The Relationship between Protein Source and Nitrogen Metabolism in the Trout

Timothy Hugh Atack

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Summary

Various experiments connected with the use of novel proteins (SCP's) in fish diets are described. In the first series of experiments classical nitrogen balance techniques were used. From these, MFN was found to be 175 mg N/100g diet and EUN 12.37 mg N/100g fish/day. Using this data, true NPU's were determined at various levels of N intake ranging from 36 to 140 mg/100g fish/day. True NPU decreased linearly as N intake increased, the rate varying only slightly with protein source. For herring meal (HM) these values were between 0.97 and 0.48, the others being - petroyeast (PY) 1.0 - 0.67; bacterial protein (MB) 0.71 - 0.48; algal protein (SA) 0.87 - 0.55; and fungal protein 0.79 - 0.54. Apparent NPU was practically constant regardless of N intake. A further experiment showed that the decrease in true NPU was not ameliorated by the use of high energy diets.

Growth experiments incorporating NPU determinations by carcass analysis gave the following true digestibility (%), true NPU, BV and PER values:- PY 91.6, 0.42, 0.46, 2.01; MB 93.5, 0.37, 0.40, 1.62; SA 83.1, 0.32, 0.38, 1.33; HM 91.2, 0.38, 0.41, 1.91; soyabean (SY) 43.6, 0.18, 0.41; brewers yeast (BY) 79.9, 0.30, 0.38, 1.17; casein (CS) 98.7, 0.40, 0.41, 1.97. Dietary effects on growth rate, body composition and food intake were also noted. For carp, the equivalent values were:- PY 96.6, 0.47, 0.49, 2.08; MB 95.5, 0.49, 0.52, 2.54; SA 87.1, 0.36, 0.41, 1.15; HM 80.3, 0.64, 0.79, 2.82,; SY 83.7, 0.42, 0.5, 1.35; CS 93.0, 0.49, 0.52, 2.54. The results are discussed in relation to the amino composition of the diets.

Food preference trials with demand feeders gave inconclusive results as trigger preference was often prevalent. Additional work on photoperiod and demand feeding rhythm showed that trout at 12°C exhibit peaks of feeding every 8 hours.

Key words: PROTEIN NITROGEN METABOLISM TROUT

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GLOSSARY

BV Biological Value - $\frac{\text{Protein retained}}{\text{Protein absorbed}}$

or $\frac{\text{NPU x 100}}{\text{Protein digestibility}}$

BY Brewers yeast

Conversion ratio - $\frac{\text{Weight fed}}{\text{Weight gained}}$

CS Casein

DM Dry matter

ENE Endogenous nitrogen excretion

EUN Endogenous urinary excretion

FCE Food conversion efficiency - $\frac{\text{Weight gained}}{\text{Weight fed}}$

FM Fishmeal

GPV Gross protein value

HE High energy

HM Herring meal

LE Low energy

MB Methanophilic bacterium

MFN Metabolic faecal nitrogen

NFE Nitrogen free extractive

NNV Net nitrogen value - NPU x N content of food

NPR Net protein retention

NPU Net protein utilisation

- apparent = $\frac{\text{Protein retained}}{\text{Protein fed}}$

- true = $\frac{\text{Protein retained + ENE}}{\text{Protein fed}}$

NPV Net protein value

OMP Oregon moist pellet

PER Protein efficiency ratio - $\frac{\text{Weight gained}}{\text{Protein fed}}$

PPV Productive protein value

PY Petroyeast

SA *Spirulina* alga

SCP Single cell protein

SY Soyabean meal

TD True digestibility

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CHAPTER 1

Introduction - Novel Proteins in Animal Nutrition

1.1 General Introduction

The concept of using microorganisms as a source of protein was first investigated some sixty-five years ago, when yeast was employed as a dietary supplement during the food shortages of the First World War. Since that time, the world protein shortage has reached crisis point, and so obviously renewed attention has been paid to ways in which this situation can be relieved. Several ways of either increasing the amount of protein or increasing the quality of food proteins (which would then reduce the quantitative requirement) have been proposed by the President's Science Advisory Committee (1967). These are by (a) fortifying cereal grains, (b) improving cereals by genetic means, (c) increasing the amount of animal protein, (d) recovering oilseed protein, (e) increasing ocean fishing, (f) increasing inland fishing, (g) producing fish protein concentrate, (h) microbial protein, (i) leaf protein, (j) algae.

The fortification of cereal grains (most of which are deficient in the indispensible amino acid lysine) has proved to be quite successful, though there is still a limit to the amount of cereal that can be produced from the limited amount of agricultural land available. A similar constraint can be applied to any attempt to expand world fisheries. Overall, from the biological point of view, microbial protein seems to have definite advantages in that large amounts of agricultural land

are not required to produce it, and microorganisms will grow on relatively simple growth media and also have a high specific growth rate, (Table 1).

TABLE 1 - Theoretical Protein Production From Various Organisms

1000 kg beef animal	1 kg day^{-1}
" " soya	100 kg day^{-1}
" " mould	28 t. day^{-1}
" " yeast	100 t. day^{-1}
" " bacteria	1×10^{11} t. day^{-1}

However, the obvious massive amounts of microbial protein that could be produced are not the only consideration, since the economics of production, the palatability, nutritive value and presence of any toxic substances should also be considered. Whilst it will no doubt be necessary to use some microbial protein as a direct addition to human food, thus keeping the food chain as short as possible, there will still be a need for high quality animal protein and this is the way in which most single-cell protein (SCP) produced in the near future will be used. Consequently, in recent years a considerable amount of research has been aimed at discovering the nutritive value, palatability and toxicity of SCP to both laboratory and farm animals.

There are three ways in which unicellular organisms could be incorporated in a diet:

(1) as the sole protein source

(2) as a supplement to other dietary proteins

(3) as a vitamin, lipid, carbohydrate, mineral etc. source.

Since this treatise is only concerned with the nutritive value of the protein of single-cell organisms, its use under section three will not be discussed.

The evaluation and comparison of various workers' results poses some problems since there is such variability in the basal diets employed as regards energy, mineral, vitamin and protein levels. All these factors, especially protein level, can significantly affect the biological value of the protein under investigation. Also the culture conditions under which the microbial protein is produced can in some cases alter its protein content and amino acid profile (Stokes and Gunness, 1946). So unless different workers use identical culture conditions, the results they obtain are not essentially directly comparable. In addition, there seems to be little standardisation of the method of denoting the nutritive value of a protein, the choice of Protein Efficiency Ratio (PER = weight gained ÷ protein fed), Net Protein Utilisation (NPU = protein retained ÷ protein fed), Biological Value (BV = protein retained ÷ protein absorbed), appearing to be a matter of personal

preference. A further difficulty arises from Kjeldahl nitrogen determination, which inevitably overestimates the amount of protein present (Anderson and Jackson, 1958), but because of its relative simplicity this still is the most commonly used method of protein determination.

The microorganisms studied in nutritional experiments can be divided into four main groups - yeasts, algae, fungi and bacteria; thus these will now be considered individually.

1.2 Yeasts

Yeasts were possibly the first form of SCP to be utilised in animal diets, mainly because yeast has been available in large quantities as a by-product of the brewing and baking industries. More recently yeasts have been specially grown on other media such as various hydrocarbons and waste sulphite liquor from the paper pulping industry.

Food yeast contains protein in an amount equal to approximately 50% of its dry weight (Peters, 1945; Inskeep et al, 1951) though this is slightly variable depending on several factors. Akopjan (1961) found that the proximate composition of yeasts grown on three different media (straw, barley bran, and sulphite liquor) was very similar and that they contained identical amounts

of 17 amino acids, which contrasts with the results of Stokes and Gunness (1946) and Al-Aswad (1972). Yeasts are deficient in the sulphur-amino acids methionine and cystine (Chiao and Peterson, 1953), and to a lesser extent, tryptophan (Stokes and Gunness, 1946). An analysis carried out by Nelson et al. (1960) showed that usually lysine is found in 5 - 6 times the quantity of methionine, whilst the methionine itself is normally 1.5 times the tryptophan content, these levels not varying in respect to one another when the actual amount of protein in the cell is varied. Overall Pillai et al. (1972b) contend that apart from methionine yeasts amino acid profile is comparable to the FAO reference pattern. Since methionine and tryptophan are indispensible amino acids for all the animals whose requirements have been discovered, to date the low levels found in yeasts limits the usefulness of yeast protein as the sole source of protein in a diet (Carter and Phillips, 1944). For this reason, very few experiments have been performed on such diets. However, the biological value of a yeast hydrolysate made from distillery sludge was 65% with a digestibility of 89% when fed to rats (Rastogi and Krishna Murti, 1961), compared to casein which had values of 66 and 88% respectively.

Much more research work has been aimed at replacing presently commonplace protein portions of a diet (for example, wheat, fishmeal, etc.) with varying amounts of yeast protein. For instance, when the diet of growing boiler chickens, which usually contains fishmeal as the main protein portion, was adjusted by replacing some or all of the fishmeal with yeast, it was discovered that 70 - 100% replacement of the fishmeal actually increased the growth rate of the birds. Feed consumption also showed advantageous results, though overall no remarkable differences in growth rate were noted for any of the fishmeal/yeast diets tested (Ito and Kurihara, 1971). Since yeast is deficient in methionine, the possibility of improving the biological value of its protein by supplementing it directly with S-amino acids has been attempted. Pillai et al (1972a) working with albino rats found that on supplementing a diet containing the yeast Endomycopsis lipolytica with methionine, PER, weight gain, protein digestibility and haematological responses of the rats were comparable with controls fed casein (commonly used as a reference protein). Other sulphur amino acids can be used. 2% cystine added to yeast protein again gives a growth promoting value equivalent to milk protein (Hock and Fink, 1943). Sljanin (1959) increased the biological value of yeast protein from 63 - 68% to 70 - 77% by the addition of cystine. An experiment similar to that of Pillai et al. was performed by Elmadfa and Menden (1973), except that

they supplemented the diet with glutamic acid as well as methionine. The results indicated that addition of 0.3 gms of methionine/100 gms of diet gave a PER equivalent to that obtained with casein + 0.3% glutamic acid.

D'Mello (1973) has carried out further tests on chick diets supplemented with yeasts and amino acids. The basal diet used was maize, soybean oilmeal and 0.16% added methionine. Other chick groups had similar diets with 10 or 15% BP yeast with or without 0.1, 0.2, 0.3 or 0.4% methionine added. There were no significant differences in respect to weight gain or feed efficiency between groups. In a second experiment, D'Mello gave seven day old chicks a diet of 22.75% yeast unsupplemented or supplemented in factorial design, with 0.15, 0.3, or 0.45% arginine and 0.12, 0.24, 0.36 or 0.45% methionine. Weight gain and feed efficiency on yeast alone were less than controls. Weight gain improved, but not to control levels, after the addition of 0.12% of methionine to the diet. A significant improvement was found when the feed consisted of yeast, 0.3% arginine and 0.24% methionine. Plasma amino acid levels also fell as dietary methionine increased (a further method examining protein utilisation).

Klose and Fevold (1945) and Mekada et al. (1972) have also shown the benefit of supplementing yeasts with methionine, but perhaps the most striking example is given by Dwivedi et al. (1972). They found that a meat substitute made from brewers yeast and isolated soy protein had a digestibility of 92% (true) compared to 95.5% for whole egg. However, PER, true BV and average weight gains were much less than for whole egg until the meat substitute was fortified with 1% methionine, whereupon they became comparable to those of whole egg. However, it must not be concluded that addition of methionine to a yeast compounded diet inevitably increases growth. Cousin (1973) showed that rabbits fed a diet with yeast added at the 7% level (with or without choline or methionine) did not exhibit a growth rate any different from controls. This was probably due to the low rate of yeast protein to total dietary protein.

The effects of yeast in a diet can alter with the age of the test animal. New-born pigs (72 hours old) were given control diets or control diets plus 1.5, 2.0, or 2.5% yeast by Veum and Bowman (1973). Whilst 1.5 and 2.0% yeast did not alter weight gain or feed efficiency throughout the trial (2 - 70 days), 2.5% added yeast did depress both these parameters. However, if the pigs were given the control diet from days 2 to 28, then 2.5% yeast gave increased weight gain and feed conversion for the rest of the trial. A similar type of experiment

again by Bowman and Veum (1973) but using the weight of the pigs instead of their age as a fundamental parameter, gave different results. From 34 - 100 kg, control diets (16 or 18% crude protein) gave more efficient conversion than those diets with added yeast, though yeast supplemented feeds were better up to 34 kg. It is very difficult to extract any really meaningful facts from these experiments but overall it seems to be advisable to reduce the yeast levels as the pigs develop, but of course this may not be necessary if a different basal diet is utilised.

If SCP diets are to be supplied to animals for most of their lifetime then other effects of its consumption must be evaluated. For instance, its effects on the egg production and viability could be important, since decreased egg production could make a control diet more attractive economically, even if the growth rate of the hatchlings were lower. For chickens Yoshida et al (1973) concluded from their experiments with yeast diets that hatchability, mortality and viability of eggs and chicks were similar to controls. On top of this weight gain, feed conversion, egg production and average egg weight were not altered. However, second and third generation chicks given yeast reached sexual maturity 3 days earlier than controls though this was probably due to differences in the energy and protein content of the diets, thus emphasising the need for experimental diets for protein

evaluation to be isonitrogenous and isocalorific. With rats more pronounced differences between groups fed differently over several generations can be seen, as shown by the work of Proll (1959). The diets tested were either basal, basal plus 4, 20 or 40% yeast. No significant differences in weight were noted in male rats except at the 40% yeast level, these rats being 50 gms lighter after 10 months. In the F1 generation, males and females were 130 and 50 gms lighter after 10 months on the 40% yeast diet. This also occurred in the F2 generation and some differences in the females' lactation was noted. In later generations, rats given the 40% and later the 20% yeast diets showed disturbances of lactation, cannabalism and sterility. This experiment introduces another factor that has to be evaluated in the feeding of SCP, ie. its effects on normal metabolism. Rats can live and grow normally for at least 150 days on a diet in which 48% of the protein is derived from food yeast (Irving and Schwartz, 1946) though more liver glycogen is probably accumulated with high yeast diets (Bickel, 1942). A more comprehensive examination by Bender and Doell (1960) indicated no differences in weight gain, food consumption, constituents of blood and urine or fertility of rats given diets including up to 21% yeast (Torulopsis utilis, Zygosaccharomyces lactis and Candida arborea).

Neither were any obvious abnormalities of tissues apparent. This contradicts certain workers such as Saidkov and Sinitzuin (1936) who concluded that separation of yeast proteins from nucleic acids (a large proportion of the nitrogen of yeasts occurs as nucleic acids) is essential for the large scale utilisation of yeasts in animal nutrition. With most animals the nucleic acids do not seem to cause much of a problem since with a diet containing yeast Ritzman (1946) found that blood uric acid levels of cattle were not affected. This may be due in part to the total breakdown of purines and pyrimidines in the rumen.

The addition of yeasts to animal feedstuffs has been thought to cause liver and kidney lesions as noted previously. However, on an 80% yeast protein ration, severe liver and kidney damage could be prevented in rats by the addition of 2% cystine (Hock and Fink, 1943). Schwartz (1951) produced dietary necrotic liver damage in rats using American Torula yeast but this was linked with low vitamin E levels and a deficiency of cystine. Similar fatal results of liver necrosis have been obtained by Lindan and Work (1951) though they had to artificially deplete their test animals of vitamin E since non-tocopherol deficient rats did not die during the experiment. Such a gross deficiency of vitamin E is unlikely to occur in everyday feeding of yeasts to animals.

The palatability of the feed is also another important factor to be considered in the use of microbial protein. Young pigs given a choice of diets (wheat or wheat plus 3, 5 or 10% yeast) ate 53% more of the diet containing 10% yeast than any of the other diets (Salmon-Legagneur and Rettagliati, 1960). Calet et al. (1962) found that yeast autolysate (ie. yeast with the cell walls removed) produced a better growth rate in chicks due to its increased palatability compared to controls. An interesting point here is that they found that the way in which the yeast is prepared does not alter its efficiency, this being more of a function of its amino acid content.

Overall Block and Bolling (1943) maintain that yeasts show a balanced though not perfect mixture of the essential amino acids whilst Sljanin (1959) concludes that they are similar to fishmeal in their protein content and biological value. They are also readily eaten and do not flavour meat or eggs whereas fishmeal can. Sljanin also gives a list of recommended daily intakes of yeast for selected animals and calculated that one metric tonne of yeast will give 5,000 litres of milk, 1 metric tonne of veal or 750 kg of pork.

1.3 Algae

Unicellular algae were first looked at as a source of protein as part of the space programme, the intention being to utilise waste materials and sunlight to synthesise edible protein. From this beginning, experimentation with algal diets has spread across the whole spectrum of animal nutrition and probably most work is now directed towards chickens as the main potential consumers of algal protein, rather than man directly. Algae have definite advantages as a means of producing protein since they do not need a carbohydrate source due to their photosynthetic abilities. However, the actual culture of large quantities of algae is fairly difficult since large surface areas exposed to sunlight are required. Also the growth medium does tend to become contaminated with other organisms. This can affect the results of feeding trials (Erchul and Isenberg, 1968), digestibility, feed efficiency of contaminated algae being lower than is found with sterile algae (Vanderveen *et al*, 1962).

Several different genus of algae are commonly used in feeding trials, the main types being *Chlorella* (strain 71105), *Scendesmus*, *Spirulina* and *Microcystis*. *Spirulina* contains approximately 60% protein with the sulphur amino acids again being first limiting (Clement *et al*., 1967), whereas *Chlorella pyrenoidosa* is also deficient in histidine for the rat (Leveille *et al*., 1962).

Algal cells like those of other microorganisms can contain up to 30% non-protein nitrogen, and their amino acid composition is also highly variable (Leveille et al., 1962). However, the amino acid content of most algae is said to be similar to that of milk powder (Meffert, 1961). Okumura and Tasaki (1973) assign a biological value of between 65 and 67% to Chlorella, digestibility being 74%. They also compare these values with those of depigmented Chlorella and found that removal of the chlorophyll and other pigments reduced the biological value to 46 - 55% whilst having no real effect on the digestibility. The metabolizable energy values were also reduced by depigmentation. The PER of Chlorella 71105 for rats at 2.19 is higher than that of most vegetable and cereal proteins including soy protein (Lubitz, 1962). However, Cook (1962) found that the PER of an unidentified algal culture grown from waste material had a PER of only 67% of that of casein, ie. about 1.67, but that protein efficiency ratio could be brought up to the level of casein (2.5) with a 3:1 casein/algal mixture.

As described above, depigmentation of the algae has not been shown to increase its nutritive value, but several other treatments can be applied which will achieve this end. Chickens given a diet of Scendesmus obliquus with 10% Chlorella grew slightly more slowly than chickens given no algae but when the experiment was repeated with algae which had been stripped of their cell membranes

there was no difference in nutritive value (Angelova et al., 1973). Boiling for 30 minutes is another method of increasing the biological value (Cook, 1962).

Algae have been used to replace fishmeal in the rations of certain species, this being especially successful with pig feeds (Meffert, 1961). However, with broiler chickens given feeds with part or all of the fishmeal replaced by 5, 8, or 11% Scendesmus from 1 day old to 56 days old the results are not quite so good (Beremski et al., 1973a). Up to 5% alga in the meal was satisfactory from 36 days old but with more algal meal or algal meal from day 1 the growth of the animals was much poorer than with fishmeal alone. Feed utilisation was not significantly affected by algal meal. An interesting point to notice from this experiment was the fortunate side-effects of algal diets for chickens in that with 8% algae carotenoids in the skin improved the appearance of the carcass. Unfortunately, the side-effects are not always so welcome. Lubitz (1962) notes that 92% Chlorella may have some possible growth retarding effects on rats, which can possibly be allied to the occasional appearance of yellow or fatty liver. Algae also have an unpleasant odour and flavour for human consumption (Cook, 1962), but this does not seem to affect the appetite of any of the laboratory or farm animals so far tested, though rats have seen to refuse certain algal diets for the first few days of a test period. Gastro-intestinal disorders can occur in certain

species especially man, but mice had a normal bowel habit on a diet of 100 parts dried algae/40 parts of maize starch/3 parts cellulose powder (Bowman et al., 1962).

Algae have been used in the diets of laying hens with variable results. Beremski et al., (1973b) report that when 6, 12, or 16% Scendesmus meal replaced similar amounts of soya bean oilmeal in the diets of Leghorn laying hens, egg yield, hatchability, thickness and weight of eggshells were better than controls. However, in other trials where 20% Chlorella replaced 20% soya bean oilmeal, feed intake of laying hens was reduced resulting in decreased egg production (Okumura and Tasaki, 1973), though the poor results were not attributed to a nutritional deficiency in the Chlorella.

Summing up, it can be said that algae have a high biological value (up to 96%) compared to yeasts (about 48%) and also on average contain smaller amounts of nucleic acids, and so do not produce high levels of uric acid in the blood (Muller-Wecker and Kofranyi, 1973). A table of the nutritional values of several algae is shown overleaf (Table 2).

TABLE 2 - Nutritional Value of Algae

	BV	DIG	NPU	PER	CAL VAL	CHEM SCORE
1 Chlorella	-	86	-	2.19	5.16	-
2 Unidentified	54.3	-	35.5	1.67	-	-
3 Scendesmus	96.0@	-	-	-	-	-
4 Spirulina	62.5	76	47.7	-	-	43-56

@ protein only

1 Lubitz, 1962

2 Cook, 1962

3 Muller-Wecker and Kofranyi, 1973

4 Clement et al., 1967

1.4 Fungi

The need for the production of fungi as a nutrient source has been outlined by Gray (1961). Fungi have several advantages over bacteria and yeasts. They have a better amino acid profile, are easier to recover from their growth medium and their filamentous structure is useful in the making of foodstuffs because it negates the necessity to extract and spin the protein (Spicer, 1971).

The composition of fungi is highly variable, depending mainly on species. The crude protein content (15 - 60%) is rather low in comparison to bacteria and yeasts and

Kjeldahl determinations of nitrogen may still be too high since fungi contain much non-protein in the form of polymerised N-acetylglucosamine (chitin). This substance can constitute 6 - 16% of the dry weight of the mycelium.

As is found with other microorganisms, fungi are deficient in methionine (Stokes and Gunness, 1946; Pillai and Srinivasan, 1956), and this is reflected in the biological value of fungal feeds. Fink et al., (1953) found that the mycelium of Penicillium notatum could be eaten by rats without injurious effects, but protein digestibility was only 58%. In a diet where 32.4% dried mycelium supplied 77% of the protein intake, the BV was 59.8% which they conclude shows fungal protein to be approximately equal in biological value to Torula utilis or potatoes. However, if the NPU is calculated from their data it is found to be about 35% which is very low compared to other proteins. Albino rats given one of three levels of protein from either rye bran, Aspergillus niger mycelium (with or without spores) of a mixture of the two, have shown this low value of fungal protein (Columbus and Rojahn, 1961). At low protein levels, bran gave similar results to the mixture, whilst at higher protein levels growth rate was greater with the mixture than with rye bran alone. Fungal mycelium alone repeatedly gave a low growth promoting value. Miglietta et al., (1972) using four different strains of Aspergillus niger (DBH5, 33, 95 and 96), compared the

growth rate of young chickens on a 10% fungal/90% commercial chick diet to controls given either 10% maize or 10% lucerne in place of the fungus. All the fungal strains except DBH33 produced slower growth than the controls, thus emphasising that microbial strain may be an important factor in feeding experiments. These workers also noted that the results were the same whether the A. niger was grown on liquid medium or steeped maize, so nutritive value may be somewhat independent of culture conditions, at least in the case of fungi.

An alternative way in which fungi can be used as a protein source, is to mould a conventional diet. Columbus et al., (1958/59) moulded a 4:1 mixture of barley and wheat offals (together with ammonium nitrate at 2.3% of the dry weight), with A. niger. This effectively increased the protein content of the feed. When groups of pigs were fed with the moulded diet, weight gains were significantly greater than with unmoulded diets, and there was also a corresponding increase in feed utilisation. The reverse was found when chickens were used as the test animals, feed utilisation and egg production being 20 and 30% lower respectively with the moulded rations.

Fungal protein can be improved by the addition of the sulphur amino acids (Fink and Schlie, 1956). In experiments on rats, these workers found that a diet containing Psalliota bispora caused the rats to lose weight. On addition of cystine, the rats soon began to grow normally, whilst with methionine an initial weight gain eventually gave way to a weight loss. However, when cystine and methionine were added together, the rats not only grew well but also showed less incidence of liver necrosis. Why this should be so is not clear since cystine is not normally considered to be a dietary essential for rats, except when methionine is absent.

Obviously much more work needs to be done on fungal feeds before they can become commonplace in animal nutrition.

1.5 Bacteria

Bacterial cells contain large quantities of protein, up to 87% in the case of Lactobacillus fermentans (Ogur, 1965). They can be grown on a wide variety of substrates, and because of this it is difficult to obtain a pure culture for feeding experiments. Bacteria which could prove to be useful in feedstuffs due to their relative ease of culture are the thermophilic types, such as Bacillus stereothermophilus. This bacterium has an optimum growth rate at temperatures between 55 and 60°C, thus

helping to maintain asepsis and to cut down the cost of cooling the growth medium. It also has a reasonably good amino acid profile, its proteins being high in lysine (6.9 gms/100 gms amino acids) though in keeping with most bacterial species they are low in both methionine and cystine (2.7 and 0.5 gms/100 gms amino acids respectively). The amino acid composition, especially that of Gram +ve types, can be affected by amino acids in the growth medium, though with E. coli and Aerobacter aerogenes varying the growth conditions does seem to have this effect (Freeland and Gale, 1947).

That bacterial proteins are deficient in certain amino acids has been shown by Tannenbaum and Miller (1967). In their experiment on rats, all the diets contained 10% protein furnished solely by either whole or broken cells of B. megaterium, or by casein supplement with 2.5% methionine. Unfortunately, the diets were not isocalorific which may have had a detrimental effect on the results. Whilst whole cells had very little nutritive value, homogenisation did significantly increase the protein digestibility, BV and NPU. In fact, it was the increase in digestibility which was the real factor affecting all the other values. Overall, with broken cells 67% of the amino acids were biologically available.

There are very few experiments similar to the one above where the test protein is used as the sole source of protein in the diet. Most workers use complex compounded feeds which unfortunately serve to obscure the actual nutritive value of the microbial protein.

1.6 Novel Proteins in Aquaculture

Experiments on many fish species of commercial interest have demonstrated that in all cases the requirement for dietary protein is considerably higher than that of mammals, (Delong et al., 1958; Nail, 1962; Cowey et al., 1970; Ogino and Saito, 1970; Nose and Arai, 1973; Dabrowski, 1977). At present, most of the protein in commercial rations is derived from fish meal but in recent years the supply and therefore costs of this ingredient has been somewhat labile, thus stimulating interest in various novel proteins as a replacement for this component. Superficially most novel protein sources (and here we can include soyabean and plant protein concentrates), would appear to be very suitable for this purpose since their high protein content allows them to be included at high levels, without causing a decrease in the overall protein content of the diet.

Of the Single Cell Proteins, petroyeasts have so far attracted most attention as a protein source for

fish. Results indicate that they have a high digestibility, of the order of 86.4 - 94.1% (Ogino and Chen, 1973b; Cowey et al, 1974), a fact which has been linked to breakdown of yeast chitin by gut microflora (Minami et al., 1972).

There have now been several studies on the effect on growth of incorporating yeasts into fish diets, though the results presented do appear to be slightly variable. In general it has been shown that diets formulated with yeast as the sole protein source, even when fortified with methionine tend to reduce both weight gain and feed efficiency (Beck et al., 1977) though in a later review (Tiews et al., 1978) they suggested that the feed efficiency value of such a diet was slightly better than a mixed protein control ration; their paper (Beck et al., 1978) gives a PPV (apparent NPU) for this yeast diet of 34.4% compared to 29.8% for the control ration. In plaice, Pleuronectes platessa, a petroleum yeast (+ methionine) diet did not compare particularly favourably with fishmeal based diets, growth being slower with an NPU value of 0.38 compared to 0.42 for freeze dried cod muscle (Cowey et al, 1974). In rainbow trout (Salmo gairdneri), Matty and Smith (1978) have shown that food conversion efficiency is slightly, but not significantly, reduced with a petroyeast diet when compared to a commercial ration, though in fact growth was slightly better due to increased feed intake. Vallet et al.

(1970) report that with mullet (Mugil sp.) conversion ratio was unaffected by using yeast as opposed to albumen or mixed protein diets, though the protein contents of the diets were rather low (25%). In comparison, it has been shown by Nose (1974) that unsupplemented petroleum yeast gave a growth rate of only two-thirds of that found with a white fish meal diet (1.18%/day compared to 1.81%/day). Interestingly, they also found that supplementation with methionine had little effect on growth whilst cystine greatly improved it, but still not to the level achieved with the control ration. When petroleum yeast is used as the sole protein source addition of calcium to the diet seems to be an effective way of increasing growth rate (Arai et al., 1975), indicating that factors other than amino acid composition have to be taken into account in SCP trials.

Most of the work on yeasts has been confined to evaluating them as a replacement for other dietary ingredients, rather than their use as a sole protein source. Cowey et al., (1971) in their work on plaice, have shown that both growth and PER are lower for diets containing 23.5% cod meal and 33.5% petroleum protein (Toprina) than for those with 47% cod meal as the sole protein source. However, Hoshiai (1972) in his review of Japanese work concludes that for most species, up to 45% of the diet can consist of petroyeast, though in this case the yeast used was from a different source, and

also the fishmeal was probably somewhat inferior to the very high quality cod meal used by Cowey et al. This highlights the fundamental problem of such replacement type work, since it is difficult to compare the values given by different researchers due to this variability in basal diets, this being on top of all the other normal variables encountered in fish nutritional work. Despite this, there appears to be some consensus that, at least for rainbow trout, around 30 - 40% of the diet can consist of yeast material. Andrueto et al., (1973) report that petroleum yeast can replace up to 30% casein-gelatin mix without detrimentally influencing food conversion. Added to this, Shimma and Nakada (1974) have shown that a 38% fishmeal:30% petroyeast diet can give a growth and feed efficiency similar to a commercial ration which a later, longer term, experiment (Shimma et al., 1976) confirmed. At slightly lower levels of dietary yeast (24%), Shimma and Nakada (1975) were able to demonstrate that feed efficiency was in fact better than with the control ration whilst growth was unaffected. More recent work by Beck et al. (1977), again with rainbow trout, confirms the usefulness of yeasts as a dietary ingredient since it could be used as a complete replacement for fishmeal in a simple compounded ration (basal diet 35% fishmeal and 35% of a mixture of poultry by-product meal and feather meal).

In results obtained with salmon (Oncorhynchus kisutch) diets Spinelli et al. (1978) showed that 25% of fishmeal in OMP rations could be replaced by yeasts whilst Bergstrom (1978) has demonstrated that a mixture of 36% fishmeal and 36% Pekilo protein (yeast grown on a paper processing waste) can give similar growth to the fishmeal controls. However in her trials, another yeast, at the same level (Symba yeast) which gave good growth initially, did not do so during the second year of feeding, perhaps indicating that SCP feeding trials should if possible be continued somewhat longer than is normal at present, as in fact Shimma et al. (1976) have done, though the many limitations encountered on the setting up of such long term work mean that for the present short term work will probably be the main source of information.

Other species for which compounded diets incorporating yeasts have been used include sea-bass (Alliot et al., 1978), and shrimps and prawns (Forster, 1976; Deshimaru and Shigueno, 1972). In all these experiments around 15 - 20% yeast gave good results.

One specific area in which certain yeasts are proving exceptionally useful is in the replacement of live food for certain larval fishes. Candida utilis has been used by Applebaum and Dor (1978) as a complete replacement for artemia in the early rearing of mirror

carp fry, with no decrease in growth rate. Similar work with carp has been performed by Sharma and Kulshreshtha (1974) and Chackrabarty and Kar (1976), though not with marked success.

Compared to yeasts, bacterial proteins have been little studied in fish diets mainly because they have not been available in large quantities until fairly recently. Some early work has been carried out on various sludge diets, which are usually a mixture of various SCP's and other materials, eg. Brewers single cell protein BSCP (Windell et al., 1974) which could be included at 10 - 15% dietary level without a decrease in the growth rate of rainbow trout. More recently, Tacon (1978) has shown that sewage sludge can be included at fairly high levels again in trout rations, whilst Orme and Lemm (1973) found a decrease in growth rate and increase in feed conversion when sludge from paper processing wastes was used to replace fishmeal in trout rations. However, such materials are by their very nature rather variable in quality, though this does not appear to limit their usefulness in commercial rations.

Recently, there has been much interest in a methanophilic bacterium (Methylophilus methylotropus) produced by Imperial Chemical Industries Ltd., (marketed as "Pruteen"). This material, when used as the sole protein source in trout rations does not seem to perform as well as some other SCP's, especially petroyeast. In

one trial (Matty and Smith, 1978), the highest PER value obtained for bacterial protein was only 0.88 compared to 2.12 for BP petroyeast. Beck et al. (1978), on the other hand, did not find such large differences between the control rations and a diet where bacterial protein supplied all the protein, though when they repeated the trial in fresh, rather than brackish water, the growth obtained with this diet was only 59% of that obtained with the control and this could not be ameliorated by amino acid supplementation. They conclude that with their rations the bacterial protein could be used to provide up to 50% of the total dietary protein without adversely affecting the growth rate. Experiments with salmon indicate that somewhat smaller quantities of bacterial SCP can be tolerated. Spinelli et al. (1978) replaced various amounts of the fishmeal in a OMP type diet and concluded that 25% could be replaced without affecting growth and food conversion, whilst 50% replacement decreased growth to 80% and food conversion to 87% of that achieved with the control ration. Total substitution of fishmeal reduced growth to one half the control level. Bergstrom (1978) used a 24% Pruteen and 48% fishmeal diet in Atlantic salmon, with good results, though during the second year of feeding some gut abnormalities appeared though there is no real evidence to link this directly with the SCP as other variables may have been involved.

Like bacteria, algae have not frequently been used as a protein source in fish diets. This can again be correlated with their low availability though there now seem to be efforts to produce Spirulina maxima in some quantity. There have been various trials in which algae have been fed in their natural state to various species (Ahmad, 1966, 1967; Gupta and Ahmad, 1966; Stanley and Jones, 1976) though this really falls outside the scope of this treatise, as does the use of algae in feeding natural filter feeders such as molluscs (eg. Masson, 1977). The reports published on the use of algae in compounded feeds are not particularly definitive. Meske (1976) found formulated fish meal free diets for carp, based on whey with added Scendesmus sp. alga gave a growth response similar to that obtained with a commercial ration though the diets did not appear to be particularly well defined. In a later paper (Reimers and Meske, 1977), the effect of algal supplementation on body composition was evaluated with the conclusion that overall, this effect was beneficial in increasing the proportion of edible to total fish weight. Again, with carp Hepher et al. (1978) have shown that several algae are less well digested than fishmeal, apparent digestibility for Spirulina sp. being 90% and Scendesmus 81%, but he concludes from his experiments that algae are a rather better quality protein source than soyabean meal. Matty and Smith (1978) found food conversion of Spirulina diets was good but growth and food conversion efficiency (FCE) was only 38% and 48% of the commercial control ration.

As noted previously, in the context of aquaculture, and especially the culture of carnivorous and omnivorous species, vegetable proteins (for example, soyabean) can be considered as novel protein sources especially when they are used at relatively high levels. Quite variable results have been achieved in nutritional work on soya bean, probably for the most part attributable to the method of preparation of the soyabean meal which greatly affects the level of trypsin inhibitor present. Sandholm et al. (1976) have shown that in trout the digestibility of soyabean protein increases from 45 - 80% when the activity of this inhibitor is decreased from 57 - 6% of its normal value, with much the same effect being observed in carp trials (Shcherbina, 1971). Other values given for the digestibility of soyabean protein by rainbow trout range from 70% (Kitamikado et al., 1964a) to 80.9% (Inaba et al., 1962), whilst Ogino and Chen (1973b) reported a value as high as 95.5%, only slightly lower than that of casein. In plaice, the digestibility appears to be about 68% (Cowey et al., 1974) similar to the figure given by Kitamikado et al.; in general it can be concluded that the digestibility of soyabean protein is somewhat lower than that of casein or fishmeal.

