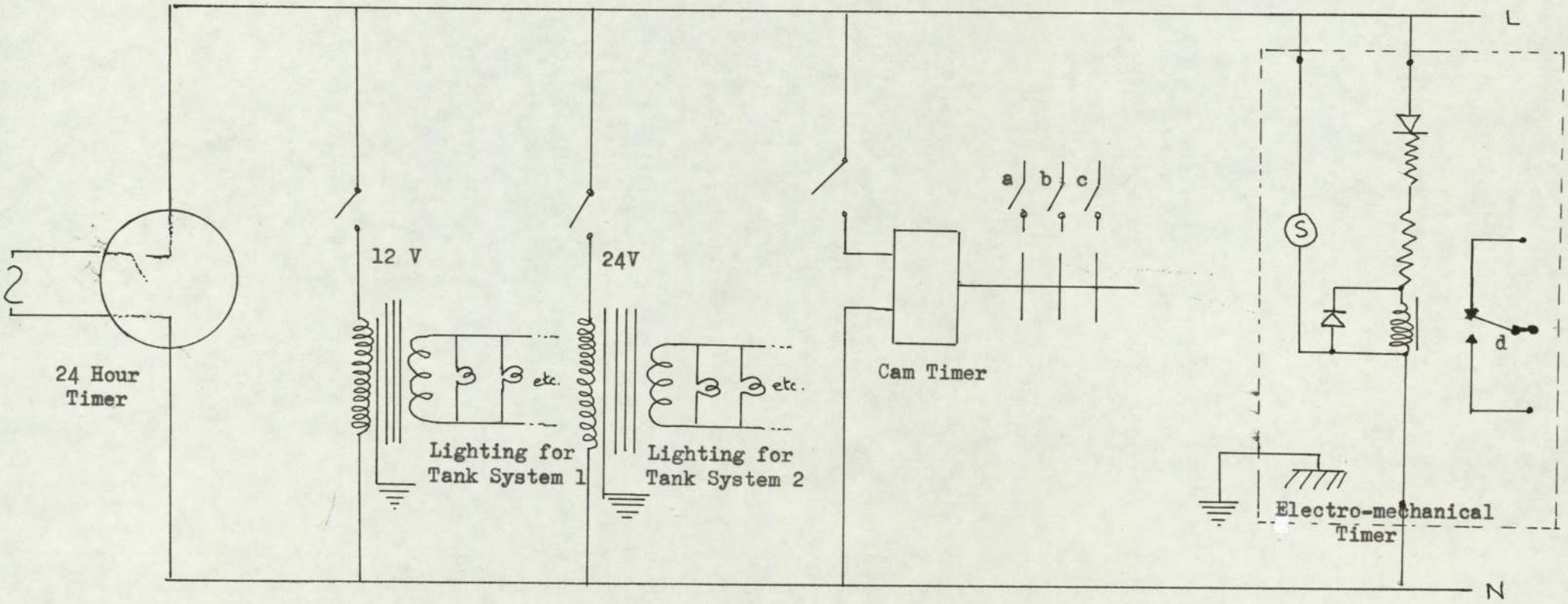
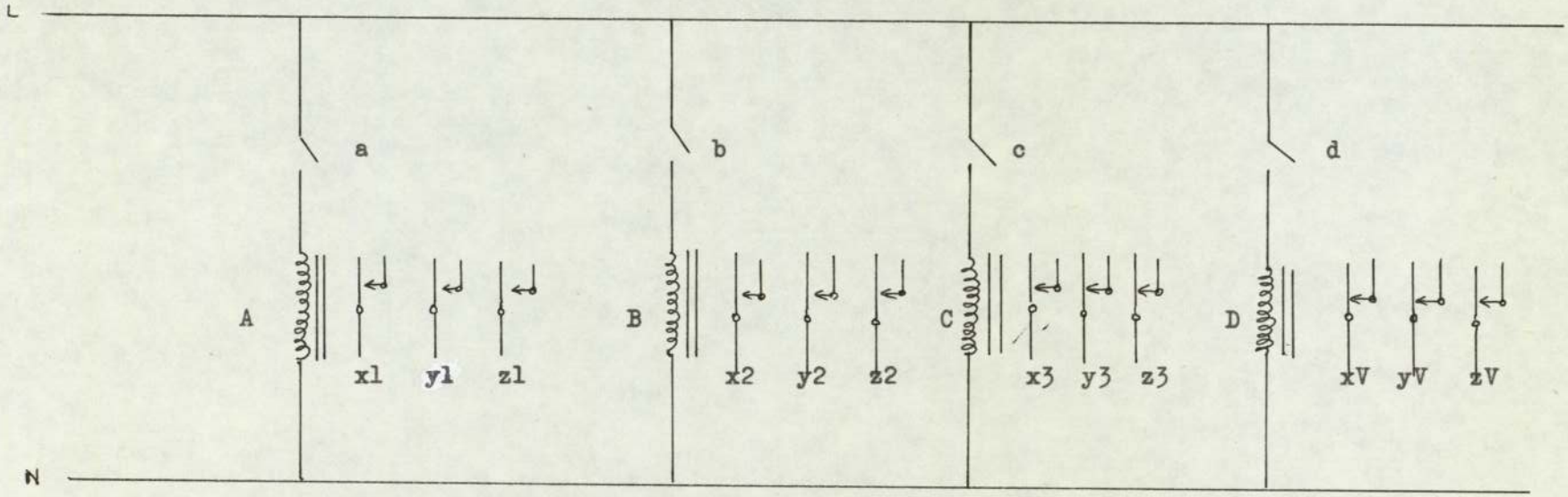


