THE USE OF EARTHWORMS AS A FEED FOR RAINBOW TROUT (*SALMO GAIRDNERI*)

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by

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ABSTRACT

The nutritional and chemical characteristics of five earthworm species: Lumbricus terrestris; Allolobophora longa; Eisenia foetida; Dendrobaena veneta and Dendrodrilus subrubicundus were assessed on the basis of crude protein and amino acid composition, lipid and fatty acid composition, and ash and mineral composition. Analyses indicated that all the earthworm species evaluated possessed a high quality protein and lipid fraction suitable for use in fish feeds which was somewhat similar in composition to that of fish meal.

During experimental feeding trials (50-84 days in duration) each earthworm species was nutritionally evaluated, on the basis of fish growth performance, feed utilization efficiency and gross carcass composition, as a complete feed (frozen slices of whole worm) for rainbow trout (Salmo gairdneri). A dried 'earthworm meal' derived from each of the species E.foetida, D.veneta and D.subrubicundus was similarly evaluated as a potential replacement for fish meal in trout diets.

Fish fed frozen slices of earthworm, with the exception of fish fed E.foetida, achieved growth rates and feed utilization efficiency comparable to fish fed a control, fish meal based ration. Fish fed solely on frozen slices of E.foetida achieved little or no growth over the experimental period. The possible reasons for the reduced palatability of frozen E.foetida to the fish are discussed, pre-treatment processes applied and a significant improvement in the palatability of frozen E.foetida was achieved by blanching.

High dietary inclusion levels of earthworm meal (replacing ≥ 50% of the fish meal protein) resulted in depressed feed intake and growth of the fish. At reduced levels of inclusion, dried E.foetida meal (constituting 5-30% of a production salmonid diet) and dried D.subrubicundus meal (constituting 7-36% of a semi-synthetic trout diet) adequately replaced the dietary fish meal component without loss in fish growth and feed utilization efficiency.

The possible uptake of potentially toxic trace elements (Fe, Zn, Mn, Pb, Cu, Cr, Ni, Co and Cd) into the fish carcass through the ingestion of contaminated earthworm diets was also investigated and the levels of certain elements, in particular Pb, were observed to increase in the carcass of fish fed high levels of earthworm in the diet. However, in no instance did the results indicate any harmful or toxic effect of including earthworms in the diets of rainbow trout.

CHAPTER 1

GENERAL INTRODUCTION

1. GENERAL INTRODUCTION

Scientific interest in earthworms (Phylum Annelida, Class Oligochaeta, Order Haplotaxida) has, until recently, principally focused upon the beneficial effect that earthworm activity has within the soil. The burrowing and casting habits of earthworms have been demonstrated to accelerate the decomposition of organic material, improve soil structure, aeration and fertility, and consequently promote plant growth and development (Darwin, 1881; Guild, 1955; Barley, 1961; Satchell, 1967; Edwards and Lofty, 1977).

In recent years the large scale culture of earthworms within organic waste substrates (vermiculture) has received increased attention as a possible alternative to conventional methods of waste disposal (Tsukamoto and Watanabe, 1977; Hartenstein et al., 1979a). Harnessing the earthworms natural habits in waste management would not only alleviate many of the problems associated with organic waste disposal (since earthworm activity effectively converts organic waste materials into a soil conditioner with potential market value, Edwards, 1983), it also results in the production of a novel protein source (earthworm biomass) which is likely to be of value in feeds for farmed animals, including fish.

Vermiculture is foreseen as a multi-purpose operation (Figure 1.1) and in view of this a section of this introductory chapter briefly assesses the role that earthworms can play in waste management and includes a short review of the life cycles and substrate preference of the British Lumbricidae. This should provide an insight into the species of earthworm likely to be

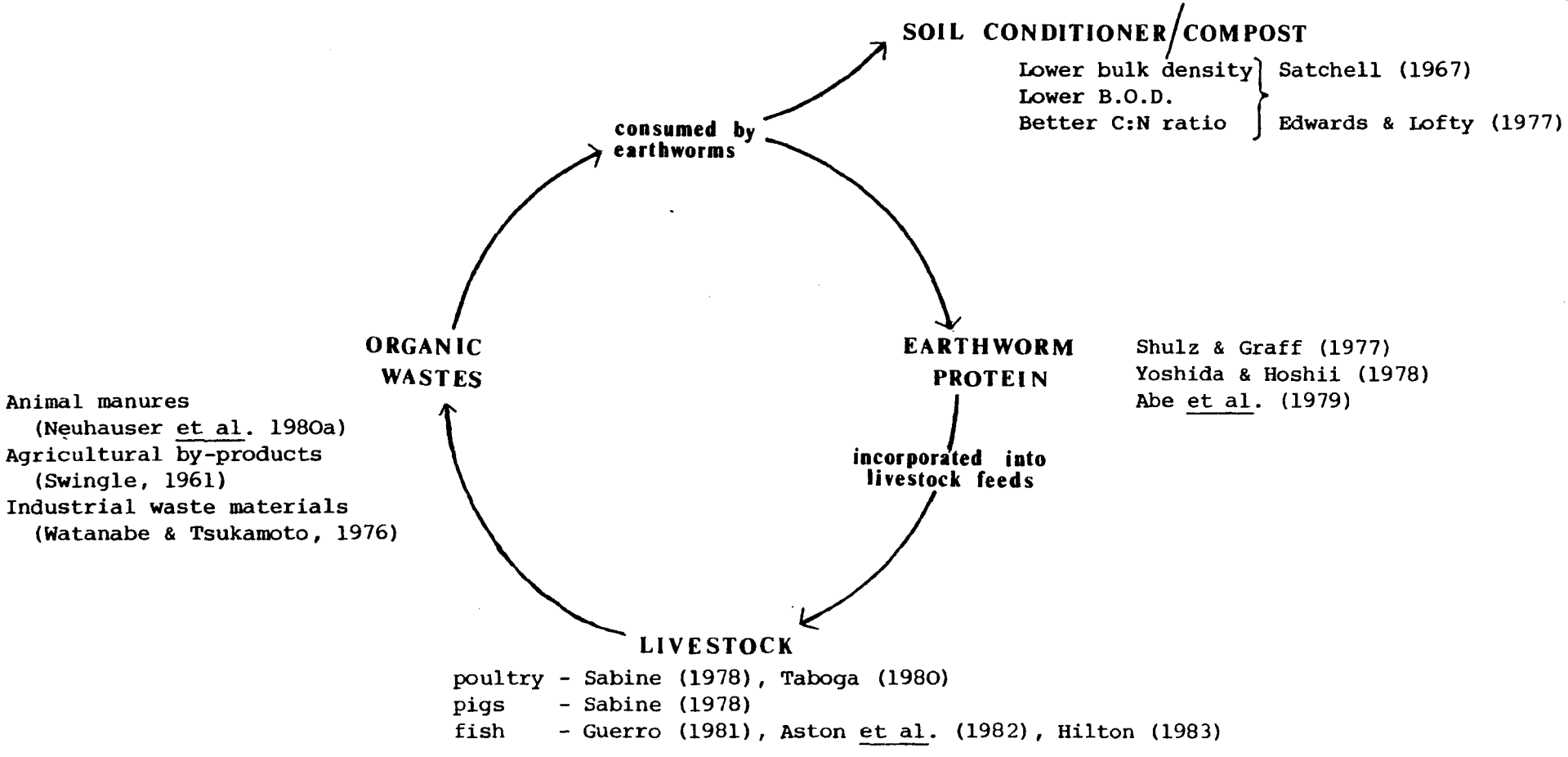


Figure 1.1: The potential rôle of earthworms in waste management

cultured commercially and therefore worth evaluating nutritionally as a feed for rainbow trout (Salmo gairdneri).

1.1 Earthworms in Waste Management

Intensive livestock farming practices generally involve the dependence upon high energy inputs from non-renewable sources and the output of only one or two marketable products. Other products of the system, with marginal market value are termed "by-products" and those products without market value are termed "wastes". Wastes from agricultural systems and food production processes represent a valuable resource in terms of energy, nutrient and mineral content and the utilization of such wastes as inputs in secondary production systems would provide a greater diversity of marketable products, a more ecologically efficient system and also reduce the problem of waste disposal.

Recovery of nutrients from livestock excreta and food processing by-products has generally been achieved either by directly incorporating the nutrient rich wastes into formulated diets and re-feeding to livestock (Cunningham, 1976; Ichhponani and Lodhi, 1976; Fontenot, 1981) or by utilizing the organic wastes as a substrate for single cell protein production (Calvert, 1974), a process which is believed to occur naturally when direct fertilization of fish ponds with organic wastes is practised (Waddington, 1963; Tortell, 1979; Wohlfarth and Schroeder, 1979; Edwards, 1980).