The low digestibility factor will almost certainly affect the results of trials where soyabean is used as the sole protein source, though it is of course only one

of the many influences on the utilisation of this material. Catfish do not grow well on soyabean diets (Dupree and Sneed, 1966; Krishnandi and Shell, 1967; Andrews and Page, 1974) with both growth and food conversions being adversely affected in comparison to casein or fishmeal diets. Supplementation of the diet with free amino acids seems to have little effect and it has been postulated that there is some growth factor in the proteinaceous portion of fishmeal that is not present in soyabean (Andrews and Page, 1974; Viola, 1975) though this may not be the only, or true, explanation of its poor value. The results found with catfish are reflected in other experiments, with both Nose (1971) and Reichle and Wunder (1974), reporting that trout were unable to grow when fed soyabean diets, Nose ascribing a Biological Value of only 25% to this material compared to 50.8% for casein. This is even lower than the BV of 34% given by Cowey et al. (1974) for soyabean in plaice diets, though whether this is a species or ingredient effect is difficult to determine.

As stated previously, amino acid supplementation of soyabean protein when it is the sole protein source appears to be ineffective, but it has been demonstrated that food conversion in trout can be greatly improved by supplementation with minerals (Ketola, 1975) though the best value obtained (2.61) is still rather poor.

Since soyabean is unattractive as a sole protein source, various workers have looked at its value as a replacement for various other dietary proteins, especially fishmeal. Again, rather variable results have been obtained. With plaice, Cowey et al. (1971) have reported that when soyabean supplies 45% of the total dietary protein (the diet contained 36% soya and 24% low temperature cod meal) weight gain and PER were significantly lower than controls, whilst with similar diets (38.8% soya and 31.6% herring meal) Alliot et al. (1978) were unable to show a detrimental effect on either the growth or the food conversion of sea bass (Dicentrarchus labrax) compared to their controls. In catfish, the work of Andrews and Page (1974) supports the results obtained with plaice in that replacement of fishmeal with soyabean meal decreased growth and food conversion. On the other hand, the sea bass results are similar to earlier work published by Cho et al. (1974) where fish meal could be decreased from 35% to 18% and soya increased from 10 to 39% of the diet without influencing growth or food conversion, though in this experiment these two ingredients were not the only dietary protein sources. However, other work (Dabrowska and Wojno, 1977) has also shown that soyabean can effectively replace fishmeal though here supplementation with cystine and tryptophan was necessary to prevent decreased protein utilisation.

The concensus appears to be that in general only partial replacement of fishmeal is possible. Tiews et al.

(1976) indicate that only 25% replacement of fishmeal is advisable for rainbow trout which is confirmed by Spinelli *et al.* (1978) where 50% replacement decreased food conversion and growth, and Higgs *et al.* (1978) only recommend about 15% soyabean meal in compounded rations for coho salmon. Interestingly, the omnivorous carp, *Cyprinus carpio*, like catfish, does not utilise soyabean particularly well (Viola, 1975).

The results reviewed here demonstrate that the situation regarding the utilisation of single-celled and other novel proteins is rather confused. Differences in the composition of compounded rations mask the effects of the substituted proteins, though of course such trials are useful in their extrapolation into possible commercial application. However, ideally more studies with semi-purified rations are necessary, to provide basic data on the true value of each protein source. With such information, it would be easier to plan later experiments where, depending on their intrinsic value, only appropriate quantities of test protein would be included into compounded rations.

As described, some semi-purified diet trials have already been performed, though there has been a lack of consistency in the description of protein performance. Hence, in this thesis, an attempt will be made to examine the basic parameters of novel protein utilisation.

It is proposed that such work will include measurement of the various indices of protein utilisation, the effect of protein intake on some of these indices, and also the effect of the protein source on diet palatability and intake.

The novel protein ingredients to be tested include a hydrocarbon-substrate yeast (Toprina), a methanophilic bacterium ("Pruteen"), an algal protein (Spirulina maxima), extracted soyabean protein ("Newprod"), brewers yeast ("Yeast Blende"), and a fungal protein (Fusarium sp.). The suppliers of each of these ingredients are given in Appendix IV. Fishmeal and/or casein were used as controls.

CHAPTER 2

Nitrogen Balance Experiments

2.1 General Introduction

In the general introduction, several terms were used in order to describe the nutritive value of a protein, but before continuing it is perhaps fruitful to consider these terms in a little more detail.

Essentially the nutritive value of a test protein can be determined in three main ways, that is by chemical, microbiological and biological methods. The chemical method involves an analysis of the protein for its constituent amino acids and comparing the levels found with those of a reference protein such as whole egg protein, FAO reference protein, or the amino acid requirements of the test animal. The disadvantages of this method are that it ignores amino acid availability, imbalance, toxicity or antagonism. Therefore, it is only normally used as a comparison with results achieved by other means.

Microbiological methods are rarely used but they are based on the fact that some microorganisms have amino acid requirements similar to those of higher animals. Unfortunately, some pretreatment of the protein is often necessary, so results can not be used to predict the protein value for higher organisms with any real confidence. However, the method has found great use in predicting the availability of certain specific amino acids such as methionine and lysine.

The biological testing procedure encompasses a large number of individual methods which can be subdivided into two main groups. The first of these involves measuring the weight gain of test animals and then linking this to the quantity of protein ingested to give values such as Protein Efficiency Ratio (PER), Gross Protein Value (GPV) and Net Protein Retention (NPR). The disadvantages of these growth methods are well known (McDonald et al., 1973), the main ones being that weight gain is not necessarily directly related to protein retention, and neither is the protein used for maintenance estimated. Thus it is common, at least in mammalian experiments to determine protein value by nitrogen balance studies, which involves measuring the quantity of nitrogen retained and comparing it with the nitrogen intake. The proportion of nitrogen retained can be measured either indirectly by estimating the nitrogen lost (Rippon, 1959; Henry and Toothill, 1962) or by measuring the increase in body nitrogen of a group of animals (Bender and Miller, 1953).

Hence for NPU, we have several formulae as reviewed by Pellet (1973):

$$NPU = \frac{N_r}{N_i} = \frac{N_i - (N_f + N_u)}{N_i} \qquad (1)$$

$$\text{or} = \frac{B - B_o}{N_i} \qquad (2)$$

where N_r = nitrogen retained

N_i = nitrogen intake

N_f = faecal nitrogen

N_u = urinary nitrogen

B = body N at end of test period

B_o = body N at zero time

Although simple, the above equations only give the apparent NPU, this being the proportion of ingested nitrogen retained. This is adequate normally but it is not a true representation of the value of the protein since some of the ingested nitrogen is used to replace endogenous losses and thus is in fact utilised. Hence, these endogenous losses should be subtracted from the other nitrogen excretions. Equations (1) and (2) then become:

$$\text{True NPU} = \frac{N_i - (N_f - N_m) - (N_u - N_e)}{N_i} \quad (3)$$

$$\text{or} = \frac{B - B_k}{N_i} \quad (4)$$

where N_m = metabolic faecal nitrogen

N_e = endogenous nitrogen excretion

B_k = body N at end of trial of non-protein fed group.

In fish trials, it is the carcass analysis method which is most commonly used to determine the value for NPU (Nose, 1962; Cowey et al., 1972; Zeitoun et al., 1974; Dabrowski, 1977) as it can easily be combined with growth studies and requires little extra analysis. However, there are some disadvantages. Originally, it proved difficult to persuade fish to voluntarily accept a non-protein diet, though this restriction now seems to have been overcome (Ogino and Chen, 1973a; Wang, 1974; Nose, 1967). Added to this, endogenous nitrogen excretions are a function of the metabolic size so, as the body weight of the non-protein fed group decreases, they lose a smaller quantity of this nitrogen than the growing test group. Again, this error appears to have been overcome to a certain extent by Dabrowski (1977). Rather than using a non-protein diet, it is possible to

feed a low protein diet and correct for this by adjusting the NPU equation to account for this (Bender and Miller, 1953):

$$NPU = \frac{B - B_k + N_k}{N_i} \qquad (5)$$

where N_k = the nitrogen intake of the low protein group

B_k = final body N of the low protein group fish

This formula, however, is designed to account for the very low levels of nitrogen in a non-protein diet, not for the feeding of a low protein diet. With (5), it has to be assumed that the small quantity of protein in the diet is completely digested and that all the absorbed protein is utilised to replace endogenous excretions (ENE), ie. it has an NPU of 100. Whilst previous workers have shown that NPU does increase as protein level decreases, it seems unlikely that 100% utilisation is ever achieved, especially as digestibility does not appear to increase with reduced protein consumption (Nose, 1967; Ogino and Chen, 1973b). These criticisms could be obviated by finding the endogenous losses by another method as shall be described later. however, body analysis experiments have to be performed over a long period and also depend on the analysis of large numbers of fish.

Alternatively, the classical nitrogen balance method holds many pitfalls as collection of metabolic excretions of fish is difficult. The method was designed for use with terrestial animals where such excretions can easily be collected separately in a metabolism cage, whereas fish, of course, excrete into their immediate environment, and excretion from various sources become intermixed. Soluble faecal nitrogen leeches into the water, and also fish excrete around 80% of their waste metabolic nitrogen into the water via the gills as ammonia rather than as urea from the kidney. In order to study the metabolizable energy of a diet, it is important to separate all these excretions, and it has proved possible by using single fish in a divided chamber (Post et al., 1965), though bacterial contamination and stress to the fish are disadvantages. For nitrogen balance determinations, this divided chamber is unnecessary as can be seen by inspection of equation (1), which shows that NPU is described as Nitrogen retained ÷ nitrogen intake. In order to find N retained, the total amount of nitrogen lost to the water has to be measured and subtracted from the intake. Hence it is of no consequence to the NPU determination whether this nitrogen is lost from the gills, kidney or faeces thus enabling them to be collected as if they were a single excretion,

$$\text{ie., app NPU} = \frac{\text{N intake - total N lost from all sources}}{\text{N intake}} \qquad (6)$$

Of course, this still leaves the problem of ascribing a value to the endogenous nitrogen excretions so that a true NPU value can be determined.

$$\text{True NPU} = \frac{\text{N intake - total N lost all sources + ENE}}{\text{N intake}} \qquad (7)$$

The only alternative to feeding a non-protein or low protein diet is to feed graded levels of protein, measure the nitrogen retention at each level and then extrapolate back to find the retention at zero protein intake, a method which has been found to be reliable at least for metabolic faecal nitrogen determination in various mammalian species (Mitchell and Bert, 1954). Ogino and Chen, (1973a) have used this method for MFN determination with fish diets, and Gerking (1955b) has calculated the maintenance requirements of bluegill sunfish in a similar fashion. Furthermore, examination of a later paper (Gerking, 1971) also allows calculation of values for total endogenous nitrogen excretions (ENE).

Total ENE determined in this way can be used directly in formula (7) without the need to determine separate values for either EUN or MFN. However, as MFN varies with the amount and quantity of the diet,

whilst EUN is independent of the food used, it seemed wise for the following experiments where various feed intakes will be used, to ascribe distinct values for MFN. It is then possible by subtracting this from a total ENE determination to obtain a value for EUN. Thus the total endogenous losses can be calculated for fish of a similar size under similar experimental conditions and at various feeding levels.

2.2 Experiment I - Determination of Metabolic Faecal Nitrogen

2.2.1 Introduction

Whilst the metabolic faecal nitrogen excretion of trout has already been determined (Nose, 1967), it seemed advisable to repeat this work for a number of reasons. Firstly, to ensure that the value was applicable to the size and stock of fish and to the temperature regime to be used throughout subsequent nitrogen balance determinations. Secondly, and probably more importantly, since MFN is related to the level of dietary fibre which affects the rate of passage of food through the gut, its value had to be determined with diets of a similar quality to those used in later experiments.

2.2.2 Materials and Methods

4 x 40 l tanks of RS1 (See Appendix X) were used in this experiment, each one being stocked with a batch weighed group of 20 rainbow trout (Salmo gairdnerii Rich) of approximately 40g average weight, to give a total known weight of fish in each tank. The batch weighing procedure consisted of netting each group of fish from a holding tank, allowing the water to drain from them for 20 seconds before placing them in a tared bucket of water on a balance of ± 1g accuracy. Previously,

repeated weighings of a single group of fish had shown that this method of weighing gave reproducible results. The lighting regime was 12 hL/12 hD, and the temperature maintained at 12 ± 0.6°C.

The diets were based on a fairly simple formulation, a mixture of casein and gelatin providing the protein source. Both these ingredients were assumed to be pure protein which later analysis proved to be incorrect (see Appendix I). However, an attempt was made to equilibriate the digestible energy levels of each diet, using values of 21.3 kJ/g for protein, 33.6 kJ/g for lipid, 6.863 kJ/g for starch and 15.4 kJ/g for dextrin (this assumes 17.158 kJ/g for carbohydrates and starch and dextrin 40 and 80% digestible respectively). The level of cellulose (fibre) was maintained at 10% though the 1% binder included would have had the effect of increasing total fibre to 11%. The diets were pelletised and dried as described in Appendix VII.

The diets were fed for 14 days at a rate of 1% of the total weight of fish per day (dry diet/whole fish), supplied as two equal portions morning and evening. Each diet was fed to one tank of fish.

TABLE 3 - Diet Formulations for MFN Trial

Diet	CG 10	CG 20	CG 30	CG 40
Ingredient:				
Casein	7	14	21	28
Gelatin	3	6	9	12
Cod liver oil	26	19.7	13.3	7
Potato starch	47	39.6	32.3	25
Dextrin	-	3.7	7.4	11
α Cellulose	10	10	10	10
Mineral mix (a)	4	4	4	4
Vitamin mix (b)	1	1	1	1
Chromide oxide	1	1	1	1
Binder (c)	1	1	1	1
Total	100	100	100	100
Digestible kJ/g	14.06	14.06	14.06	14.06
mg Kjeldahl N/g	15.3	29.5	43.9	56.0

(a) See Appendix V

(b) See Appendix VI

(c) Sodium carboxymethyl cellulose (high viscosity).

At the end of 14 days, the fish were removed from the tanks, anaesthetised in 50 ppm. MS 222 (Sandoz) and the faeces stripped from the final portion of the alimentary tract of each fish, onto a circle of Whatman No. 1 filter paper. Great care was taken not to contaminate the

faecal samples with urine, which could easily be prevented by gently squeezing the urine out of the fish prior to stripping the faeces. Only a small quantity of faeces was removed from each fish as it has been shown that removal of faecal samples which include material from higher up the gut can affect the results of digestibility trials (Austreng, 1978a).

Samples from each tank were pooled in order to be able to obtain sufficient quantities for analysis. After drying at 105°C for 24 hours, both the diet and the faeces were analysed for total nitrogen by the micro-Kjeldahl method (Horowitz, 1970) and for chromic oxide marker by the wet ashing method of Furukawa and Tsukahara (1966). A standard curve had previously been determined for chromic oxide using samples of known Cr_2O_3 content.

2.2.3 Results

The fish fed well throughout the experiment, and no mortalities occurred.

(a) Metabolic Faecal Nitrogen

Faecal nitrogen was calculated in relation to food intake using the formula below:

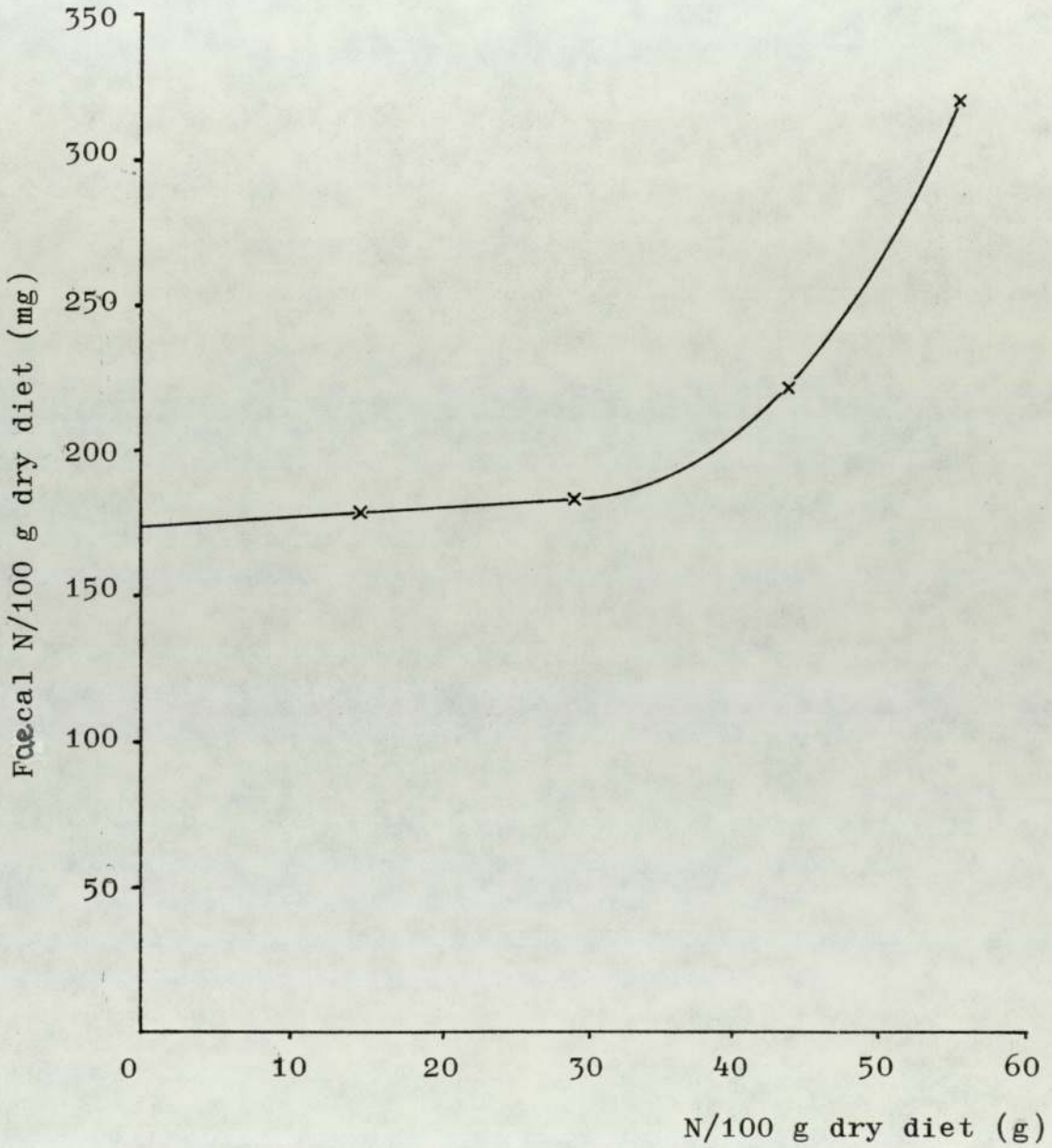
$$\text{mg Faecal N/100g dry diet} = \text{mg N/g Faeces} \times \frac{\text{mg } Cr_2O_3 \text{ diet}}{\text{mg } Cr_2O_3 \text{ faeces}} \times 100$$

The results are plotted against nitrogen intake in Fig. 1. A curve was fitted to the points by eye, it being assumed that the relationship was linear at low nitrogen intake. By back extrapolation of this straight line, the nitrogen lost in the faeces at zero N intake was found to be 175 mg/100 g dry diet consumed.

(b) Digestibility

Using the above data, it was possible to calculate the apparent and true digestibilities of the casein/gelatin mix (Table 4).

Fig.1. Estimation of metabolic faecal nitrogen



Apparent digestibility =

$$\frac{\text{mg N/100g dry diet} - \text{mg faecal N/100g dry diet}}{\text{mg N/100g dry diet}} \times 100$$

True digestibility =

$$\frac{\text{mg N/100g dry diet} - \text{mg faecal N/100g dry diet consumed} + \text{MFN}}{\text{mg N/100g dry diet}} \times 100$$

TABLE 4 - Digestibility of casein/gelatin protein

N intake (mg/100g fish)	Apparent Total N Digestibility (%)	True Total N Digestibility (%)
15.3	88.1	99.6
29.5	93.8	99.7
43.9	95.0	99.0
56.0	94.2	97.4

2.2.4 Discussion

The value found for metabolic faecal nitrogen is slightly higher than that determined for trout by Nose (1967) whose values ranged from 50 - 150 mg N/100g dry diet consumed. Whilst this may be due to experimental error it could also be attributed to the level of α-cellulose (10%) in the present diets being higher that used in his formulations. For carp, Ogino and Chen (1973a) have ascribed MFN values of 144 ± 27.7 and 180 ± 29.4 mg N/100g diet at 20°C and 27°C respectively.

Whilst recalculation of the data of Wang (1974) gives the much lower values of 66.6, 86.58 and 103.23 mg N/100g diet consumed at 16-20, 21-25 and 26-30°C respectively. In both these experiments, only 55% α-cellulose was included, thus overall it does appear possible that 175 mg N/100g diet is a true value when considered in relation to the fibre level of the test diets.

The digestibility values are of interest in that they demonstrated that true digestibility was inversely related to nitrogen intake, which differs from the results of Ogino and Chen (1973b), who found no decrease in the digestibility with increasing protein intake, confirming earlier work on bluegill sunfish by Gerking (1955b). Kitamikado et al. (1964b) have shown that at constant protein levels, protein digestibility falls with increasing quantities of dietary starch, but in this experiment the starch level was lowest when the protein level was maximal, so starch could not have been the influencing factor. The quantity of lipid in the diet decreased with increasing protein but Kitamikado et al. showed that this ingredient had no effect on digestibility. It has to be concluded then that true protein digestibility, at least of a casein/gelatin mixture, is inversely related to dietary protein level; but whether it is a significant effect cannot be deduced from this experiment.

2.3 Experiment II - Determination of Total Endogenous Nitrogen Excretion

2.3.1 Introduction

As described in the introduction to this section, it is possible to determine the total endogenous nitrogen excretion (EUN + MFN) by two main methods, either by feeding a non-protein diet or by back extrapolation of the nitrogen losses of fish fed increasing levels of protein. ENE evaluation by force feeding has been performed previously by Gerking (1955b) and Savitz (1969), and more recently by ad libitum feeding of non-protein diets (Nose, 1967; Wang, 1974). However, consumption of protein free diets appears to be quite variable, and thus ENE can easily be affected if the fish refuse to consume sufficient ration to cover their basic energy requirements. The results obtained for ENE reported in the literature vary quite considerably and so, as for MFN, it was decided to evaluate this parameter of nitrogen metabolism with the stock of fish available in the laboratory and at the temperature to be employed in further N balance experiments.

2.3.2 Materials and Methods

The tank system used was similar to that described by Atherton and Aitken (1970) and Smith (1976) but again slightly modified. It consisted of a total 16 x 10 litre conical shaped tanks (Appendix VIII) made from inverted acid bottles with the bottom removed, and covered in plastic mesh to prevent the fish escaping. These were fitted into two water baths set up in a light proof insulated cabinet in which the photoperiod was maintained at 12 hours light/12 hours dark. For each tank, the inflow supply pipe consisted of 5 mm plastic tubing connected to a 1.5 cm common supply line lying inside the water bath. Outflow water passed from the bottom of the tank via a faecal trap (made from a 250 ml glass bottle) and up through a swing arm device which regulated the tank water volume. A further length of plastic pipe containing an air lift pump connected the faecal trap to a second tank inflow point. The contents of the faecal trap could be drained off through a simple tap constructed from used plastic syringes. Water issuing from the swing arm was collected in plastic guttering and delivered to a biological filter, this being a plywood tank 1m x 1m x 0.6m deep, fitted with a false bottom of perforated corrugated plastic covered with 0.3m of 1 cm diameter gravel. A layer of crushed oyster shells was deposited on top of the gravel in order to buffer the normal drop in pH noted in recycled water (Spotte, 1970). Make-up water (0.3 l/min) was added to

the filter and excess system water passed to waste through an overflow point 10 cm above the level of the gravel, so the filter material remained submerged at all times. This overlying water was strongly aerated to ensure that oxygen was not limiting during filtration. Since the nitrogen load on the filter was very low, little oxygen was consumed by the nitrifying bacteria enabling the water to be pumped directly from the bottom of the filter to the fish tanks without the need for intermediate aeration. The oxygen content of this water was measured regularly and found to be above 90% saturation at all times. Other water quality parameters (pH, NO_2, NO_3, NH_4^+) were also regularly monitored, and found to be within the tolerable limits for rainbow trout at all times. Each tank was initially stocked with 5 rainbow trout of 40g average weight. These were fed with a commercial diet (BP Nutrition Limited) at 1% body weight per day (an amount they would consistently consume) for 14 days in order to acclimate them to the system. Mortalities during the first few days were rather high, as the fish did not appear to adapt well to the conical shaped tanks, tending to 'burrow' head first into the tank outlet. This was eventually overcome by fitting a plastic insert over the outlet so that the tanks effectively had a flat bottom. This reduced first stocking mortalities dramatically when a new batch of fish were restocked. To cover for random mortalities, the replacement fish were stocked at a rate of 7/tank

which was evened out to 5/tank after the 14 days of acclimation, at which time they were weighed.

The ingredients for the test diets were mixed to the formula shown in Table 5, pelletised (Appendix VII) and analysed for moisture by drying at 105°C for 24 hours for the total nitrogen by the micro-Kjeldahl method (Horowitz, 1970). These diets were then fed to replicate tanks of fish at a rate of 1% of their body weight/day (dry food/whole wet fish) divided into two equal portions given at 10am and 6pm. Great care was taken to ensure that all pellets were consumed by offering them very slowly, and as the feeding rate was reasonably low for trout at 12°C the food was consumed very readily with no visible waste. Added to this, the diet was consumed almost instantaneously, there being little chance for any leeching of the nutrients to take place, especially as the pellets proved to be particularly hard. At the end of 7 days, the fish were reweighed and the amount of food corrected to 1% body weight.

After a further 4 days, the balance trial could be started. At 0800 hrs., the pipework of each tank system was thoroughly cleaned out with a long handled test tube brush in order to remove any fungus or faeces attached to it. The system was then run normally for 2 hours to allow the fish to recover from any disturbance they may have been caused by the cleaning procedure. The

fish were then fed the first half of the daily ration which was readily consumed. The tank inflow was removed and placed in the surrounding water bath so that the temperature of the tank water remained within its normal limits. The water which collected in the surrounding bath returned to the filter via the bath overflow pipe. As there is always a small head difference between tank water level and outlet water level in any system where water is flowing into a tank, 1 minute was allowed for excess water to flow out of the tanks before the air lift pumps, connecting each faecal trap to its respective tank, were turned on; each one adjusted to give an inflow rate of 0.8 l/min. The start time for each tank was staggered by 10 minutes so that sufficient time was allowed at the end of the experiment for final water and faecal samples to be taken. At the same time as the air lift pump was started, a 200 ml sample of water (initial sample) was removed from each tank for total nitrogen analysis (see Appendix IX). This was necessary as the normal recycled water contained background levels of various nitrogenous compounds from bacterial degradation of ammonia, as well as nitrogen excreted directly by the fish.

Each air lift pump cycled the water through its respective tank for 24 hours, the fish being fed the second portion of their daily ration at the normal time. After 23.75 hrs., the rubber pipe between the tank and

the faecal trap was clamped off and the water drained from the faecal trap into a Buchner funnel in which a circle of dried and weighed Whatman No. 3 filter paper had previously been placed. After filtration under vacuum, the filtrate was returned to the tank, the clamp removed and the water recycled again for 15 minutes to ensure that the filtrate was thoroughly mixed with the remaining tank water. 500 ml of this mixed water (final sample) were then removed and stored in a sealed bottle for subsequent analysis of total nitrogen as before. By adding the filtrate to the tank water, there was no net nitrogen loss from the system, Since the method of nitrogen balance employed only required the total nitrogen loss to be determined, it was irrelevant to the method whether this water contained only nitrogen excreted directly into the water or whether it was also contaminated with leeched faecal nitrogen. In fact there was no need as such to filter out the faeces; they could have been homogenised with the total amount of water in the system and sub-samples taken for nitrogen analysis. However, it would have proved difficult to obtain a truly homogenous mix, and therefore a representative sample, so filtration of the faeces was deemed to be more accurate.

Following removal of the final water sample, the fish were carefully netted from the tank ensuring that as little water as possible was removed with them.

Each tank was drained off and the volume of water measured to the nearest 10 ml. The 500 ml sample was added to this figure so the total volume of water in the system during the balance trial was known. It was assumed that no water was lost by evaporation or leakage during the run.

The filtered faeces were dried for 24 hrs. at 105°C together with the filter paper and then weighed. The dried faeces were scraped from the filter paper, ground to a fine powder with a pestle and mortar and samples analysed for nitrogen by the usual micro-Kjeldahl method.

2.3.3 Results

The nitrogen lost per tank of fish was calculated from the equation:

mg N lost/day = (mg N/g dry faeces x g dry faeces) +
((mg N/l final sample - mg N/l initial sample) x vol. of tank in litres)

The nitrogen excreted over the 24 hour period was then assumed to be directly related to the nitrogen intake over the same period, though of course some nitrogen will have arisen from metabolism of protein ingested the previous day. However, as feeding times were fixed, this should have been exactly balanced out by the fish

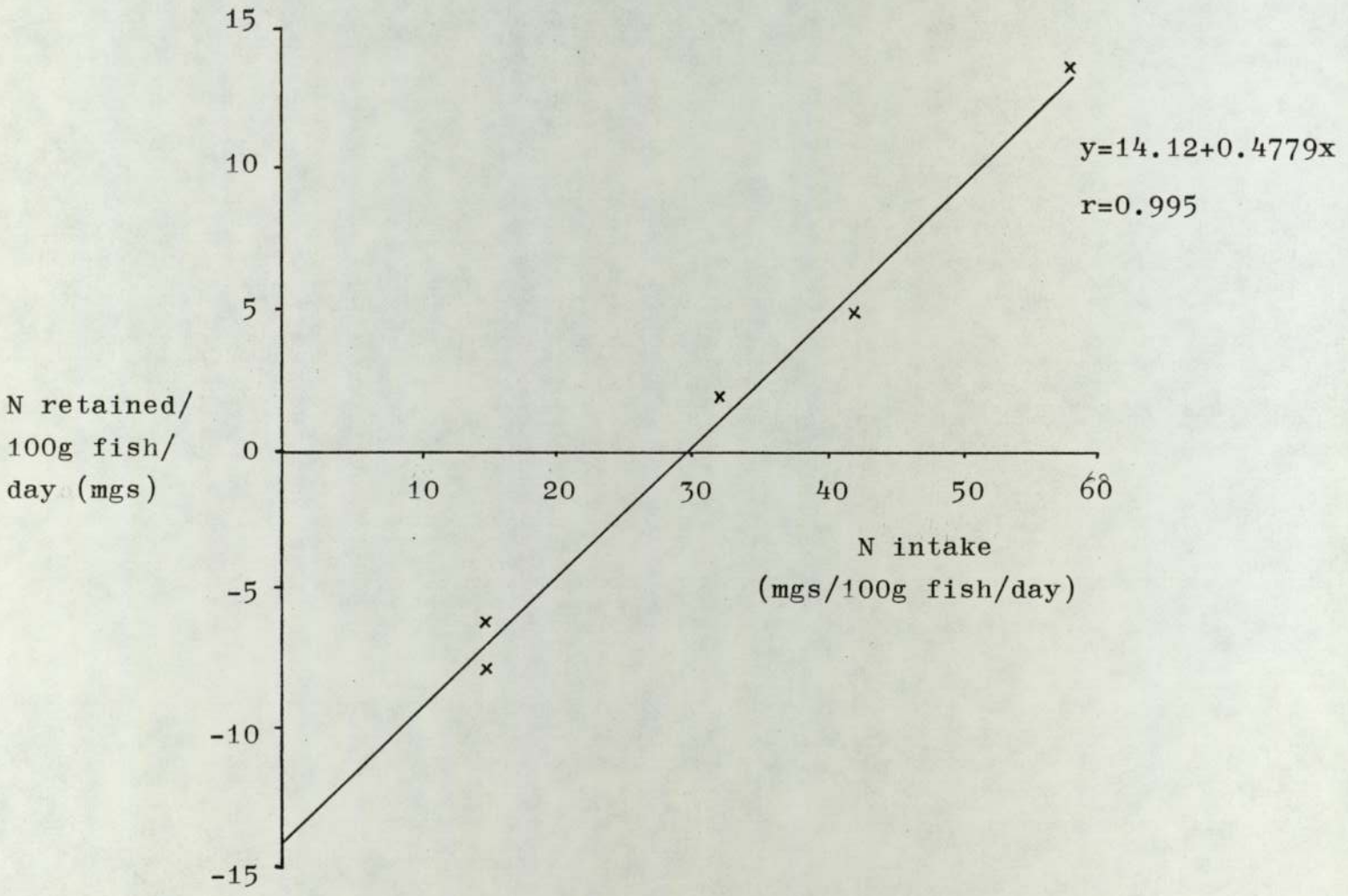
failing to excrete some of the nitrogen consumed during the experimental run until after the run had terminated. As Rychley and Marina (1977) have shown that nitrogen excretion is cyclical over a 24 hour period, this assumption must hold true and it is not important where the experiment is started in relation to the cycle as long as it ends at the same point 24 hours later, and also as long as feeding is continued at the times that gave rise to that cycle. Hence the nitrogen intake related to the nitrogen lost is merely calculated as:

mg N/g dry food fed/day x g dry food fed/day

In order to account for small differences in the total weight of fish in each tank, the N intake and N lost figures were expressed as mg N/100g fish. These were then plotted in Fig. 2. It will be noticed that only five points were obtained, this being due to the loss of three tanks of fish after an air line failure. The five points were considered to be sufficient for further analysis as they appeared to be closely linked by a straight line. Hence a linear regression was fitted to them by the method of least squares. The correlation coefficient (0.995) showed that the straight line was a good fit to the points. The intercept on the y axis is equivalent to the nitrogen loss at zero nitrogen intake, ie. the total ENE. This was found to be:

14.12 mg N/100g fish/day

Fig.2. Estimation of endogenous nitrogen excretions using casein as the protein source.



As the MFN determined in Experiment 1 had a value of 1.75 mg N/g diet fed and the fish in this experiment were fed at 1% of their body weight/day, then:

EUN = 14.12 - 1.75 mg N/100g fish/day

= 12.37 mg N/100g fish/day

2.3.4 Discussion

There is very little data available from the literature on the endogenous nitrogen excretion of rainbow trout. Nose (1962) found that trout fed a non-protein diet at 17°C lost 13.9 mg N/100g fish/day. As this was calculated from body analysis data, it is in fact a value for total ENE, which compares to the 14.12 mg N found in the present experiment. However, as the fish were much smaller and the temperature appreciably higher, the two figures should not be as closely related as they appear to be, and perhaps indicates that the present value is rather high.

Most of the other data on ENE values relates to various warm water species and it is perhaps interesting to compare the ENE of trout to these, though, of course, both species and environmental differences have to be taken into consideration. Both Gerking (1955b) and Savitz (1969) have studied the endogenous nitrogen excretion of bluegill sunfish fed a non-protein diet.

As neither of these workers appear to have included any analysis of faecal nitrogen, they were in fact measuring only the endogenous urinary excretion and not the ENE. At 23.9°C, Savitz assigned a value for EUN of 11.54 mg N/100g fish similar to Gerking's formula which provides a value of 11.24 mg N/100g body weight at a roughly equivalent temperature (25.9°C). However, in another paper on the effect of rate of feeding and body weight on protein metabolism of sunfish (Gerking, 1971), the y intercept on a graph of nitrogen consumed against nitrogen retained is equivalent to the ENE and is in effect a somewhat similar method of determining this value as that described here. For fish of 85.2g weight, this intercept gives a value for total ENE of 3.77 mg N/100g fish/day at 24.5°C which is significantly different from the value reported in his other paper above. Although the methodology was different, such a large discrepancy would not be expected.

The endogenous nitrogen excretions of carp have also been studied both by water and body analysis methods. The daily nitrogen losses of carp estimated by Creach et al. (1971) were 27.3 ± 7.6 mg N/100g body weight though the temperature is not stated. Ogino and Chen (1973a) using a protein free diet, give a value for total metabolic nitrogen excretions, ie. ENE (expressed in mg N/100g fish/day) of 10.5 at 20°C, 11.2 at 22°C, 11.9 at 24°C, 12.7 at 26°C and 13 at 27°C. Wang (1974)

has calculated both MFN and EUN. By addition of these values, the ENE (again mg N/100g fish/day) was 12.9 ± 2.4 at 16-20°C, 20.9 ± 1.4 at 21-25°C and 49.8 ± 12 at 26-30°C. Infante (1974), again using carp, measured the ammonia and Kjeldahl N excretion (probably equivalent to EUN) at 20°C and arrived at a value of 13.8 mg N/100g body weight. Thus, for warm water fish, the daily ENE values ascertained by various methods appear to be reasonably comparable at similar temperatures, the majority being in the region of 10-13 mg N/100g fish at around 20°C. Though a lower value (8.4 mg N/100g fish/day) has been given for sea bass by Guerin-Ancey (1976), this does not include any MFN so his results are comparable to those for carp and bluegill sunfish. Thus, if it is assumed that any species differences are overridden by the effect of temperature, then the value obtained here at 12°C is a little high, as was indicated by comparison with the data of Nose (1962). Dabrowski (1977) has reported very high ENE values for grass carp fry (54.9 mg N/100g fish/day), but, in view of the small size of the fish, this could be expected and cannot therefore be considered as an indication of any species differences.