Fig. 48 Electrical Circuit - Incorporating Lighting and Feeder Timing Systems.

was reprogrammed to provide a one minute 'on-period' on each of three cams in succession at one minute intervals. The cams of the timer engaged microswitches. The three microswitches, (a, b and c in Fig48), were connected in series with a relay (A, B and C Fig49) having three on/off contacts (X, Y and Z). Each of these was connected in series with a solenoid operated valve, Fig50. A toggle switch (T1, T2 and T3) was also connected in series with each relay contact, Fig50 . The result was that each of three solenoids could be independently operated, up to three times per hour at one minute intervals, by running the cam timer and switching as many of the toggle switches to the 'on' position as was required. A fourth solenoid valve was connected in parallel to one of the other three, as shown in Fig 50. This could not be operated independently, except on manual overide (see below).

For the purpose of providing an electrical impulse at variable intervals, one requires a timing mechanism with an automatic reset. Electronic timers with such a function are normally expensive. The same function, however, was achieved for less than half the cost by using an electromechanical delay timer (e.g. as supplied by Elremco Ltd) combined with a very simple air operated toggle switch.

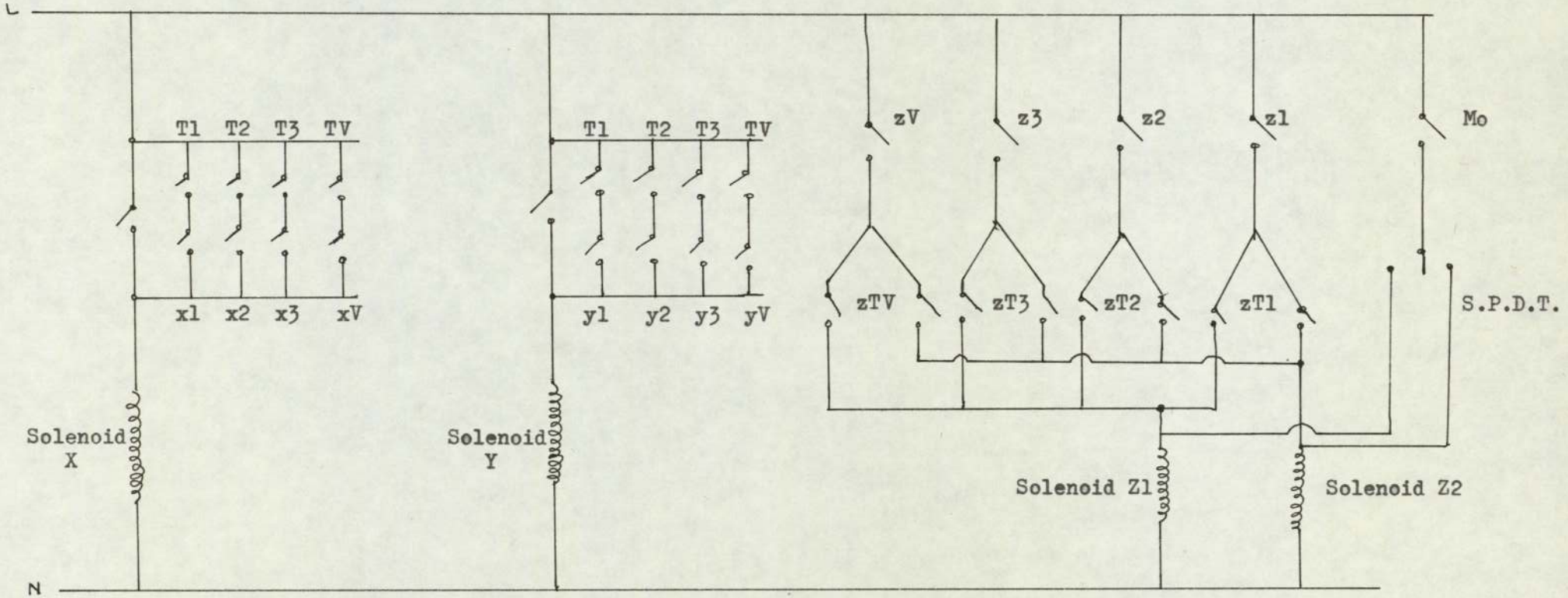
The delay timer, with a range of 0-6 hours at 12 minute intervals, was connected to the output from the Venner timer. On the expiry of the timed interval set on the dial, a relay was energised in the timer, operating a set of changeover contacts, d in Fig48 . This was connected in series with the fourth relay, D, as shown in Fig49 . When energised, this operated three changeover contacts each connected separately to one of the solenoid systems. When the appropriate toggle switches (TV, Fig50) were in the 'on' position, the solenoids operating the air valves were energised, supplying power to the feeding systems as previously described. An air supply was also taken to an air-operated toggle switch made from a 20ml syringe, the plunger of which was connected to a standard toggle switch Fig51 . Air entering the syringe advanced the plunger and consequently 'switched off' the supply to the