Recently the possible production of detritivorous invertebrates, grown on organic waste substrates, has been investigated. House fly lavae and pupae (Calvert et al., 1969), soldier fly lavae (Hale, 1973; Bondari and Sheppard, 1981),

chironomids (Shaw and Mark, 1980) and various oligochaetes, including earthworms (Hartenstein et al., 1979a; Edwards and Densem, 1980; Aston and Milner, 1981/1982; Aston et al., 1982a) are all organisms which have the potential for concentrating nutrients present in the waste into their body tissue. This system presents the additional advantage that production may be carried out "on site" with the possibility of utilizing these novel protein sources directly as feed supplements.

Apart from the recovery of nutrients present within the waste substrate it has been shown that the primary effect exerted by earthworms in waste management is to accelerate decomposition of the organic fraction (Mitchell, 1979; Mitchell et al., 1980). This may be achieved indirectly as a result of the substrate passing through the earthworm gastrointestinal tract and is believed to be due to an increase in the ratio of surface area to volume of the material egested and also due to the burrowing activities of earthworms creating pore spaces and translocating nutrients (Waugh and Mitchell, 1981). Thus the presence of earthworms facilitates oxygen penetration of the waste, thereby increasing the activity of other organisms present. Mitchell et al. (1980) reported an increase in the population of bacteria and bacteriophagic nematodes (active at lower oxygen tensions than earthworms) as a result of innoculating sewage sludge with the earthworm Eisenia foetida.

The increase in aerobic metabolism within the waste material (sewage sludge) elevates the redox potential (Mitchell et al., 1980). This has been demonstrated to affect microbial sulphur transformations resulting in a decline in the proportion

of reduced sulphur compounds (for example methanethiol and hydrogen sulphide) which may be toxic and are partially responsible for the malodour resulting from anaerobic decomposition. Under aerobic conditions the proportion of sulphate compounds increases and these are readily utilized in the formation of sulphur amino acids (Waugh and Mitchell, 1981).

Microbes and microbial products within the decomposing waste which would otherwise be subject to putrefaction may be assimilated by the earthworms. Thus the nutritional constituents dispersed throughout the substrate are concentrated into earthworm biomass, effectively reducing the biological oxygen demand (B.O.D.) of the waste material (Mitchell et al., 1982). By a similar process, and through the increased surface area available for evaporation, the moisture content of the earthworm substrate is also significantly reduced, resulting in a decrease in the bulk density of the waste material (Hatanaka et al., 1978; Hartenstein et al., 1979a; Waugh and Mitchell, 1981).

As a result of the activity of earthworms within the waste material the B.O.D., odour, moisture content and bulk density can be significantly reduced, the availability of phosphorus may be increased (Satchell and Martin, in press) and the resulting material represents a potentially marketable product, as a soil conditioner for use in the horticultural industry (Edwards, 1983).

1.2 The Selection of Earthworm Species Amenable to Culture in Biodegradable Wastes

1.2.1 Earthworm life cycle and fecundity

Until recently, studies on the life cycles of British Lumbricidae were designed to provide information on the activity

of earthworms in the soil, and for this reason experimental conditions were held as close as possible to field conditions. However, during the past decade the potential of various earthworm species for large scale culture has been assessed. Experimental conditions have been controlled and monitored, additional factors such as age and population density recorded, and mathematical models devised for application in managed systems. Evidence from this research suggests that manipulation of the culture conditions can significantly increase earthworm fecundity and biomass production, thereby increasing the rate at which organic wastes are converted to soil conditioner.

The typical life cycle of an earthworm is depicted in Figure 1.2. Earthworms are capable of reproduction once the clitellum (a glandular swelling of some anterior segments) has formed. The number of cocoons produced per year; the incubation time necessary before worms hatch; the number of juvenile worms emerging from each cocoon and the period required before juvenile worms become clitellate depends upon the earthworm species, and environmental variables such as temperature, moisture, nutrition, age and population density.

1.2.1.1 Cocoon production: The rate of cocoon production for eleven species of earthworm cultured at ambient temperatures with optimal soil moisture and fed bullock droppings is shown in Table 1.1.

The effect of temperature on cocoon production was first investigated by Evans and Guild (1948) who demonstrated that a 10°C increase in the temperature at which A.caliginosa was held resulted in a four-fold increase in cocoon production. Subsequently, Graff (1974) reported the production of up to

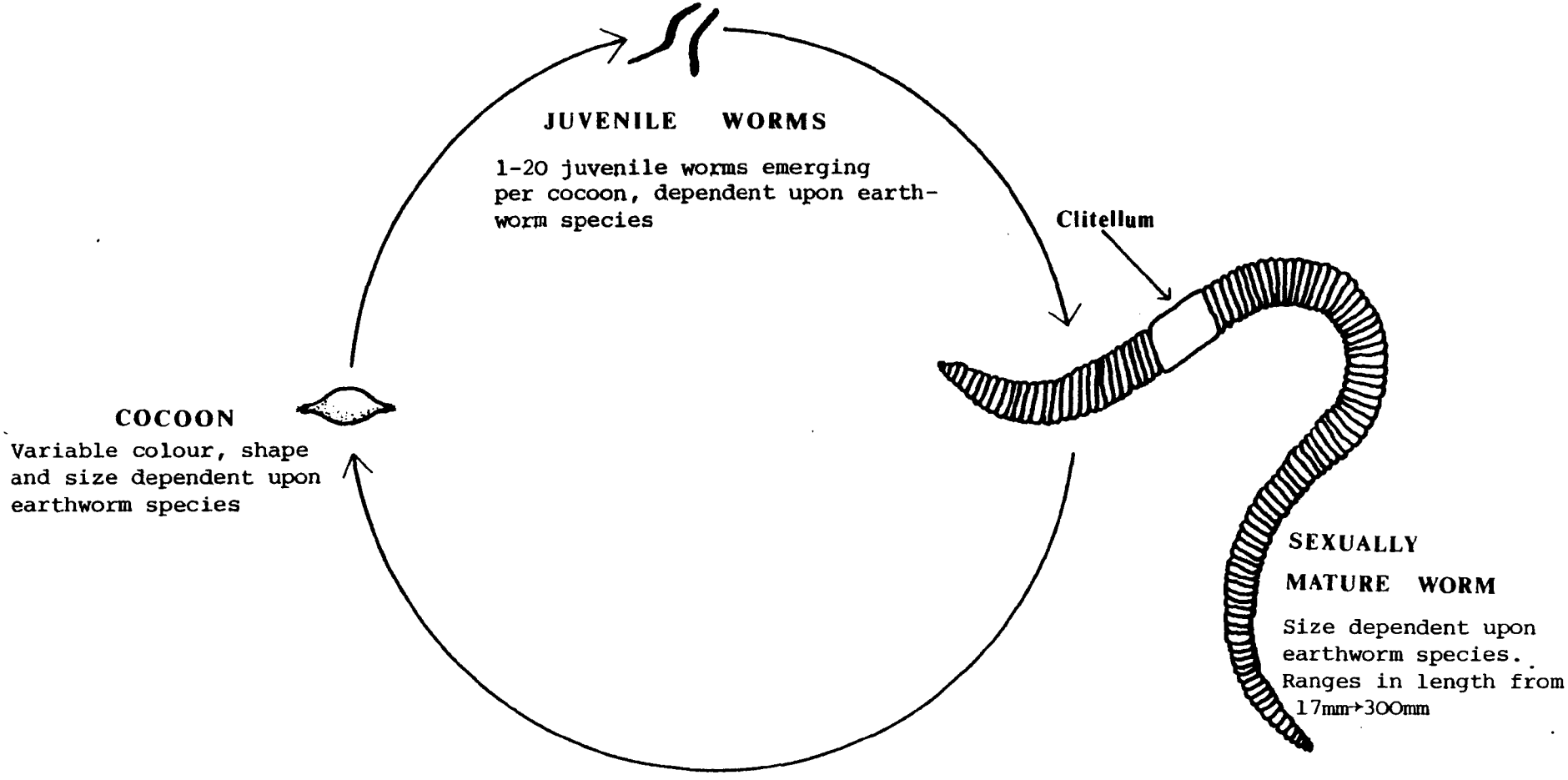


Figure 1.2: Generalized 'life cycle' of an earthworm

Table 1.1: The annual production of cocoons by various species of earthworm[1].

Species	Number of cocoons/year
Lumbricus rubellus	79
Lumbricus castaneus	65
Dendrobaena subrubicunda	42
Allolobophora caliginosa	27
Allolobophora chlorotica	25
Dendrobaena mammalis	17
Octolasium cyaneum	13
Eisenia foetida	11
Allolobophora rosea	8
Allolobophora longa	8
Allolobophora nocturna	3

1. Source: Evans & Guild (1948).

3 cocoons/individual/week (159 cocoons/year) by E.foetida held at 25°C as compared with the production of 11 cocoons/year recorded for this species by Evans and Guild (1948, Table 1.1). The threshold temperature below which no cocoons are laid lies between 3°C and 10°C (Edwards and Lofty, 1977; Gerard, 1967).