Of course it is possible that the balance methodology used in the experiment was the source of the error, so it is probably worth considering what these could be. One of the major assumptions made is that the nitrogen lost during the experimental run was directly related to

the nitrogen fed over the same period. As described previously, it would appear that over a 24 hour period this should hold true, otherwise it would indicate that fish retained a greater percentage of their food on one day than on another, which is somewhat unlikely as the feeding intervals were regular and the amount of food constant at each feed. Whilst it may have been prudent to run the experiment for longer than 24 hours to negate this possibility, this could have led to other problems; such as the accumulated ammonia excretions becoming auto-toxic, or particulate matter in the water adversely affecting the gills. In fact, most of the possible errors in the methodology would have given rise to a lower figure for endogenous nitrogen excretion than that found. For instance, any loss of ammonia from the water would have given this effect but Gerking (1955b) has shown that this is not a problem at the normal pH of fish rearing systems. Added to this, there are indications that unionised ammonia in the water tends to reduce the nitrogen excretion of the fish (Fromm and Gillette, 1968). If this had been a factor in the present experiment, the overall result would have again been to reduce the estimated value of the ENE. However, unionised ammonia levels in the tank water would have been very low due to the low pH, the comparatively small amounts of nitrogen excreted/litre of water, and the effects of bacterial breakdown, and also the short exposure time. The bacterial degredation of ammonia to nitrate would not

have affected the results as the nitrogen analysis method included all forms of nitrogen in the water.

One possible reason for what may be a slightly high ENE value could be that the method used would also estimate mucus nitrogen lost from the fish, which should in essence be considered as an endogenous loss since it is nitrogen which has been utilised but which is routinely replaced. It can be allied to perspiration/ skin nitrogen losses which are sometimes included in mammalian balance studies as an added endogenous loss.

Hence, for all the above reasons, the figures found here for ENE and EUN will be used in the following experiments to calculate true NPU values.

The implications of the shape and slope of the curve in Fig. 2 will be discussed in relation to the results obtained in the following series of nitrogen balance experiments.

2.4 Experiment III - Determination of True NPU Values for Fishmeal, Petroyeast, Bacterial, Algal and Fungal Proteins

2.4.1 Introduction

Experiment II demonstrated that the determination of nitrogen balance by water and faecal analysis is feasible, thus it was decided to use this method to determine the utilisation of several different proteins by rainbow trout. The intention was to test each protein at four levels of intake, in order to study the relationship between nitrogen intake and nitrogen retention, and also to evaluate whether the relationship is similar for all the proteins to be studied. Ogino and Chen (1973c) have examined this relationship for casein, white fishmeal, dried egg yolk and corn gluten, and have concluded that utilisation decreases as protein intake increases. However, they also noted that the rate of decrease was similar for all the proteins tested, indicating that differences in protein value are evident at all comparable levels of nitrogen intake. On the other hand, Dabrowski (1977) has shown that the relationship between NPU and protein intake for casein, fed to grass carp, is curvilinear, which agrees with some of the results obtained in mammalian studies (reviewed by Pellett, 1973), though in view of the differing protein requirements of mammals and fish, great

care has to be taken in making any comparisons of this nature. In addition to the above literature, there is also some evidence that apparent protein utilisation remains constant at all normal levels of intake (Gerking, 1955a; Nail, 1962; Gerking, 1971; Savitz et al., 1977), though there are obvious errors in using apparent, rather than true, protein utilisation values. In the following experiments, both these parameters will be studied for the five different protein sources under consideration.

2.4.2 Materials and Methods

The methodology was essentially identical to that used in evaluating the endogenous nitrogen excretion. 7 x 40g fish were initially stocked in each tank of the nitrogen balance system (Appendix VIII), this number being reduced to 5/tank after 14 days. Water quality was also maintained, as closely as possible, within the limits described in Experiment II, as were the temperature and photoperiod.

The diets used are shown in Tables 5 - 9, these being isoenergetic on a calculated digestible energy basis. As the diets were semi-purified, they were a little more complex to formulate than the purified casein-gelatin diets used previously. From the proximate analysis of each protein source (Appendix I), it was

possible to calculate the quantity of dry meal that was required to give a 10, 20, 30 or 40% protein level in each diet. Using these values, the quantity of lipid, ash, and NFE provided by the meal in each diet could be calculated. The ash level was then balanced out by the addition of a mineral mix (Appendix V). Similarly, sufficient cod liver oil and soyabean oil were formulated into each diet to bring the overall lipid up to the desired percentage. Since there was no measure of the fibre levels in the proximate analysis, it was not possible to balance this factor, so a fixed amount of α-cellulose was incorporated into all the diets. Vitamin supplement (Appendix VI) and binder levels were also fixed. Summing the quantities of test meal, added lipid, mineral mix, α-cellulose, vitamin mix and binder, and subtracting these from 100, gave the percentage of carbohydrate required to be added to each diet.

Trial formulations had shown that a digestible energy level of 14.06 kJ/g was about the maximum that could be achieved in both high and low protein diets. Thus, for each diet, the calculated digestible energy from the protein (21.302 kJ/G) and lipid (33.606 kJ/g) were subtracted from the total amount of energy required. The energy supplied by the NFE of the meal was also subtracted from this figure assuming it to have a calorific value equivalent to starch (40% digestible) of 6.8634 kJ/g. Hence, both the amount of energy and the

quantity of carbohydrate that could be included to provide this energy, were known. Then, as in Experiment II, potato starch and dextrin (assumed 6.863 and 13.727 kJ/g digestible energy respectively) were mixed to give a carbohydrate mix of intermediate calorific value sufficient to complete the diet. The formula below was used to compute the required levels of each:

$$\frac{(\frac{c}{w}) - 6.863}{6.863} \times w = \text{wt of dextrin}$$

and w = wt dextrin = wt of starch

c = kJ required/100g diet

w = wt of added carbohydrate required/100g diet.

The diets were then prepared according to the procedure in Appendix VII. Following preparation, they were analysed for moisture and for total nitrogen as described previously. Each diet was then fed to two tanks of fish at a rate of 2% of their body weight/day (dry diet/whole fish). The feeding rate was increased to 2% (rather than the 1% used in the ENE evaluation experiment) so that the quantity of nitrogen ingested would be more comparable to the ad libitum intake, and also to ensure that adequate quantities of energy were consumed to cover the animals' daily requirements. It was also hoped that the larger quantities of nitrogen ingested and excreted would also reduce any possible errors in their estimation. The fish were given two equal sized feeds at 10 am. and 6 pm.

TABLE 5 - Formulation of Fishmeal Diets for Experiment III

Ingredient (Moisture free)	Protein Level %			
	10	20	30	40
Fishmeal	12.34	24.69	37.03	49.38
Cod liver oil	12.21	9.41	6.61	3.82
Soya oil	12.20	9.40	6.61	3.81
Mineral mix	8.74	7.49	6.23	4.97
α-cellulose	10.00	10.00	10.00	10.00
Binder	2.00	2.00	2.00	2.00
Vitamin mix	1.00	1.00	1.00	1.00
Potato starch	32.09	28.10	24.14	20.15
Dextrin	9.42	7.91	6.38	4.87
Total D.M.	100.00	100.00	100.00	100.00
Kjeldahl N/g (mg)	18.16	36.64	54.96	70.29

TABLE 6 - Formulation of Petroyeast Diets for Experiment III

Ingredient (moisture free)	Protein Level %			
	10	20	30	40
BP Petroyeast	17.19	34.37	51.56	68.75
Cod liver oil	12.26	9.52	6.78	4.03
Soya oil	12.26	9.51	6.77	4.03
Mineral mix	8.01	6.02	4.03	2.03
α-cellulose	10.00	10.00	10.00	10.00
Binder	2.00	2.00	2.00	2.00
Vitamin mix	1.00	1.00	1.00	1.00
Potato starch	27.85	19.72	11.48	3.31
Dextrin	9.43	7.86	6.38	4.85
Total D.M.	100.00	100.00	100.00	100.00
Kjeldahl N/g (mg)	17.88	34.72	50.64	67.62

TABLE 7 - Formulation of Bacterial Diets for Experiment III

Ingredients (moisture free)	Protein Level %			
	10	20	30	40
ICI Bacterial Protein	12.80	25.60	38.40	51.20
Cod liver oil	11.88	8.77	5.65	3.00
Soya oil	11.88	8.76	5.64	2.05
Mineral mix	8.62	7.24	5.86	4.48
α-cellulose	10.00	10.00	10.00	10.00
Binder	2.00	2.00	2.00	2.00
Vitamin mix	1.00	1.00	1.00	1.00
Potato starch	32.40	28.71	25.05	21.39
Dextrin	9.42	7.92	6.40	4.88
Total D.M.	100.00	100.00	100.00	100.00
Kjeldahl N/g (mg)	18.12	34.07	52.44	65.32

TABLE 8 - Formulation of Algal Diets for Experiment III

Ingredient (moisture free)	Protein level % 10	20	30	40
Spirulina meal	15.96	31.89	47.83	63.79
Cod liver oil	9.54	6.59	4.14	3.36
Soya oil	9.54	6.59	4.14	-
Mineral mix	8.26	6.51	4.77	3.03
α-cellulose	10.00	10.00	10.00	10.00
Binder	2.00	2.00	2.00	2.00
Vitamin mix	1.00	1.00	1.00	1.00
Potato starch	14.77	16.86	3.00	-
Dextrin	28.93	18.56	23.00	16.82
Total D.M.	100.00	100.00	100.00	100.00
Kjeldahl N/g (mg)	18.13	36.55	53.01	71.19

TABLE 9 - Formulation of Fungal Diets for Experiment III

Ingredients (moisture free)	Protein Level %			
	10	20	30	40
Fusarium meal	19.36	38.74	58.12	77.49
Cod liver oil	9.76	7.52	6.28	4.04
Soya oil	9.76	7.52	6.28	4.04
Mineral mix	8.81	7.61	6.42	5.23
α-cellulose	10.00	10.00	10.00	5.00
Binder	2.00	2.00	2.00	2.00
Vitamin mix	1.00	1.00	1.00	1.00
Potato starch	10.42	2.13	-	1.20
Dextrin	28.89	23.48	9.90	-
Total D.M.	100.00	100.00	100.00	100.00
Kjeldahl N/g (mg)	19.47	35.53	49.47	62.27

The experimental run and all analysis was conducted in an identical manner to that already described with water samples being taken at the start (initial sample) and end (final sample) of the 24 hour period.

2.4.3 Results

As in the previous experiment, the nitrogen lost and nitrogen fed per tank of fish were calculated from the equations:

mg N lost/tank/day = (mg N/g dry faeces x g dry faeces) + ((mg N/l final water sample - mg N/l initial sample) x vol of tank in litres)

mg N fed/tank/day = mg N/g dry food x g dry food fed/day.

For ease of further calculation, these values were again expressed as a mg nitrogen per 100g of fish weight/day. Apparent NPU could thus be calculated from the simple formula:

$$\text{Apparent NPU} = \frac{\text{mg N fed/100g fish/day} - \text{mg N lost/100g fish/day}}{\text{mg N fed/100g fish/day}}$$

To calculate true NPU's the quantity of endogenous nitrogen lost/day had been included in the formula. Experiment II had given the EUN lost/day as 12.37 mg N/100g fish/day, and Experiment I had shown that MFN amounted to some 1.75 mg N/g diet consumed. Obviously, as 100g of fish were consuming 2g of dry diet (ie. 2% of their body weight), then total endogenous excretions equalled:

12.37 + (1.75 x 2) mg N per 100g fish weight/day = 15.87 mg N

Then:

$$\text{True NPU} = \frac{\text{mg N fed/100g fish/day} - \text{mg N lost/100g fish/day} + 15.87}{\text{mg N fed/100g fish/day}}$$

These were calculated for each tank of fish giving 2 points at each level of intake (although occasionally a tank of fish was lost due to system malfunction).

(a) Fishmeal Diets

The true and apparent NPU values are tabulated in Table 10. They clearly show that both true and apparent NPU's decrease as nitrogen intake (ie. protein content of the diet) increases. When this data was plotted in Fig. 3, it appeared that the decrease was in fact linear and so straight lines were fitted to the two sets of data by the method of least squares. The correlation coefficients were - 0.98 and - 0.97 respectively, indicating that the negative linear regression provided a particularly good measure of the relationship between protein level and NPU.

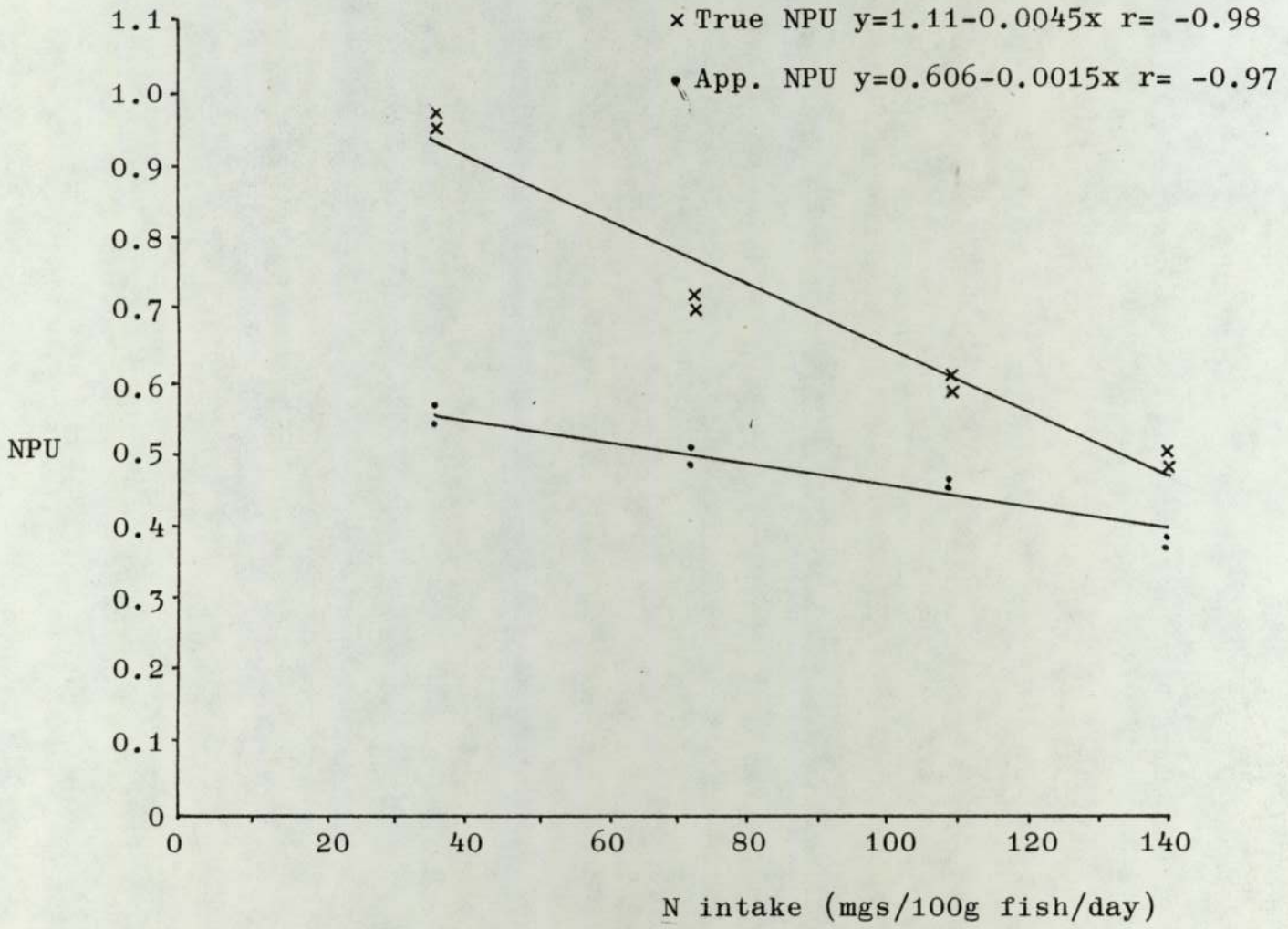
TABLE 10 - True and Apparent NPU with Fishmeal as the Protein Source

N intake/100g fish day (mg)	True NPU	Apparent NPU
36.32	0.97	0.54
36.32	0.99	0.55
73.27	0.71	0.49
73.27	0.72	0.50
109.91	0.61	0.47
109.91	0.59	0.45
140.57	0.50	0.39
140.57	0.48	0.37

Especially notable from the above data is the fact that true NPU approached 1.0% at low levels of protein intake though, of course, at such low levels a small error in the ENE value would have had a rather profound effect on the accuracy of this value. This aspect will be discussed more fully later.

The slope of the regression line is of great interest since it gives a measure of the rate of decrease in protein utilisation. Using this line, a figure comparable to the Net Protein Value (NPV) can be calculated, NPV being the product of NPU x % crude protein content of the diet. It is equivalent to the factor Protein Accumulation Rate x % crude protein used by Ogino and Saito (1970), and is a measure of the amount of protein available for metabolism. Hence the point at which this

Fig.3. Fishmeal - NPU at various levels of nitrogen intake.



value becomes maximal is the point at which the protein intake is optimal, ie. where further increases in protein intake do not increase the total amount of protein that can be used by the fish. For this experiment, the NPV can be determined from the equation:

True NPU x N fed/100g fish/day

However numerically it is not the same as NPV although it follows the same pattern and should perhaps be termed the Net Nitrogen Value or NNV. In Table 11, the NNV has been calculated from the true NPU regression equation shown in Fig. 3.

TABLE 11 - NNVs for Fishmeal Protein

	Nitrogen Intake (mg N/100g fish/day)						
	80	90	100	110	120	130	140
NNV	59.43	62.78	65.23	66.77	67.40	67.13	65.95

The results clearly show that the Net Nitrogen Utilisation Value reaches a maximum at around 120 mg N intake/100g fish/day. This can easily be converted to a protein level, as the fish were fed 2% of their body weight/day. Thus, to achieve this figure, each gramme of diet would have to contain 60 mg of nitrogen which converts (N x 6.25) to 375 mg protein/g of diet (ie. 37.5% protein). For fishmeal, it would appear that under these experimental conditions a 37.5% diet is optimal for maximal growth in terms of total N utilisation, though

not necessarily overall maximal growth, as this encompasses increases in all body compartments.

(b) Petroyeast Diets

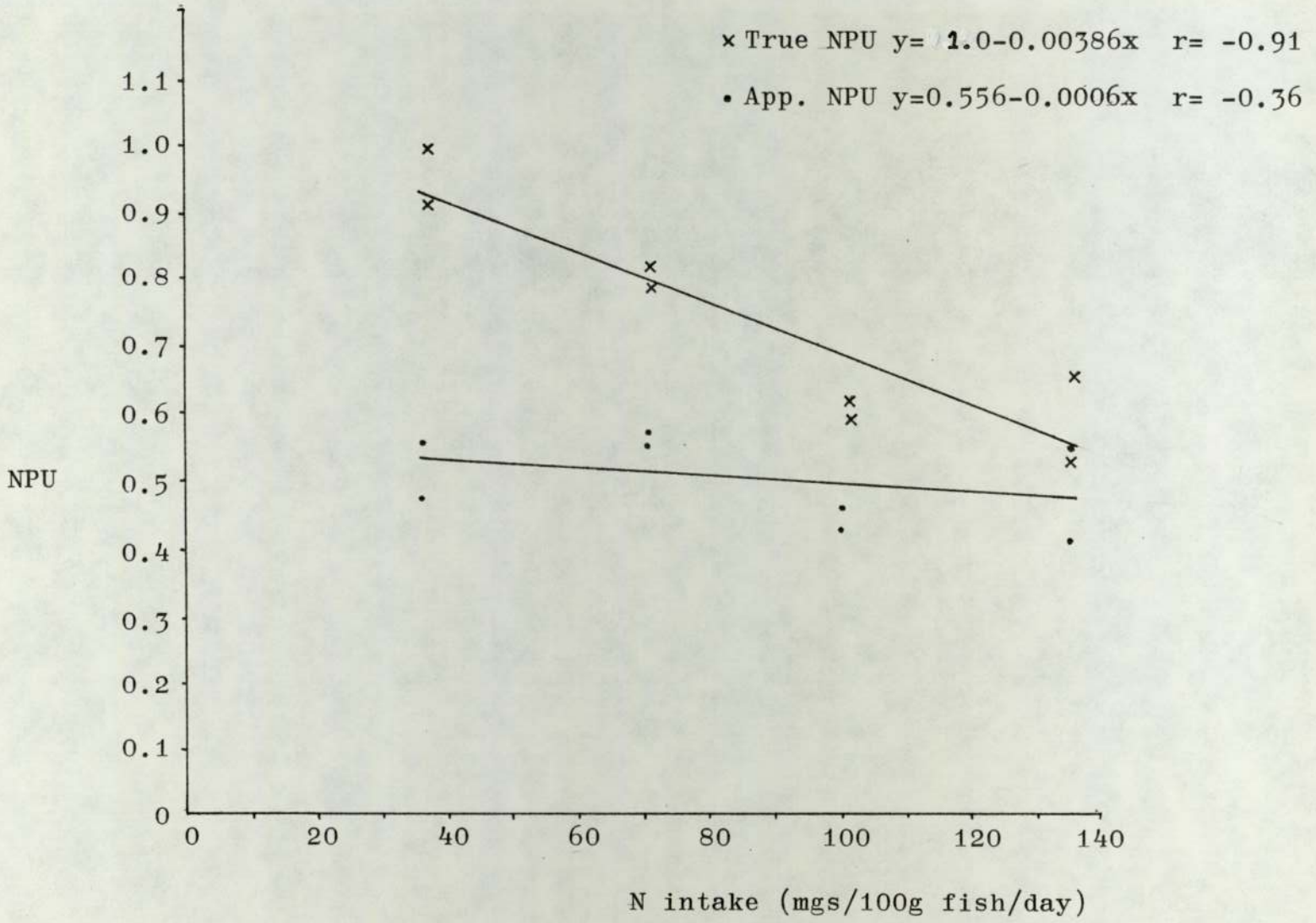
The results obtained with this protein source are shown in Table 12.

TABLE 12 - True and Apparent NPU with Petroyeast as the Protein Source

N intake/100g fish/day (mg)	True NPU	Apparent NPU
35.75	1.00	0.56
35.75	0.92	0.48
69.68	0.80	0.58
69.68	0.79	0.56
99.96	0.59	0.42
99.96	0.62	0.46
135.43	0.53	0.41
135.40	0.67	0.56

True NPU ranged from 1.0 at low N intake to 0.53 at high intake. Since the values decreased with increasing nitrogen intake in much the same manner as was found with the fishmeal, a linear regression was again fitted to the points (Fig. 4), this giving an r value of - 0.91 (a good correlation) and a slope of - 0.00386. However, the apparent NPU's did not exhibit such a distinct

Fig.4. Petroyeast - NPU at various levels of nitrogen intake.



relationship with intake and the regression coefficients of the line fitted to these points gave a value for r of only - 0.36 indicating that in this case apparent NPU is not correlated with nitrogen consumption (Fig. 4).

The NNV figures calculated from the regression of true NPU against N intake (Table 13). A figure was also included for an intake of 150 mg N/day as it was not

TABLE 13 - NNV's for Petroyeast Protein

	N intake (mg N/100g fish/day)							
	80	90	100	110	120	130	140	150
NNV	60.71	64.83	68.17	70.74	72.54	73.57	73.82	73.30

obvious whether the NNV value had in fact reached its maximum at the highest dietary nitrogen level used in the experiment (140 mg N/day). This extrapolation of the regression has to be regarded as statistically suspect since there is of course no evidence to confirm that the curve remains linear beyond 140 mg N/100g fish/day. It did show, however, that NNV had reached a maximal level at an N intake of 140 mg/100g fish/day which for a diet fed at 2% of the fish body weight/day under these experimental conditions gives a calculated optimum protein level of 43.75%, substantially higher than that found with the fishmeal protein.

(c) Bacterial Diets

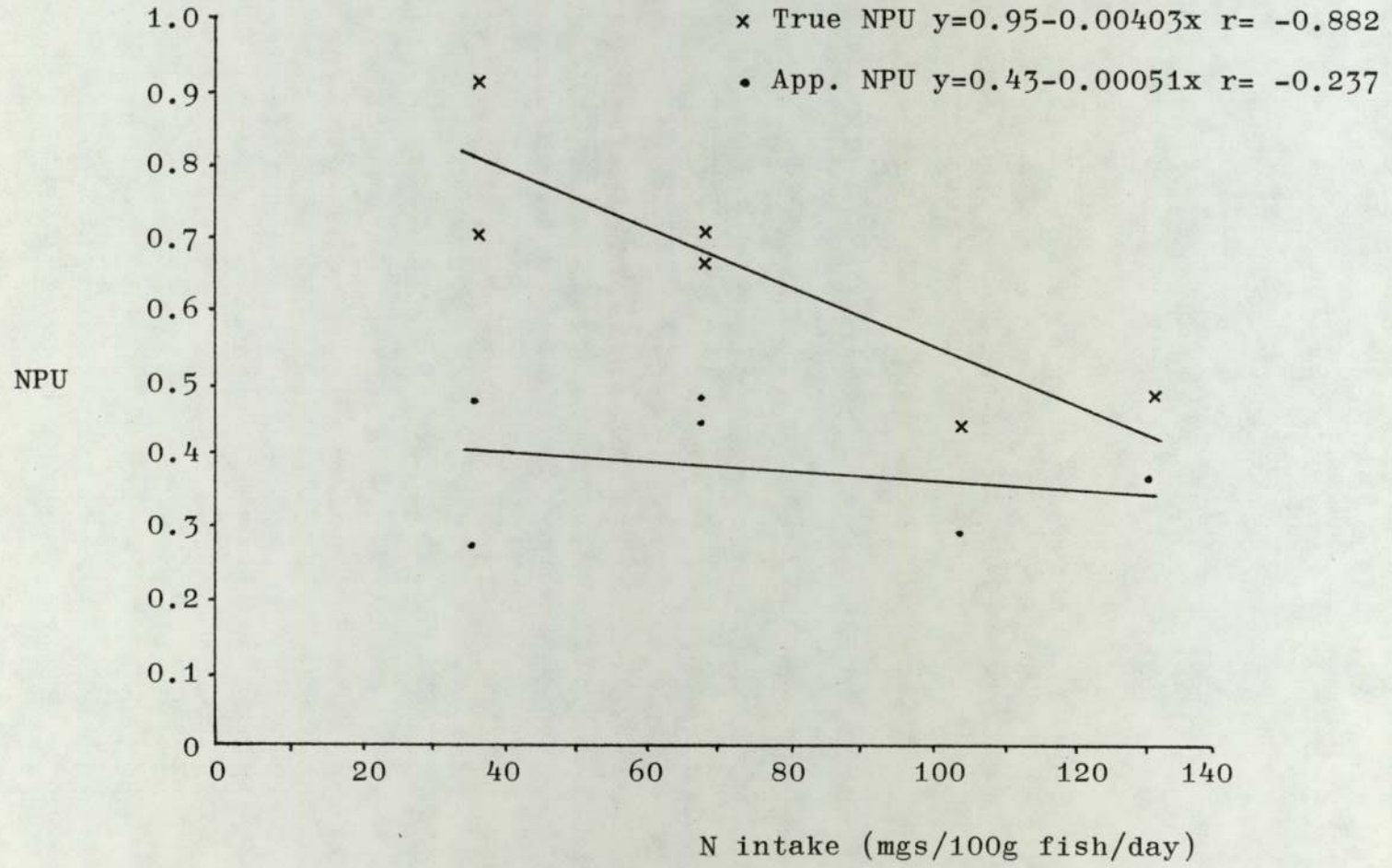
In this test, the two low protein groups gave two rather different results, and added to this 2 tanks of fish were lost during the experiment due to air line failure. Hence, there was a little less evidence upon which to construct a linear regression or indeed to ascertain whether the results warranted such treatment.

TABLE 14 - True and Apparent NPU with Bacterial Meal as the Protein Source

N intake (mg/100g fish/day)	True NPU	Apparent NPU
36.26	0.71	0.27
36.26	0.92	0.47
68.20	0.67	0.44
68.07	0.71	0.48
104.67	0.45	0.30
130.60	0.48	0.36

In view of the results obtained with the previous two test proteins a least square regression was fitted and is shown in Fig. 5. The correlation coefficient was found to be only - 0.882 which is probably a little low but can still be taken as evidence of a fairly strong negative relationship between protein level and true NPU. The actual true NPU values tended to be rather lower than those found for fishmeal and petroyeast especially for the low N intake groups. At high N intakes, the differences are not as apparent.

Fig.5. Bacterial protein - NPU at various levels of nitrogen intake.



The variability between replicates and the small number of points also led to a very low correlation ($r = -0.237$) between apparent NPU and protein intake, (Fig. 5), though this coefficient is rather similar to the results obtained in the two previous trials.

The Net Nitrogen Values, which were again calculated from the regression of NPU on N intake (Table 15) show that the total quantity of nitrogen which can be employed is maximal under these circumstances, when intake is around 120 mg/100g fish/day. For a 2% feeding rate, this is equivalent to 60 mg/g diet or a 37.5% protein level. The value is thus similar to fishmeal but somewhat lower than petroyeast.

TABLE 15 - NNV's for Bacterial Protein

	N intake (mg N/100g fish/day)					
	80	90	100	110	120	130
NNV	50.51	53.20	55.08	56.15	56.42	55.89

(d) Algal Diet

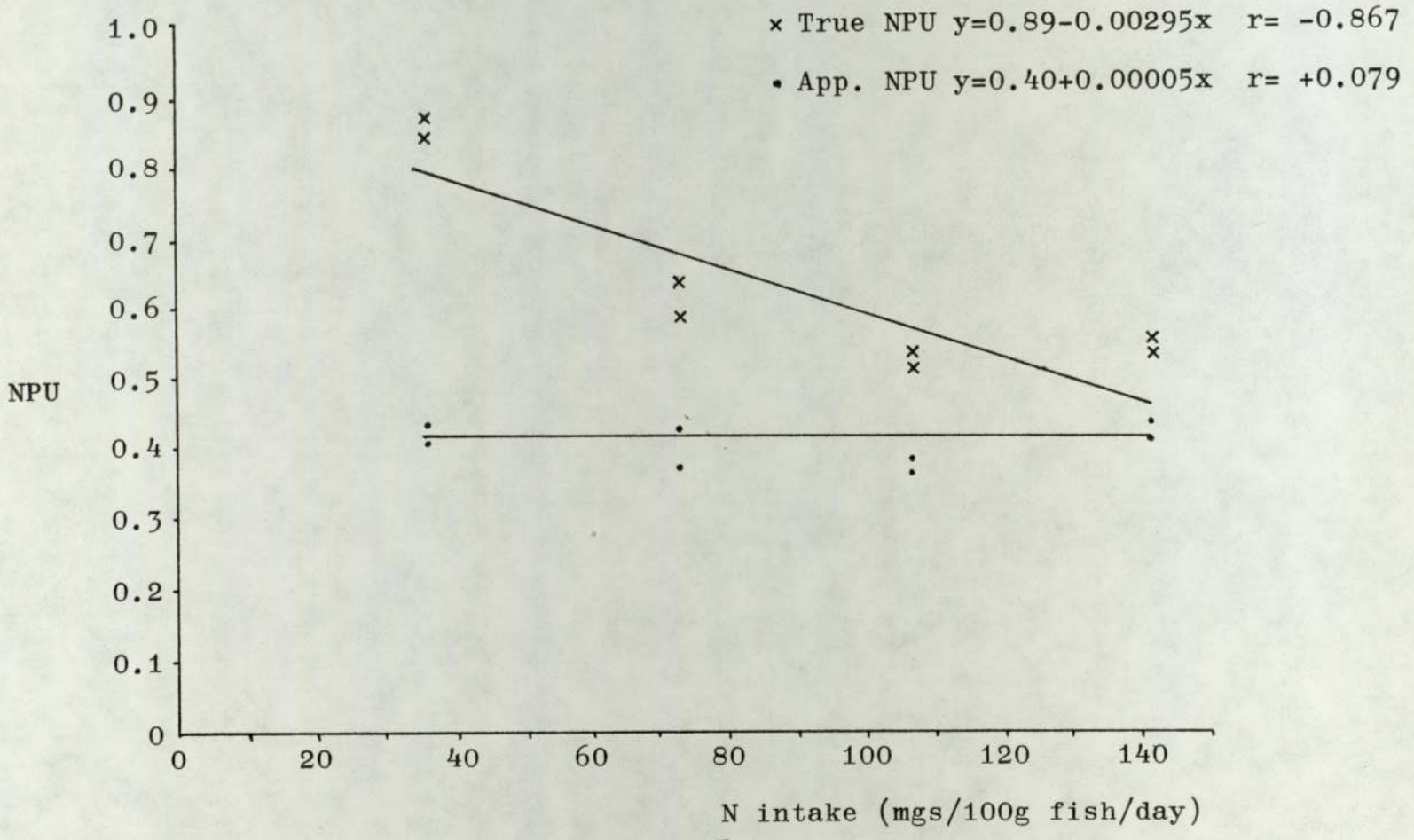
With this group of diets replicate treatments proved to be very comparable (Table 16), though the results obtained were open to two different interpretations regarding the type of relationship between N intake and NPU. A negative curve is one possible linkage, though

its shape would indicate that NPU first decreases and then increases as protein consumption increases. Providing that energy is not limiting in the intermediate protein level diets, this would seem unlikely as a representation of the true trend. This becomes obvious from the definition of NPU which is described as protein utilised divided by protein fed. Thus, once the level of absorbed protein that can be metabolised by the fish has reached a maximum then NPU has to decrease as the quotient (protein fed) increases. However, the second possibility, a linear regression, was again fitted to the points (Fig. 6), as it was assumed to be the best overall link between datum points, which the value of - 0.867 confirmed.

TABLE 16 - True and Apparent NPU with Spirulina alga as the Protein Source

N intake (mg N/100g fish/day)	True NPU	Apparent NPU
36.26	0.87	0.43
36.26	0.84	0.41
73.10	0.59	0.37
73.10	0.64	0.42
106.02	0.52	0.37
106.02	0.53	0.38
142.38	0.54	0.42
142.38	0.55	0.44

Fig.6. Algal protein - NPU at various levels of nitrogen intake.



The true NPU at low protein intake was not as high as that found for the fishmeal and petroyeast proteins being in fact more comparable with the bacterial protein diets. The slope of the regression, however, showed that the rate of decrease of NPU was slower than for all three previous test diets. Hence, the NNV's calculated from this regression line (Table 17) showed that the total quantity of nitrogen available to the fish had not reached an obvious maximum at the highest experimental intake and so two further projected values were calculated for intakes of 150 and 160 mg N/day. These indicated that maximal NNV occurred at 150 mg N intake equivalent to a 46.9% dietary protein level.

Regarding the apparent NPU, a linear regression (Fig. 6) again demonstrated that the values were unaffected by the amount of protein ingested since the r value was only + 0.079 and the slope + 0.0005.

TABLE 17 - NNV's for Spirulina Alga Protein

	N Intake (mg N/100g Fish/Day)								
	80	90	100	110	120	130	140	150	160
NNV	53.06	57.04	60.43	63.22	65.43	67.05	68.08	68.52	68.36

(e) Fungal Diets

The true NPU's in this trial (Table 18 and Fig. 7) decreased linearly as protein consumption increased the correlation coefficient (- 0.959) indicating that this was a highly significant link. The actual NPU values were closely allied to those given by the algal protein and indeed the slopes of these two regressions were very similar (- 0.00304 for Fusarium and - 0.00295 for Spirulina). For this reason, the protein level which gave an optimal NNV value (Table 19) was the same as that found for the algal protein, being of the order of 46.9% of the dry diet (ie. 150 mg N/100g/fish/day at a 2% feeding rate.