where a, b, c and d are micro-switches of the cam-timer in Fig. 48

A, B, C and D are relays, each with 3 c/o contacts: x,y and z

Fig. 49. Circuit Diagram - Feed Control Systems.



where x1,2,3,V; y1,2,3,V; z1,2,3,V are contacts of the relays shown in Fig 49.

T 1,2,3,V are s.p.s.t. toggle switches.

zT 1,2,3,V are d.p.s.t. toggle switches.

Fig. 50. Circuit Diagram - Switch System for Feed Control

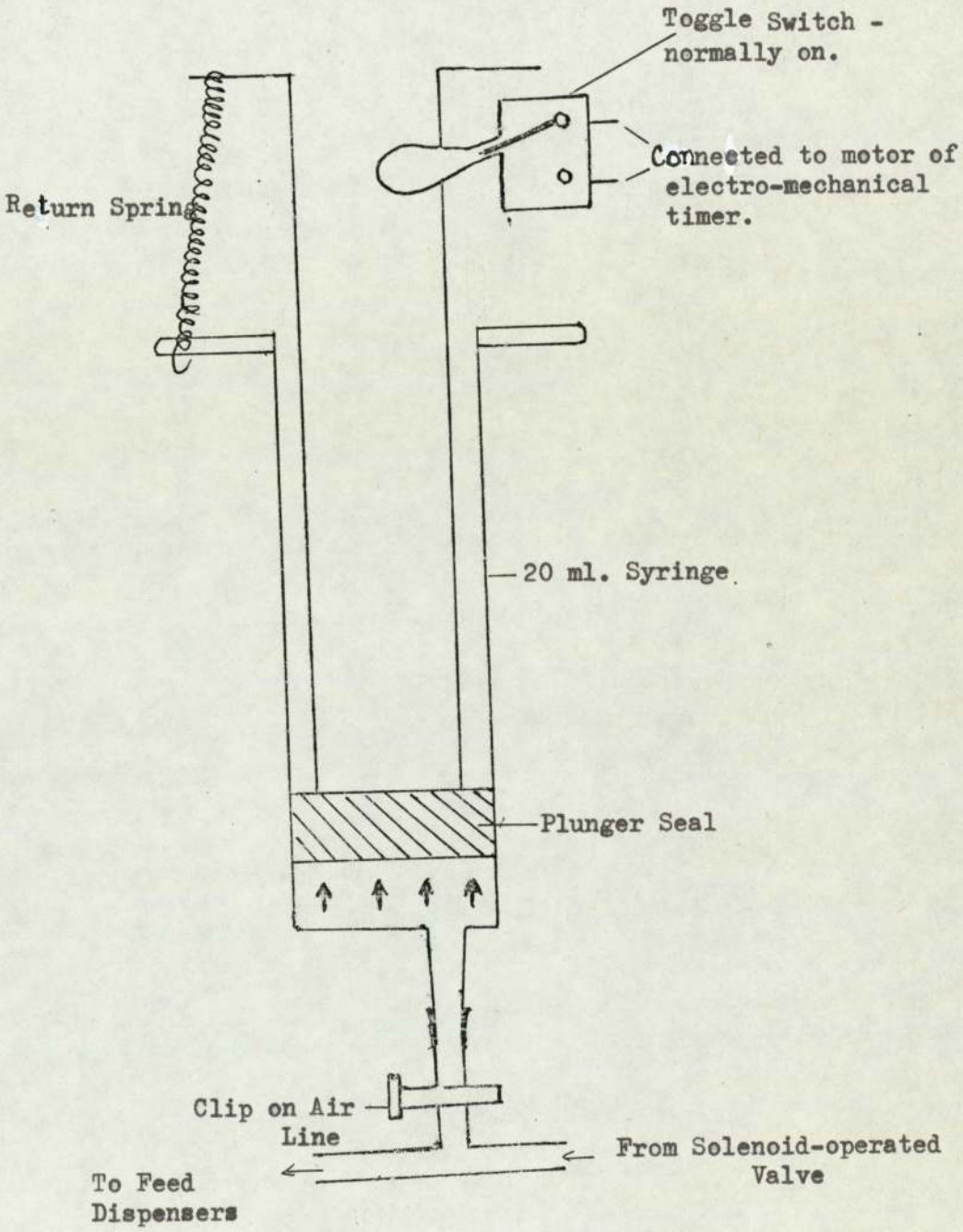


Fig. 51. Air Switch

motor of the delay timer. This in turn disengaged the clutch which reset the timer and consequently turned off the supply to the solenoid. An extension spring inserted between the syringe body and plunger returned the plunger to its original position when the air was exhausted, the toggle switch thus being turned back on for a further timed period. A clip on the air line supplying the 'air-powered switch' could be adjusted to alter the speed at which the switch was turned on. The greater the resistance on the air line, the longer it took for the air pressure to build up sufficiently to operate the plunger against the elastic force of the spring. By suitably adjusting the clip, the period for which the solenoid valve was open could be set such that one ensured that all feeders, no matter how remote from the control box, had operated.

The use of both the ex-washing machine programmer and the electronic delay timer with air powered toggle switch, provided versatile timing mechanisms for a number of independently operated feeding systems, at a comparatively low cost. Further, the design was relatively simple incorporating the use of 'macro'-electric components familiar to a layman, rather than more complicated 'micro'-electronic components.