The moisture content of the substrate and the type of substrate consumed by clitellate earthworms also influences cocoon production (Evans and Guild, 1948). The maintenance of an adequate moisture level in the substrate becomes more critical under controlled conditions at higher temperatures and a substrate moisture level of 70-85% has been suggested as optimum when culturing E.foetida (Tsukamoto and Watanabe, 1977; Hartenstein, 1983).

Cocoon production also varies with both the age and population density of the earthworms. Hartenstein et al. (1979b)

demonstrated that 5-10 weeks after hatching, E.foetida (at 25°C) showed increasing rates of cocoon production, but 10-27 weeks after hatching a decline in cocoon production was evident. Since population density affects the size of adult worms and this in turn affects the number of progeny, a relationship between population density and expected number of progeny can be calculated. From experimental evidence and by mathematical inference maximum cocoon production (at 25°C) may be expected from a population of eight E.foetida (aged 5-27 weeks) per 300cc of substrate (horse manure; Hartenstein et al., 1979b).

Certain species of earthworm (Eisenia spp, Allolobophora spp, Octolasium spp) undergo a period of suspended activity termed "diapause". This is thought to be related to environmental conditions and interrupts cocoon production (Evans and Guild, 1948). However, Gerard, (1967) suggested that under optimum culture conditions diapause would not occur.

1.2.1.2 Incubation periods for cocoons: Under natural conditions this is dependent upon the time of year at which cocoons are deposited, a flexible incubation period being adaptive to adverse conditions (Evans and Guild, 1948). The collation of results in Table 1.2 clearly illustrates the effect of temperature on the incubation periods of cocoons from various earthworm species.

1.2.1.3 Number of juvenile worms emerging per cocoon and hatchability:

Juvenile earthworm production per cocoon ranges from 1-20 in lumbricids and varies according to species (Stephenson, 1930). Of fourteen species cultured by Evans and Guild (1948) only two species: E.foetida and D.subrubicunda regularly produced more than one worm per cocoon (Table 1.3) and these

results were later confirmed in studies with Japanese earthworm species (Nakamura, 1973).

Table 1.2: The effect of temperature on the incubation periods of cocoons of various earthworm species

Species	Temperature (°C)	Incubation period (days)	Reference
E.foetida	10	85.5	Tsukamoto & Watanabe, 1977
A.chlorotica	10	112	Gerard, 1967
E.foetida	11	77	Evans and Guild, 1948
A.chlorotica	11	87.5	" " "
D.subrubicunda	11	59.5	" " "
A.longa	11	70	" " "
A.chlorotica	15	50	Gerard, 1967
E.foetida	15	45.6	Tsukamoto & Watanabe, 1977
Dendrobaena veneta	18	42	Dawkins, unpubl.
A.chlorotica	20	36	Gerard, 1967
E.foetida	25	19.2	Tsukamoto & Watanabe, 1977
E.foetida	25	21	Neuhauser et al., 1980a

Tsukamoto and Watanabe (1977) varied the temperature at which cocoons and earthworms (E.foetida) were held and demonstrated the multiple emergence of young worms decreasing with increasing temperatures from 10-25°C. Within the same temperature range the hatchability of cocoons also decreased with increasing temperature. Thus, optimum conditions for waste conversion may not always comply with conditions for maximum earthworm reproduction.

Hartenstein et al. (1979b) demonstrated a close association between the weight of adult E.foetida and the weight

Table 1.3: Number of worms emerging from isolated cocoons, expressed as a percentage of viable cocoons.[1] (Culture conditions given in text)

Species	Number of viable cocoons	% of cocoons with 1-8 worms 1	2	3	4	5	6	7	8
A.longa	89	97							
A.nocturna	56	93	7						
A.caliginosa	131	100*							
D.subrubicunda	340	62	33	4½	½				
A.chlorotica	142	100*							
E.foetida	139	20	30	27	15	2	3	2	½
Eisenia rosea	42	100*							
Eisenia tetrahedra	198	95½	4½						
L.castaneus	182	100							
Lumbricus festivius	50	100							
L.rubellus	105	100*							
Lumbricus terrestris	82	100							
O.cyaneum	40	90	10						
Octolasium lacteum	50	100							

* indicates two worms occasionally emerged

1 Source: Evans and Guild (1948)

of cocoons produced, and also between the weight of the cocoon and the number of juveniles emerging. The size of adult E.foetida is itself a reflection of the quality of the waste substrate as a source of nutriment.

1.2.1.4 Growth period to reach sexual maturity: The period of development from the emergence of the juvenile worm to the appearance of the clitellum is also predominantly influenced by temperature (Table 1.4).

Table 1.4: The effect of temperature on the growth period of various species of earthworm from hatching to sexual maturity

Species	Environmental conditions (°C)	Growth period (weeks)	Reference
L.terrestris	natural field conditions	52	Satchell, 1967
D.subrubicunda	Feb.-Mar. air raid shelter	22 - 42	Evans & Guild,1948
E.foetida	Jan.-Feb. "	70 - 74	"
E.foetida	July "	47	"
A.longa	July "	58	"
A.longa	Dec.-Feb. "	38 & 40	"
A.chlorotica	Nov.-Mar. "	29 - 42	"
A.caliginosa	Apr.-July "	54 - 58	"
A.caliginosa	natural field conditions	25	Gerard, 1967
A.chlorotica	" " "	21	"
A.chlorotica	15	17 - 19	Graff, 1953
A.chlorotica	18	13	Michon, 1954
E.foetida	18	9½	"
D.veneta	21	7½	Dawkins, unpubl.
E.foetida	28	6½	Michon, 1954

Hartenstein et al. (1979b) showed that the time taken for 50% of a population of E.foetida (at 25°C) to become clitellate was independent of population density and substrate (horse manure and sewage sludge). However, when E.foetida were fed substrates as widely different as dung and tilia leaves, the onset of sexual maturity was significantly affected (Michon, 1954).

1.2.2 Earthworm habitat and substrate preference

Several authors have classified the British Lumbricidae according to habitat and substrate preference. Satchell (1967) divided the earthworm species according to the strata of soil inhabited: either surface dwelling or deep burrowing. Bouché (1972) also classified the earthworm species indigenous to France according to the soil horizon colonised and the feeding habits of the worms. These classifications correlate well with those of Arthur (1965) and Piearce (1983) separating groups of earthworms according to their size and locomotive ability; Graff (1953) and Svendsen (1957a & b) who classed the earthworms as pigmented (Eisenia spp, Lumbricus spp and Dendrobaena spp) or unpigmented (Allolobophora spp and Octolasium spp); and Bornebusch (1930) and Piearce (1972) distinguishing acid tolerant, acid intolerant and ubiquitous species.

Satchell (1980) proposed that these divisions could be explained as a result of the basic principles of r and k selection (MacArthur and Wilson, 1967). Different survival strategies evolving in response to the nature of the environment, whether constant, 'k selection' (as in the mineral soil horizon) or unpredictable, 'r selection' (as in the surface litter layer).

1.2.2.1 Surface horizon: Earthworms colonising this horizon are relatively small (Arthur, 1965; Figure 1.3), pigmented (Graff, 1953, Svendsen, 1957a & b) and usually acid tolerant (Bornebusch, 1930). Evidence also suggests that these species have a higher optimum temperature for growth and are generally more fecund: producing numerous offspring per generation and many generations per year (Graff, 1953).