TABLE 18 - True and Apparent NPU with Fusarium as the Protein Source

N Intake (mg N/100g fish/day)	True NPU	Apparent NPU
38.94	0.79	0.38
38.94	0.79	0.38
70.70	0.68	0.46
98.94	0.54	0.38
98.94	0.66	0.49
124.54	0.52	0.39
124.54	0.54	0.42

Fig.7. Fusarium protein - NPU at various levels of nitrogen intake.

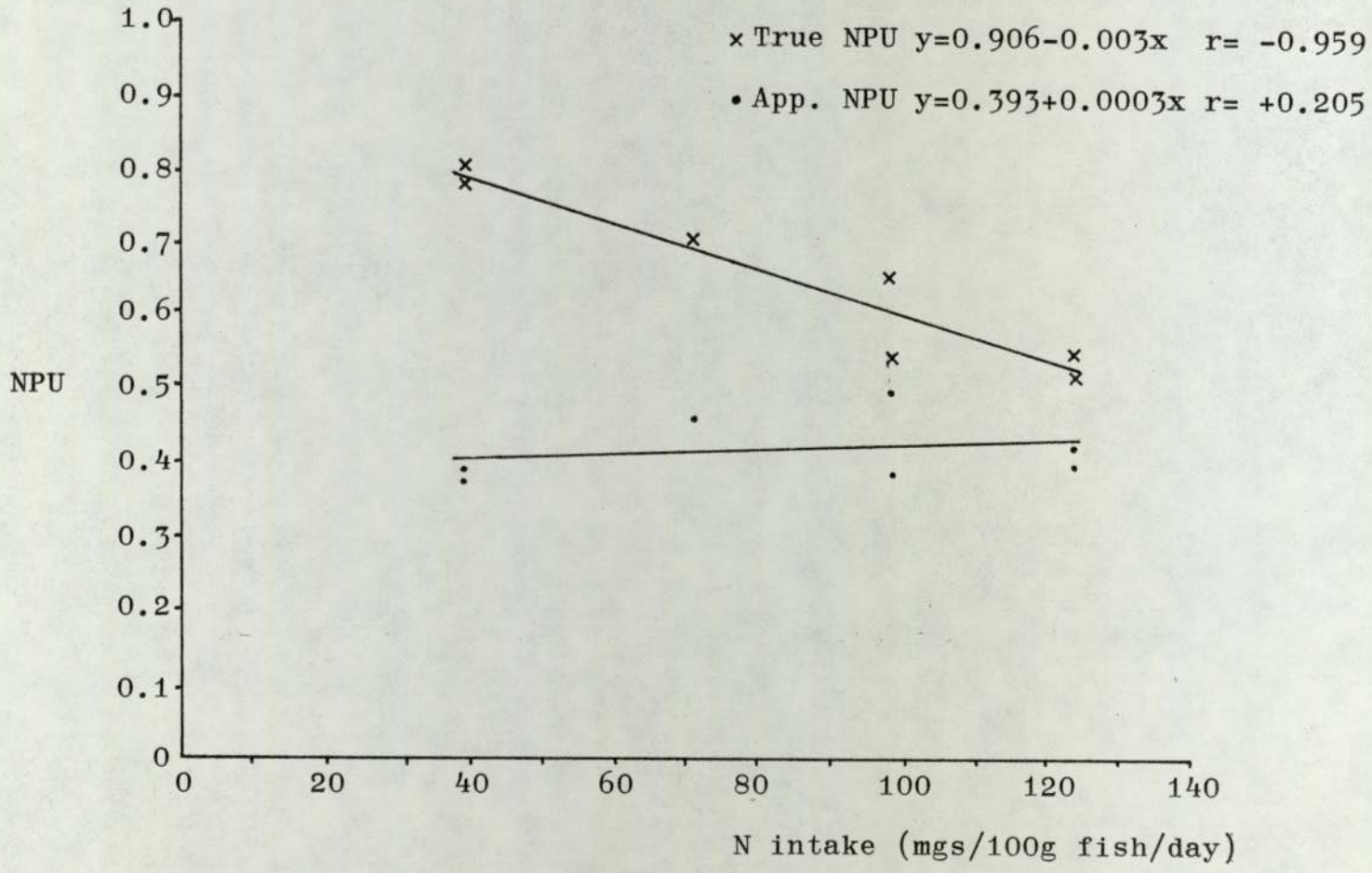


TABLE 19 - NNV's for Fusarium Protein

	N intake (mg N/100g fish/day)						
	100	110	120	130	140	150	160
NNV	60.45	62.82	64.88	66.34	67.18	67.42	67.06

The apparent NPU's did not follow as clear a trend as the true values. The least squares regression of apparent NPU on nitrogen intake gave a line with a positive slope which had only been found in one of the previous trials. The r value of this line was only + 0.205 and so it cannot be regarded as a significant correlation. Thus it was concluded that here again apparent NPU was unaffected by dietary protein level.

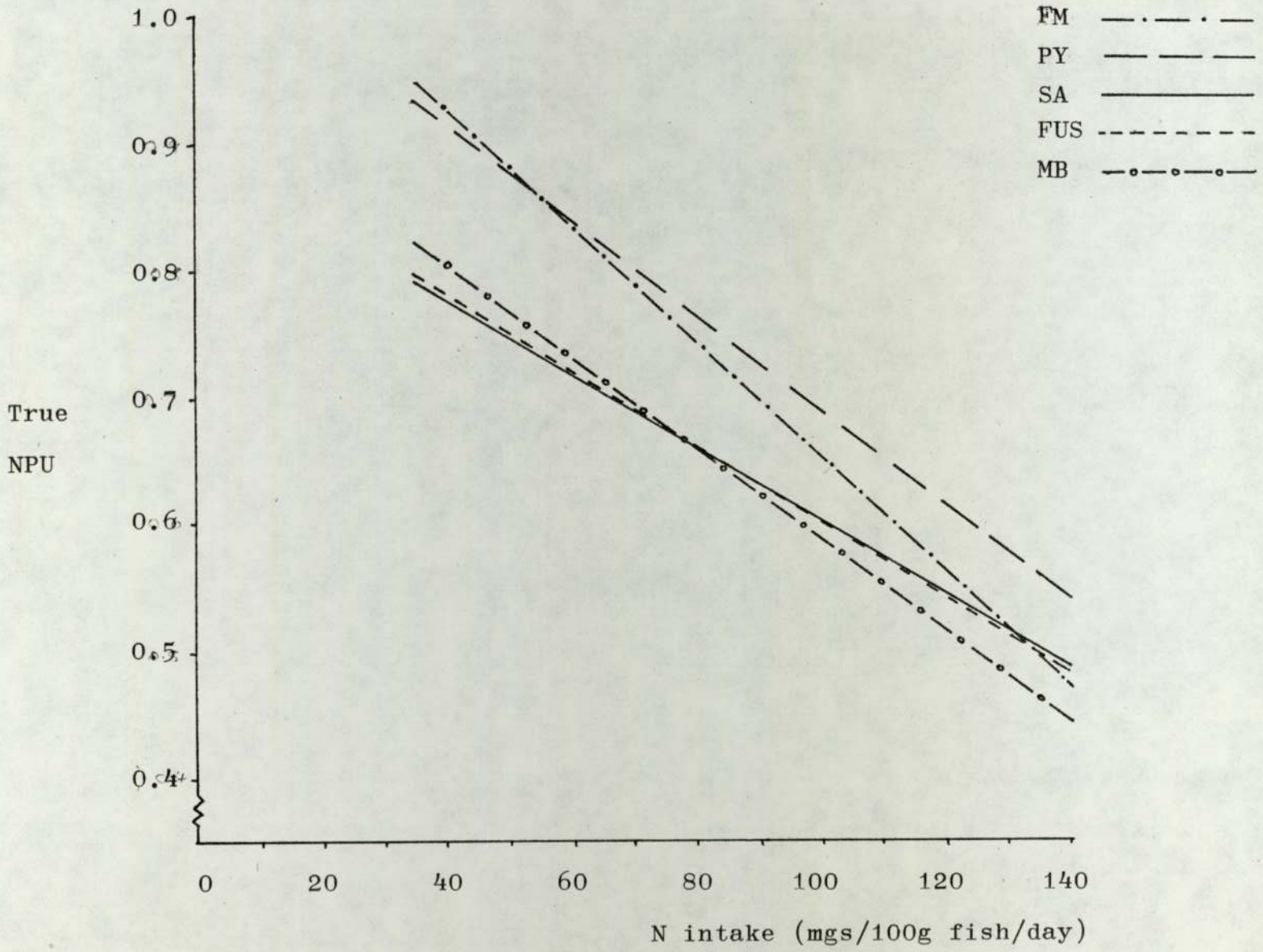
2.4.4 Discussion

This series of trials produced some interesting results, though rather large variability was noted between some replicates. This seems to be a fairly common feature of nitrogen balance experiments even when they are performed by body analysis methods (Gerking, 1955a; Gerking, 1971; Nose, 1971). In these experiments, the variability, whilst possibly biological, was probably due to very small errors in the nitrogen analysis method at such low nitrogen levels. Added to this variability in replication, in certain situations a repeatable NPU value of greater than 1.0 was noted. This effect has

also been noted by Nose (1971) who attributed it to the large effect small errors in the estimation of endogenous nitrogen losses at such low intakes. This seems feasible in consideration of the amount of nitrogen analysis that has to be performed in order to obtain a balance. Overall, however, the method appeared to give reasonable and essentially repeatable results.

Before considering further the absolute values for NPU for each of the dietary proteins tested, it is important to study further the relationship between protein intake and degree of utilisation. It can be seen from Fig. 8 that this relationship is negative, at least in respect to the true Net Protein Utilisation. This result differs from those of early workers such as Gerking (1955a) who showed that a plot of N intake against N retained was linear indicating that NPU was constant. However, the results presented here agree with more recent work by Nose (1962) who showed that NPU was not constant at various levels of protein intake. His data showed that Biological Value was maximal (0.93) with a 10% protein diet and minimal (0.096) with a 30% protein diet. At a higher protein intake (40%) the BV fell between the above two values, indicating that the degree of utilisation was not related to protein intake. However most recent work has demonstrated that the degree of protein utilisation is inversely linked to protein intake and this seems to hold true for a wide variety

Fig.8. Comparison of the true NPU values from Figs.3-7.



of protein sources and fish species. (Ogino and Saito, 1970; Nose, 1971; Cowey et al., 1972; Ogino and Chen, 1973c; Cho et al., 1976; Ogino et al., 1976.) Most authors also seem to agree that the relationship is also linear but Dabrowski (1977) found the relationship to be best described by a negative hyperbolic equation, as described by some other authors working on mammals (reviewed by Pellett, 1973). The results obtained in the present experiments tend to confirm the linear rather than the hyperbolic relationship. There are several possible reasons why the results of Dabrowski differ from those found here. Firstly, he used a different species (Ctenopharyngodon idella) and their average initial weight was only 0.15 - 0.2 g. Since the protein requirements of fry are usually considered to be much higher than those of large individuals, their protein metabolism is obviously somewhat different and this may be reflected in their reaction to varying protein levels. Added to this a large proportion of the energy in his diets was supplied in the form of carbohydrate, and this again may affect protein utilisation, though it would be expected that a species such as grass carp would metabolise this nutrient quite successfully. Alternatively, his curve may have resulted from his rather more comprehensive calculation of NPU than that normally used, in that it took into account the increasing size of the test fish during the experiment. However, this could not explain the variance from the

results presented here since in the balance trial NPU was measured directly over a very short period. Overall, a hyperbolic equation is more likely than a linear one when the derivation of NPU (N utilised ÷ N fed) is considered. Once the fish have reached a point where they are utilising as much nitrogen as physiologically possible, further increase in nitrogen will cause the NPU to fall asymptotically (since N utilisation is constant). However, up to the maximum retention point (optimum protein level) either equation is theoretically possible. For this reason, a linear equation was fitted to these results as it appeared to give the best fit to the data points obtained.

Cowey and Sargent (1972) have asserted that NPU decreases by virtue of the fact that at low protein intakes the amino acid composition of the protein is limiting, whilst at higher intakes a greater proportion of the protein is burnt for energy. In this experiment, it was found that two protein sources (petroyeast and fishmeal) gave NPU values of around 1.10 at a 10% protein level, despite the fact that petroyeast is obviously limiting in the sulphur amino acids, and fishmeal slightly deficient in arginine (Appendix II). One conclusion that could be drawn from this is that the amino acid requirements (as a percentage of the dietary protein) for maintenance are rather different to those required for growth. Alternatively, it is possible that certain amino

acids may be synthesised from non-essential amino acids in the required quantities for maintenance. These two explanations, coupled with the high calorie to protein ratio could thus provide a reasonable explanation of the elevated NPU levels. From this point, the NPU could decrease linearly due to both the changing amino acid requirements, the lowering calorie to protein ratio, and the added effects of high substrate levels (ie. amino acids) on the enzymatic metabolism of these substrates. Whilst conservation of essential amino acids at maintenance has been suggested, Cowey (1975) has concluded that this does not seem to occur to any great extent at least in plaice.

Fig. 8 shows that the rate of decrease in NPU was similar, but not identical for the various proteins tested. Ogino and Chen (1973c) showed that for several dietary proteins the Biological Values did in fact decrease at the same rate, that is, the BV versus protein intake curves were parallel. In these experiments, the main deviant from this parallelity was the fishmeal protein. This may have been a consequence of the diets being semi-purified and thus to an extent only partially defined, and also due to the fact that various diets were not identical in their levels of lipid and digestible carbohydrate because of formulation difficulties. Added to this, the results obtained may have been a reflection of a decreased digestibility caused by the high levels of

starch included in the fishmeal diets as shown by Kitamikado et al. (1964b), though the bacterial diets with similar starch levels showed lower NPU slope. In essence, the results do not substantially disprove those of Ogino and Chen (1973c).

If the NPU decrease lines are in fact parallel, it indicates that the optimum dietary protein level does not vary with the quality of the protein. Here the slopes deviated sufficiently from a parallel course to give slightly different estimates of the optimum protein requirements of trout. With the fishmeal and bacterial proteins, the optimal dietary level, ie. the point at which NNV became maximal, was about 37.5%. The NNV did not reach a maximum level with the other three protein sources tested, but extrapolation indicated that this would have occurred at about 44% with the petroyeast and 47% with the algal and fusarium proteins. It appears therefore that there is some evidence that optimum protein level is linked to protein quality as the poorer quality proteins as measured by amino acid analysis (Appendix III) tended to give higher requirement levels. This would agree with mammalian work done by Hegsted and Chang (1965), whose lactalbumen equivalent method of estimating protein quality naturally infers a variability in optimum protein quantity with changes in protein quality. In fish nutrition this kind of result is of great interest since it indicates that it may be possible to reduce the level of

dietary protein in commercial fish rations, for fish of this size, to 35% or even lower, and that previous experiments which set the protein requirements of salmonids at 40% or more, on which present day rations are based may have been the result of poor protein quality. Indeed there is now some evidence that trout only require 30 - 35% protein diets, (Luquet, 1971; Ogino et al., 1976) when either protein quality and/or dietary energy levels are high. Added to this, much of the work on protein quantity has been performed on fish smaller than those used in the present experiments (Delong et al., 1958; Satia, 1974). However, from the commercial point of view it may be more feasible economically to retain the higher protein level regardless of fish size, rather than to upgrade the quality of the protein.

The NNV values also demonstrate that at supra-optimal protein levels the amount of nitrogen available for growth is slightly reduced. This can be ascribed to the reduced quantities of non-protein energy in such diets, which resulted in the fish having to catabolise protein to meet their daily energy requirements. This means that, in practice, the previously described relationship where nitrogen retention remains constant above optimum protein levels does not hold true in practice, and therefore the NPU/N intake curve may not follow the hypothetical asymptotic path even at these levels.

Turning to the absolute NPU values found, the fact that there is no definite value for each test protein makes comparison with the few previously reported by other workers rather difficult. However, it can be said that for most of the protein sources, the NPU's are slightly higher than those described in the literature, even for compounded rations. Cowey et al. (1974) gives the true NPU of several dietary proteins for plaice, but comparison is difficult since they were included at a 50% dietary protein level and the diets were fed ad libitum. Hence the values obtained here cannot be directly compared as the highest level of protein used was 40%. The general indications are that the values for fishmeal and petroyeast on an equivalent basis would be somewhat higher than theirs.

Several authors have reported protein utilisation figures either directly as true or apparent NPU's or as equivalent values (eg. protein conversions or protein coefficients which are reciprocals of apparent NPU). Most of this work suggests that under reasonable conditions a protein retention of 30 - 40% is not unusual (Page and Andrews, 1973; Zeitoun et al., 1974; Garling and Wilson, 1977; Macleod, 1977). The apparent NPU's reported here for most of the protein sources are also of this order, suggesting that the methodology of nitrogen balance used gives results equivalent to those achieved by other means.

The regression lines fitted to the apparent NPU's and the very low regression coefficients found, demonstrated that unlike the true values, apparent NPU is unaffected by the level of inclusion of protein in the diet. This agrees with work published by Nail (1962) who found that protein conversion (reciprocal apparent NPU) was statistically consistent with increasing dietary protein level in channel catfish diets. On the other hand, Zeitoun et al. (1974) discovered a peak in apparent NPU at about a 40% protein level in coho salmon experiments. However, Gerking (1955b) showed that true NPU (from his graph of N fed versus N retained) was constant. For this reason, the apparent NPU (his N efficiency) followed an asymtotic path. This was also shown later by Savitz (1977). However, in both these experiments isoenergetic diets were not used, the fish being fed with varying quantities of wet fish. Under these conditions, a low N efficiency could be expected at low protein intakes as there was some likelihood that protein would have been used as a basic energy source.

Interestingly, the results obtained in this series of experiments do not agree with those found in Experiment II. In that experiment, a linear relationship was found for casein when N intake was plotted against N retained. The slope of this line, the true NPU, therefore remained constant for all intake levels. This may have been the result of the very low feeding rate used and therefore the comparatively low protein and energy intakes.

Alternatively, the small number of points on which the graph was based, or the particular formulation of these diets may have biased the results. Whilst it may have been a characteristic of casein in particular, this seems rather unlikely. The true NPU value (0.48) was in fact rather low in comparison to those presented here, which would indicate that some factor did limit the utilisation of casein.

One further consideration in the interpretation of the NPU results is the effect that non-protein nitrogen may have on the accuracy of the NPU's obtained with novel protein sources. Such ingredients can contain 15% or more non-protein nitrogen. There is no data on the extent to which these substances (nucleic acids, glucosamines, chitin, etc.) can be absorbed and utilised by fish. If they are not absorbed to any extent, or are excreted directly, then the utilisation of the protein, or true NPU would have been higher than that estimated here. However, in view of the lack of data, there can be no justification for arbitary correction of the NPU to account for the influence of non-protein nitrogen. Basing utilisation values on only the true protein (rather than the Kjeldahl protein) intake may also lead to errors if non-protein nitrogen does have any protein sparing effect.

2.5 Experiment IV - Effect of Energy Level and Feed Intake on the NPU of Fishmeal Protein

2.5.1 Introduction

In the previous series of experiments, it was shown that Net Protein Utilisation decreases linearly as protein intake increases. The rate of this decrease was slightly variable, the proteins tested falling roughly into two groups. As considered in the discussion of those results the reason for the variation was not immediately apparent though it seemed possible that it may have been due to energetic, rather than protein effects. There is now a good body of evidence to show that, to a certain extent, protein energy can be spared for growth by both dietary carbohydrate and lipid. In the case of carbohydrate, the ability to spare protein varies considerably between species and between carbohydrate sources. In general, omnivorous species such as carp and catfish can digest and utilise carbohydrates especially polysaccharides, to a greater extent than carnivores such as trout and salmon. For instance, Garling and Wilson (1977) suggest that at ratios between 0.45 and 4.5, carbohydrate can be substituted for lipid in the normal physiological energy ratio of 2.25:1. Carbohydrate appears to be especially effective in low protein and low energy diets (Dupree, 1975). On the other hand, Luquet *et al*. (1975, 1976) and Austreing *et al*. (1977) have shown that elevated

carbohydrate levels tend to reduce the utilisation of protein for growth in salmonids rather than increase it. Furukawa (1976) has described a decrease in protein digestibility with high carbohydrate diets and Palmer and Ryman (1972) stated that fish show an oral intolerance to glucose. However, Pieper and Pfeffer (1978), also working with rainbow trout, have reported that additional energy in the form of carbohydrate does in fact increase the utilisation of dietary protein and Cowey et al. (1975) have also shown the beneficial effects of carbohydrates in plaice diets even when they are used to replace lipid. Overall, the picture of carbohydrate utilisation is therefore unclear, but generally it is recommended that the level of digestible carbohydrate should be restricted in diets for fish such as rainbow trout.

The situation regarding lipids is rather clearer. Most fish species tolerate and utilise high levels of fats. Again there appear to be some differences between the warm water omnivores and cold water carnivores. For trout and salmon, lipid levels around 15 - 20% appear to give good results in growth trials (Higashi et al., 1964; Watanabe et al., 1978; Austreng, 1978b; Takeuchi et al., 1978). However, the above figures are slightly higher than the optimum reported for species such as catfish. Stickney and Lovell (1977) found an increase in protein deposition with lipid levels of up to 8%, but further increases to 16% did not spare further

quantities of protein, whilst Dupree (1969) showed that at this level protein utilisation in this species actually decreased. In a later report (Dupree et al., 1978), a level of 15% lipid was suggested as optimum for this species. In carp also, lipid spares protein successfully, at least at sub-optimal protein levels (Sin, 1973b).

In reference to the effects of dietary energy on NPU values, the work on Ogino et al. (1976) is probably most applicable. Their data indicated that rainbow trout utilise lipid more than carbohydrates as an energy source, this being reflected in the slope of the NPU curves. However, they did not attempt to show the effect of variation in the quantity of lipid at each dietary protein level, and hence the following nitrogen balance experiments were undertaken to study this effect and its possible implications in relation to the results obtained previously with novel protein diets. The experiment was repeated at two different feeding levels to ascertain whether the results obtained with casein in Experiment II could have been due to the feeding rate used.

2.5.2 Materials and Methods

The apparatus used throughout this series of experiments, the methodology of fish acclimation, sampling and analysis were all identical to those used in the balance experiments described previously. However, the fish used were slightly larger, being of the order of 50 - 55g each. All 16 of the nitrogen balance tanks (Appendix VIII) were utilised so the experiment was in fact conducted as two trials. In the first trial, the low and high energy diets were compared at a 2% feeding level (dry diet/whole fish) whilst in the second experiment, a 1% feeding level was used.

The diets were prepared according to the formulations shown below (Tables 20 and 21).

TABLE 20 - Formulation of Diets for Experiment IV

(a) High Energy Fishmeal Diets

	HE10	HE20	HE30	HE40
Fishmeal	12.34	24.69	37.03	49.38
Mineral mix (App. V)	8.74	7.49	6.23	4.97
Vitamin mix (App. VI)	1.00	1.00	1.00	1.00
Binder	1.00	1.00	1.00	1.00
Cod liver oil	19.41	18.82	18.22	17.62
Glucose	15.00	15.00	15.00	15.00
Potato starch	10.00	10.00	10.00	10.00
α-cellulose	32.51	22.00	11.52	1.03
Total	100.00	100.00	100.00	100.00
calc. dig kJ/g	12.09	14.23	16.36	18.50
calc. mg P/kJ	8.12	13.86	18.40	21.51
calc. % total dry E as P energy	17.5	29.9	39.1	46.0

TABLE 21 - Formulation of Diets for Experiment IV

(b) Low Energy Fishmeal Diets

	LE10	LE20	LE30	LE40
Fishmeal	12.34	24.69	37.03	49.38
Mineral mix (App. V)	8.74	7.49	6.23	4.97
Vitamin mix (App. VI)	1.00	1.00	1.00	1.00
Binder	2.00	2.00	2.00	2.00
Cod liver oil	24.41	18.81	13.22	7.63
Potato starch	32.09	28.10	24.14	20.15
Dextrin	9.42	7.91	6.38	4.87
α-cellulose	10.00	10.00	10.00	10.00
Total	100.00	100.00	100.00	100.00
calc. dig kJ/g	14.06	14.06	14.06	14.06
calc. mg P/kJ	7.17	14.34	21.51	28.43
calc. % dig E as P energy	15	30	45	61

The low energy diets were essentially the same as those used in Experiment III, though the added lipid in this case composed of cod liver oil alone, not a mixture of cod liver oil and soyabean oils as employed before. The digestible energy values were calculated as before on the basis of 21.302 kJ/g for protein, 33.606 kJ/g for lipid, 17.159 kJ/g for glucose, 13.727 kJ/g for dextrin and 6.863 kJ/g for starch.

The high energy diets had to be formulated in a slightly different manner in order to ensure that the diets were not unphysiological or unpelletable. The overall level of lipid (including that in the fishmeal) was maintained at 20% of the dry diet. Glucose replaced part of the starch and alpha cellulose was used to provide the remaining bulk. This in fact meant that diets HE10 and HE20 had a lower digestible level than the comparable low energy diets. At the other end of the range diets HE30 and HE40 contained 2.30 and 4.44 kJ/g more energy than LE30 and LE40 respectively.

The diets were prepared and pelletised as described in Appendix VII, and then analysed for total nitrogen and moisture in the usual way. In each experiment, the fish were fed their daily ration in two equal portions at 10am and 6pm. All the diets proved to be acceptable to the fish, being consumed almost as soon as they entered the water. Temperature throughout the experiment ranged from 11.6 to 12.5°C.

2.5.3 Results

The true NPU values were calculated from the MFN and ENE figures described previously. At a 2% feeding level total ENE was calculated as 12.37 + (2 x 1.75) = 15.87 mg N/100g fish/day. At a 1% feeding level, the equivalent figure was 12.37 + 1.75 = 14.12 mg N/100g fish/day.

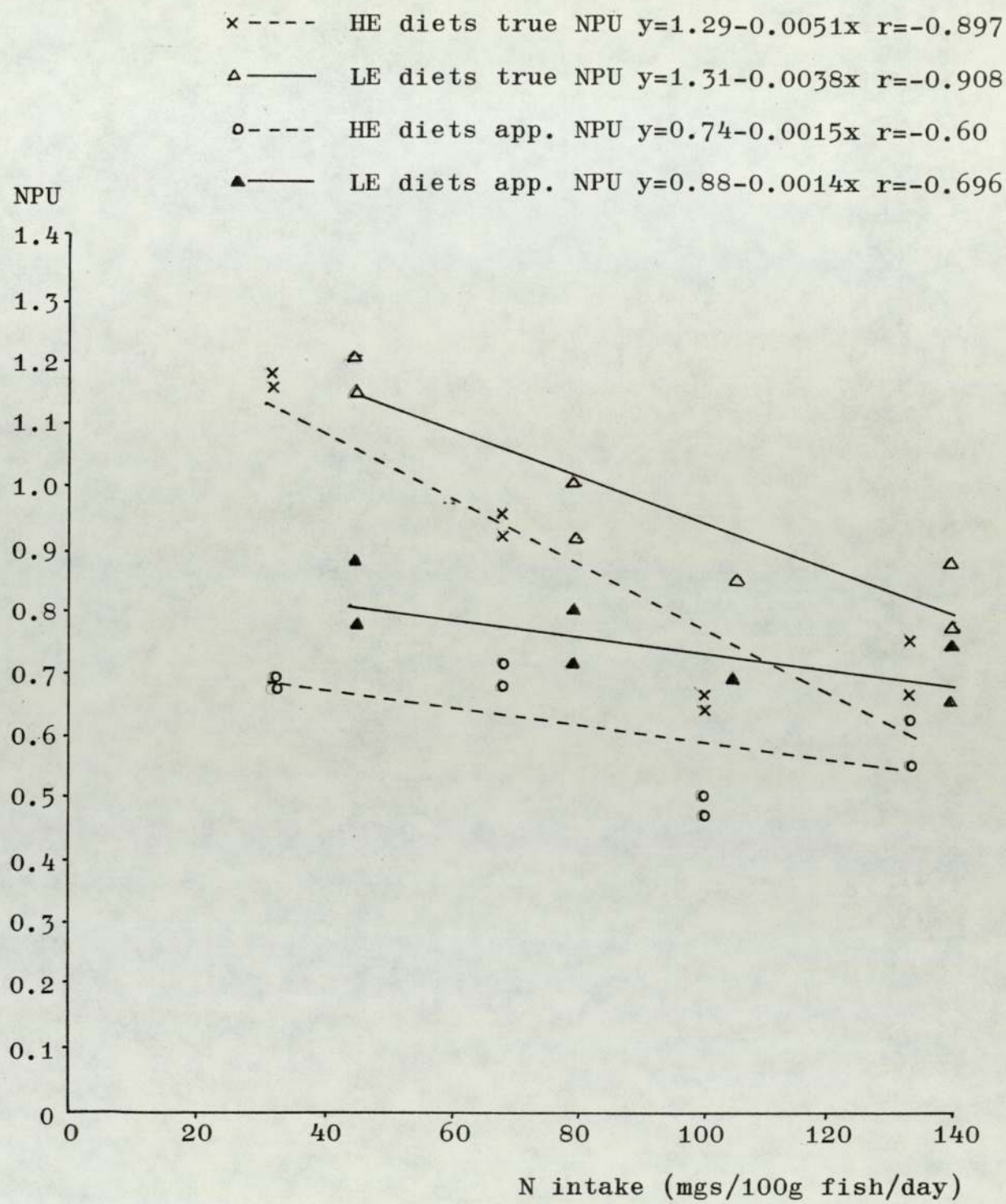
The results obtained with the various diets are shown in Tables 22 and 23. During the 2% feeding trial one group of fish fed diet LE30 was lost, so no data could be included for this group.

These results are expressed graphically in Figs. 9 and 10 respectively.

TABLE 22 - NPU Values for HE and LE diets at a 2% Feeding Level

	Intake mg N/100g fish/day	True NPU	App. NPU
HE10	31.9	1.18	0.68
"	31.9	1.18	0.68
HE20	67.76	0.96	0.72
"	67.76	0.92	0.68
HE30	99.40	0.62	0.47
"	99.40	0.66	0.50
HE40	132.83	0.75	0.63
"	132.83	0.67	0.55
LE10	45.33	1.21	0.89
"	45.33	1.16	0.78
LE20	78.86	1.11	0.80
"	78.86	0.91	0.71
LE30	105.43	0.84	0.69
LE40	139.33	0.77	0.65
"	139.33	0.87	0.76

Fig.9. Effect of energy level on NPU values of fishmeal based diets fed at 2% fish body weight per day.



(a) 2% Feeding Level

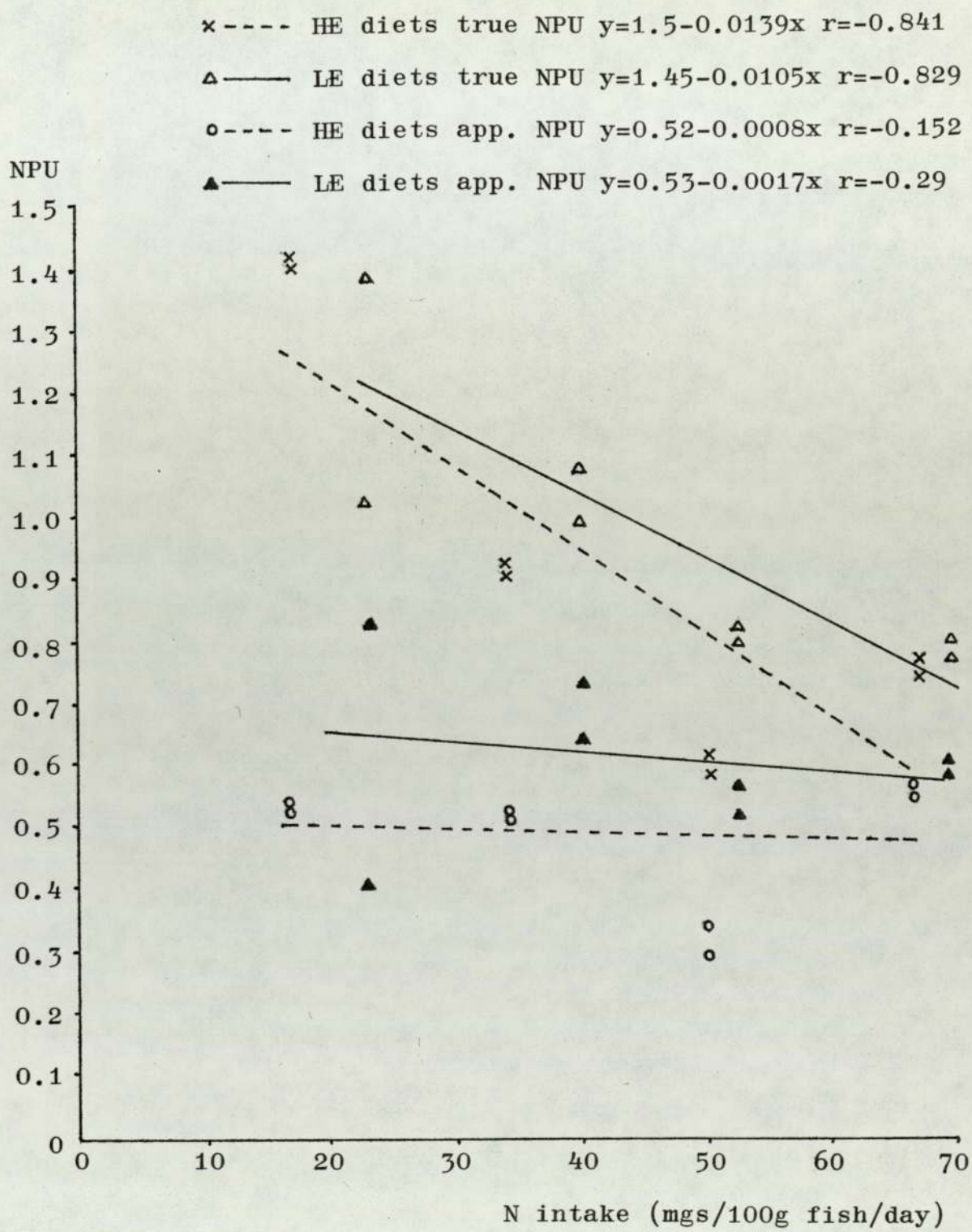
As noted in previous trials, the true NPE decreased with increasing protein intake, and since this appeared to be a linear relationship for both diets a least squares regression was fitted to the points. The slopes of these regressions were - 0.0051 for the HE diets and - 0.0038 for the LE diets. The points were in fact rather variable, and also the NPU values obtained were somewhat high especially when compared to those obtained with fishmeal previously. In fact, several values were well above 1.0 which seems to occur quite regularly in this type of nitrogen balance work. The greater slope of the HE regression line would indicate that the true NPU tends to decrease at a faster rate for these high energy diets in comparison to the LE diets. The correlation coefficients for the two lines were - 0.897 and - 0.908, so the lines can be regarded as an acceptable fit to the points.

The apparent NPU of course showed similar differences, although the values were practically constant for each group of diets, the regression line slopes being - 0.00145 and -0.00138 for the HE and LE groups respectively. Compared to the results found previously with fishmeal (Experiment III), the apparent NPU's, which were in the region of 0.60 - 0.80, were rather high.

TABLE 23 - NPU Values for HE and LE Diets at a 1% Feeding Level

	Intake mg N/100g fish/day	True NPU	App. NPU
HE10	15.96	1.41	0.53
"	15.96	1.43	0.55
HE20	33.89	0.94	0.52
"	33.89	0.94	0.52
HE30	49.70	0.63	0.35
"	49.70	0.57	0.29
HE40	66.38	0.77	0.56
"	66.38	0.77	0.56
LE10	22.67	1.04	0.42
"	22.67	1.46	0.84
LE20	39.42	1.01	0.64
"	39.42	1.10	0.74
LE30	52.74	0.78	0.52
"	52.74	0.84	0.57
LE40	69.72	0.78	0.58
"	69.72	0.79	0.59

Fig.10. Effect of energy level on NPU values of fishmeal based diets fed at 1% fish body weight per day.



(b) 1% Feeding Level

Apart from the LE10 diets, replication of the results at each level of protein intake was fairly good, although the correlation coefficients for both HE and LE lines were lower than those found at the 2% feeding level. Again, at low intakes, the true NPU's were rather high, but they decreased very rapidly as N intake increased, the slopes being - 0.0105 for the LE and - 0.0138 for the HE diets. Hence the pattern found for the higher feeding rate was repeated, with high energy diets giving lower NPU values than the corresponding low energy diets. Compared to the 2% feeding rate, true NPU's were lower at equivalent nitrogen intakes (eg. 60 mg N/100g fish/day) with the 1% feeding level, ie. when the daily energy intake per fish was reduced.