Each of the solenoid valves could be operated automatically three times an hour from the cam timer or at preset variable intervals from the electronic timer. However, another toggle switch was connected into each solenoid circuit to by-pass the timing mechanisms and provide a manually operated overide, as shown in the circuit diagram. (Mo in Fig50).

Manual Control Valves for 'Ad Libitum'Feeding

Manual control valves were incorporated into the feeding system to enable 'ad libitum' feeding to be carried out as described in Section 4. Three master control valves were incorporated to by-pass the solenoid valves and supply air directly to the ring mains, thus avoiding

excessive use of the solenoids. The function of the three valves was as follows: valve 1 allowed air into the ring main via the solenoid operated valve; valve 2 allowed air to enter the ring main direct from the main air supply, thus by-passing the solenoid valves; valve 3 prevented the backflow of air through the solenoid valve exhaust when valve 2 was open. An air flow diagram for the feeding system is shown in Fig 52. It was necessary to have valve 1 and all the individual feeder valves open during automatic operation, whilst during manual operation of individual feeders, valves 2 and 3 should be open and the feeder valves should be closed. The sequence of switching from automatic to manual was

1) Close feeder valves.
2) Close control valve 1.
3) Open control valve 3.
4) Open control valve 2.

The order was reversed for switching from manual to automatic.

Demand Feeding

A demand feeding system was designed incorporating the feed dispenser unit and solenoid actuated valve previously described. Additional components of the system included a contact switch, an electronic circuit including a monostable multivibrator circuit, and two recording devices, one being a simple electromechanical counter, the other an air operated pen-recording system.