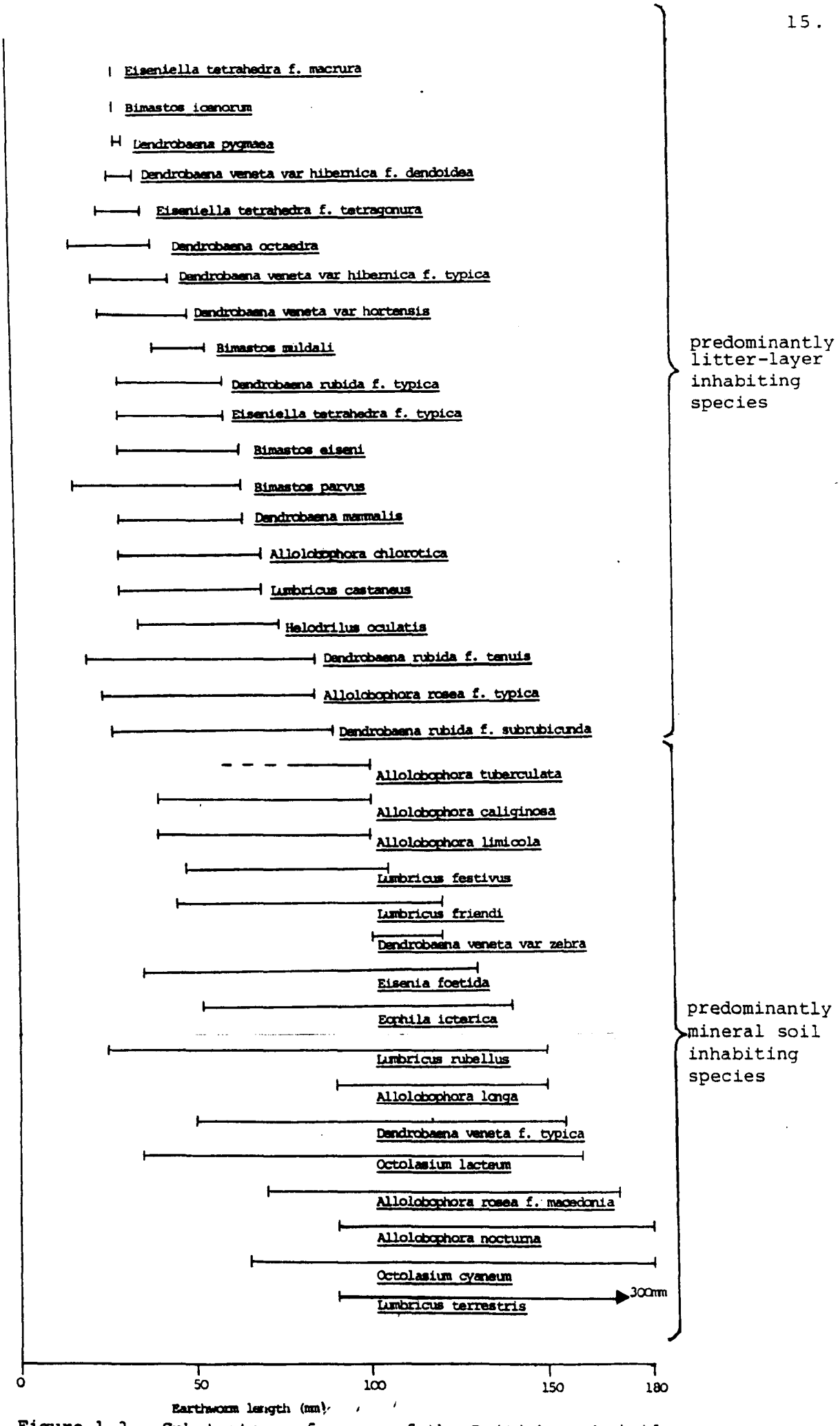


Figure 1.3: Substrate preference of the British Lumbricidae

The size of these earthworms restricts them to this region their length to width ratio making them incapable of burrowing deeply (Arthur, 1965). These earthworms feed almost exclusively on litter, ingest very little mineral soil and produce casts consisting almost entirely of litter fragments (van der Drift, 1961). The anatomy of the earthworm gut is adapted to the amount of mineral soil ingested; those species ingesting a high proportion of mineral soil possessing a longer gut with more folded typhlosole (Semonova, 1966).

The pigmentation may serve as a protection against predators (Satchell, 1967) and possibly from the harmful effects of ultraviolet light (Zeilenska, 1913; Merker and Braunig, 1927).

Being acid tolerant, these species would be adapted to survive the relatively low pH often encountered in this humus rich layer. The distinction between acid tolerant litter dwelling species and acid intolerant burrowing species made by Bornebusch (1930) was extended by Satchell (1955) to include a third group more ubiquitous and diverse in habit. Piearce (1972) further modified this classification to include the activity of the calcium glands in influencing the ability of earthworms to tolerate low pH. Piearce (1972) divided the earthworms into the following eco-physical groups:-

(i) Pigmented litter feeders - the ubiquitous species living in soils of wide pH and having very active calcium glands.

(ii) Unpigmented top-soil feeders - acid tolerant species with mineral soil a major part of the diet and having intermediate calcium gland activity.

(iii) Unpigmented compost feeders living in neutral or basic soil of high calcium content and having inactive calcium glands.

1.2.2.2 Mineral-soil horizon: The larger, deep burrowing species generally inhabit the mineral soil layer (Arthur, 1965; Satchell, 1967; Figure 1.3). These earthworms have a greater proportion of soil and less organic matter in their casts (Arthur, 1965). Within this horizon the earthworms are usually non-red or unpigmented (Graff, 1953; Svendsen, 1957a & b), these species may feed in the upper parts of the soil profile and have been commonly reported in fields, pasture and forest but not in compost. These species are apparently unable to tolerate high temperatures, are less fecund than species present in the litter layer and may be liable to aestivate through summer thereby interrupting reproduction (Evans and Guild, 1948; Graff, 1953).

Obviously, there is no clean cut division between groups of earthworms and consequently some species exhibit characteristics common to more than one group.

The characteristics associated with earthworm species occurring in the litter layer: ingesting a high percentage of organic matter, with high fecundity, activity at higher temperatures and adaptation to a wider range of pH makes them more suitable for use in waste management.

1.3 Earthworm Culture

Due to the natural occurrence of E.foetida in "manure, compost heaps, under dead leaves and generally in soil rich in organic matter" (Gerard, 1964) most of the research conducted in vermiculture has concentrated on this species. Some success has also been achieved growing Perionyx excavatus in India (Kale et al., 1982) and Eudrilus eugeniae in North America (Neuhauser et al., 1979), Germany (Graff, 1982) and Britain (C. A. Edwards, pers.comm.).

Hartenstein (1983) summarised the current research with E.foetida: under ideal conditions (at temperatures of between 18-25°C pH 6.0-7.0, within aerobic manures and sludges of moisture content 80-85%, rich in micro-organisms and with a nitrogen content of c. 1.2%) this earthworm species produced 1-10 juvenile worms per cocoon and 1-5 cocoons per week. At 20-25°C, 45-60% of the cocoons hatched after 3 weeks and 50% of the juvenile worms achieved sexual maturity after a further 4-6 weeks. Where growth was not limited by food supply or population density a 5% conversion ratio could be expected: 100 gms of organic substrate could be expected to yield 5 gms of earthworm biomass (on a wet weight basis, 80-85% moisture).

In the laboratory and under ideal experimental conditions Hartenstein (1981) achieved maximum earthworm biomass production of 2 gms live weight gain over a 7 week growth period in a surface area to volume of 24 cm^2:110 cm^3 (horse manure as food on 20 gms of soil). This rate of production could theoretically be extrapolated to a value of 6685 Kg protein/ha/year. Although a direct comparison cannot be made, the average output of animal-protein producing systems in North America was only 13 Kg protein/ha/year in 1980 (Pimental et al., 1980).

Animal manures and sewage sludges contain an optimum nitrogen content to support earthworm growth (Swingle, 1961; Gaddie and Douglas, 1975; Abe et al., 1979). However, freshly voided faeces and anaerobically digested sludges must undergo a period of biological and chemical alteration before earthworms will feed and survive in them (Neuhauser et al., 1980b; Mitchell et al., 1977; Mitchell et al., 1978). Earthworms have been

successfully grown on a wide range of organic wastes including kitchen wastes (Bhat et al., 1974; Neuhauser et al., 1980b), green manure (Bhat, 1974; Guerro, 1981), paper and cardboard (Gaddie and Douglas, 1975), agricultural processing by-products (Swingle, 1961; Neuhauser et al., 1980b) and industrial by-products, for example pulp refuse, (Tsukamoto and Watanabe, 1977). Under laboratory conditions simple nutrients such as proteins and carbohydrates did not support weight gain, nutritional benefit being derived solely from cellular mass (Neuhauser et al., 1980b).

1.4 Nutritional Evaluation of Earthworms

Earthworms are known to provide an essential link in many natural food chains, being readily consumed by numerous species of birds and small mammals for example shrews and moles (French et al., 1957; McInroy, 1971). Although earthworms may not form such a link in the food chain of fish a long term association between earthworms and fish is well established due to their use as an excellent bait in sport fishing. Furthermore with the expansion of commercial fish farming in Britain the use of live foods, such as earthworms, has been advertised as encouraging the feeding response of stressed fish and as an intermediate feed for juvenile fish (Nightingale, 1980). The provision of live foods for marine fish larvae is considered essential to ensure the survival and commercial production of these fish species (Bromley, 1979) and the aquatic oligochaetes Tubifex tubifex and Lumbriculus rivalus have been employed as a fish feed in this context.

The use of invertebrates in fish farming is not without historical precedent; and in various countries invertebrate

larvae and insects have been used to supplement the diets of cultured fish. In the early days of trout culture in North America livestock carcasses were hung above the raceways and the trout diet supplemented with maggots falling from the rotting flesh (Bowen, 1970). In Japan and China silkworm pupae have been utilized as a feed supplement in carp culture (Hickling, 1962) and the use of lights to attract aerial insects above fish ponds is described by Heidinger (1971). On an experimental scale, pupae of the face fly (Musca autumnalis, De Greer) have been successfully fed to channel catfish, Ictalurus punctatus (Loyacano, 1974) and soldier fly larvae (Hermetia illucens, L) satisfactorily used in feed for channel catfish and tilapia (Bondari and Sheppard, 1981). Earthworms of the species P.excavatus (Guerro, 1981), E.eugeniae (Hilton, 1983) and E.foetida (Aston et al., 1982b) have also been evaluated in feeding trials with tilapia (Oreochromis niloticus), rainbow trout (Salmo gairdneri) and eels (Anguilla anguilla) respectively.