Apparent NPU's for both LE and HE groups were again practically constant regardless of N intake, the regression line slopes being - 0.0017 and - 0.00083 respectively. The correlation coefficients of - 0.29 and - 0.152 were also rather low.

As in the previous experiments, a Net Nitrogen Value (NNV), equivalent to Net Protein Value, was calculated from the NPU regressions for each diet at both feeding levels. The results of these calculations are shown in Tables 24 and 25.

TABLE 24 - NNV's for LE and HE diets at a 2% Feeding Level

N intake (mg/100g fish/day)	40	60	80	100
NNV LE diets	46.34	64.92	82.44	92.90
NNV HE diets	43.45	59.05	70.58	78.03

N intake (mg/100g fish/day)	120	140	160	180
NNV LE diets	102.29	108.62	111.90	112.11
NNV HE diets	81.40	80.68		

TABLE 25 - NNV's for LE and HE diets at a 1% Feeding Level

N intake (mg/100g fish/day)	20	30	40
NNV LE diets	24.84	34.11	41.28
NNV HE diets	24.60	32.76	38.42

N intake (mg/100g fish/day)	50	60	70
NNV LE diets	46.35	49.34	50.22
NNV HE diets	40.98	40.63	37.73

For the LE diets at a 2% feeding level, the maximum NNV could only be found by extrapolation of the regression line to an intake of 180 mg N/100g fish/day, equivalent (at N x 6.25) to a 55% dietary protein level. At the same feeding level, the HE diet showed its optimum NNV value at an intake of 120 mg N/100g fish/day (a 37.5% protein).

A similar difference in optimum protein level was noted at a 1% feeding rate, the values being 70 mg N and 50 mg N/100g fish/day for LE and HE diets respectively, these being equivalent to 44% and 31% dietary protein levels. However, whilst it appears superficially that the dietary protein requirements of the fish given high energy diets are lower than those on the low energy feeds, the NNV data also shows that the total amount of nitrogen available for metabolism was substantially reduced when the high energy diets were used.

2.5.4 Discussion

The very high true NPU values, found at low protein intakes is again very perplexing. The answer may lie in small inaccuracies in the measurement of low nitrogen excretion rates. This could be one of the main factors, as the high NPU's had a tendency to be somewhat out of line with the general trend of the regressions. Added to possible inconsistencies in N measurement, a small

error in the estimation of endogenous nitrogen loss would also have had a great effect, especially noticable at low nitrogen intakes. Such an error would also affect the slope of any true NPU regression, overestimation increasing, and underestimation decreasing, this slope. The reproductability of low balance measurements would indicate that the ENE estimation, rather than the analysis of nitrogen lost, was in error, unless of course any analytical error was constant and reproducible.

At a 2% feeding rate, there were obvious differences in the utilisation of the two groups of diets. However, it was rather surprising to note that it was the high energy diets which were poorly utilised. This would indicate that excessive energy has an inhibitory effect on protein utilisation. One possibility is that dietary lipid interfered with protein absorption, though this is unlikely in view of the data given by Kitamikado et al. (1964b), who showed that fats have little effect on protein digestibility, whereas the same is not true for starch. If this is correct, a lower digestibility could have been expected for the protein in the LE diets, which contained relatively large quantities of potato starch.

Another influencing factor may have been the high levels of glucose in the HE diets. Luquet et al. (1975) showed that high levels of digestible carbohydrate

(sucrose) led to reduced weight gains with 35% protein diets, and Alliot et al. (1978) working with sea bass, noted poor growth and high liver glycogen with elevated carbohydrate levels. Austreng et al. (1977) have also noted a decrease in protein deposition with high dietary levels of digestible carbohydrate, whilst Ringrose (1971) has concluded that energy levels above 75 Kcal (313 kJ) kg/% protein tend to reduce weight gain and, therefore, it may be inferred, protein retention. In this series of experiments, all the diets were above this energy level, though Ringrose's data may still be taken as an indication that supra-optimal energy levels have a detrimental effect on protein deposition. On the other hand, many workers have found that high lipid levels (the HE diets contained 20%) are not detrimental to a wide variety of fish species, and in fact recommend such quantities (Higashi et al., 1964; Takeda et al., 1975; Takeuchi et al., 1978; Watanabe et al., 1978). Thus the 20% lipid in the HE diets may have been expected to have a beneficial effect, though when compounded with the large dietary quantities of digestible carbohydrates, the overall effect may have been detrimental. This argument is supported by the fact that the LE and HE regressions tended to diverge as protein level increased and differences in dietary lipid became maximal.

Interestingly, the pattern was almost identical when the diets were fed at 1% of the fish body weight. As the daily energy intake at this rate was reduced, it has to

be concluded that the effect of protein to energy ratios on protein deposition is at least in part independent of the total quantities of protein and energy absorbed, though for both LE and HE groups deposition was lower at equivalent nitrogen intakes when feed intake (and therefore energy intake) was reduced. The effect of daily energy intake is especially noticable in the slopes of the regression lines, true NPU decreasing at a much greater rate when the feeding rate was restricted to 1%, indicating that the energy intake was a major limitation at high dietary protein levels.

The data presented here regarding the optimum dietary protein level is also worth noting, in that it highlights some of the difficulties in assessing this value, and the advantage of using NNV (or NPV) in its determination. Essentially four optima were found, depending on the diet and the feeding level, and each one was a true optimum for that group of diets at that particular feeding level, and superficially it would appear that high energy diets give a lower optimum protein level. However, the true optimal protein level should be defined as the amount that provides the largest quantity of protein which is available to the animal for maintenance and growth, that is, the greatest NPV (this optimum is not necessarily the same as that which gives the greatest growth). On this basis, the optimum lay within the low energy groups of diets at the 2% feeding level. However, at a different

feeding level, or with different diet formulations, it is very possible that a lower optimum could be achieved, and so the figure given here should not be taken as an absolute value.

The 1% feeding level experiment did not produce results which correlated with the ENE experiment where casein was fed at a rate of 1% of the fish body weight per day. In that experiment, NPU remained constant regardless of N intake and it was postulated that this could have been due to the low feeding level/energy intake. Hence, the reason for those results is still unexplained, but it is likely that the straight line fitted to the points, although a good fit, was not a true representation of the relationship between N intake and N retained, and that a curvilinear response may have been a better description. This could also explain why some true NPU's found were greater than 1.0 since they were calculated from an ENE determined from this assumed straight line.

Overall, the results presented here show that the decreases in true NPU with increasing dietary levels of several novel proteins was not caused, in the main, by limitations in the dietary energy level and that in fact NPU tends to decrease at a greater rate if energy level is raised significantly.

CHAPTER 3

Growth Experiments

3.1 Experiment V - The Determination of Protein Efficiency Ratio, Net Protein Utilisation, Biological Value and True Digestibility of some Novel Proteins in Rainbow Trout Diets

3.1.1 Introduction

As mentioned in Section 1.6, there have now been several studies in recent years on the possibility of replacing the fishmeal component of fish feeds by various lower cost or more readily available ingredients such as plant or novel protein materials. However, most previous work has concentrated on the partial replacement of fishmeal, which makes interpretation of the basic nutritional value of the supplementary protein rather difficult. The present investigation was therefore designed to evaluate the test proteins as the sole protein sources in various semi-purified rations, so that comparative digestibility, PER, NPU and BV values could be ascertained. The ingredients under investigation comprise the methanophile bacterium (MB), the petroyeast (PY), the Spirulina algal protein (SA), the brewers yeast (BY), and the extracted soyabean protein (SY), with Fishmeal (HM) and casein (CS) as controls.

3.1.2 Materials and Methods

The experiment was conducted in three identical water recycling systems (Appendix X), each one consisting of eight 40-litre circular tanks supplied with 2.0 l/min of biologically filtered water, maintained at $12^{o} \pm 0.5^{o}C$. A constant 0.5 l/min of fresh water was added to each system in order to replace evaporation and leakage losses and also in order to maintain nitrate levels below 20 ppm. Ammonia was efficiently removed by the filter, with unionised ammonia always lower than 0.01 ppm. The pH remained at 7.1 ± 0.3. The photoperiod regime was 12 hL/12 hD.

Twenty-five rainbow trout of approximately 30 gm weight, obtained from Burwarton Trout Farm, Salop, were stocked into each tank. They were then fed for 2 weeks with a commercial diet in order to acclimate them to the system. At the end of this period, they were batch weighed, allowing 20 sec. for the water to drain from them before placing them in a tared bucket of water. During the experiment, the fish were weighed at bi-weekly intervals. At the end of the experiment, three fish were removed from each treatment and taken for carcass analysis.

The semi-purified diets (Table 26) were formulated to be isonitrogenous and isocalorific (on a digestible energy basis). The dietary protein level was set at 30%

TABLE 26 - Formulation of Diets for Experiment V

	PY	MB	SA	HM	SY	BY	CS	Z
Test meal[(a)]	53.75	42.02	52.28	40.38	47.17	73.53	38.23	-
Cod liver oil	13.55	13.27	12.27	13.22	14.76	13.76	15.00	23.00
Mineral mix (App. V)	5.53	7.54	6.27	7.73	9.21	2.24	7.75	11.50
Glucose	5.00	5.00	5.00	5.00	5.00	5.00	5.00	10.00
Potato starch	13.99	25.20	18.25	26.64	16.39	-	28.11	4.84
Dextrin	-	-	-	-	-	-	-	32.16
α-cellulose	7.87	7.87	7.87	7.87	7.87	7.87	7.87	16.00
Binder [(b)]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Vitamin mix (App. VI)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Chromic oxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
TOTAL	102.19	103.40	104.40	103.34	102.90	104.90	104.46	100.00
TOTAL D.M.	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Calculated digestible energy value 14.00 kJ/g for each diet

(a) PY = Petroyeast; MB = methanophilic bacterium; SA = dried Spirulina alga; HM = herring meal; SY = extracted soybean; BY = brewers yeast; CS = commercial casein; Z = zero protein

(b) Sodium carboxymethylcellulose (high viscosity)

as this level appeared to give optimal PER values in previous studies (Matty and Smith, 1978) and also in an attempt to minimise the utilisation of protein as an energy source by the fish. Total ash was balanced out by the addition of mineral mix (Appendix V) to a level of 12% of the dry diet. Nitrogen Free Extract (NFE) was balanced by the addition of starch and the ether extract (EE) by supplementation with cod liver oil. The vitamin mix was as described in Appendix VI. Chromic oxide was included in the diet in order to obtain digestibility data by the method of Furukawa and Tsukahara (1966) used previously. A zero protein diet was formulated to contain the same energy value as the test diets though some of the cod liver oil was lost from the diet during preparation. The diets were pelletised as described in Appendix VII. Triplicate groups of fish were fed each diet "ad libitum" twice a day but were not fed on the day prior to weighing. The amount of food consumed each day was noted.

Diets and fish were analysed for protein by the micro-Kjeldahl method (Horowitz, 1970) using the conversion factor N x 6.25; for fat by ether extraction; for ash by incineration at 550°C and for moisture by oven drying at 105°C to constant weight.

Experimental results were analysed statistically by the Multiple range test of Duncan (1955).

3.1.3 Results

(a) Average Weight Gain

The change in the average weight of the fish during the experiment is shown in Fig. 11. The groups on the herring meal (HM) and petroyeast (PY) diets grew quickly, with the commercial casein (CS) showing equivalent growth for the first four weeks, though this declined towards the end of the experiment. Fish fed the dried Spirulina alga (SA) and methanophilic bacterium (MB) diets grew at approximately the same rate, this being lower than with the HM, PY or CS diets. Brewers yeast (BY) and extracted soyabean (SY) feeds produced very poor growth.

Fish fed the non-protein diet were able to maintain their weight for about two weeks, probably due to the accumulation of fat, though they lost weight as expected during the rest of the experiment.

(b) Food Consumption

The quantity of dry food consumed was calculated as a percentage of the mean wet body weight of each tank of fish at the mid-point of every feeding period. The results, together with statistical analysis, are shown in Table 27.

Fig.11. Change in average fish weight during Experiment V.

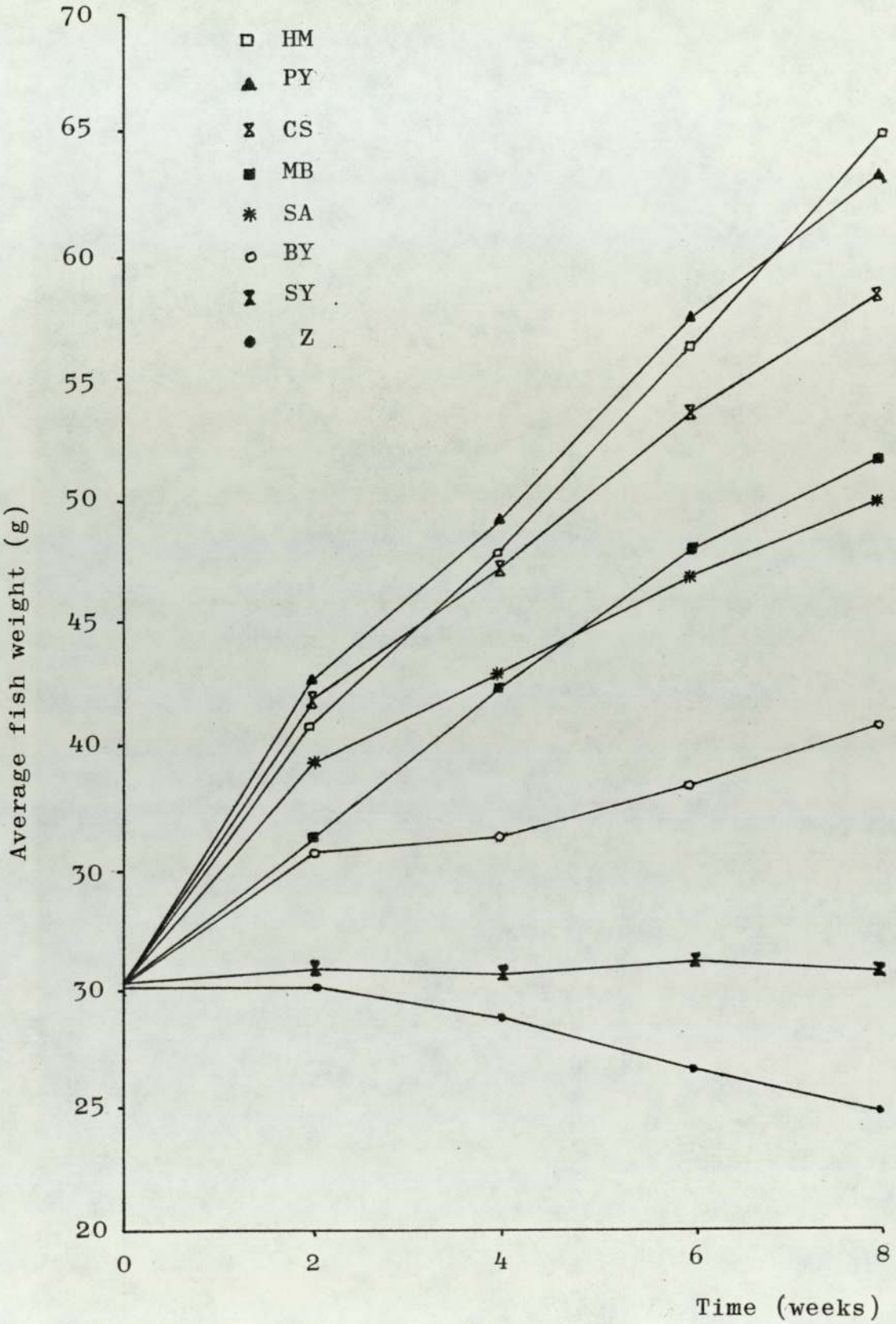


TABLE 27 - Food Consumption Rates during Experiment V

	DIET FED							
	PY	MB	SA	HM	SY	BY	CS	Z
Consumption	2.07^{b}	2.01^{b}	2.15^{b}	2.47^{a}	1.52^{c}	1.72^{c}	1.98^{b}	1.27^{d}

Figures with common superscripts are insignificantly different ($p < 0.05$). Data expressed as weight of dry food consumed per 100g fish/day.

TABLE 28 - Body Composition of Fish at end of Experiment V

	DIET FED							
	PY	MB	SA	HM	SY	BY	CS	Z
Body Composition								
Water	71.24^{e}	74.93^{g}	72.83^{ef}	71.89^{ef}	73.64^{fg}	72.90^{ef}	72.28^{ef}	77.92^{h}
Protein	15.49^{i}	15.42^{i}	15.20^{i}	15.30^{i}	14.11^{i}	14.69^{i}	15.13^{i}	12.22^{j}
Ether Extract	9.75^{m}	5.91^{k}	8.29^{m}	9.17^{m}	8.42^{m}	8.61^{m}	8.95^{m}	6.11^{k}

Figures with common superscripts are insignificantly different ($p < 0.05$). All figures expressed as percentage of wet body weight.

Since all the diets were essentially isocalorific, the food consumption data can be an indication of the palatability of the various diets, if all other parameters are equal, though any differences may be attributed to ingredients other than the proteinaceous portion.

Trout presented with the fishmeal based diet showed the highest food intake, significantly higher than those for the SA, PY and MB diets, although the consumption of these latter three feeds was still considered to be acceptable. The very low food intake of fish given the BY and SY formulations in part explains the poor growth associated with these rations.

(c) Body Composition

Data for the nine fish analysed on each regime was pooled in order to evaluate differences between treatments (Table 28). Body ash, and therefore NFE, were not included in the analysis as ash is normally considered to be a relatively stable parameter of fish (Phillips et al., 1966; Cowey et al., 1974; Elliot, 1976; Dabrowska and Wojno, 1977; Yu et al., 1977).

Feeding a zero protein diet led to fish having a significantly higher body water and lower body protein content than any of the other groups. Apart from this group which would have been losing body protein as endogenous nitrogen excretions, carcass protein level

did not vary with dietary treatment. This is in agreement with the findings of Cowey et al. (1974) on plaice. The main differences in body composition were observed in the fat and moisture components which varied inversely within each group, as has been noted before (Kausch and Ballion-Cusmano, 1976; Dabrowska and Wojno, 1977; Grayton and Beamish, 1977; Murray et al., 1977). Of special interest was the low fat content of fish fed the bacterial protein. The reason for this was not apparent as dietary fat content and feed intake were similar to other groups which exhibited a relatively higher fat content. The low carcass fat of the non-protein fed group can be attributed to their very low feed intake during the latter part of the experiment.

(d) Protein Efficiency Ratio (PER)

The PER's are shown in Table 29. The value for soybean was not included as it was negative and thus of little significance. The HM, PY and CS diets gave the highest figures, being in the region of 1.91 - 2.01. The MB protein showed an intermediate value at 1.62 with BY the poorest at 1.17. It has to be considered that in fish, as with other animals, PER tends to vary with protein intake (Ogino and Saito, 1970; Cowey et al., 1972) and as in this experiment some of the diets were consumed less readily than others, the PER values are less comparable to one another than if a fixed feeding regime had been employed. Despite this, the PER's for

CS, HM and SY diets are fairly comparable to those found by Nose (1971) where casein, white fish meal and soybean had values of 2.2, 2.6 and -0.53 respectively. However, the PER for casein was lower than that reported by Ogino et al. (1976) which was about 4.0 at a 30% protein level.

(e) Digestibility

Since a non-protein diet was included in the trial, it was possible to calculate the metabolic faecal nitrogen (MFN) and hence the true digestibility of the dietary protein under test. The metabolic faecal nitrogen amounted to 176 mg N/100g of diet consumed. This is slightly higher than the 50 - 150 mg N/100g diet reported by Nose (1967) but very similar to the figure reported in Experiment I.

The casein protein was extremely well assimilated (98.7%), the digestibility being similar to the values determined by Nose (1967) for trout and Ogino and Chen (1973b) for carp. The MB, PY and FM proteins were also well absorbed. The good digestibility of PY proteins has been ascribed to the presence of chitin decomposing bacteria in the guts of fish (Minami et al., 1972). Indeed both plaice and carp also digest petroprotein yeast as well as the trout did in this experiment (Cowey et al., 1974; Ogino and Chen, 1973b).

The two plant proteins tested varied markedly in their digestibilities. The dried Spirulina alga (SA diet) gave a high value of the same order as brewers yeast. This was probably due in some degree to the disintegration of cell walls during its manufacture. For a similar reason, a higher digestibility could have been expected for the soybean meal, but the low value found suggests that the trypsin inhibitor normally present in this material had not been completely removed during the protein extraction procedure. Previous workers using various soybean meals have reported significantly higher values than that found here (Kitamikado et al., 1964a; Nose, 1967; Nose, 1971).

(f) Net Protein Utilisation

The true net protein utilisation (NPU) was calculated using the formula of Bender and Miller (1953) employing the body analysis data from Table 28.

As above, a value was calculated for each tank of fish and statistical analysis applied (Table 29). It can be seen that some degree of variation between replicates led to large standard errors and so small differences in NPU could not be detected statistically. Hence, the PY, CS, MB and HM proteins were found to be insignificantly different from one another, but were significantly higher than the BY and SA proteins. The soybean gave the lowest value (0.18), highlighting its poor digestibility.

TABLE 29 - Protein Utilisation Data (Experiment V)

Protein \ Index	PER	True Digestibility	NPU	BV
PY	2.01^{p}	91.6	0.42^{s}	0.46^{y}
MB	1.62^{q}	93.5	0.37^{s}	0.40^{xy}
SA	1.33^{r}	83.1	0.32^{t}	0.38^{x}
HM	1.91^{p}	91.2	0.38^{s}	0.41^{xy}
SY	-	43.6	0.18^{u}	0.41^{xy}
BY	1.17	79.9	0.30^{t}	0.38^{x}
CS	1.97^{p}	98.7	0.40^{s}	0.41^{xy}

Figures with common superscripts are insignificantly different ($p < 0.05$)

In general, the results are comparable with those found by Cowey et al. (1974) with plaice but much lower than those calculated from the data of Ogino and Chen (1973c) with carp.

(g) Biological Value

The biological values (BV) were calculated from the NPU and digestibility data where BV equals the NPU divided by the protein digestibility x 100. The results of this calculation are shown in Table 30.

The BV's were remarkably similar; the only significant difference that could be detected was between the PY and BY and SA proteins. Here again, the results were much lower than those reported by Ogino and Chen (1973c) in carp.

3.1.4 Discussion

One of the main problems encountered during protein evaluation, especially in fish, is the large effect dietary and environmental conditions have on the results obtained. In this trial, an attempt was made to overcome some of these difficulties by maintaining a similar stable environment for all fish and by using iso-nitrogenous and iso-energetic diets. However, the "ad libitum" method of feeding employed (in order to obtain data on diet acceptability) did lead to some difficulty in comparing the results for different protein sources. This is seen most obviously in the NPU's and BV's. For instance, the soybean protein which gave very poor growth showed a biological value very similar to those of the higher quality proteins tested, whereas Ogino and Chen (1973c) have demonstrated that differences in BV exist even at the high protein levels used in fish diets. The explanation for the similarity in BV values found here appears to be linked to the low intake and poor digestibility of this protein. This meant that, over a given period, fish fed this diet metabolised less absorbed

nitrogen when compared to those given a more acceptable and digestible diet. The overall effect was therefore akin to feeding non-isonitrogenous diets which has a consequent effect on NPU and BV values, the latter increasing as protein level (or absorbed protein) decreases as discussed previously. A fixed feeding regime would have counteracted this to some extent but is impractical when one diet is very unacceptable to the fish. However, even if this were possible, the digestibility effect would probably still be apparent in the protein utilisation values obtained.

Added to this, the ingredients and diets in this study were analysed by the Kjeldahl technique using the conversion factor N x 6.25. Since most SCP's contain high levels of non-protein nitrogen (mainly nucleic acids), the utilisation of true protein may well have been much higher, especially for the petroyeast and bacterial diets.

In Table 30, an attempt has been made to compare the biological values of the test proteins with various chemical predictions of protein quality, calculated from the amino acid profiles given in Appendix II. Only one group, the chemical scores based on whole egg protein, gave results which were comparable in magnitude to the biological values, all the others being somewhat high. Comparisons of ranking of the test proteins is rather

TABLE 30 - Comparison of the BV's Found With Various Chemical Predictions of Protein Quality

BV	EAAI(1)	CS(2)	CS(3)	CS(4)
PY^{y} (0.46)	HM (74)	HM (58)	HM(110)	HM (93)
HM^{xy} (0.41)	SY (71)	MB (47)	MB (87)	MB (75)
SY^{xy} (0.41)	PY (68)	CS (46)	SY (82)	PY (63)
CS^{xy} (0.41)	SA (68)	SA (42)	PY (80)	SY (63)
MB^{xy} (0.40)	MB (66)	PY (40)	CS (74)	BY (54)
SA^{x} (0.38)	BY (64)	SY (40)	SA (70)	CS (53)
BY^{x} (0.38)	CS (61)	BY (34)	BY (70)	SA (53)

Chemical predictions taken from Appendix III.

(1) Essential Amino Acid Index - reference, whole egg protein

(2) Chemical score - reference, whole egg protein

(3) Chemical score - reference, amino acid requirements of carp (Nose, 1978)

(4) Chemical score - reference, amino acid requirements of chinook salmon (Mertze, 1969)

fruitless as many of the BV's found were insignificantly different statistically and therefore have to be ranked in groups rather than individually. However, it should be noted that fishmeal did not perform as well as all the chemical analyses predicted. In general, a lowering of the intake of nitrogen by the fish would appear necessary in order to achieve a closer correlation between

biological value and most chemical indices of protein quality. This could be achieved either by decreasing the dietary protein level or by restricted feeding (provided the daily energy requirement was met). However, any results obtained with an extremely limited protein intake would be of less value in trials such as the one presented here where the purpose was to evaluate the proteins in semi-practical fish rations.

The two plant proteins tested were not well utilised by the trout. The low value of soybean protein found here agrees with the results of several other workers with various fish species (Nose, 1971; Andrews and Page, 1974; Reichle and Wunder, 1974; Viola, 1975; Koops et al., 1976). Andrews and Page (1974) attribute this to the absence of growth factors in soybean which are present in fish meal, but in this experiment it appeared to be due to a combination of the low food intake and digestibility. On the other hand, the algal diet was comparatively well accepted and digested, but the fish given this diet still showed poor growth, indicating an amino acid limitation. Another alga (Scendesmus sp.) has been shown to be quite a useful ingredient in carp rations (Meske and Pruss, 1977; Reimers and Meske, 1977) though the results are not directly comparable due to the differences in the digestive systems of carp and trout. The alga used here had the side effect of producing good colouration in the fish, probably due to

its high level of plant pigments. The brewers yeast, which is a common ingredient in commercial feeds, appears to have a low nutritional value for trout.

3.2 Experiment VI - The Determination of Protein Efficiency Ratio, Net Protein Utilisation, Biological Value and True Digestibility of Some Novel Proteins in Carp Diets.

3.2.1 Introduction

Compared to the amount of work on salmonids, the replacement of fishmeal in the diets of warm water species has received little attention. This is probably because complete compounded rations are not commonly used in the commercial culture of such fish, though the trend towards the intensive farming of species such as carp in thermal effluents must eventually lead to the production of specialised compounded feeds.

In nature carp are omnivorous, and hence their digestive system is adapted to suit that feeding habit. It therefore seems possible that they may be able to utilise protein sources of a quality somewhat inferior to those normally included in trout and salmon rations, a subject that has provoked a little research (Terao, 1960; Hoshiai, 1972; Ogino and Chen, 1973b, 1973c; Viola, 1975; Meske and Pruss, 1977; Hepher et al. 1978; Omnae et al., 1978). However, as for trout, there is little definitive information on the basic nutritional value of various proteins in carp feeds. Hence the following experiment was designed in order to evaluate the degree of utilisation of some novel proteins when

used as the sole protein source in semi-purified rations. It was envisaged that the results of this trial could be compared to those obtained previously with trout to determine whether species differences affect absolute and relative nutritive values.

3.2.2 Materials and Methods

Fingerling mirror carp (Cyprinus carpio L., obtained from the Cotswold Carp Farm, Gloucestershire, England) were graded so that the experimental fish were as similar in size as possible. 10 fish were stocked in each tank of system RS 2 (Appendix XI) and acclimated to the experimental conditions for two weeks, being fed for this time on a commercial trout ration (BP Nutrition Ltd.). Prior to the experiment, they were batched weighed, allowing 20 seconds for the water to drain from them before placing them in a tared bucket of water. Throughout the experiment, they were weighed in this way every 10 days, no feed being provided on the day prior to weighing. At the end of the trial, 2 fish were taken from each tank for carcass analysis.

The protein sources to be tested were the BP Petroyeast (Toprina), ICI methanophilic bacterium ("Pruteen"), Spirulina alga, and the extracted soybean protein concentrate ("Newprod") from the same batches as those used in Experiment V. The control diets consisted

of commercial grade casein and herring meal respectively. A low protein diet (10% casein) was also included so that Biological Value (BV) and Net Protein Utilisation (NPU) values could be obtained by the method of Bender and Miller (1953) and also that the true digestibilities of the various dietary proteins could be evaluated by the Furukawa and Tsukahara method (1966). Whilst a non-protein diet could possibly have been used, such a diet had posed acceptability problems in the previous trout experiment.

The diets (Table 31) were formulated to be similar, though not identical, to those used in Experiment V being isonitrogenous (30% protein) and as far as possible isoenergetic on a digestible energy basis. Although the protein level was below the 38% recommended for carp (Sin, 1973a) it was used in an attempt to minimise the utilisation of protein for energy and also so that some degree of comparison could be made between the results obtained for carp and trout.

The diets were prepared in the usual way (Appendix VII). Unfortunately later proximate analysis showed that an error in formulation had led to the fishmeal control having a lower protein content, and the bacterial diet a higher protein content than intended. The effects of this error are considered in the discussion of the results.

TABLE 31 - Formulation of Diets for Experiment VI

	CS LOW	CS	PY	MB	SA	HM	SY
Test Meal[(a)]	11.13	33.78	51.56	38.62	47.84	37.03	44.27
Caplin oil	20.00	15.00	13.55	13.27	12.27	13.22	14.76
Mineral mix	10.25	7.75	5.53	7.54	6.27	7.73	9.21
Binder[(b)]	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Vitamin mix	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Glucose	20.15	5.00	5.00	5.00	5.00	5.00	5.00
Starch	28.10	28.10	13.99	25.20	18.25	26.64	16.39
α-cellulose	7.87	7.87	7.87	7.87	7.87	7.87	7.87
Chromic oxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50
	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Proximate Analysis of Diets (% of Dry Diet)

	CS LOW	CS	PY	MB	SA	HM	SY
Crude Protein	10.30	30.24	30.84	35.19	30.21	25.08	29.93
Ether extract	18.22	15.77	14.23	16.17	14.24	13.69	16.01

(a) On a moisture free basis.
CS LOW = Low casein; CS = casein; PY = petroyeast; MB = methanophilic bacterium; SA = Spirulina alga; HM = herring meal; SY = soyabean meal.

(b) Binder - sodium carboxymethylcellulose (high viscosity)

Due to the inherent problems in feeding carp to a true satiation level, they were fed on a fixed feeding regime of 4% of their body weight (dry food/whole fish) per day, divided into 4 equal feeds, though with certain diets the fish often showed signs of being unwilling to consume the whole days ration. If this occurred, they were fed only the amount they would eat within a 30 minute feeding period. Each diet was fed to duplicate tanks of fish. All statistical analysis was carried by the multiple range method of Duncan (1955).

3.2.3 Results

(a) Growth

The increase in average weight of fish throughout the experiment is shown in Fig. 12, whilst the average specific growth rate for the whole period is tabulated in Table 32. Statistical analysis showed that the methanophilic bacteria (MB) gave significantly the highest growth, followed by the casein (CS) and herring meal (HM) diets. The petroyeast (PY) diet gave a significantly lower growth than the above feeds but higher than the soyabean (SY) and algal (SA) diets. Fish on these latter two diets grew no faster than those on the 10% casein protein feed.

Fig.12. Change in average fish weight during Experiment VI

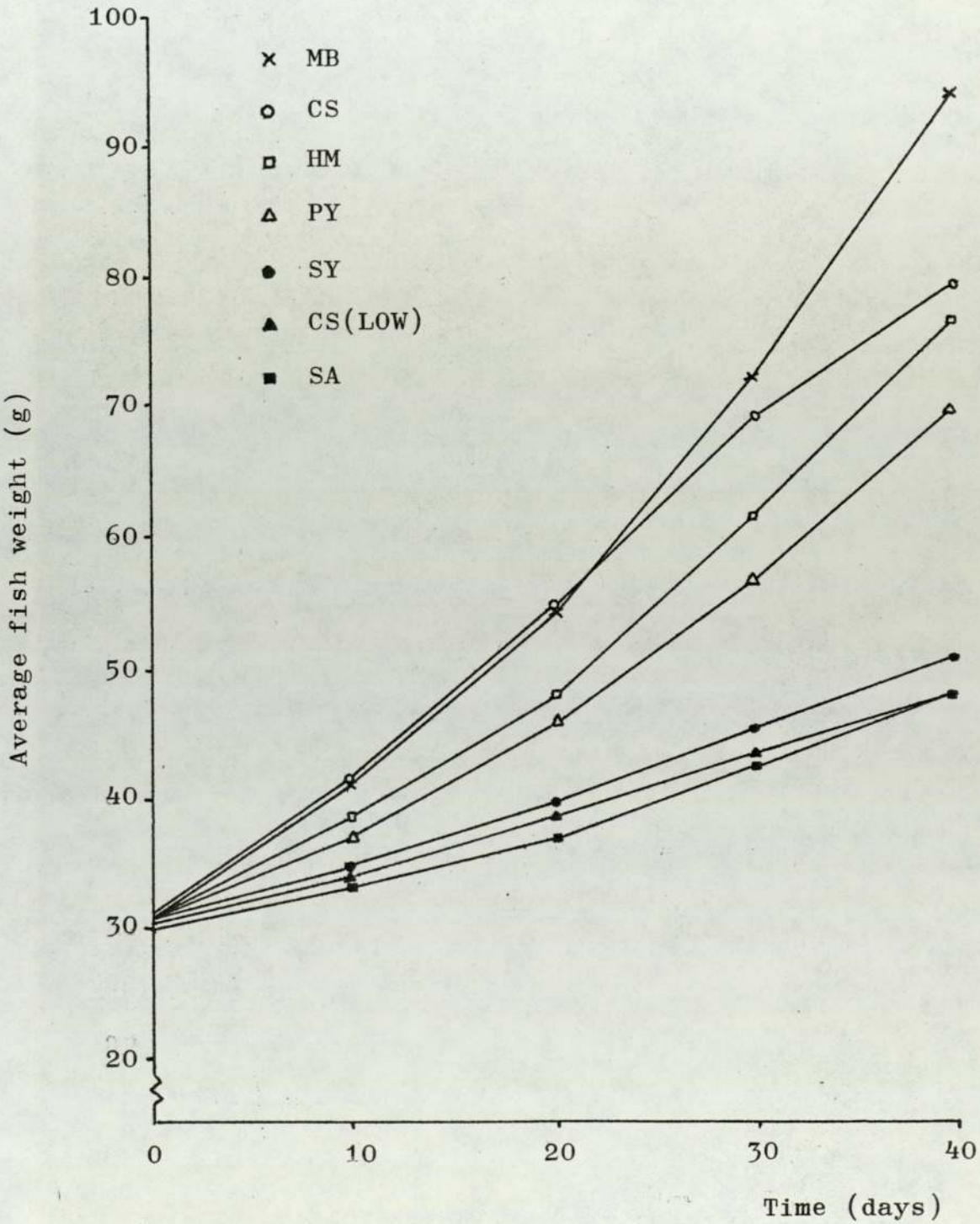


TABLE 32 - Average Specific Growth Rate during Experiment VI

Dietary Treatment	Specific Growth Rate (%/day)
CS (LOW)	1.13 [a]
SA	1.18 [a]
SY	1.24 [a]
PY	2.08 [b]
HM	2.26 [c]
CS (HIGH)	2.40 [c]
MB	2.78 [d]

* Figures with common superscripts are insignificantly different $P = < 0.05$.