Contact switch

The switch was shown in Fig 38. It was made from two 10ml syringes. The plunger was removed from one of the syringes and the bottom was cut off the outer case. The solid rubber seal from the end of the plunger was then removed. A small hole was burned in the seal and a 6" length of insulated rigid wire was pushed through the hole. The wire was held tightly in the rubber. When the wire was pushed laterally

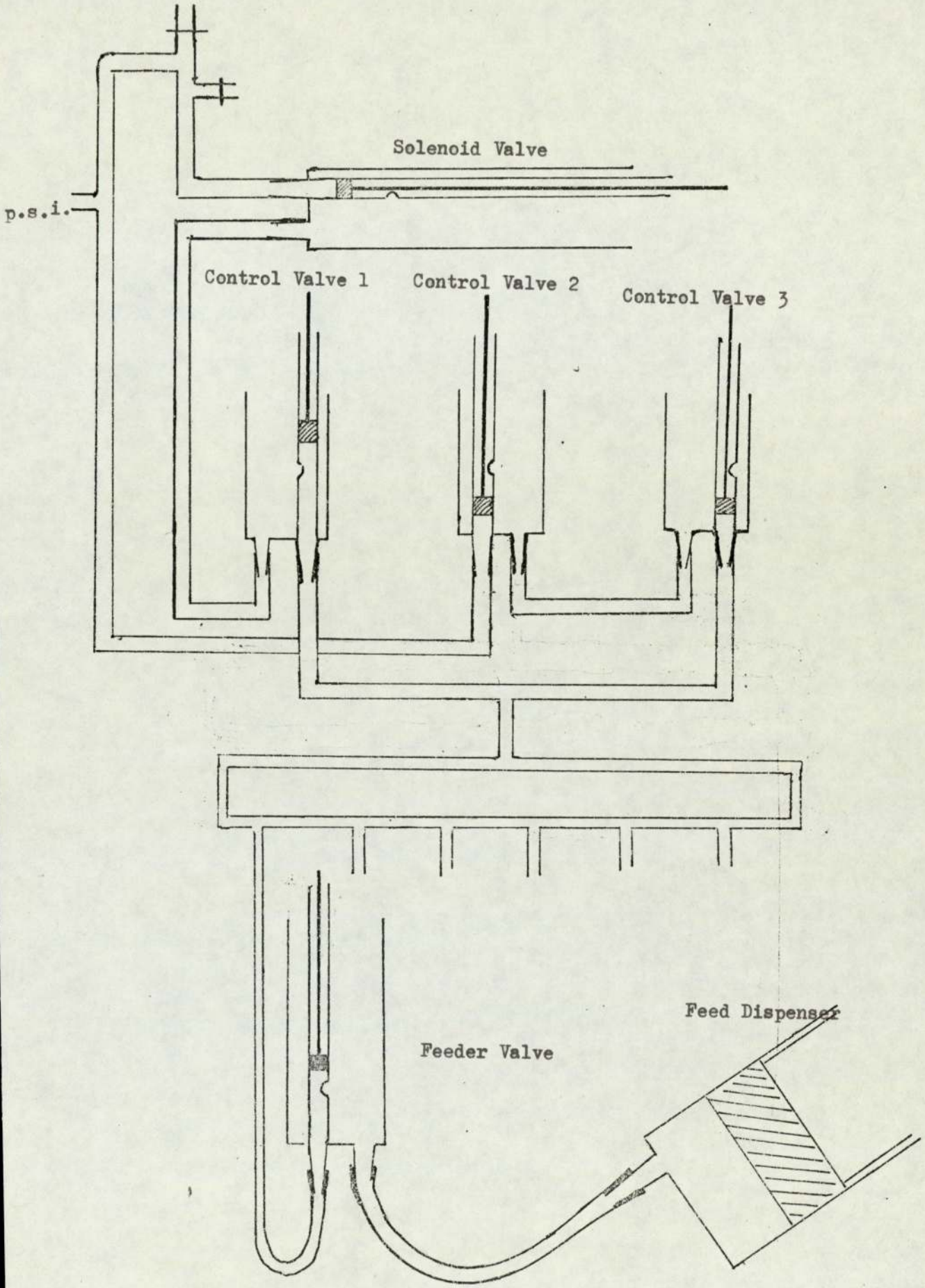


Fig. 52. Manual - operated Valve System for Satiation Feeding.

at one end, there was an equal and opposite movement at the other end. When released the wire returned to a central position by virtue of the elasticity of the rubber seal. The rubber seal was then pushed into the bottom of the syringe case, where it fit tightly, sealing the bottom end.

A rubber seal was removed from a second syringe. A hole, large enough to accommodate a 1" length of $\frac{1}{4}$" plastic rod, was burned in it. The end of a short length of plastic rod was filed enabling a very short length of $\frac{1}{4}$" copper tube to be forced onto it. An insulated copper wire was then pushed through a second small hole burned in the rubber seal, adjacent to the first. The wire was soldered to the outside of the copper ring. A second insulated copper wire was pushed through a third hole in the rubber seal and soldered $\frac{1}{2}$" from the top end of the 6" length of rigid wire. The rubber seal was then forced into the top of the 10ml syringe case. The position of the long rigid wire was adjusted so that it was just inside the copper ring and a small movement in any direction was sufficient to make an electrical contact.