Nutritional evaluation of earthworm biomass has indicated that on a dry weight basis, E.foetida, the earthworm species commonly grown in vermiculture, contains c. 60% crude protein and 10% lipid. Apart from a possible deficiency in the sulphur containing amino acids, methionine and cystine (Yoshida and Hoshii, 1978; Amerio, 1983; Hori et al., 1983) the amino acid composition of E.foetida compares favourably with that of fish meal protein the ingredient most commonly used to supply the dietary protein requirement in livestock feeds (Table 1.5).

The fatty acid composition of earthworm lipids (A.caliginosa and L.rubellus) extensively investigated by Hansen

Table 1.5: The essential amino acid composition of dried E.foetida and fish meal protein (values expressed as g/100g protein)

Amino acid	E.foetida[1]	Fish meal[2]
Arginine	6.1 - 7.0	7.8
Cystine	1.4 - 4.2	1.0
Histidine	2.2 - 4.3	2.6
Isoleucine	4.2 - 6.3	4.2
Leucine	7.8 - 8.7	7.1
Lysine	6.6 - 8.7	7.9
Methionine	1.5 - 3.6	3.1
Phenylalanine	3.5 - 4.6	3.6
Threonine	4.7 - 5.3	4.0
Tryptophan	1.2 - 1.5	1.1
Tyrosine	2.2 - 4.4	3.4
Valine	4.5 - 5.9	7.9

1. Maximum and minimum value given for each amino acid were obtained from the following sources: McInroy (1971); Schulz & Graff (1977); Sabine (1978); Yoshida & Hoshii (1978); Satchell (1980); White (1982).

2. Source: Tacon (1982).

and Czochanska (1975) revealed a high proportion of poly-unsaturated fatty acids, including the essential fatty acids linolenic acid and linoleic acid, of particular importance in fish nutrition (Castell, 1979). These results have been confirmed for other species of earthworm including E.foetida by Cerbulis (1967) and Amerio (1983). However, it should be emphasised that there is also some evidence to suggest that the fatty acid composition of earthworm lipids may vary according to species, substrate, age and season (Hansen and Czochanska, 1975; C. A. Edwards, pers.comm.).

Biological evaluation of earthworms has been achieved by feeding earthworms either live to poultry (Taboga, 1980) and eels, Anguilla anguilla (Aston et al., 1982b) or as a dried 'worm meal', included within a balanced feed ration for mice (McInroy, 1971); rats (Amerio, 1983); poultry (Sabine, 1978; White, 1982; Amerio, 1983; Hori et al., 1983); pigs (Sabine, 1978); fish (Guerro, 1981; Hilton, 1983) and humans (McInroy, 1971; Guerro, 1981).

The majority of feeding trials, conducted to evaluate earthworm meal in livestock feeds have used the earthworm species E.foetida, due to its availability and the comparative ease with which it may be produced in sufficient quantity. In feeding trials, no significant difference in growth was observed between broiler chickens fed a diet containing 15% protein supplied by E.foetida meal and those chickens fed a control diet in which the protein was supplied by meat meal (Harwood, 1976; Sabine, 1978).

Growing chickens fed diets containing 8% E.foetida meal exhibited significantly lower growth rates than birds fed a similar diet with 8% fish meal and no worm meal. However, when

the diets containing worm meal were supplemented with the amino acids methionine and glycine, no such difference arose (Hori et al., 1983). Amerio (1983) found E.foetida meal unsatisfactory as a feed for broilers: 7-8 days after initiating the feeding trials, the birds had intense diarrhoea. This response was also reported with rats fed 15% earthworm meal in the diet, even after sterilization of the worm meal (Amerio, 1983).

In contrast to these findings, McInroy (1971) fed mice on diets containing 43.4% steam bath dried E.foetida without deleterious effect. No significant difference occurred in the growth rates of these mice and mice fed diets containing crude edible caesin.

White (1983) assessed the biological value of dried E.foetida and L.terrestris force fed to mature cockerels. Both worm meals exhibited a high amino acid retention and had true metabolizable energy contents comparable to other dietary components. Live and powdered E.foetida and L.rubellus were reported to be palatable to chickens. However, a vitamin D deficiency was evident in birds not exposed to direct sunlight (Taboga, 1980).

A comparison of the growth of pigs fed starter and grower rations containing either worm meal or meat meal as the protein source revealed no significant difference which could be associated with the inclusion of E.foetida in the diets (Sabine, 1978).

With fish, Guerro (1981) reported a significant increase in the growth of O.niloticus fed diets containing 15% earthworm meal: 10% fish meal compared with fish fed diets containing 25% fish meal. The earthworm species used for this study, P.excavatus

was sun dried before being ground to a meal and incorporated into the fish diets.

In a comparison of the growth rates of eels, A.anguilla, fed various oligochaete worms (E.foetida, chopped, live; Lumbricillus rivalis, live; a mixture of Tubifex tubifex, Limnodrilus hoffmeisteri and Limnodrilus cervix, live) and a commercially available eel food, Aston et al. (1982b) reported highest growth rates by eels fed L.rivalis, the lowest percentage weight increase occurring with eels fed E.foetida.

Hilton (1983) evaluating an earthworm species indigenous to North America (E.eugeniae) reported a decrease in the growth rate and feed utilization efficiency with increasing dietary inclusion levels of worm meal from 50-100% of the protein source, in feeds for rainbow trout.

The primary aim of the present research was to evaluate various species of terrestrial lumbricid worms, as a feed for rainbow trout. This was approached in two ways:-

(i) Frozen slices of whole earthworms were offered to the fish as a sole feed for the duration of the feeding trial and the performance of fish fed these diets was compared with fish fed a commercially available trout pellet. The earthworm species Lumbricus terrestris, Allolobophora longa, Eisenia foetida, Dendrodrilus subrubicundus and Dendrobaena veneta were evaluated in this way.

(ii) Dried 'worm meals' derived from the earthworm species Eisenia foetida, Dendrodrilus subrubicundus and Dendrobaena veneta were assessed as a potential fish meal replacer in pelleted trout rations.

In addition, investigations were carried out during several of the experimental feeding trials into the possibility of heavy metal accumulation by the fish through the ingestion of contaminated earthworm diets. Levels of selected heavy metals were monitored in the diets, fish carcasses and various fish tissues throughout the experimental period.

CHAPTER 2

THE NUTRITIONAL EVALUATION OF FROZEN EARTHWORMS AS A SOLE FEED FOR RAINBOW TROUT

EXPERIMENT 2.1

THE NUTRITIONAL EVALUATION OF THE EARTHWORM SPECIES LUMBRICUS TERRESTRIS, ALLOLOBOPHORA LONGA AND EISENIA FOETIDA AS A FEED FOR RAINBOW TROUT

2.1.1 INTRODUCTION

A preliminary seventy-day feeding trial was conducted with rainbow trout to assess the acceptability of three earthworm species: Lumbricus terrestris, Allolobophora longa and Eisenia foetida to the fish. The nutritional value of these worms was measured in terms of growth response, feed utilization efficiency and whole fish carcass composition at the end of the experiment. A commercially available trout pellet served for comparison as the control.

These particular earthworm species were chosen for evaluation since they were available in sufficient quantities for the period of this feeding trial. However, of the three earthworm species tested, only E.foetida has been demonstrated to have the potential for commercial culture (Watanabe and Tsukamoto, 1976; Hartenstein et al., 1979a). For example, L.terrestris has never been successfully bred in the laboratory (Hartenstein, 1983).

When earthworms are cultured as part of an integrated agricultural/aquacultural system it is likely that they will be fed directly to fish, without any form of pre-treatment, either as a supplementary or complete feed. In the experiment described in this chapter earthworms are fed as the sole diet for rainbow trout during the trial period. Any nutritional deficiencies within the earthworms should become quickly apparent under these circumstances.

It has been suggested that since earthworms are liable to accumulate toxic mineral elements from their surrounding substrate into their tissues (Van Hook, 1974; Gish and Christensen,

1973; Ireland, 1979; Ash and Lee, 1980; Hartenstein et al., 1980a) a potential hazard exists when they form part of a food chain. Therefore in this experiment levels of Ca, P, K, Na, Mg, Fe, Zn, Mn, Cu, Pb, Cr, Ni, Co and Cd have been measured in diets and fish maintained on these diets during the experimental period.

2.1.2 MATERIALS AND METHODS

2.1.2.1 Diets

All earthworms used in this study were supplied by Dr. C. A. Edwards and J. R. Lofty, Rothamsted Experimental Station, Harpenden, Herts. L.terrestris and A.longa were collected from rough pasture and E.foetida from a local sewage works. The earthworms were frozen directly after collection and not stored on an inert substrate to void their gut lumen.