Of interest was the gradual decline in the growth rate of the casein control group, this being especially notable from days 30 - 40. The reason for this is not immediately apparent, but the same effect was noted in the previous trout experiment. Growth was practically constant throughout the experiment for all the other treatments.

(b) Body Composition

The gross body composition (Table 33) was little affected by the dietary regime except in the group fed the low protein diet. These fish showed a slightly higher body fat content due to the high level of lipid

in the diet. Consequently body water was also lower than for the other groups, body fat and water being inversely related as has been noted in previous experiments (Kausch and Ballion-Cusmano, 1976; Dabrowska and Wojno; 1977; Grayton and Beamish, 1977; Murray et al., 1977). This low protein group, together with those given the algal protein also exhibited a slightly lower protein content than the herring meal controls. Body ash of the carp was unaffected by the dietary regime, as has been noted with other fish species (Phillips et al., 1966; Cowey et al., 1974; Elliot, 1976; Dabrowska and Wojno, 1977; Yu et al., 1977).

(c) Food Conversion

The bacterial protein diet gave the best food conversion (Table 34), though this can be ascribed to its relatively higher protein content in comparison to the other diets. The yeast, herring meal and casein feeds also allowed good conversions, but the soyabean and algal diets proved to be very poor in this respect. Hoshiai (1972) has reported a food conversion of 1.11 for a petroyeast based carp diet, slightly better than that found here, whilst the casein value is very similar to that found by Ogino and Saito (1970).

TABLE 33 - Gross Body Composition of Carp at the End of Experiment VI

Dietary Treatment	Body Composition % *			
	Water	Fat	Protein	Ash
CS (LOW)	72.80^{c}	9.46^{d}	12.10^{g}	3.19^{h}
HM	76.85^{ab}	5.25^{e}	13.84^{f}	2.90^{h}
MB	76.83^{ab}	5.90^{e}	13.24^{fg}	3.19^{h}
CS (HIGH)	75.99^{ab}	5.72^{e}	13.54^{fg}	2.96^{h}
PY	75.99^{b}	6.84^{e}	13.20^{fg}	3.02^{h}
SY	76.82^{ab}	6.34^{e}	13.02^{fg}	3.00^{h}
SA	77.32^{a}	6.18^{e}	12.32^{g}	3.04^{h}

* Figures in each column with common superscripts are insignificantly different. ($P < 0.05$)

TABLE 34 - Protein Evaluation Data from Experiment VI

Dietary Treatment	Food Conversion	PER	NPU	Digestibility	BV
CS (LOW)	3.04^{a}	3.24^{e}	-	-	-
HM	1.42^{c}	2.82^{f}	0.64^{j}	80.3	0.79^{n}
MB	1.14^{d}	2.54^{g}	0.49^{k}	95.5	0.52^{p}
CS (HIGH)	1.39^{c}	2.48^{g}	0.49^{k}	93.0	0.52^{p}
PY	1.55^{c}	2.08^{h}	0.47^{k}	96.6	0.49^{q}
SY	2.86^{a}	1.35^{i}	0.42^{l}	83.7	0.51^{pq}
SA	2.50^{b}	1.15^{i}	0.36^{m}	87.1	0.41^{r}

* Figures in each column with common superscripts are insignificantly different ($P = < 0.05$)

(d) Protein Efficiency Ratio (PER)

Average PER values for the various diets are shown in Table 34. The low protein feed gave a very high PER, which was to be expected as Ogino and Saito (1970) have shown that in carp PER increases as the dietary protein level decreases. In fact the absolute values noted for both the high and low level casein diets in this experiment are very similar to those reported by Ogino and Saito (1970).

Both the plant proteins, Spirulina and soyabean, gave rather poor ratios (1.15 and 1.35 respectively), significantly lower than that for the petroyeast (2.08). The bacterial diet was found to be comparable to the casein control but neither performed as well as the fishmeal feed.

(e) Net Protein Utilisation (NPU)

A true NPU value was calculated, for each protein, from the body analysis data in Table 33, assuming the low casein diet to have a NPU of 100. Since the body composition of the various groups of fish was fairly stable, the results in general reflected the PER values, with the Spirulina and soyabean exhibiting the lowest values (0.36 and 0.42 respectively). The petroyeast (0.47), bacterial (0.49) and casein (0.49) had intermediate values whilst the herring meal showed significantly the highest degree of utilisation (0.64).

The value for casein is higher than that found by Ogino and Saito (1970) of about 0.40 at a similar protein level, and all diets exhibited higher values than those reported by Sin (1973b) for a compounded ration.

(f) Digestibility

The true digestibility of each protein (Table 34) was determined by analysis of faecal samples obtained by hand stripping the contents of the terminal portion of the intestine of each fish, these being pooled in order to obtain sufficient material for analysis. The protein of the low casein diet was assumed to be 100% digestible, which thus allowed the metabolic faecal nitrogen to be calculated. This was found to be 217 mg N/100g of diet fed, which is a little higher than that reported by Ogino and Chen (1973a) of 170 mg N on a non-protein diet and 144 mg N with protein containing diets. This may be an indication that the 100% digestibility assigned to the diet was incorrect. In fact, if the 93% value found for the higher casein level is used then the MFN becomes comparable to those previously reported. However, for the purposes of this experiment, such small differences are of little significance.

Most of the test proteins showed good digestibility, the petroyeast and bacterial proteins being exceptionally well assimilated at 96.6 and 95.5% respectively. The plant proteins showed good but comparatively lower

digestibility, though both these proteins were more available than the fish meal protein. The value for Spirulina is similar to that reported by Hepher et al., (1978).

The poor absorption of the fish meal protein can be ascribed to the rather coarse nature of the meal used in the feed.

(g) Biological Value (BV)

The degree of utilisation of absorbed protein (Biological Value, Table 34) was obtained by dividing each NPU x 100 by its respective digestibility. Most of the values thus obtained proved to be very similar, though statistically significant differences could be detected between certain of the protein sources. The Spirulina alga gave the lowest figure (0.41) and fish meal the highest (0.79).

3.2.4 Discussion

One problem in evaluating the results obtained lies with the varying protein contents of the diets used. For instance, since all the protein levels were below the optimum requirements of carp, the higher growth obtained with the bacterial diet in comparison to the herring meal can be ascribed to its higher protein content. Equivalent protein levels may have led to the bacterial protein giving growth more comparable to the casein and petroyeast diets whilst the herring meal would possibly have ranked somewhat higher.

Most of the other evaluation data can be considered in the same way, as protein level has a significant effect on its utilisation. Thus a 30% protein level in all of the diets may have resulted in the herring meal protein exhibiting lower and the bacterial protein higher PER and NPU and BV values than those found, though the extent to which this would tend to negate or reverse the differences found between these and the other proteins is difficult to judge. However, it seems likely that overall the bacterial protein would be ranked as being of intermediate value between casein and herring meal.

Diet palatability could not be measured in this experiment though it was found that it was for the most part impossible to feed the designated quantities of the soyabean and algal proteins to the fish, indicating

some acceptability problems. All the other diets proved to be highly acceptable.

An attempt was made to correlate the biological values obtained in the experiment with various chemical indices of protein utilisation calculated from the amino acid profiles of the test proteins (Appendix II). The results (Table 35) show that in absolute terms none of the chemical methods accurately predicted the biological values found. The explanation of this probably lies in the fact that the biological value of a protein varies with the level of that protein in the diet and also varies in relation to other dietary and environmental characteristics. This tends to overshadow any correlation with amino acid composition. So for any one protein source, there is, if all other parameters are stable, only one level of protein ingestion that would tend to give a biological value similar to that predicted by the chemical score.

However, as the biological values of various proteins appear to decrease at an equivalent rate (Ogino and Chen, 1973c) they should always be ranked in the same order provided they are fed at equivalent rates, and this ranking should essentially be dependent on their amino acid composition. In fact, Table 35 shows that all the chemical analysis methods used correctly predicted the herring meal as the best protein source but overall the chemical scores were more consistent with the experimental results

than the EAAI. The chemical score based on whole egg protein incorrectly placed the algal protein whilst that based on the amino acid requirement of carp misplaced the casein, and the chinook salmon score wrongly ranked the casein and algal proteins.

Since similar diet formulations were used in the previous experiment on trout (Experiment V), it is of interest to compare the protein utilisation data for the two species, though of course the effect of the slightly different formulations, feeding regimes and environmental conditions has to be considered. Similarly diet ingredients other than the proteinaceous portions, especially carbohydrates, are used to different extents by the two species and so the diets would not have been equivalent on a metabolic energy basis. For instance, carp can digest crude cellulose to a certain extent (Shcherbina and Kazlauskene, 1971) and also digest starch more efficiently than trout (Chiou and Ogino, 1975).

However, the comparison (Table 36) does produce some interesting results. The absolute PER values for fish meal, bacterial protein and casein are all higher than their corresponding values for trout whereas the petroyeast and algal proteins exhibited almost identical values. As some carp growth did occur on the soyabean diet, a positive PER could be determined for this protein whereas in trout a loss of weight led to a negative PER. In

TABLE 35 - Comparison of the BV's found with various chemical predictions of protein quality

BV Experimental	1 EAAI	2 Chemical Score	3 Chemical Score	4 Chemical Score
HM $(0.79)^{n}$	HM(0.74)	HM(0.58)	HM(1.10)	HM(0.93)
MB $(0.52)^{p}$	SY(0.71)	MB(0.47)	MB(0.87)	MB(0.75)
CS $(0.52)^{p}$	PY(0.68)	CS(0.46)	SY(0.82)	SA(0.67)
SY $(0.51)^{pq}$	SA(0.68)	SA(0.42)	PY(0.80)	SY(0.63)
PY $(0.49)^{q}$	MB(0.66)	SY(0.40)	CS(0.74)	PY(0.63)
SA $(0.41)^{r}$	CS(0.61)	PY(0.40)	SA(0.70)	CS(0.53)

1. Essential amino acid index - reference whole egg protein
2. Chemical score - reference whole egg protein
3. Chemical score - based on amino acid requirements of carp (Nose,1978)
4. Chemical score - based on amino acid requirements of chinook salmon (Mertz,1969)

TABLE 36 - Comparison of the results obtained with carp with those previously obtained with trout.*

Diet	PER		NPU		Digestibility		BV	
	Carp	Trout	Carp	Trout	Carp	Trout	Carp	Trout
HM	2.82	1.91	0.64	0.38	80.3	91.2	0.79	0.41
MB	2.54	1.62	0.49	0.37	95.5	93.5	0.52	0.40
CS (HIGH)	2.48	1.97	0.49	0.40	93.0	98.7	0.52	0.41
PY	2.08	2.01	0.47	0.42	96.6	91.6	0.49	0.46
SY	1.35	-	0.42	0.18	83.7	43.6	0.51	0.41
SA	1.15	1.33	0.36	0.32	87.1	83.1	0.51	0.38

* Trout data from Experiment V

general, the ranking of proteins according to their PER is fairly similar, though with trout petroyeast ranks similar to casein whilst in this carp trial, it was the bacterial protein that proved to be more closely related to the above control.

Again, as for PER values, the NPU's rank the proteins in roughly the same order of value for both species, though the absolute values are appreciably higher for carp, probably due to the dietary energy effect. Both plant proteins were poorest in quality for both carp and trout.

Digestibilities were also similar though soyabean protein was approximately twice as well digested by carp as by trout, whilst the herring meal was slightly less well assimilated.

Biological values for trout were remarkably constant, whilst for carp significant differences could be determined between many of the values, which again may be due to the greater available energy level of the carp diets.

As the total nitrogen digestibility was high for both these ingredients, then it appears that at least a portion of the nucleic acids are absorbed. If it is then assumed that these are not utilised to any great extent then the retention of true dietary protein

may have been appreciably greater than the values shown here. However, as there is a lack of information on the metabolic fate of large quantities of dietary nucleic acids and especially any protein sparing effect they may have, protein utilisation values based on crude protein analysis may be assumed to be a sufficiently close estimate of their true values.

CHAPTER 4

Demand Feeding Experiments

4.1 Experiment VII - Determination of the Palatability of some Novel Protein Rations by the Use of Demand Feeders

4.1.1 Introduction

In Experiment V, the differences in food intake noted between groups of fish fed by hand twice a day were taken as an indication of the palatability of the various rations. However, this is not strictly true. In order to determine palatability, two major restrictions have to be overcome. Firstly the food intake should be truly ad libitum, that is, regulated by the fish itself rather than by any restriction imposed by the experimenter, although it has been shown that twice daily hand feeding does appear to give near maximal food intake by trout (Grayton and Beamish, 1977). For terrestrial animals, ad libitum feeding can readily be achieved merely by making excess food available to the animal at all times. With fish, the problem is a little more complex as modern compounded feeds cannot be left in the tank for any length of time. This can be overcome by the use of demand feeders, where the fish are trained to press a lever which causes a small quantity of food to be dispersed into the tank. Such devices have now been used by several workers in order to investigate various aspects of the feeding habits of fish (Rozin and Mayer, 1961; Rozin and Mayer, 1964; Hepher, 1972; Adron et al.

1973; Landless, 1976). Hence these devices should enable ad libitum intake to be assessed, though of course it is possible that instigating a conditioned response imposes external limitations on the food intake.

The second limitation that has to be removed is that which links factors other than diet palatability to the voluntary food intake of the fish, such as variations in temperature, water quality, fish size, etc. This was achieved by Adron et al. (1973) who employed two demand feeders in one tank, each containing a different diet. Hence all environmental and physiological variables were nullified and only the acceptability of the two diets should have defined the amount of each that was taken, provided of course that there was no preference for one particular feeder. Adron et al. overcame this added problem by reversing the feeders during the trial. Using this procedure, they were able to show differences in acceptability between certain diets.

In the following series of experiments, a methodology similar to that of Adron et al. was used to determine the relative palatability of some novel protein based diets. The trials were deemed necessary as it seems possible that palatability limitations may affect the quantity of a feed ingredient that can be readily included in a commercial feed. However, it has to be

borne in mind that using semipurified rations ingredients other than the one under scrutiny may affect the palatability of the diet.

4.1.2 Materials and Methods

25 rainbow trout averaging 150.8g in weight taken from the main laboratory stock and which had previously been trained to use a demand feeder, were stocked in the 1m tank of the demand feeding system (Appendix XII). The water temperature was maintained at 8 ± 1°C and the photoperiod at 12 L/12 D. The triggers for the two demand feeders used were placed in the tank immediately adjacent to their respective feeders. Initially, the feeding mechanisms were placed diametrically opposite so that the two sides of the tank were as far as possible mirror images but it was noted that one feeder was consistently activated more frequently than the other. By a process of trial and error with a control diet (BP "Mainstream" Trout No. 4 floating) it was found that both feeders were used equally when placed close together as shown in Fig. 28 Appendix XII. The fish were then allowed to acclimate to the system for one week before the experiments commenced. The same group of fish were used throughout the trials because laboratory stocks of this sized fish were limited and replacement of a portion of the group could have caused social interaction problems leading to undesirable effects

on their demand feeding behaviour. For the same reason, mortalities were not replaced.

The iso-nitrogenous and iso-calorific diets used are shown in Table 37. They were pelletised in the usual manner (Appendix VII) though a 5 mm die was used to form the pellets. Since it was thought that colour rather than palatability preference could arise, the diets were made as far as possible iso-chromatic by the addition of sufficient quantities of a green dye (Edicol green, D.F. Stansted and Co., Billericay, Essex) so that all the diets were similar to the algal diet, which had the strongest natural colour. A small quantity of dye was also added to this diet in case it had any effect on palatability. Whilst every effort was made to produce diets which were of an identical texture, this was impossible to achieve due to the inherently different characteristics of the test proteins. All the diets were analysed in the usual way (Horowitz, 1970).

Originally it was planned to compare each of the test proteins to each of the others but unfortunately there proved to be insufficient time to achieve this. However, this basic method had been decided upon, rather than a simple comparison to a standard reference diet such as herring meal, since comparison to this fishmeal alone would not allow the novel proteins to be ranked against each other. For example, if in two separate

TABLE 37 - Formulation of Diets for Experiment VII

	PY	MB	SA	SY	HM
Test meal[a]	71.67	56.03	69.71	61.89	53.84
Cod liver oil	8.07	7.70	6.36	9.69	7.63
Mineral mix (App. V)	2.04	4.72	2.92	6.95	4.98
Dextrin	4.10	4.12	4.02	3.49	3.81
Starch	6.40	24.42	13.46	10.14	27.10
α-cellulose	9.58	9.58	9.58	9.58	9.58
Binder[b]	2.00	2.00	2.00	2.00	2.00
Vitamin mix (App. VI)	1.00	1.00	1.00	1.00	1.00
Total	104.86	109.57	109.05	106.54	109.94
Total D.M.	100.00	100.00	100.00	100.00	100.00

Proximate analysis (%)	PY	MB	SA	SY	HM
Protein (N x 6.25)	41.76	40.86	41.03	42.67	40.71
Lipid	9.36	10.92	9.73	11.53	9.19
Moisture	9.40	10.60	10.17	9.20	9.60

(a) All ingredients formulated on a "wet" basis

PY = petroyeast; MB = methanophilic bacterium;

SA = Spirulina alga; HM = herring meal; SY = soybean

(b) Sodium carboxymethylcellulose - high viscosity

trials, two diets, A and B, were only taken at half the rate of herring meal, this would not necessarily indicate that when compared to each other they would be consumed equally.

At the start of each 14 day trial, 100g of each of the two diets to be compared were placed in their respective feeders. The fish were then not disturbed until the following day when the amount of each diet consumed was calculated and the hoppers refilled. After 7 days, the food/feeder relationship was reversed. At both start and changeover times, a check was made to ensure that equal quantities of food were dispersed by each feeder for a single press of the trigger. This quantity averaged 0.3g.

4.1.3 Results

For each trial, the amounts of the two diets consumed on a dry weight basis were calculated each day as a percentage of the total food intake on that day and the results plotted in Figs. 13 - 19. The results were calculated in this way both to ease presentation and also to regulate any fluctuations in overall daily food consumption. No comparison was made between the total amount of food dispensed in separate trials since, as described before, this could have arisen for reasons other than differences in palatability.

Although the trials were run in such a way that no diet was used sequentially, this stricture could not be maintained at the end of the series, and the MB diet had to be used in successive trials. However, for clarity and ease of comparison, the experiments are reported in a more logical order. The broken vertical line on each figure shows the point at which the diets were reversed in their feeders.

(a) Herring Meal (HM) vs. Petroyeast (PY) diets - Fig. 13

The average daily consumption of fish meal diet was 46.6 and 56.2% of the total daily food intake before and after feeder interchange respectively. Apart from a sudden drop in the intake of HM diet just prior to changeover, probably caused by a feeder or trigger jamming, the intake remained relatively constant. The results would thus indicate that whilst there was no great difference in acceptability between the two diets, there was possibly a slight bias towards the herring meal.

(b) Herring Meal (HM) vs. Bacterial (MB) diets - Fig. 14

Here there appeared to be a definite preference for the fishmeal diet at least during the first seven days. On changing the feeders, the percentage of HM diet taken was reduced to about 50% of the total daily intake indicating that the fish had become accustomed to, and associated one trigger with, this diet and were still

Fig.13. Food preference trial - HM vs PY diets.

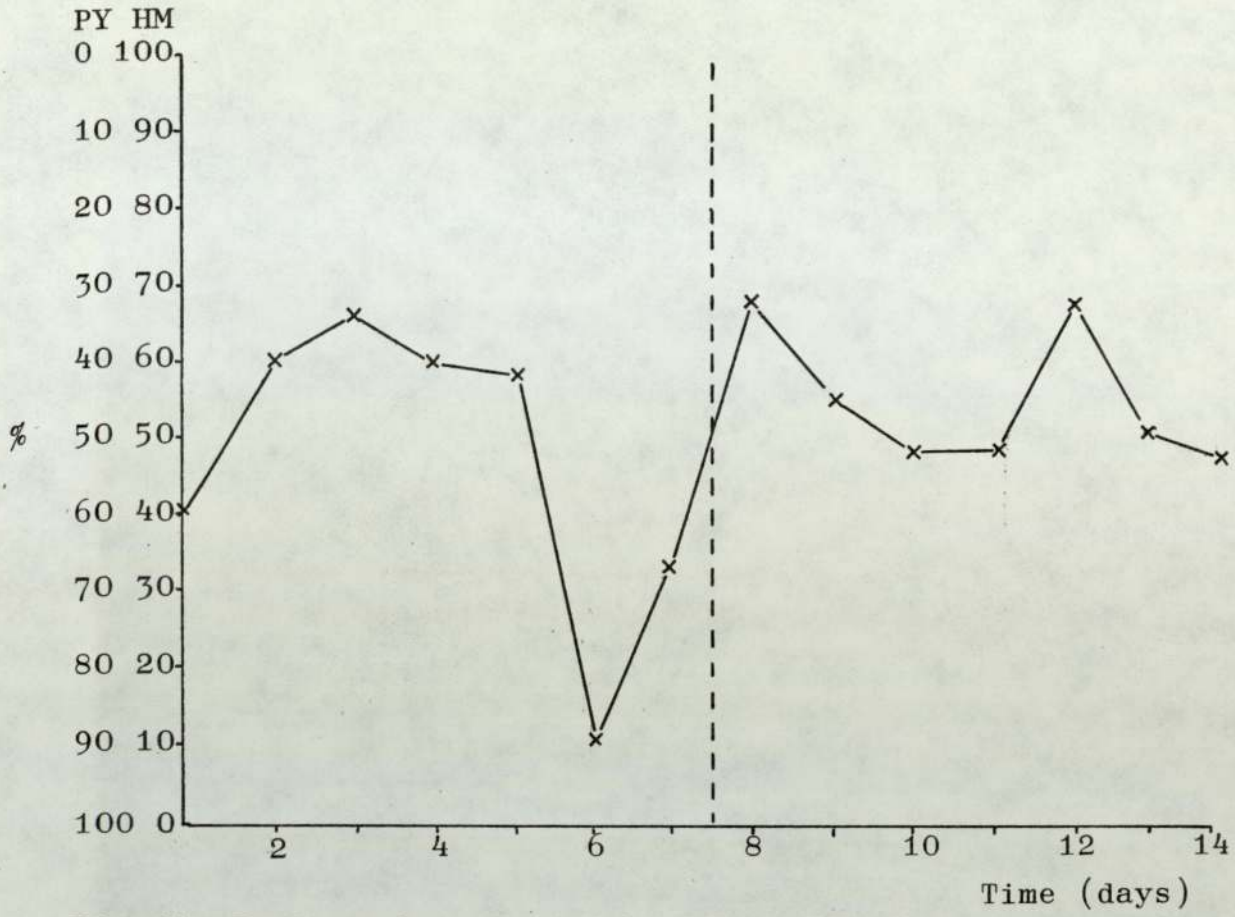
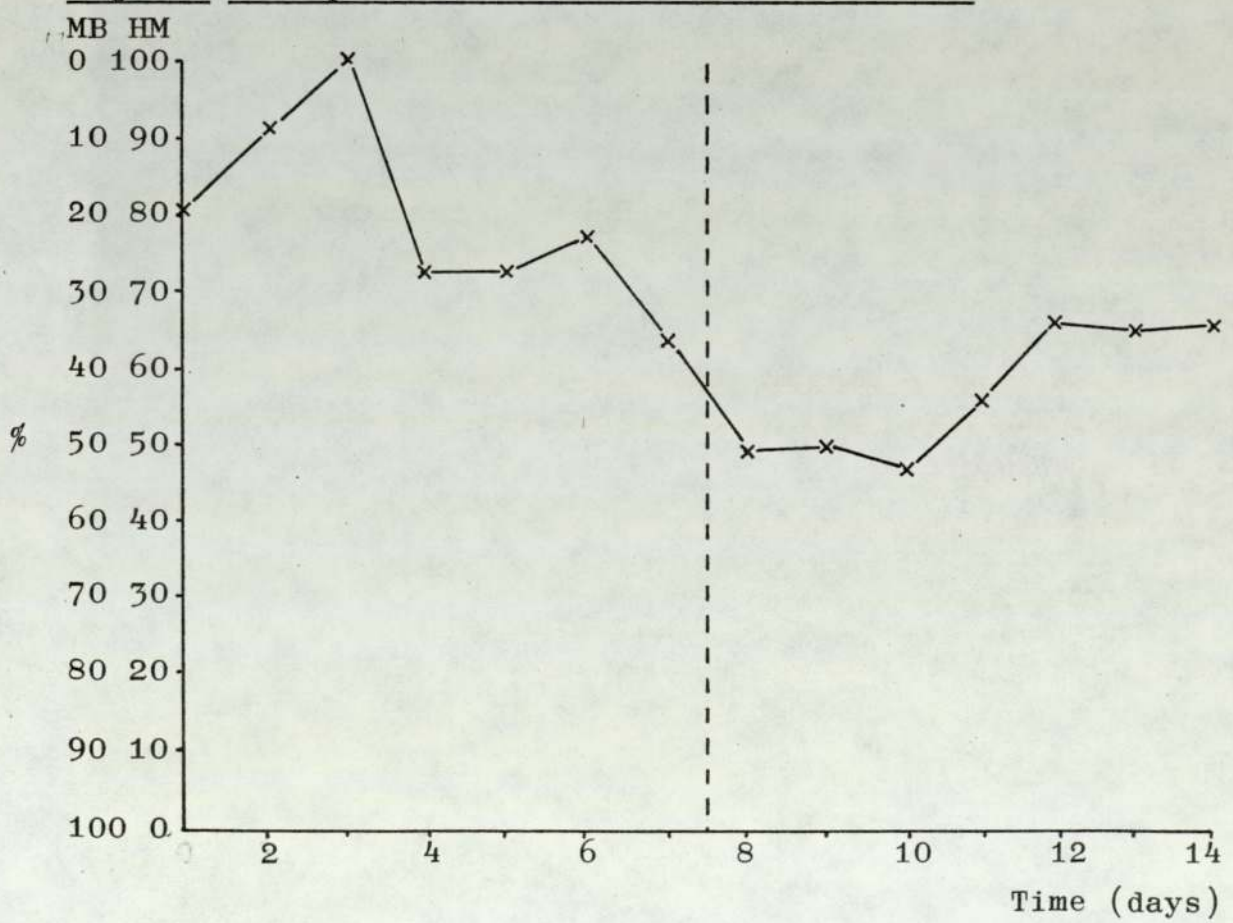


Fig 14. Food preference trial - HM vs MB diets



attempting to obtain it even after changeover. Although the relative amount of herring meal taken did increase as the trial progressed, it did not reach its previous level and so some trigger preference is assumed to have been involved.

(c) Fishmeal (FM) vs. Spirulina alga (SA) diets - Fig. 15

On day one of this trial, equal quantities of the two diets were dispersed. However, the fish quickly gained a preference for the herring meal dispenser and by day seven, it comprised 92% of the total amount of food released daily. On changeover, there was a dramatic fall in the percentage release of this diet, which as noted above, suggests a marked trigger preference. By day 11, much of this preference had been overcome though again the proportion of HM diet dispensed did not approach its previously recorded levels.

(d) Fishmeal (FM) vs. Soyabean (SY) diets - Fig. 16

The fishmeal diet again proved to be the more acceptable during the first seven days of the trial, though after changeover the soyabean feed was dispensed in greater proportions, and this situation was maintained until the end of the trial, which had to be shortened by two days due to a feeding system breakdown.

Fig.15. Food preference trial - HM vs SA diets.

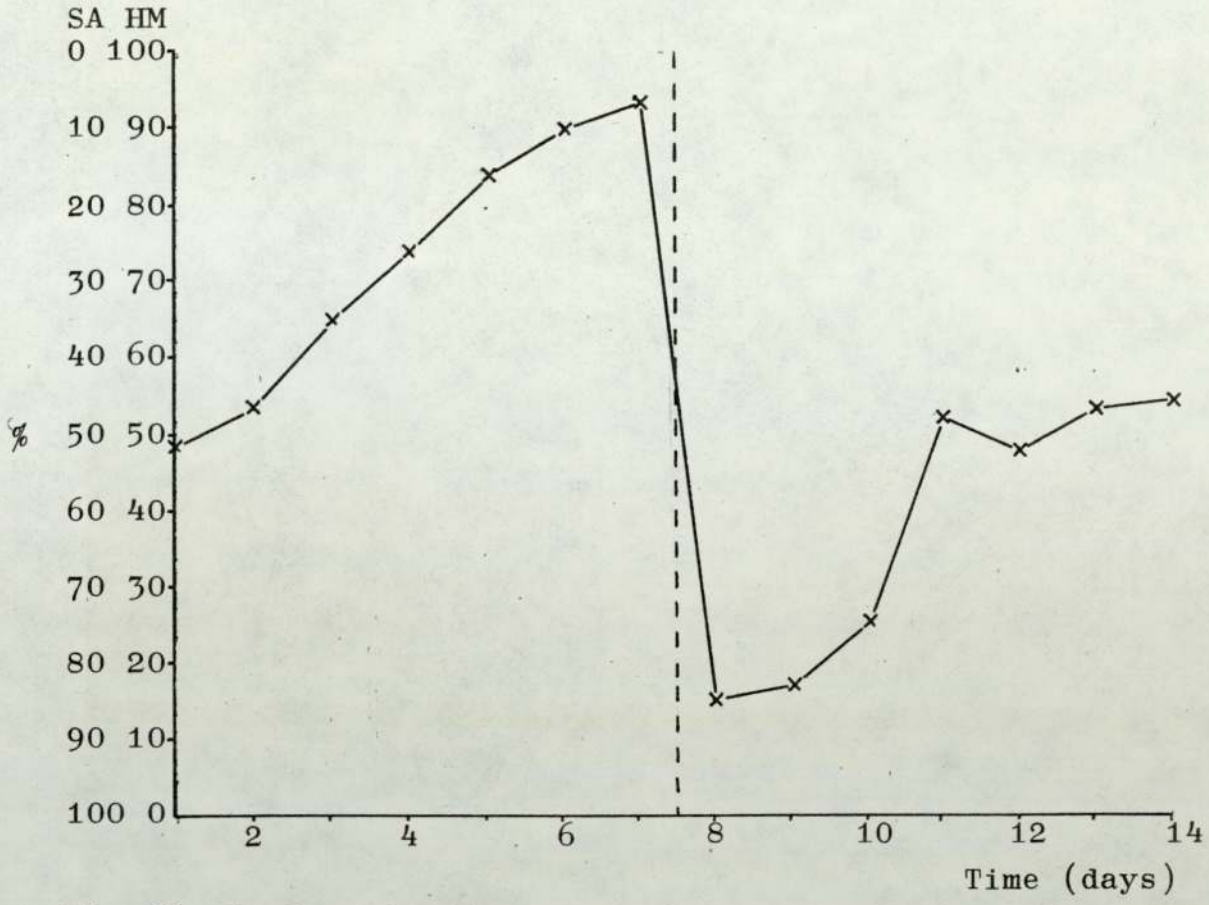
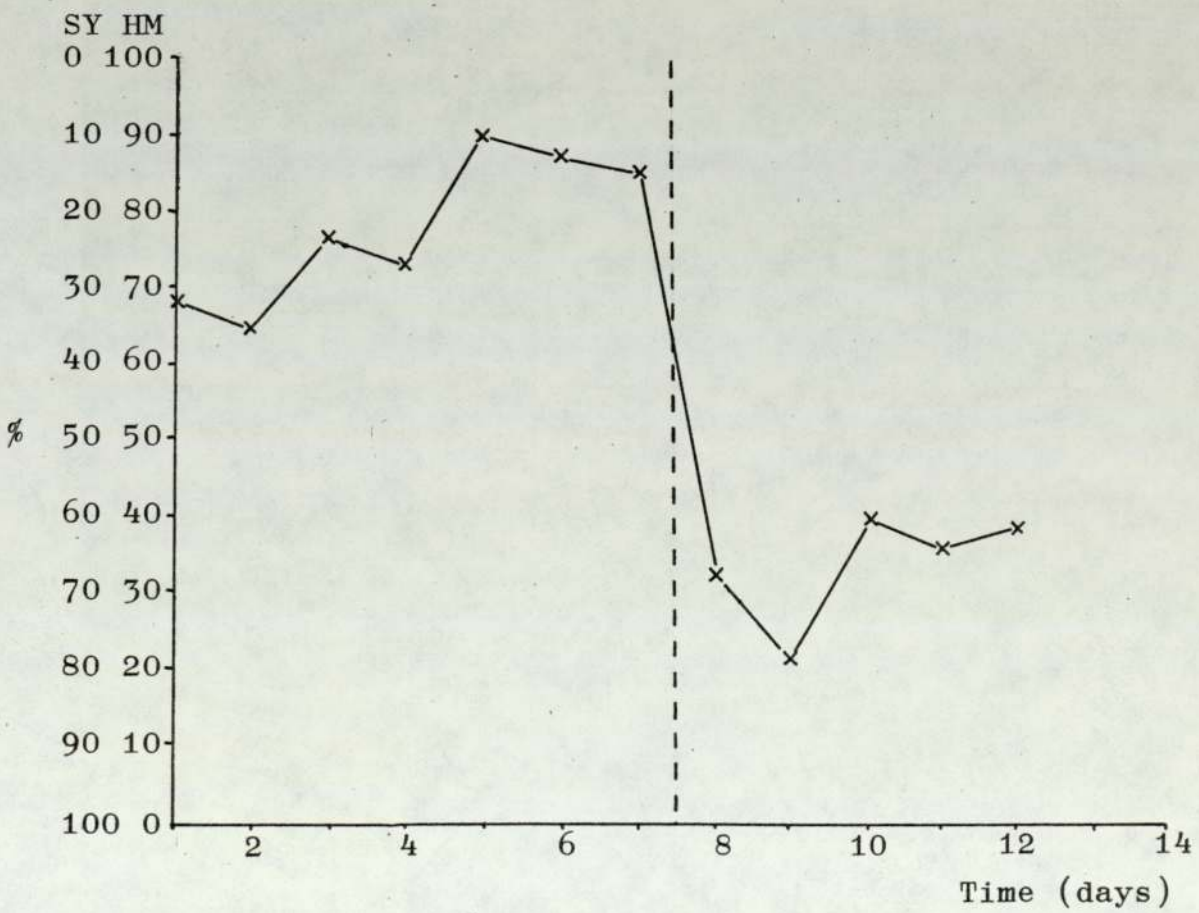


Fig.16. Food preference trial - HM vs SY diets.



(d) Petroyeast (PY) vs. Bacterial (MB) diets - Fig. 17

In this run trigger preference appeared to predominate as the two portions of the figure are virtual opposites. The large dip in the line before changeover may have again been due to a trigger or feeder malfunction, as such a large decrease in relative intake was well out of line with the general trend.

(e) Petroyeast (PY) vs. Spirulina alga (SA) diets - Fig. 18

The proportion of PY diet dispersed increased sharply on day 2 of the trial and remained at a high level (80 - 90%) with changeover whence it decreased and in fact it continued to decrease up to day 12. Although there was some restoration towards its original level on days 13 and 14, the trial terminated before it could be determined whether this trend would have been sustained.

(f) Bacterial (MB) vs. Spirulina alga (SA) diets - Fig. 19

From the start of the experiment, 90% of the daily total of food dispersed comprised of the bacterial diet and this proportion was maintained over the following 6 days. However, at changeover, the situation was completely reversed as noted in the PY/MB trial. A small increase in the percentage of MB diet dispersed was noted on days 12 and 13 but on day 14 the amount was again reduced.

Fig.17. Food preference trial - PY vs MB diets.