Electrical circuit

The switch was connected in series with a relay operated from a 12V D.C. supply as shown in the circuit diagram, Fig **53**. A solenoid operated valve was connected through one of the contacts of the relay to a 240V A.C. supply. A mechanical counter was connected through the other contact to the 12V D.C. supply. When contact was made at the switch, the relay contacts changed over thus completing the independent solenoid and counter circuits, both of which therefore operated. The operation of the solenoid valve allowed air to enter a feed dispenser unit as previously described.

The system worked reasonably well, provided that the length of air tube between the valve and feed dispenser was relatively short and large fish were used. In this application of the air operated feeding system, air power was a disadvantage in that a finite time was required

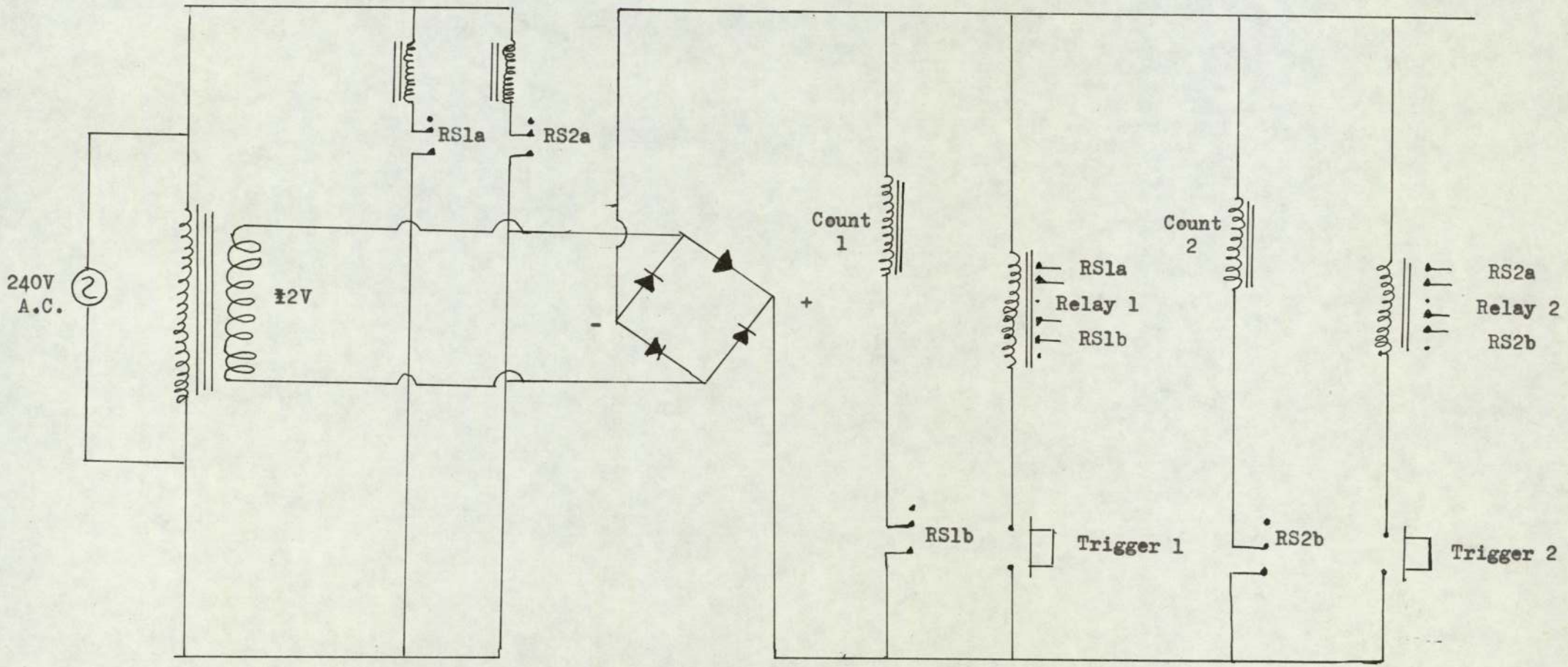


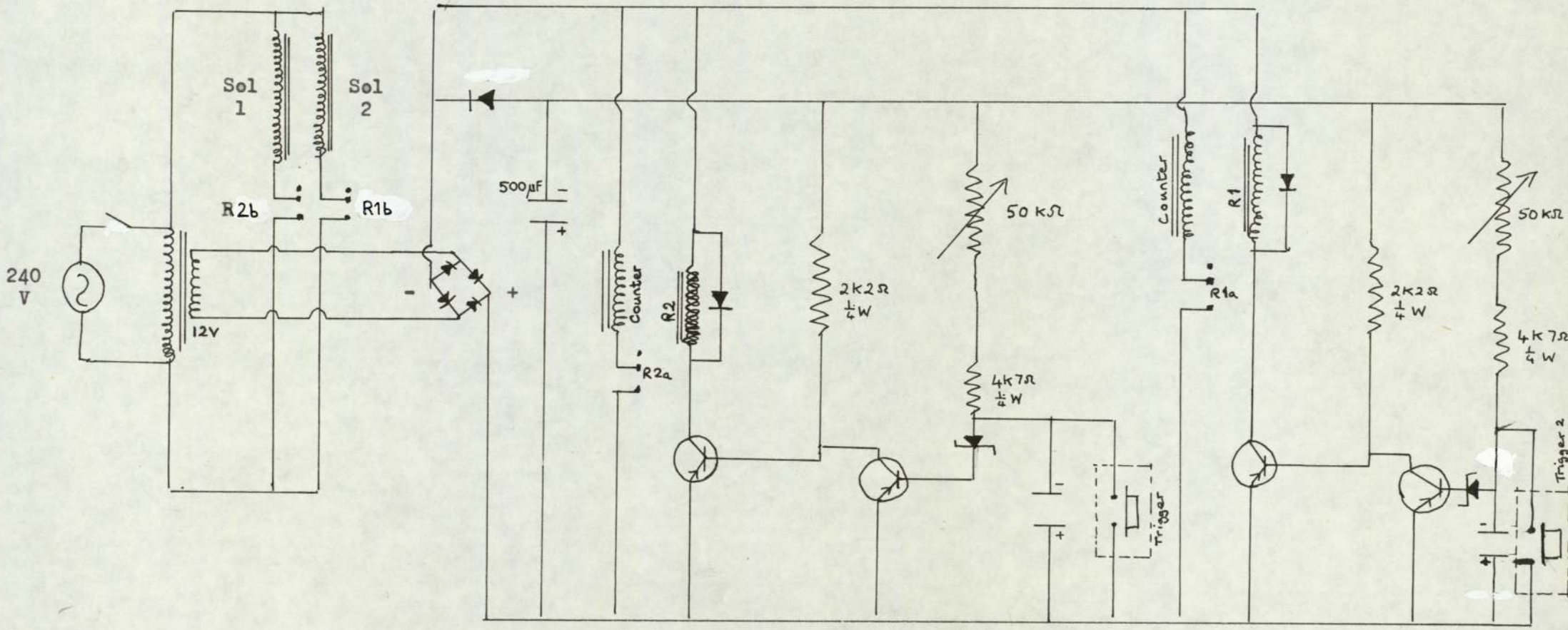
Fig. 53. Demand Circuit 1.

for the air pressure to build up in the system and operate the dispenser plunger. Larger fish were able to operate the switch with sufficient force and maintain contact for a sufficient length of time to allow the operation of the dispenser plunger. Small fish, however, made only momentary contact as shown by a comparatively large number of counts per unit feed dispensed.

A number of methods to get round this problem were considered. Monostable multivibrator circuits are frequently used for this purpose in electronics (standard electronics textbook) and Simms (1974, unpublished project), with the guidance and advice of an electrical engineer, was able to modify the electrical control system and incorporate such a delay circuit. Fig 54 shows the complete circuitry for the operation of two demand feeders. In this form the variable resistor can be adjusted to give a fixed delay period when the solenoid will be activated, irrespective of the time of contact at the switch. Even an instantaneous contact was therefore sufficient to open the valve for a preset interval, during which time feed was dispensed. The delay was set at about 1 sec. In this circumstance, if fish made contact more than once during this interval, feed would be dispensed only once.