Since this experiment was designed as a direct comparison between earthworms, as a complete diet, and a commercially available trout pellet (Edward Baker Ltd., Sudbury, Suffolk) no adjustment could be made to ensure that diets were either iso-nitrogenous or iso-calorific.

The proximate and mineral composition of the experimental diets and the amino acid and fatty acid composition of the earthworm species tested is shown in Tables 2.1.1 and 2.1.2 respectively.

During the 70-day experimental feeding period six dietary regimes were tested as follows:-

Table 2.1.1: Proximate and mineral composition of experimental diets (all values expressed on a dry weight basis)

Component	A.longa	L.terrestris	E.foetida	Commercial trout pellet*1
Moisture (%)	78.29	81.09	83.26	9.00
Nutrient content (% dry weight)				
Crude protein	50.43	56.10	58.78	46.30
Lipid	1.44	2.13	9.04	7.66
Ash	35.20	28.72	17.24	13.58
Acid insoluble ash	25.97	19.18	11.79	-
Crude fibre	0.45	1.26	0.20	-
NFE*2	12.48	11.79	14.94	32.46
Energy content (kcal/100g dry wt)				
Gross energy*3	351.05	387.17	480.69	466.52
Digestible energy*4	290.07	323.25	405.14	365.36
Mineral content				
Macro-elements (g/kg dry weight)				
Ca	3.40	6.96	6.13	29.98
P	8.95	8.10	9.07	17.96
K	7.03	8.83	6.93	9.74
Na	3.38	3.57	4.52	7.79
Mg	0.73	0.82	0.63	2.34
Micro-elements (mg/kg dry weight)				
Fe	5280.69	4014.09	1228.49	123.02
Zn	300.99	304.49	252.94	102.27
Mn	282.61	248.58	18.89	74.06
Pb	24.62	23.40	20.85	9.68
Cu	12.25	13.34	25.79	11.70
Cr	10.28	8.59	6.83	3.49
Ni	7.74	8.38	13.32	3.79
Co	7.42	8.55	4.02	4.25
Cd	4.06	3.30	4.90	<0.01

*1 Edward Baker Ltd., Sudbury, Suffolk

*2 Nitrogen Free Extract (100-H_2O + crude protein + lipid + ash)

*3 Calculated on an estimated 5.7 kcal/g protein; 9.5 kcal/g lipid; 4.0 kcal/g carbohydrate

*4 Calculated on an estimated 5.0 kcal/g protein; 9.0 kcal/g lipid; 2.0 kcal/g carbohydrate (Cowey et al., 1972)

- not determined

Table 2.1.2: Amino acid and fatty acid composition of three earthworm species used as experimental diets

	E.foetida	L.terrestris	A.longa*1
Amino acid (g amino acid/100g recovered amino acid)			
Aspartic acid	9.30	9.35	11.36
γ-aminobutyric acid	0.66	0.93	-
Alanine	6.50	6.03	5.97
Cystine*2	0.70	0.66	0.68
Glutamic acid	17.70	17.56	13.66
Glycine	6.44	5.70	6.26
Proline	4.75	3.73	3.96
Serine	5.19	5.47	5.47
Arginine	5.62	6.54	7.17
Histidine	2.96	2.85	2.30
Isoleucine	4.14	4.53	5.10
Leucine	8.29	8.48	8.13
Lysine	6.52	7.26	7.81
Methionine	2.80	2.29	1.14*3
Phylalanine	3.97	4.17	6.04
Threonine	5.60	5.12	4.80
Tryptophan	0.72	0.91	-
Tryosine	3.46	3.67	4.53
Valine	4.65	4.75	5.60
Fatty acid*4 (% of total free and fatty acid g.l.c. peak areas)*5,*6			
11:0	7.87	1.62	
12:0	11.88	0.70	
13:0	2.70	-	
14:0	2.63	2.06	
14:1	4.81	2.17	
15:0	1.21	-	
16:0	5.07	5.41	
16:1	2.29	5.95	
18:0	7.36	6.65	
18:1	10.62	11.06	
18:2	6.44	8.53	
Unidentified	-	6.14	
18:3	5.89	6.62	
20:4	7.53	12.22	
Unidentified	9.87	7.76	

*1 No samples were available for fatty acid analysis.
*2 Determined as cysteic acid after performic acid oxidation.
*3 These values are low due to oxidation during hydrolysis.
*4 Figures before colon indicate number of carbons, figures after indicate number of double bonds.
*5) Averages based on 8 and 3 (E.foetida, L.terrestris) samples of
*6) worms of varying age groups and from different substrates.
- Not detected.

Treatment	Feeding regime	
	a.m.	p.m.
1	Frozen *L.terrestris*	Frozen *L.terrestris*
2	Frozen *A.longa*	Frozen *A.longa*
3	Frozen *E.foetida*	Frozen *E.foetida*
4	Commercial pellet	Frozen *E.foetida*
5	Frozen *E.foetida*	Commercial pellet
6	Commercial pellet	Commercial pellet

An accurate record was kept of the exact weight of each diet consumed by the fish during successive bi-weekly intervals throughout the feeding trial.

Fish were offered the diets twice daily, six days per week. A twice daily frequency of feeding to fish has been demonstrated to give optimum growth and feed conversion efficiency (Grayton and Beamish, 1977). At 1000 and 1700 hours all fish were fed "to appetite": pieces of food were offered to the fish until the feeding response ceased and one or two pieces of food were ignored at the base of the tank.

During feeding, earthworms not immediately being chopped and offered to the fish were stored in a beaker within a thermos flask. In this way the defrosting and refreezing of earthworms which may have encouraged bacterial spoilage was avoided.

2.1.2.2. Animals and Tanks

One hundred and forty rainbow trout, of mean weight 30g, were supplied by Howietoun Fish Farm, Bannockburn, Nr. Stirling, Scotland, and divided into seven groups ensuring that each group of twenty fish was approximately the same weight.

The fish from one group were killed by a sharp blow on the head and stored at -20°C for subsequent whole carcass proximate and mineral analysis. Each of the remaining six groups of fish were housed in a 60ℓ circular tank and assigned one of the dietary feeding regimes.

Mains city water, artifically aerated (venturi aspirator) was supplied in a throughflow system, to each of the 60ℓ tanks at a rate of 2ℓ/min/tank. A central standpipe and sleeve was designed to ensure that faeces and excess feed were rapidly carried out of the tank (Figure 2.1.1).

The water temperature during the experiment was 7.5°C ± 1°C and fish were subject to natural photoperiod.

At the start of the experiment all fish were anaesthetised using Benzocaine (ethyl para-aminobenzoate; BDH Ltd., Poole, Dorset) at a concentration of 700 mg/ℓ, and weighed individually (after being shaken free of excess water) on a top pan balance to the nearest 0.01g. At bi-weekly intervals throughout the experimental period fish were weighed by this procedure. Prior to weighing, fish were not fed for at least 20 hours. Mortalities occurring during the experimental period were recorded.

On the final day of the experiment fish were anaesthetised and weighed as described above. The fish fed each dietary treatment were killed by a sharp blow on the head and from each group ten whole fish were stored at -20°C for subsequent proximate and mineral analysis. The livers from five fish were removed and weighed for the calculation of liver somatic index (LSI):

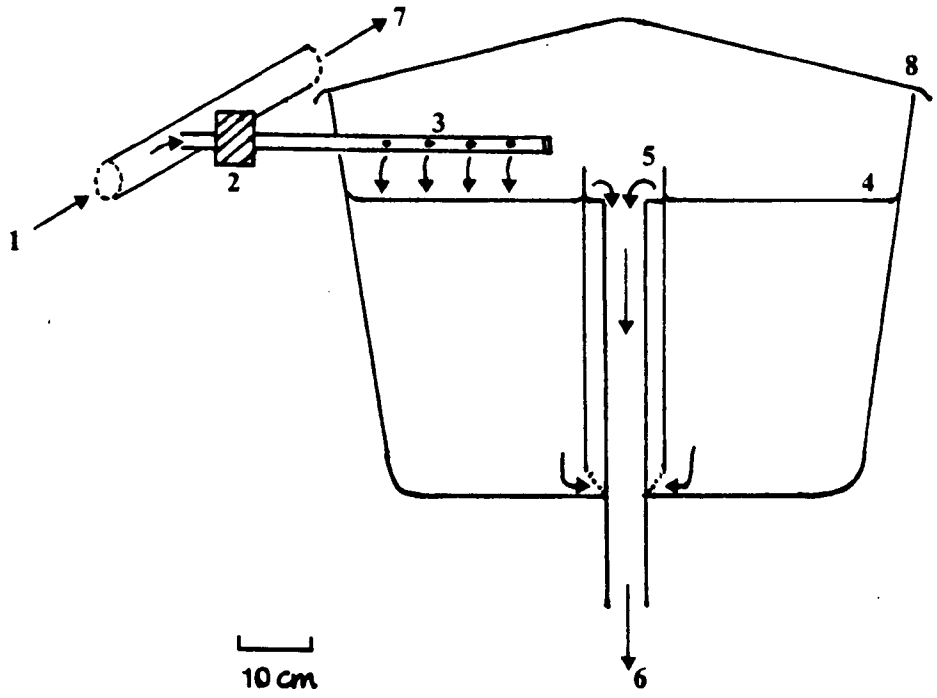


Figure 2.1.1 Diagramatic representation of one of the tanks within the fish holding system

Key:

↗	Direction of water flow
1	Inflow from mains water
2	Tap for adjusting flow rate of water into the tank
3	Water inflow into the tank
4	Level of water within the tank
5	Central standpipe and sleeve designed to carry water, feed and faeces rapidly from the base of the tank
6	Water outflow to drain
7	To remaining tanks in the system
8	Translucent fibreglass cover

$$LSI = \frac{\text{Liver weight}}{\text{Weight of whole fish}} \times 100.$$

Livers were then stored at -20°C for subsequent mineral analysis. From the remaining five fish in the group slices of liver, kidney, spleen and gill were removed and stored in fixative (10% buffered formalin) for subsequent histological examination.