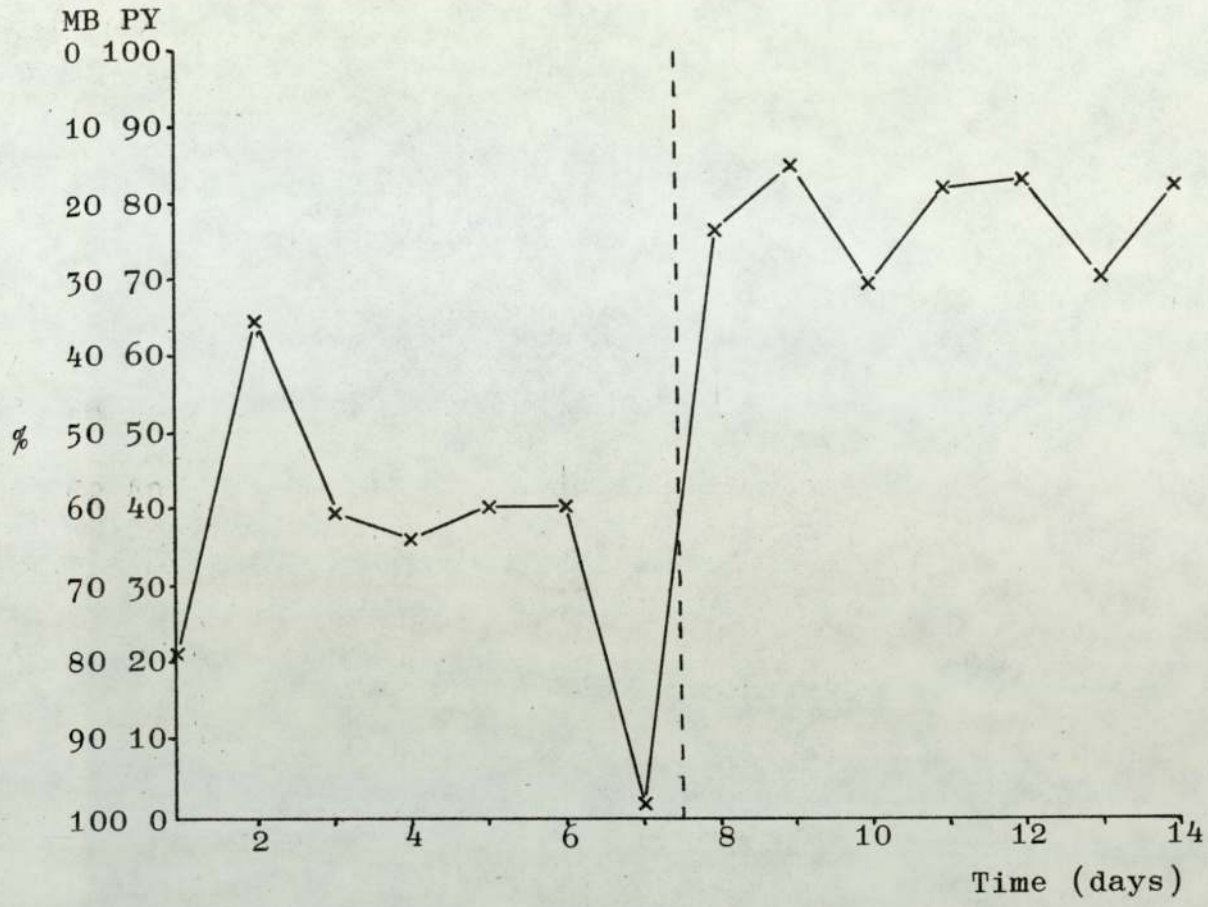


Fig.18. Food preference trial - PY vs SA diets.

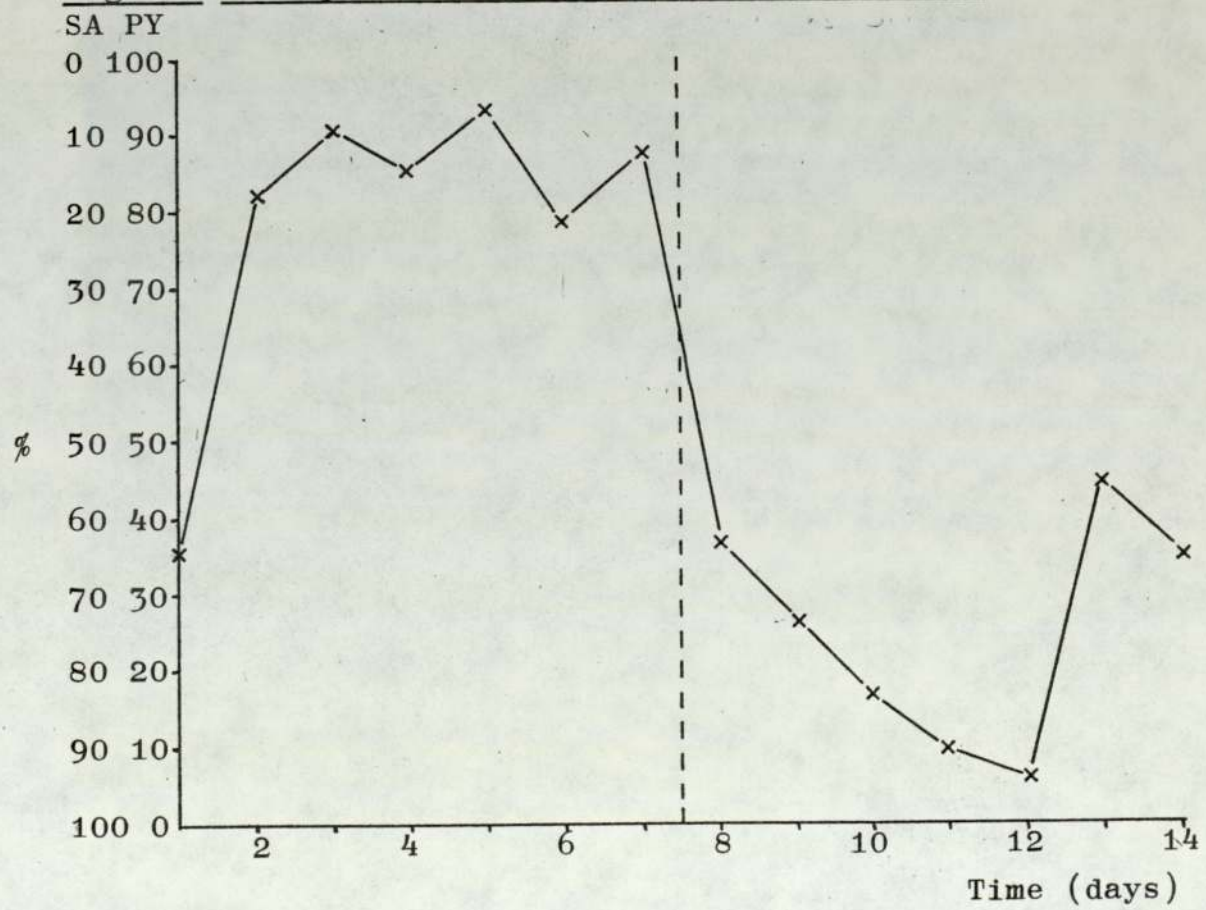
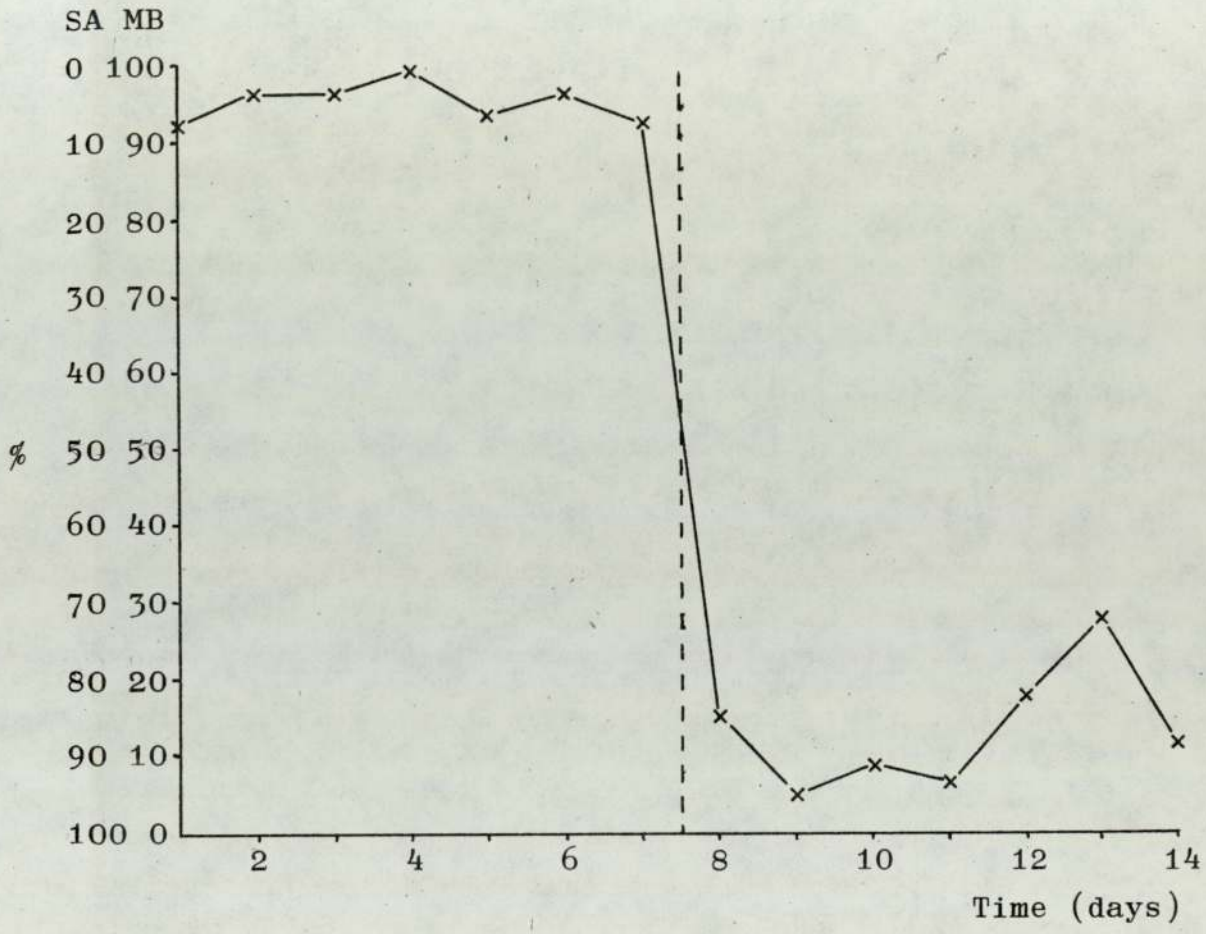


Fig.19. Food preference trial - MB vs SA diets.



4.1.4 Discussion

In general the trials did not produce many very conclusive results. Indeed trigger preference was observed more often than diet preference. The first trial described (HM vs. PY) did show, however, that trigger preference was not always a factor since equal quantities of both diets were taken before and after changeover, a situation which of course would not occur if trigger bias was dominant. The main restriction in the production of conclusive results appeared to be related to the duration of the individual experiments. In certain cases, where a trigger bias was noted, there were indications that this was gradually being overcome (eg. HM vs. SA and PY vs. SA). Hence it appears that an acquired trigger preference induced by the selection of a particular ration is usually maintained for about 4 days after the diets have been changed over. Hence increasing the duration of the trials to a total of perhaps 4 weeks each may have given more definitive results. Alternatively, such an extended period may have lead to a strengthening of an induced trigger bias, which in turn would perhaps require a longer period to be overcome.

Although not conclusively proven, the following trends may be broadly and somewhat subjectively deduced from the experimental results. Firstly, the herring meal based diet seemed to be the most attractive as often the

proportion of this taken would increase during the course of a trial. Petroyeast and bacterial diets then followed closely very little difference being noted between HM and PY, HM and MB or PY and MB. However, the Spirulina diet did seem slightly less acceptable (Note: HM vs. SA). Comparison with the SA diet also shows the small difference between the MB and PY rations, as some attraction towards PY was noted in the PY/SA trial, but not to MB in the MB/SA trial.

Since the soyabean was only tested against fishmeal, little can be drawn from this run. However, this was the only trial where uneaten food was noted in the tank after changeover. This was not quantified and any attempt to do so may have been counter productive since collection of uneaten food would have disturbed the fish, unless of course it could have been collected outside the confines of the tank. Whilst it may have been possible to achieve this, it is highly likely that much of the uneaten food would have disintegrated both before and during collection and that faeces would also have been trapped. In addition it would have been necessary to ensure that all uneaten food was of one type, which would have been difficult to ascertain after a little time in the water. Hence collection of uneaten food would have produced results of doubtful value.

The above considerations lead on naturally to criticisms of this kind of trial. Firstly, as noted, a comparison can only be made between the quantities of food delivered, not those consumed. Such differences could well have been appreciable in cases where trigger preference prevailed, but for the reasons already outlined, it was not possible to quantify this.

Secondly, it is practically impossible to formulate diets that differ in taste alone, as the various ingredients give diets of varying textures. Hence the results seen in the trials may well have been influenced by more than one factor. In addition, the levels of ingredients other than the protein meals, eg. starch and added lipids, were not fixed so that the total dietary energy could be equalised. Such ingredients may have had a significant effect on diet palatability.

The results do not compare very favourably with those obtained by ad libitum feeding in Experiment V, where a more definitive ranking was achieved. The criticism of such trials in that they do not give rise to true ad libitum intake is thus unimportant since all the test diets were subjected to the same restricted feeding regime, and environmental variables were largely negated by employing sufficient replication. Hence differences in intake should have been attributable to palatability differences. Added to this twice daily hand feeding experiments reduce food wastage problems

as feeding can be curtailed before appreciable wastage of food occurs. Whether then in fact the errors that could be attributed to environmental differences are greater or smaller than those encountered in this demand feeding trial is a matter of conjective, and there is some justification for undertaking controlled comparative trials to evaluate the two possible methods of analysing palatability.

4.2 Experiment VIII - Determination of the Effect of Photoperiod on the Feeding Rhythm of Rainbow Trout under Laboratory Conditions

4.2.1 Introduction

During Experiment VII, it was noted that the triggers appeared to be actuated more frequently at particular times of the day, and thus it was decided to study this effect in more detail to ascertain what, if any, rhythms exist in the feeding activity of trout and the relationship between any rhythm and photoperiod.

Rhythmicity is a characteristic of many physiological processes in both plants and animals. Some of these rhythms appear to be under endogenous control, although many are influenced by external factors such as solar, tidal and temperature cycles. In latitudes where seasonal changes in daylength are apparent, the photoperiod has been shown to be an important factor in the control of rhythmicity. In fish, it has been demonstrated that the daylength has pronounced effects on both seasonal and diurnal processes, but there is little data available at present on its effects on the daily feeding pattern. Studies on the diurnal activity pattern of fish, though not necessarily an indication of feeding activity, have shown that under natural conditions many species exhibit a crepuscular rhythm (Alabaster and Robertson, 1961; Young *et al.*, 1972). Such studies suffer from the lack of

control of other factors that can affect rhythmicity, such as the availability of food and the temperature. This can be overcome in the laboratory by the use of demand feeders, which allow the fish constant access to food, and temperature control, this being especially easy in recycling systems.

The present study was designed to determine the influence of four different lighting regimes on the demand feeding activity of rainbow trout.

4.2.2 Materials and Methods

35 rainbow trout which had been conditioned previously to use a demand feeder were stocked in the experimental system described fully in Appendix XII, which as before, was supplied with biologically filtered recycled water at 8 ± 1°C. Illumination was supplied by a 4 foot, 40 watt fluorescent tube giving low intensity, diffuse light at the water surface. The photoperiod was controlled by a "Venner" timer so that the transition between light and darkness and vice versa was sudden and complete.

For this experiment, the demand feeding system consisted of only one multidirectional trigger and feeder, which was adjusted to disperse approximately 1g of food for each press of the trigger. The feeding unit was

filled as necessary with commercially available floating pellets (BP "Mainstream Trout No. 5 floating). It was positioned next to the trigger in such a way that the food was carried away from the trigger by the water flow. Trigger actuations were recorded on a chart recorder run at a rate of 12 cm/hour. This allowed trigger presses more than 10 - 15 seconds apart to be easily distinguished. Great care was taken to ensure that any servicing of the equipment was performed without disturbance to the fish.

The four photoperiods studied were 6 hours light/ 18 hours dark ("short" day), 12 hours light/12 hours dark ("normal" day), 18 hours light/6 hours dark ("long" day), and continuous light. The fish were aclimated to each photoperiod for 2 weeks, the trigger actuations only being recorded over a subsequent 6 day period, except for the 12 L/12 D regime where 13 days could be included in the results, as this was the normal laboratory photoperiod to which the fish were already accustomed.

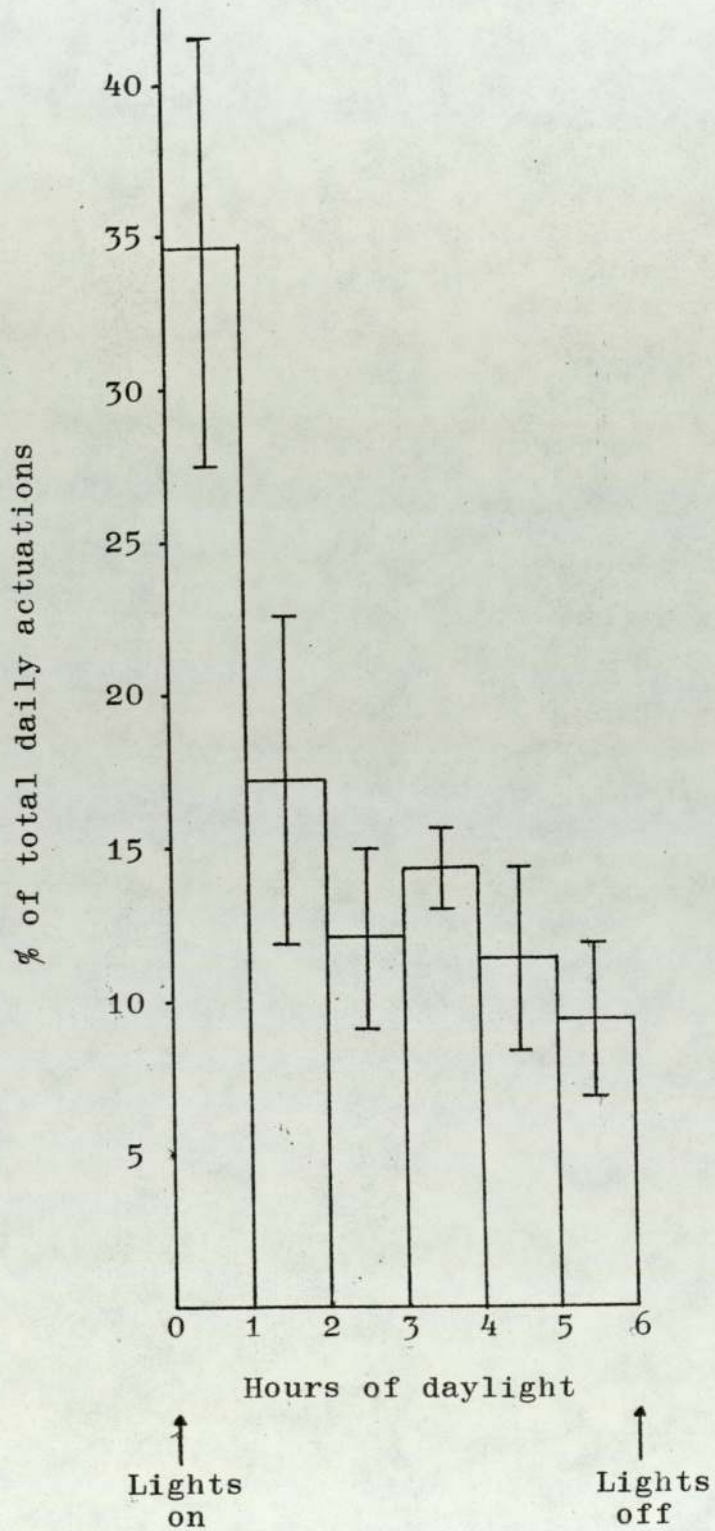
4.2.3 Results

Separate days under each lighting regime were considered to be replicate treatments. Thus, when calculated as a percentage of the total daily actuations, the number of presses occurring during similar hours on separate days could be meaned and standard errors calculated. It was necessary to express hourly actuations as a percentage to obviate small differences in the total number of presses occurring within individual days. Occasional trigger presses did occur during the night on all regimes with a dark phase, though they appeared to be random and not aggregated into groups as found during the day. It is unlikely that night feeding could have occurred since the level of light was below that required for visual feeding to take place. For these reasons, such actuations were assumed to be accidental and ignored in the analysis of results.

(a) 6 hours light/10 hours dark (Fig. 20)

The fish invariably started feeding within the first 2 minutes of light and subsequently continued to feed at a high rate throughout the first hour of the day. After this initial bout, feeding declined to a much lower level that remained fairly stable up to the end of the photoperiod.

Fig.20. Mean hourly actuations under a 6L/18D photoperiod (±S.E.)



(b) 12 hours light/12 hours dark (Fig. 21)

The means of the daily runs showed that a polyphasic pattern of feeding activity existed. Feeding commenced soon after the start of the day and reached a peak in the second hour of light. This was followed by a variable consumption rate throughout the rest of the day, but a sharp increase was noted in the last hour of light. The two feeding peaks were separated by a period of about 9 hours.

(c) 18 hours light/6 hours dark (Fig. 22)

It was found that the day was divided into 3 feeding periods by 2 periods of very low activity. The main feeding peak occurred over the first 2 hours of the day, other peaks being less obvious. It was particularly noticeable that there was no definite increase in consumption during the last hour of light in contrast to the pattern assumed in the 12 hour day. An interval of 7 hours was apparent between the median point of the first feeding peak and the start of the second feeding period, with about the same length of time elapsing between the start of the second and third feeding periods.

Fig.21. Mean hourly actuations under a 12L/12D photoperiod (±S.E.)

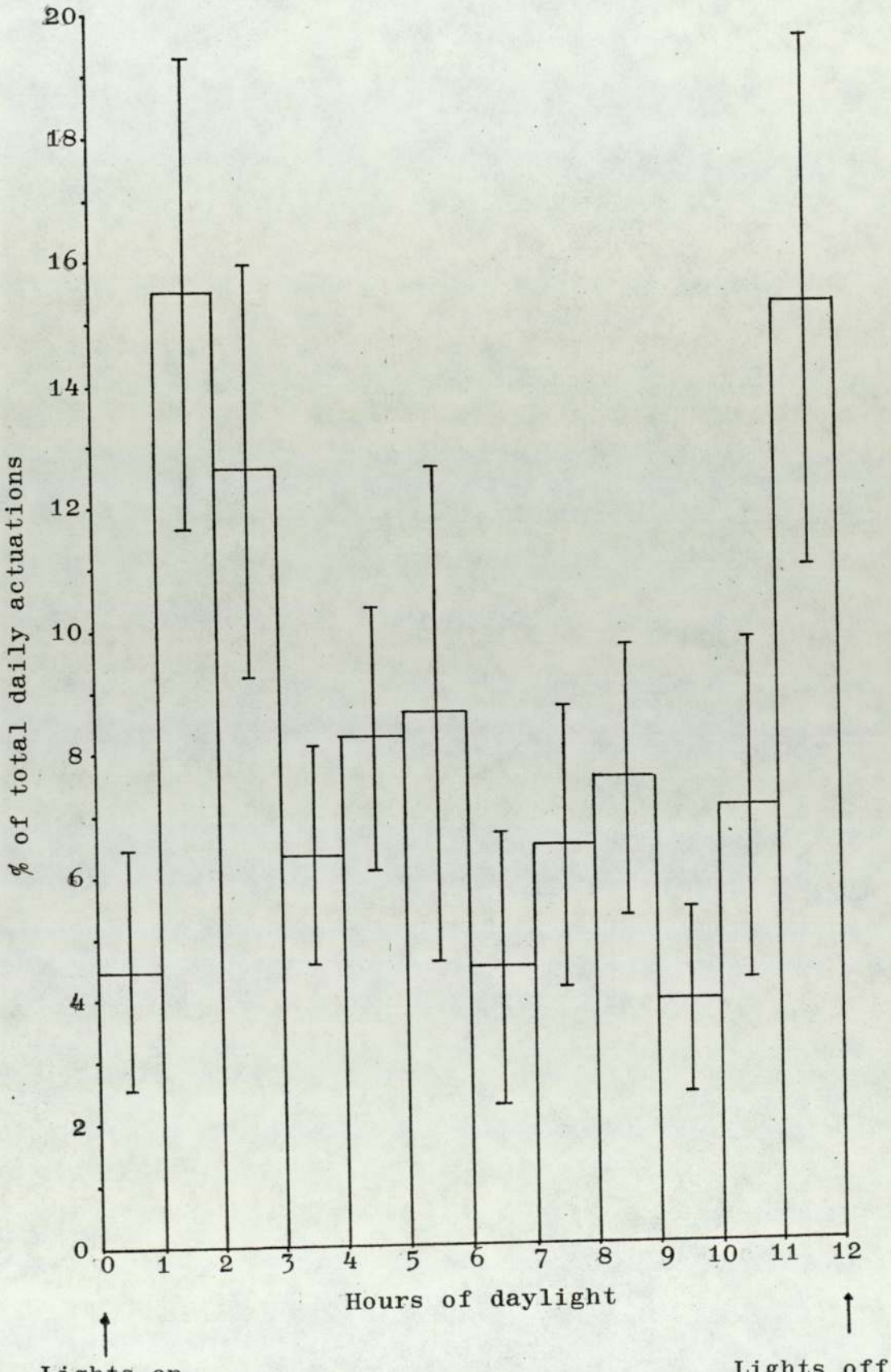
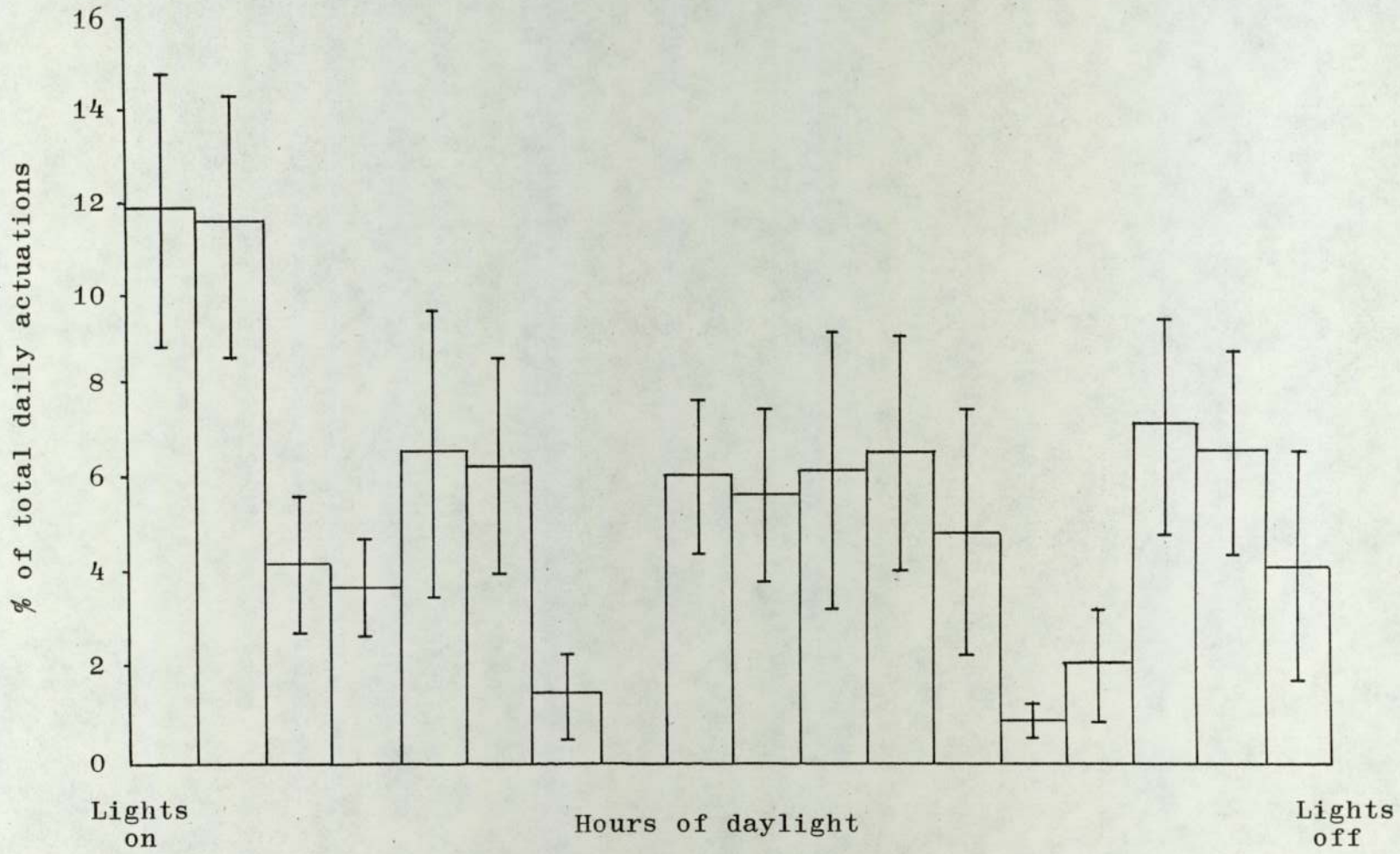


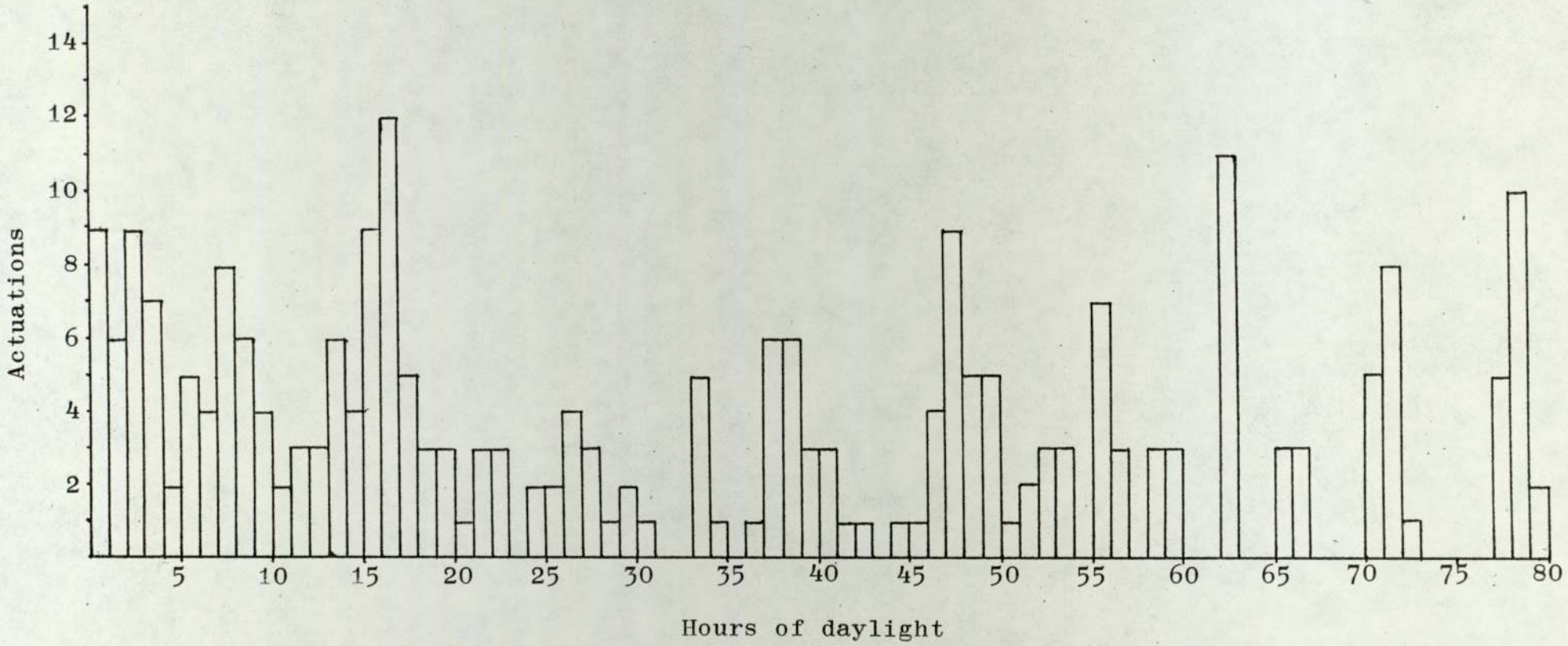
Fig.22. Mean hourly percentage actuations under a 18L/6D photoperiod (±S.E.)



(d) Continuous Lighting (Fig. 23)

For this trial, the results were plotted on the figure as trigger actuations/hour against time, since there was little to be gained from converting them to percentages as in previous figures. Once plotted in this way, a peak of feeding was considered to have occurred within any hour or group of hours where the number of actuations were greater than the hourly mean for the whole period. Peaks thus defined varied in duration, intensity and periodicity, but the mean time between the median points of consecutive peaks was found to be 7.5 hours (S.E. $\pm$ 0.5 hours). There was some change in the feeding habits of the fish as time progressed. There was a decrease in feeding between peaks and also the peaks tended to become more individual. However, the interval between them did not alter noticably, giving an overall impression of an increase in stability of the rhythm with time.

Fig.23. Hourly actuations under conditions of continuous light. A period of 80 hours towards the end of the trial.



4.2.4 Discussion

The results demonstrate that trout under suitable lighting conditions show a diurnal polyphasic rhythm of feeding activity. The interval between peaks under continuous lighting of 7.5 hours, which was also reflected under the 18 hour photoperiod, agrees well with the results of Adron *et al.* (1973) and Landless (1976), who found a feeding periodicity of about 8 hours. Adron *et al.* have linked this to the rate of gastric digestion and evacuation, that is, gut distension. If this is the case, a longer interval between peaks could have been expected in our experiments, since the lower temperature would have given rise to a slower rate of digestion (Brett and Higgs, 1970). It is possible that the effect of the slower rate of digestion may have been counteracted by the lower food intake at the reduced temperatures, thus varying only the amplitude and not the length of the feeding cycle.

Considering the other photoperiods studied, it was noticable that an initial peak of feeding always occurred within the first 2 hours of light, irrespective of the duration of the photoperiod. This correlates well with the increased activity noted at dawn amongst many species of fish in natural daylight (Alabaster and Robertson, 1961; Swift, 1962; Young *et al.*, 1972). In contrast, there was no definite increase in feeding rate associated with the last hour of light under either the 6 or 18 hour photoperiods, whereas high activity

at dusk is exhibited by fish in the wild (Alabaster and Robertson, 1961). A feeding rhythm similar to the natural crepuscular pattern was observed in fish on the 12 hour photoperiod, indicating that trout possess a sense of daylength, even in the absence of a cue such as dusk, and that this sense could be used to time feeding so that it reached a second peak immediately prior to darkness. The absence of this "dusk" feeding bout under the 6 and 18 hour photoperiods was probably related to the degree of satiation, since in both cases a feeding peak had occurred within the previous 5 hours.

Overall, our results agree with the hypothesis that the feeding rhythm of trout is mainly governed by the degree of gut distension. This is a particularly attractive explanation since goldfish, which have no storage stomach, exhibit a diurnal monophasic rhythm of feeding activity (Rozin and Mayer, 1961). In trout, light appears to be merely a requirement for feeding to take place, rather than a direct stimulus.

The results obtained in these and other experiments on the feeding rhythms of trout may have some application to fish farming. Adron et al. (1973) have shown that, compared to shorter photoperiods, food consumption and growth are increased under continuous lighting, without a loss of food conversion efficiency. It is not known whether the 7-8 hour rhythm is maintained over extended periods of continuous light or if the lack of a

scotoperiod may evenutally cause stress to the fish. Our results indicate that it may be possible to achieve a similar increase in growth rate by using an 18 hour light/6 hour dark photoperiod, since the same number of feeding peaks were exhibited as under the 24 hour lighting conditions, although a closer study of the interactions between fish size, temperature and photoperiod is required before any such practice becomes adopted.

CHAPTER 5

Conclusions

The results obtained from each experiment have already been discussed fully in their relevant sections and also comments made on the various individual methodologies employed. Hence the main purpose of this section is to take an overall view of the results and arrive at some general conclusions on the subject under discussion. However, before proceeding further, there are two important points related to the methodology which are worth noting here. The first is the fate of the non-protein nitrogen commonly present at high levels in SCP's. No account was taken of this in evaluating NPU. Strictly NPU should be based on the utilisation of true protein not Kjeldahl N. However, as already mentioned, the total N digestibility of the test materials tended to be rather high, even for those containing high levels of non-protein nitrogen, indicating that such materials are readily absorbed from the gut. Unfortunately, there is a lack of information on the metabolic fate of such non-protein N substances as nucleic acids, especially any protein sparing effect they may have. In view of this, it was felt that correction of the NPU values for non-protein nitrogen may have lead to other errors. Thus, as it is common practice in nutritional work to use Kjeldahl nitrogen as a measure of dietary protein content and degree of utilisation, this method was employed without modification.