Pen recording system

In addition to recording the number of contacts/unit time, it was considered desirable to record the distribution of contacts during a 24hr period. Some form of recording system was therefore required. Whilst it is relatively simple to incorporate a potentiometric recording device into the electrical circuit, they are generally expensive. A simple 'air-powered' recording device, however, was installed very cheaply. This involved using a 24hr motor, such as that in a humidity recorder, to turn a circular chart, in this case made from plastic-coated paper. A 20ml syringe mounted over the chart was used to power a very fine felt tip pen as shown in Fig 55 and in Plate 20 in the main text. The principle of operation is identical to that used in the



Fig, 54. Demand Circuit 2 (From Simms, 1974. Student Project)

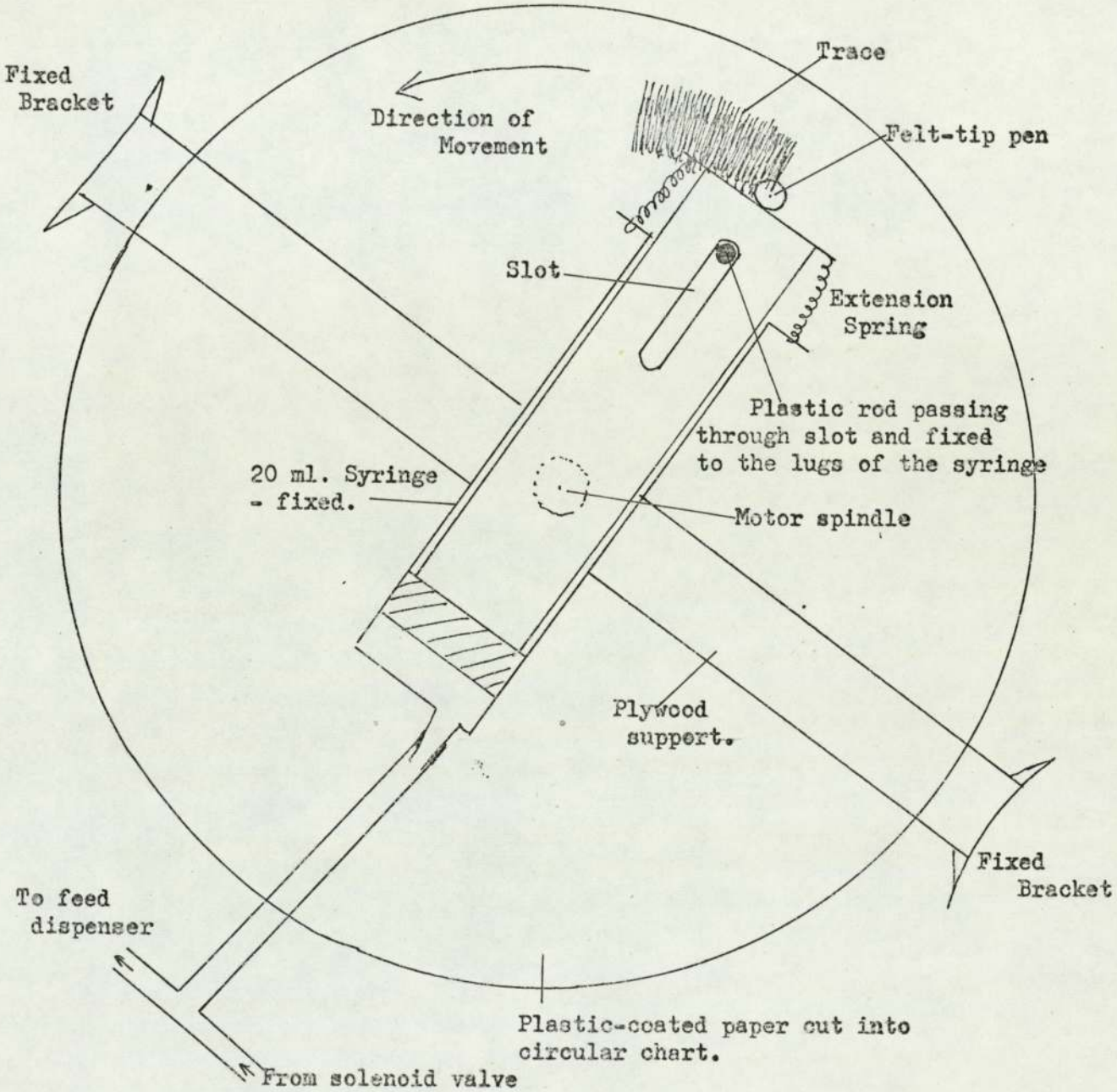


Fig. 5. Demand Feeder Recording System

feed dispenser. The air tube supplying the recorder was connected into the same supply line as the dispenser. Thus, when contact was made at the switch, the food dispenser and recorder operated simultaneously. In this way a series of short black lines were recorded around the chart each time food was dispensed. Two recorders could be mounted over the chart recording from two feeders at one time.

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