2.1.2.3 Chemical Methods

(i) Fish samples: The ten whole fish from each treatment, and the fish sacrificed at the start of the experiment, were divided into three groups onto clean, dry, pre-weighed glass petri dishes and oven dried at 105°C for 24 hours. Samples were cooled in a dessicator and the moisture content calculated as weight lost on drying. After grinding each sample to a homogenous powder in a glass pestle and mortar the following determinations were undertaken:

Crude protein - determined by the indirect method of Munro and Fleck (1969) in which total nitrogen in the sample is measured and multiplied by a factor of 6.25. This assumes that protein contains 16% nitrogen. In fact the N content of individual proteins may vary from between 12% and 19% N (Tacon, 1979).

Crude lipid - determined by the method of Korn and Macedo (1973)

Ash - determined as the inorganic residue remaining after ignition of the sample in a muffle furnace at 450°C for 16 hours (AOAC, 1980).

Mineral determination -

Techniques using "wet-ash digestion" and "ignition/dry ash digestion" are commonly employed to prepare

samples for mineral determination. The latter method is advantageous since it enables larger samples to be used and facilitates the determination of elements present at low concentrations (e.g. Cadmium). Furthermore since the organic fraction is destroyed prior to digestion with concentrated acids this method is less hazardous. However the high temperatures involved in the ignition/dry ash method may result in the loss of potentially volatile elements (Katz et al., 1981). Sub-samples of dried, ground earthworms were prepared for mineral determination by both methods and the results showed consistently lower concentrations for all trace elements except iron (where conc.HNO_3 causes an interference in the detection of this element) when samples were prepared by "dry ash" digestion.

The following procedure was therefore adopted to prepare samples for mineral determination:- Replicate sub-samples (moisture free) of finely ground carcass and livers from fish fed dietary treatments 1, 2, 3 and 6 were digested with 5 ml concentrated HNO_3 at room temperature for 48 hours. The HNO_3 was then refluxed with the samples at 120°C until brown fumes ceased to evolve. After cooling 3 ml of 70% $HClO_4$ was added and refluxing continued for a further 30 minutes. On cooling the sample was filtered through ashless filter paper, made up to volume with de-ionised water and stored in acid washed polyethylene bottles. Analysis of Ca, K, Na, Mg, Fe, Zn, Mn, Cu, Pb, Ni, Cr, Co and Cd was carried out using a Perkin Elmer 373 Atomic Absorption Spectrophotometer according to the manufacturers' instructions. For the determination of Ca and Mg, lanthanum chloride was added to give a final concentration of 0.75%.

The determination of phosphorus was carried out by the method of Eisenreich et al., (1975). This method is adapted for use with acidified solution and therefore samples digested as described above with $HNO_3/HClO_4$ were diluted with the appropriate volume of double distilled water before adding the mixed reagent (antimony - H_2SO_4/molybdate). Phosphorus standards were adjusted to contain the same percentage by volume of acid as the samples.

(ii) Diets: Triplicate samples of each of the experimental diets were analysed for moisture, ash, crude lipid, crude protein and minerals as described above. Due to the exceptionally high ash content of earthworms used as diets in this experiment the acid insoluble ash content (a fraction generally considered unavailable to fish) was determined. The inorganic fraction remaining after ash determination was refluxed with 10% w/v HCl for 25 minutes, cooled and filtered through ashless filter paper. After oven-drying the residue and filter paper, until moisture free, the sample was ignited in a muffle furnace at 450°C for 8 hours. The fraction remaining is designated acid insoluble ash. Nitrogen Free Extractive (NFE: 100-crude protein + lipid + ash + moisture) was determined by difference and the gross and digestible energy contents of the diets by calculation (Cowey et al., 1972).

The amino acid composition of freeze dried homogenised worm tissue was determined by ion-exchange chromatography using a Techicon TSM Amino Acid Analyser, following hydrolysis with toluene 4-sulphonic acid containing tryptamine and mercapto-ethanol (Liu and Chang, 1971)., Cystine was determined as cysteic acid following performic acid oxidation of worm tissue (Moore, 1963). For fatty acid analysis replicate samples

of E.foetida and L.terrestris were extracted with a mixture of acidified chloroform:methanol (2:1 v/v) in a Silverson "Versor" blender according to the method of Folch et al. (1957) and the crude lipid extract saponified with methanolic NaOH. The fatty acid methyl esters were prepared with 14% methanolic BF_3, the methyl esters extracted into hexane and analysed by gas-liquid chromatography using a Pye Unicam Series 105 gas chromatograph.

2.1.2.4 Histological Methods

Tissue slices of gill, liver, spleen and kidney, fixed in 10% buffered formalin were embedded in paraffin wax, cut at 5 µm, stained with haematoxylin and eosin and examined under light microscopy.

2.1.2.5 Statistical Methods

For statistical comparison analysis of variance was calculated and significant differences between the means determined by Duncans' Multiple Range test (Duncan, 1955). The standard error of the mean (± SE) has been given to indicate the range of the means.

2.1.3 RESULTS

During the first two weeks of the experiment the fish were extremely nervous, ate very little food and consequently lost weight over this period. However, for the remaining eight weeks fish maintained on diets 1, 2, 6 and, to a lesser extent, diet 4, fed aggressively and exhibited good growth. Fish fed diet 3 (frozen E.foetida twice daily) and diet 5 (frozen E.foetida a.m./commercial pellet p.m.) had a limited feeding response and showed little or no growth during the experimental period.

2.1.3.1 Growth Response and Feed Utilization Efficiency

The growth response and feed utilization efficiency of fish over the experimental test period is shown in Table 2.1.3 and the growth response is shown graphically in Figure 2.1.2.

On the basis of mean final fish weight, percentage weight gain and specific growth rate (SGR: Log_e final body weight - Log_e initial body weight/time (days) x 100, Table 2.1.3) fish fed L.terrestris and A.longa exhibited a better growth response than fish fed the commercial pellet and commercial pellet a.m./E.foetida p.m. combination. However, no significant difference ($p < 0.05$) emerged between fish fed each of these diets in terms of mean final body weight. Fish fed frozen E.foetida and E.foetida a.m./commercial pellet p.m. had significantly lower ($p < 0.05$) mean final body weights, and consequently a lower percentage weight gain and specific growth rate than fish fed the other four dietary treatments.

Fish found frozen E.foetida to be unpalatable consuming only a sufficient quantity of feed to maintain their body weight. The unpalatable nature of frozen E.foetida was such that fish fed diet 5 (frozen E.foetida a.m./commercial pellet p.m.) exhibited no feeding response when offered the commercial pellet in the afternoon. Furthermore, the growth of fish fed diet 4 (commercial pellet a.m./E.foetida p.m.) was entirely due to the fish consuming the commercial pellet since frozen E.foetida was completely rejected when offered during the afternoon feed.