A further comment must also be made on the two different methods used to determine NPU. The nitrogen balance method tended to give somewhat higher values than those obtained by carcass analysis at equivalent N intakes. Whilst this would have been due, at least in part, to the slightly different diet formulations and tank conditions used, it is more likely to be linked to the basic differences in methodology. As the nitrogen balance technique gave consistent results and also ranked the proteins in much the same order as the carcass analysis method, the higher values may have been the result of an inaccuracy in the measurement of endogenous nitrogen losses, ie. overestimation. Alternatively, with the carcass analysis method, the endogenous losses were calculated from a group of fish given a non-protein diet. As these fish lost weight, their endogenous losses would have been lower than those of the test fish, giving rise to an underestimation of their true value and therefore an underestimation of true NPU. Hence in reality the true NPU values determined by the two methods may have been more closely allied that the present results would indicate.

Overall the results show that the utilisation of novel proteins by fish is comparable to that found with terrestial animals. Of all the sources tested, the petroyeast proved to be particularly well accepted and utilised both by trout and carp. Unfortunately, for a

number of reasons, this material (Toprina) is no longer manufactured, and at present there appears to be no prospect of a resumption of production. However, a number of other yeast materials are being produced especially on sulphite-liquor from the paper processing industry, and these warrant further attention. The very poor results obtained with brewers yeast though, show that yeasts can vary widely in their nutritional value and indeed it is unlikely that this substance will find use as a major protein source in fish rations, especially as its low protein content makes its inclusion at significant levels in high protein fish rations impossible. Despite this, its current price and physical characteristics make it attractive as a supplementary protein source.

The bacterial protein (Pruteen) like petroyeast proved to be a relatively good nitrogen source, inferring that it could be used at high levels in compounded rations as a replacement for fishmeal. Results of previous workers (Beck et al., 1978; Spinelli et al., 1978) would suggest that 25 - 50% replacement of fishmeal is possible without any depression of growth rate. Of course, conclusions drawn on the possible usefulness of all the proteins tested here are based solely on biological findings not on the economics of diet formulation which include such considerations as price and quantities available.

Both the plant proteins tested gave rather poor growth rates in both trout and carp trials and are therefore of little value as major dietary protein components. In addition, the algal protein (Spirulina) is presently unavailable in large enough quantities to be commercially attractive, though there are plans for large scale production. If these come into effect, the material will probably find a place in the market equivalent to that occupied by brewers yeast. Soyabean protein is presently included at low levels in manufactured fish foods and the results presented here would indicate that a significant increase in inclusion level is not possible without adversely affecting growth rate. There also appear to be palatability problems associated with this material.

The fungal protein tested in the nitrogen balance trials did not perform particularly well and in fact was never produced in commercial quantities.

For the future, more work is required on all the protein sources used here, especially to evaluate any long term problems associated with their use. This might include such data as their affect on the long term health of the fish, on the palatability of the farmed product to the consumer and possible growth promoting effects of amino acid supplementation of SCP's.

Appendices
and
References

Appendix I Proximate Analysis of Feed Ingredients

Ingredient	Protein (N x 6.25)	Ether Extract	Ash	Moisture	NFE
Petroyeast-Toprina (BP Nutrition)	55.82	2.69	11.11	4.07	26.31
Methanophilic bacterium (ICI LTD.) 1. Pruteen	73.48	9.10	10.15	5.96	1.31
Methanophilic bacterium 2. Pruteen	71.38	4.11	9.42	8.12	6.97
Herring meal (Spillers)	74.30	4.40	9.33	8.29	3.68
Herring meal (BP Nutrition Ltd.)	66.10	7.11	13.73	7.35	5.71
Extracted soya meal (Newprod)	63.60	0.50	4.85	6.14	24.91
Spirulina meal	57.38	5.22	10.01	8.49	18.90
Brewers yeast meal	40.80	1.69	12.59	6.66	38.26
Fusarium meal	48.19	2.32	5.75	6.64	37.10

Appendix I (Cont.)

Ingredient	Protein (N x 6.25)	Ether Extract	Ash	Moisture	NFE
Casein	78.48	-	9.81	11.65	0.06
Blood meal	67.12	-	1.23	25.74	5.91
Meat and bone meal	49.32	4.35	28.05	9.42	8.86
Bone meal	41.00	11.18	40.43	6.59	0.80
Feather meal	80.76	5.14	2.29	8.44	2.73
Aspergillus niger	43.20	4.98	4.22	17.96	29.64

1. Meal supplied as a fine powder
2. Meal supplied as granules

Appendix II. The amino acid composition of the test proteins.

	Extd Soya	Brew Yeast	Casein	BP Yeast	Algal	Fish Meal	ICI Bact
Asp	11.51	9.07	5.60	10.84	9.93	8.48	9.25
Thre	4.26	4.78	3.30	5.11	5.14	4.85	5.04
Ser	5.60	4.78	4.80	5.37	4.97	4.31	3.61
Glut	18.77	12.50	18.05	15.05	16.12	12.92	10.48
Prol	5.28	3.55	9.10	4.48	3.92	4.24	3.27
Gly	4.18	4.17	1.60	4.93	4.71	6.39	5.10
Ala	4.26	5.76	2.40	6.90	7.06	6.26	7.35
Cys	1.32	0.91	0.46	0.72	0.92	0.92	0.67
Val	4.81	4.78	5.15	5.46	5.84	5.25	5.65
Met	1.21	1.25	2.45	1.77	1.74	2.83	2.31
Isoleu	4.73	4.17	3.95	4.57	5.14	4.44	4.76
Leu	7.73	6.37	7.85	7.35	7.93	7.47	7.21
Tyr	3.39	2.94	4.75	3.14	3.83	3.16	2.99
Phen	5.21	3.92	4.20	4.48	4.10	4.04	3.67
Hist	2.52	1.76	2.45	2.05	1.46	2.56	1.84
Lys	6.21	6.13	6.30	7.26	4.18	7.34	6.06
Arg	7.47	4.26	3.20	5.11	7.06	5.59	5.03

Tryptophan was not analysed

Appendix III. Various chemical indices of protein quality.

Ingredient	EAAI[1]	Chemical Score[2]	Chemical Score[3]	Chemical Score[4]
Herring meal (HM)	74	58	110	93
ICI Bacterium (MB)	66	47	87	75
Casein	61	46	74	53
Extracted soya (SY)	71	40	82	63
BP Petroyeast (PY)	68	40	80	63
Algal meal (SA)	68	42	70	53
Brewers yeast (BY)	64	34	70	54

1. Essential amino acid index - reference protein whole egg.
2. Reference protein whole egg.
3. Reference - amino acid requirements of carp (Nose, 1978).
4. Reference - amino acid requirements of chinook salmon (Mertz, 1969).

Appendix IV. Feed ingredient suppliers or manufacturers.

Name/Trade name	Description	Manufacturer/Supplier
Toprina	Hydrocarbon grown yeast	BP Proteins Ltd. Grangemouth. UK.
Pruteen	Methanophilic bacterium	ICI Ltd.,Billingham Cleveland. UK.
Spirulina maxima	Blue-green alga	Sosa Texcoco SA.,Mexico
Newprod	Extracted soya-bean meal	T.Lucas and Co.,Bristol UK.
Yeast Blende	Brewers yeast	Wrigglesworth Feedstuffs Ltd.,Hull. UK.
Fusarium	Starch grown fungus	Tate and Lyle Ltd. London. UK.
Fishmeal	Herring meal	Spillers Ltd.,Kennet.UK
Cod liver oil	-	" " "
Casein	Commercial grade	Hopkin and Williams Ltd Romford. UK.
Dextrin	-	" " " "
Starch	Potato	Fisons Laboratory Chemicals,Loughborough UK.
Glucose	-	" " " "
Alpha-cellulose	-	Sigma Chemical Co., London. UK.
Binder	Sodium carboxy-methylcellulose (high viscosity)	BDH Chemicals, Poole. Dorset. UK.

Appendix V. Mineral mix

Composition	Weight(g)
Calcium orthophosphate	13.58
Calcium lactate $5H_2O$	32.70
Ferric citrate $5H_2O$	2.97
Magnesium sulphate $7H_2O$	13.20
di-Potassium hydrogen orthophosphate	23.98
di-Sodium hydrogen orthophosphate	8.72
Sodium chloride	4.35

Trace elements	Weight(mgs)
Aluminium chloride	8.30
Potassium iodide	15.00
Manganese sulphate H_2O	80.00
Cobalt chloride $6H_2O$	100.00
Zinc sulphate $7H_2O$	150.00

This mix is essentially Western Fish Nutrition Laboratory (Cook, Washington) mix number H440, though the levels of certain trace minerals were reduced to account for the trace elements present in the vitamin mix (Appendix VI), assuming the mineral mix to be included at a nominal 4% dietary level

Appendix VI. Vitamin and trace mineral supplement.

Vitamin	per 10 kg mix	At 1% inclusion gives/kg diet
Vitamin A	12 m.i.u.'s	12,000 i.u!s
" D_3	1.5 "	1,500 "
" E	60 g	60 mg
" B_2	25 g	25 mg
" K	15 g	15 mg
Nicotinic acid	150 g	150 mg
Calcium pantothenate	50 g	50 mg
Folic acid	4 g	4 mg
Vitamin B_1	10 g	10 mg
" B_6	15 g	15 mg
" C	600 g	600 mg
Biotin	600 mg	0.6 mg
Vitamin B_{12}	20 mg	0.02 mg
Choline chloride	1500 g	1,500 mg
BHT	113 g	113 mg
Mineral		
Iron	100 g	100 mg
Cobalt	2 g	2 mg
Manganese	20 g	20 mg
Copper	3 g	3 mg
Zinc	20 g	20 mg
Iodine	5 g	5 mg
Magnesium	450 g	450 mg

Appendix VII. Diet pelletising.

The diets used in all the experiments were pelletised by wet extrusion, the only variable in preparation being the size of the extrusion plate. This varied according to the size of fish used in the experiment. For each diet all ingredients were first weighed to the nearest 10 mgs. The dry ingredients were then mixed in a Kenwood Chef mixer until an homogenous powder was obtained. The lipid portion was then gradually added from a conical flask whilst mixing continued. In order to ensure that all lipid had been removed from the conical flask, 2 sequential portions of cold water were vigorously shaken up in the flask to form an emulsion with any lipid adhering to the sides. This was added to the diet mix. The amount of water incorporated into the mix was not fixed, as each diet absorbed water to a different extent. At this point very small quantities of food dye were added to the water to ensure that all the diets were similarly coloured, in an attempt to avoid any colour preference affecting the ad.lib. intake of the fish.

After sufficient water had been added to the mix to form a stiff dough, it was extruded through a Kenwood mincer, forming long strings of diet. These were placed on a piece of muslin streched over a wooden frame and placed in a drying cabinet set at 37°C, until they were sufficiently dry to be broken up into pellets. These were graded through suitable sieves, according to the size of pellet required.

Samples were taken for proximate analysis, and the remainder sealed in plastic bags which were then stored in a freezer at $-4^{o}C$ until required.

Appendix VIII Nitrogen balance system.

The complete system and its use are described in the text as it is an integral part of the methodology. However for clarity a diagram of one tank is shown here (Fig. 24), and a key to the labelling given below.

Key to Fig. 24.

A = Recycled water inflow

B = Air inlet and tap

C = Air lift pump

D = Faecal trap

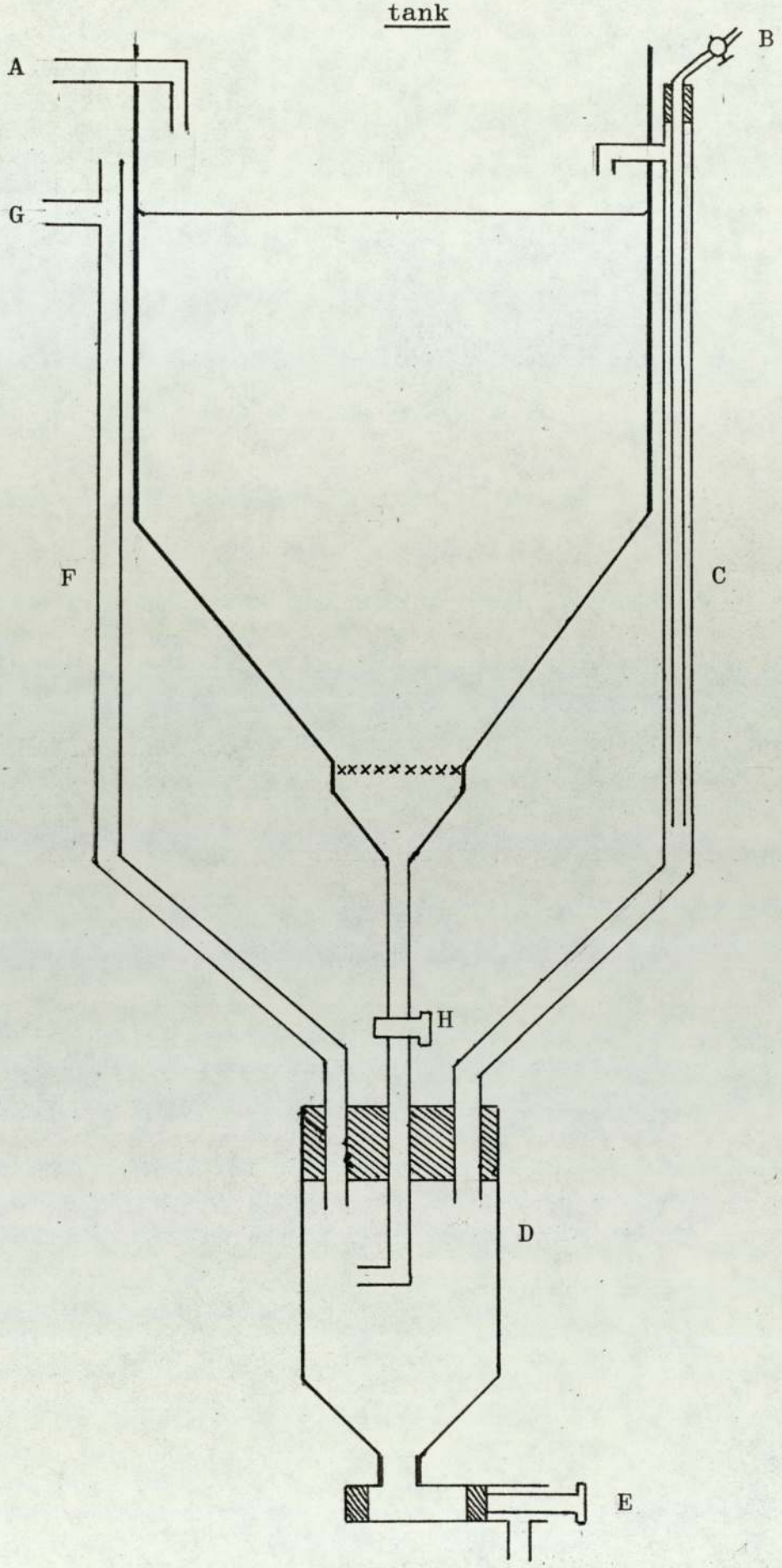
E = Drain for removal of faeces

F = Tank water level device

G = Water return to filter

H = Tap enabling faecal trap to be drained

Appendix VIII (cont) Fig.24. Design of a nitrogen balance tank



Appendix IX. Methodology and standardisation of total nitrogen analysis used for nitrogen balance determination.

Reagents. Concentrated sulphuric acid (sp.gr. 1.84)
Sodium hydroxide solution (40% w/v)
Copper sulphate solution (10% w/v)
Potassium sulphate (Analar grade)
Saturated boric acid solution
Hydrochloric acid (0.01 M)
Devarda's alloy (45% Al; 50% Cu; 5% Zn)
Tashiro's indicator (0.2% methyl red + 0.1% methylene blue in absolute alcohol).

Method

8 mls of sulphuric acid, 6g of K_2SO_4 and 0.6 mls of $CuSO_4$ solution were added to 100 mls of sample in a 500 ml macro-Kjeldahl digestion flask. This was then gently boiled down and digested until the remaining liquid changed from a brown/black to a green colour. After cooling, 150 mls of distilled water, 32 mls of sodium hydroxide solution and 100 mgs of Devarda's alloy powder were added to the digestion flask. This was connected to a macro-Kjeldahl condensor apparatus and allowed to stand for 5 minutes to ensure that all the nitrate and nitrite present had been reduced to ammonia. This was then boiled off and collected in 25 mls of saturated boric acid solution containing 2 drops of indicator. When 50 mls of distillate had been collected it was titrated against the previously standardised 0.01 M HCl, the end point of this titration being taken as the

point at which the indicator turned from green to grey. A blank was run in the same way using 100 mls of distilled water in place of the sample.

Then mgs N/litre sample= (mls HCl sample - mls HCl blank

$$\text{x } 0.14 \text{ x } \frac{1000}{100}$$

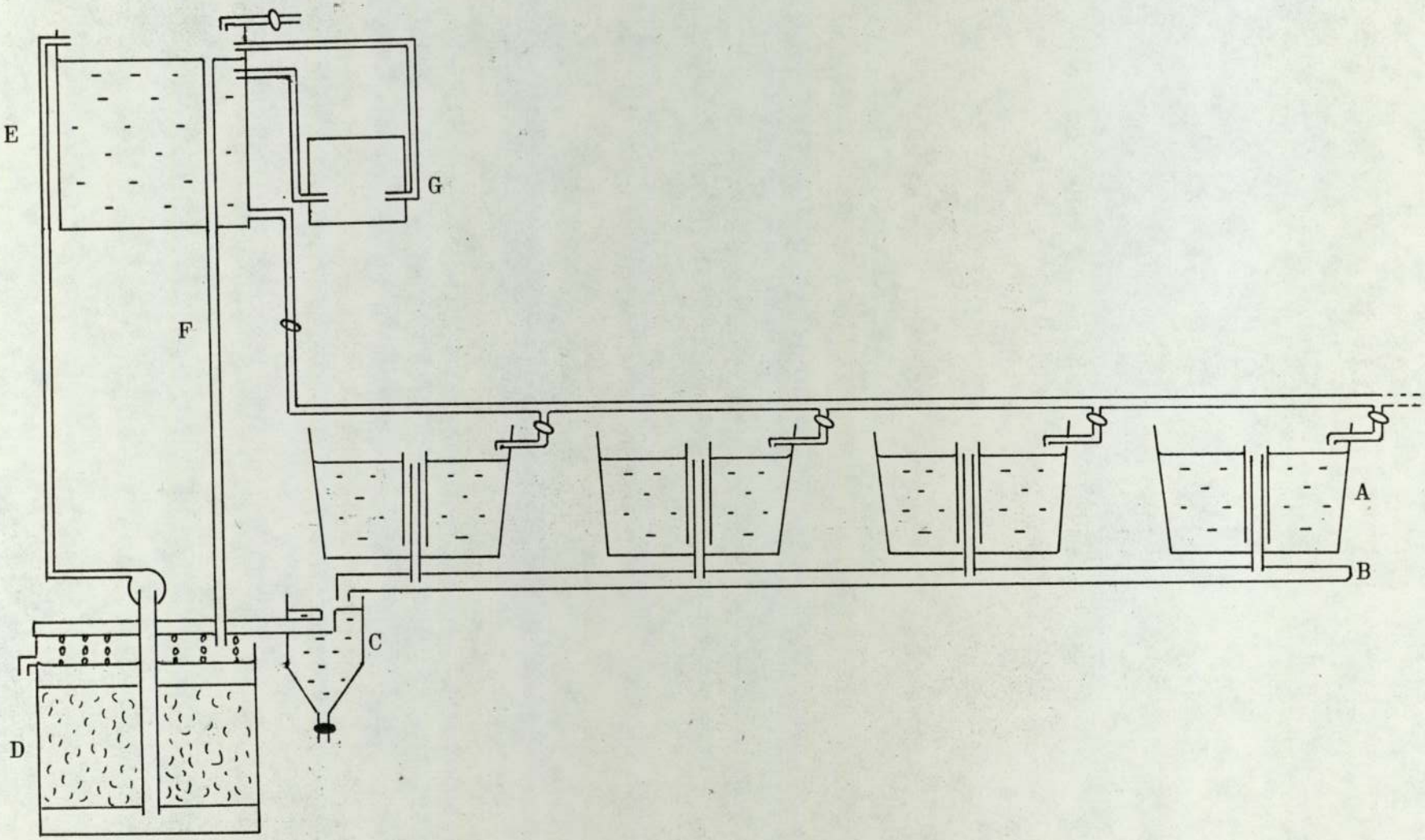
When run with a standard test solution of NH_4Cl (giving 28 mgs NH_4-N/litre), the above method gave a recovery of between 99.5 and 104.5% with an average of 100.1% (SD±2.38%). With a standard solution of $NaNO_3$ (again giving 28 mgs NO_3-N/litre) a recovery of between 90.5 and 96%, with an average of 92.8% (SD±2.32%) was found. Thus ammonia can be accurately determined by the above method, whilst nitrate estimations tend to be a little low. However, as ammonia is the main excretory product of fish and nitrate levels in the experimental samples were always relatively low, the small error in nitrate estimation would only have had a minor effect on the overall accuracy of the balance proceedure.

Appendix X. Design of recycling system RS1

Although described as one system this actually consisted of 3 identical recycle systems. Each comprised 8 circular 60 cm diameter, 40 litre, white polythene tanks (A) (Fig 25). These had a central sleeved standpipe which allowed water to flow into a drain (B) connecting each group of 4 tanks. From the drain water passed into a 10 litre conical solids settlement tank (C). This was designed in such a way that the water entered tangentially and left,through a central outlet. It was found that this flow characteristic gave optimal solids settlement before biological filtration. The filter (D) comprised a 103x69x61 cm polythene tank fitted with a false bottom of perforated plastic supported on house bricks. The filter medium used was 0.3 m^3 of 1 cm gravel covered with 3 kg of crushed oyster shell which served to buffer pH changes during filtration. A pump (Beresford PV21,Birmingham,England) drew water from beneath the gravel at a rate of 36 l/min and lifted it to a 150 litre header tank (E). A "Circotherm" heater/chiller (G) (Grant Instruments Ltd.,Cambridge, England) maintained the temperature of the system at $12\pm0.5^{o}C$. Aring main taken from the bottom of the header supplied the filtered water back to the rearing tanks. The flow to each tank was controlled at 2 l/min by a Hoffmann clip/rubber hose tap. Clean make-up water entered the header tank at a rate of 0.5 l/min.

This system proved to be very efficient. The pH remained stable at 7.1 ± 0.3 and unionised ammonia and nitrate never rose above 0.01 and 20 mgs/l respectively.

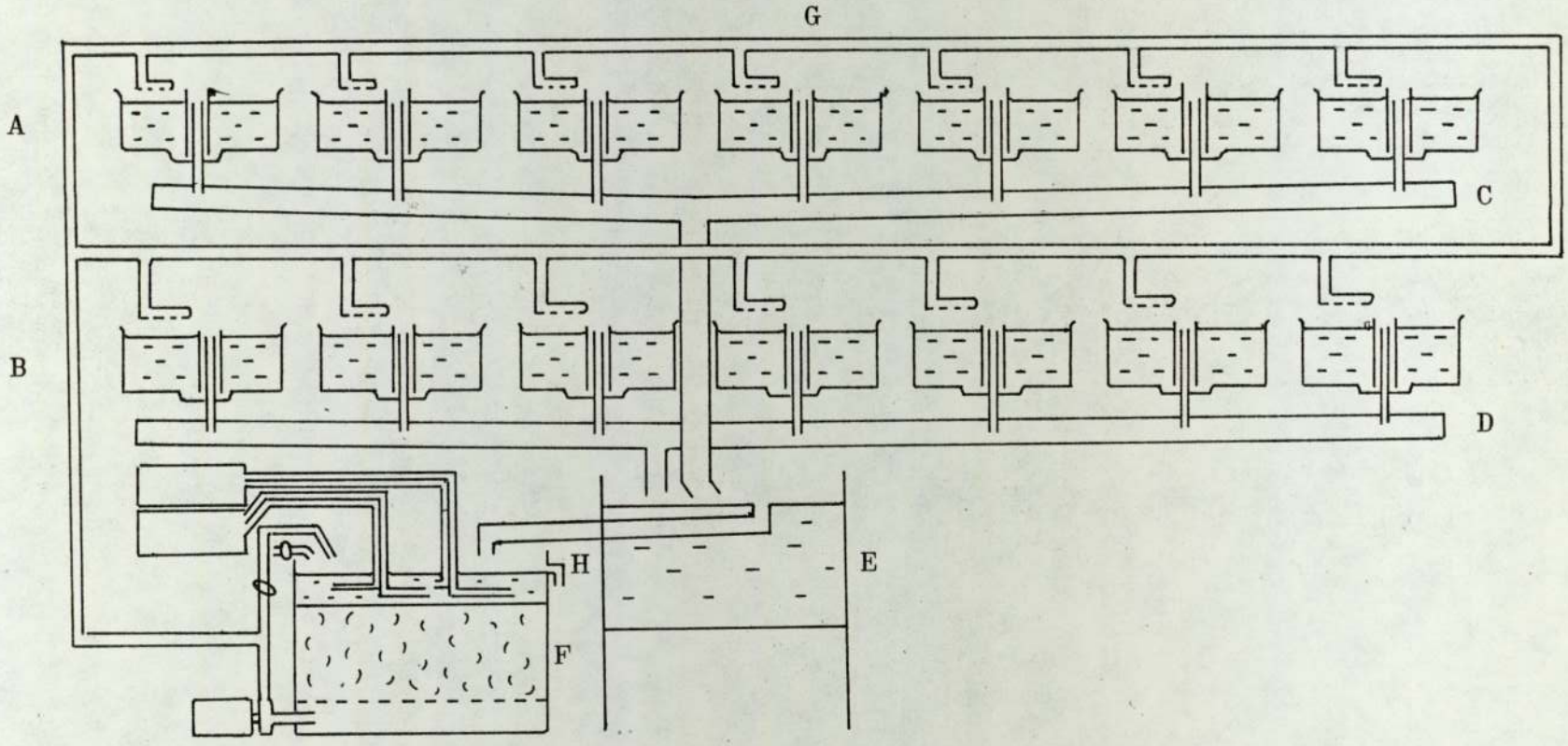
Appendix X (cont). Fig.25. Design of recycling system RS1.



Appendix XI. Design of recycling system RS2.

This warm water recycling system (Fig.26) was constructed on the same principles as system RS1, though no header tank was included due to shortage of space. Fourteen 12 litre circular tanks were arranged in two rows of 7 (A and B), one above the other. Each tank had a central standpipe drain which allowed outflow water to pass into two collecting troughs (C and D). These were connected to a 40 litre solids settlement trap (E), which could be cleaned out daily by siphon. After solids settlement, the water overflowed into the filter (F). This had a total capacity of 200 litres, 110 litres of which was occupied by 1 cm gravel. Water was pumped from the bottom of the filter round a ring main (G), which had a "bleed-off" through an aerating venturi back to the filter. This enabled control of the amount of water reaching each tank (1.5 l/min). During operation it was found that the amount of oxygen consumed by the filter was so great that re-aeration of the water by the tank inlet spraybars was insufficient. Hence supplementary aeration at a rate of 1.5 l/min was necessary for each tank to ensure adequate oxygen levels (above 5 mgs/l). The temperature of the water was maintained at $25\pm0.5^{o}C$ by two "Circotherm" units, which abstracted water from, and returned it to, the top of the filter. Make-up water was added to the filter at a rate of 0.25 l/min, excess water leaving the system via an overflow (H). Water quality was monitored twice weekly. The levels of O_2, NH_3, NO_3, NO_2 and pH were judged to be within tolerable limits for carp at all times.

Appendix XI (cont) Fig.26. Design of recycling system RS2.



Appendix XII. Demand feeding system.

This system (Figs.27 and 28) consisted of a 1 metre diameter polythene tank (A) connected to a biological filter (B). The tank was fitted with a sleeved standpipe outlet, which allowed water to pass directly from its center to the top of the filter. The filter itself consisted of a 1.0x1.0x0.6 metre plywood tank, containing a 30 cm layer of 1 cm gravel, held above the bottom of the tank by a sheet of perspex,perforated and supported as described previously. The pump (C) lifted water from the sub-gravel space and passed it directly to the tank. A spraybar (D) situated across the diameter of the tank distributed and re-aerated the water, and also caused a circular flow round the tank. The amount of water pumped could be controlled by a tap (E). Fresh water was added to the system at F at a rate of 0.4 litres/min, whilst waste water left through an overflow (G). Temperature was held at $8\pm1^{o}C$ by a heater/chiller unit (H).

The tank was surrounded by black polythene sheeting in order to minimise disturbance to the fish. Illumination was provided by a 4 ft. 40 watt fluorescent tube positioned in such a way that only diffuse light entered the tank. The length of the photoperiod was directly controlled by a "Venner" timer so that the transition between darkness and light was sudden and complete.

The demand feeding system itself consisted of a 12 volt multi-directional switch operated by a trigger lever (Fig.29)

This was placed in the tank so that the tip of the trigger just broke the water surface in order to reduce accidental contact by the fish. The trigger was connected via a delay device and relay to a 240 volt solenoid operated valve which actuated an air powered food dispenser (Fig.30) situated adjacent to the trigger. The feeder could be adjusted by turning the nuts X to dispense as much food as required at each actuation. The intricacies of the electronic control system are described by Smith (1976).

Appendix XII (cont). Fig.27. Demand feeding recycling system

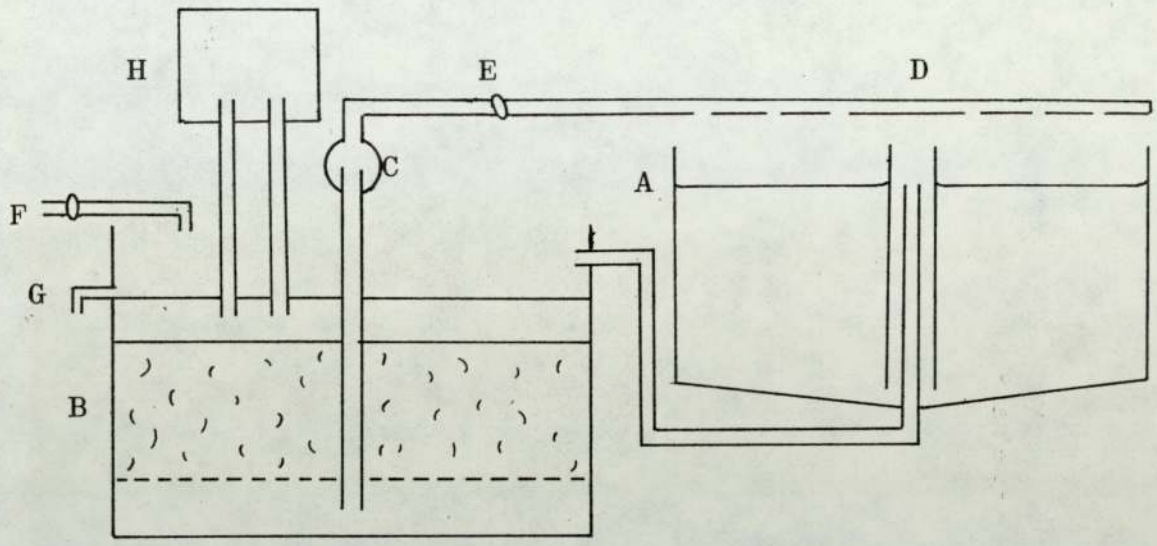
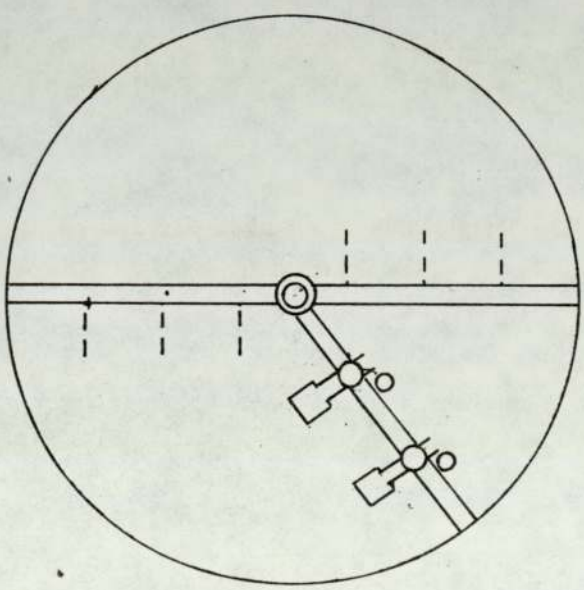
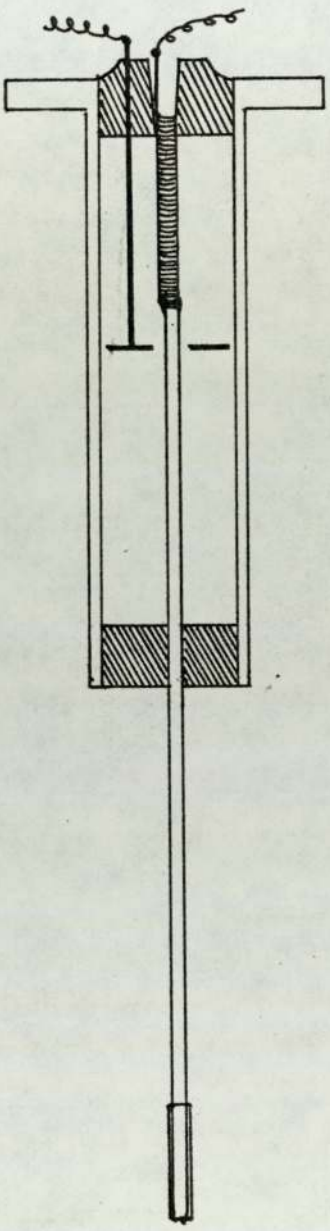


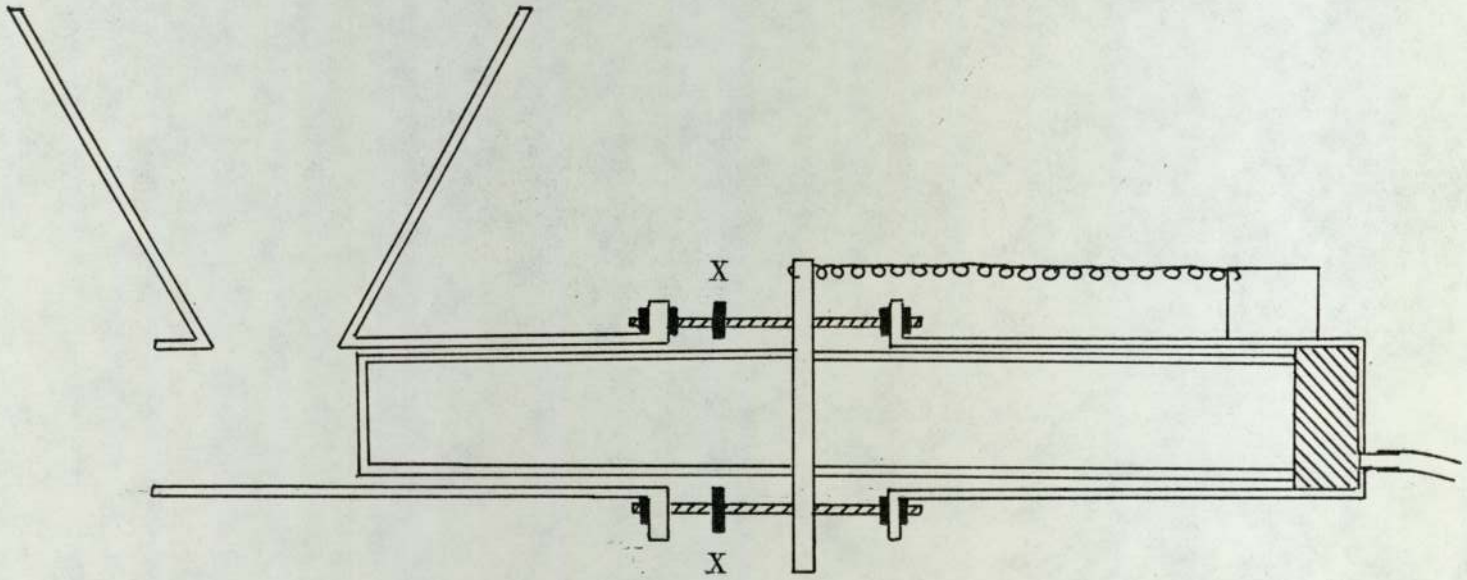
Fig.28. Trigger positions for palatability studies.



Appendix XII (cont) Fig.29. Demand feeding trigger.



Appendix XII (cont) Fig.30. Food dispenser.



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