The efficiency of feed utilization by the fish was measured in terms of the food conversion ratio (FCR = Food fed/ Weight gain). To facilitate comparison between dietary treatments

Table 2.1.3: Growth, feed utilization, liver somatic index and carcass composition of rainbow trout fed the experimental diets for seventy days

Mean values	Dietary treatments 1	2	3	4	5	6	± SE*1
Mean initial weight (g)	31.10[a]	30.90[a]	30.80[a]	31.30[a]	30.20[a]	30.40[a]	0.992
Mean final weight (g)	50.24[b]	51.72[b]	30.89[a]	45.68[b]	34.22[a]	47.70[b]	2.160
Weight gain (%)	61.54	67.38	0.29	45.94	13.31	56.91	
Specific growth rate (%)	0.68	0.74	0.04	0.54	0.18	0.64	
Total food intake (g)	2420.97	2712.65	820.18	362.38* 407.06+	212.58* 567.28+	507.74	
Food intake (mg/day,dry weight)	375	366	115	324	242	367	
Weight gain (mg/day)	274	298	<0.01	205	0.06	248	
Food conversion ratio	1.37	1.23	-	1.58	-	1.48	
Protein efficiency ratio	1.45	1.45	-	1.22	-	1.46	
Nitrogen intake (mg/day)	30.24	32.80	10.80	26.88	20.80	27.20	
Nitrogen deposition (mg/day)	7.36	8.00	<0.01	5.76	2.08	6.56	
Apparent N utilization (%)	24.34	24.39	-	21.43	10.00	24.12	
Gross energy intake (kcal/day)*2	1.316	1.417	-	-	-	1.712	
Digestible energy intake (kcal/day)*3	1.088	1.183	-	-	-	1.341	
Liver somatic index (%)	1.83[b]	1.84[b]	1.17[a]	1.60[b]	1.50[ab]	1.68[b]	0.129

Carcass composition (% wet weight)

	Initial	Final					
Moisture	74.03	76.45[b]	75.35[a]	77.49[c]	75.18[a]	76.67[b]	75.20[a]	0.280
Crude protein	15.37	15.89[a]	15.99[a]	15.50[a]	16.00[a]	16.13[a]	15.75[a]	0.376
Lipid	7.19	4.50[a]	5.60[b]	3.99[a]	6.15[bc]	4.20[a]	6.45[c]	0.231
Ash	2.37	2.69[a]	2.63[a]	2.69[a]	2.63[a]	2.65[a]	2.47[a]	0.042

*1 Standard error, calculated from residual mean square in the analysis of variance

* frozen E.foetida + commercial trout pellet

abcd Mean values for components with the same superscripts are not significantly different ($p < 0.05$)

*2 Gross energy calculated as 5.7 kcal/g protein; 9.5 kcal/g lipid and 4.0 kcal/g carbohydrate

*3 Digestible energy calculated as 5.0 kcal/g protein; 9.0 kcal/g lipid and 2.0 kcal/g carbohydrate (Cowey et al., 1972)

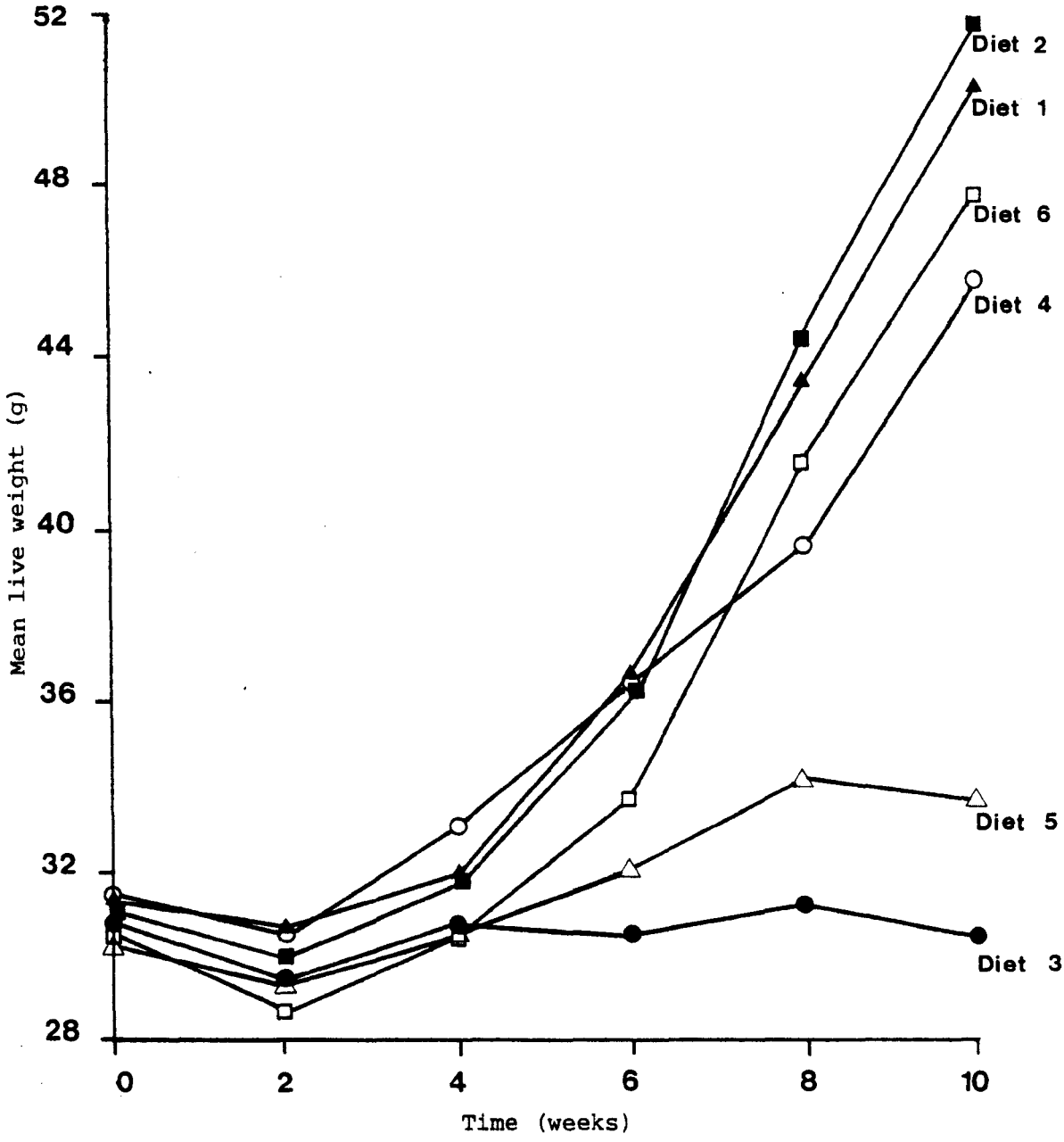


Figure 2.1.2: The growth response of rainbow trout fed the six experimental diets

the weight of food fed to the fish has been expressed on a moisture free basis and FCR calculated using the dry weight values. Fish fed diets 1, 2, 4 and 6 exhibited a good FCR and bearing in mind the high ash content of A.longa (Diet 1) and L.terrestris (Diet 2), 35.2% and 28.72% respectively (Table 2.1.1) the FCR of fish fed these two diets was particularly good.

A better assessment of the nutritional quality of the diet was calculated as efficiency with which dietary protein was utilized by the fish, termed the protein efficiency ratio (PER):

$$PER = \frac{\text{gain in fish weight (wet weight)}}{\text{weight of crude protein consumed}}$$

and the apparent nett protein utilization (NPU):

$$App.NPU = \frac{\text{N content of final fish carcass} - \text{N content of initial fish carcass}}{\text{N consumed during experiment}} \times 100$$

This last parameter provides the best indication of protein utilization since it also takes into account the body composition of the fish. There was no apparent difference between fish fed diets 1 and 2 and diet 6 in terms of these parameters (Table 2.1.3).

Despite the different moisture content of diets 1, 2 and 6 (78.29%, 81.09% and 9.00% moisture respectively), fish fed frozen A.longa (diet 1) and L.terrestris (diet 2) had achieved the same dry matter feed intake (mg/day/fish) during the experimental period as those fish fed the commercial trout pellet, diet 6 (Table 2.1.3). The gross energy intake and digestible energy intake (k calories/day/fish) was higher for fish fed diet 6 than fish fed either of the frozen earthworm diets (diets 1 and 2, Table 2.1.3).

2.1.3.2 Carcass Composition and Liver Somatic Index

The carcass composition of fish sacrificed at the start (initial fish) and end (final fish) of the experiment is given in Table 2.1.3.

Proximate composition: After 70 days there was no significant difference ($p < 0.05$) between treatments on the basis of carcass crude protein and ash composition. However, significant differences ($p < 0.05$) in carcass moisture and lipid content had developed between fish fed the different dietary treatments and an inverse relationship was evident between these two components (Table 2.1.3). Fish fed diet 3 (frozen E.foetida) had a significantly lower ($p < 0.05$) liver somatic index compared with the remaining treatments.

Mineral Composition:

(i) Diets: The mineral composition of the earthworms L.terrestris, A.longa and E.foetida and the commercial trout pellet are given in Table 2.1.1. The macro-elements Ca, P, K, Na and Mg were present at lower concentrations in the earthworms species tested than the commercial trout pellet. Conversely, the micro-elements (Fe,Zn,Mn,Pb,Cu,Cr,Ni,Co,Cd) monitored were evident at higher concentrations in the L.terrestris, A.longa and E.foetida than the commercial trout pellet.

(ii) Fish carcass: The mineral composition of the fish carcass and liver of fish fed diets 1, 2, 3 and 6 is given in Table 2.1.4 and 2.1.5 respectively. Only fish fed a single diet type were used for mineral analyses. There was no significant difference ($p < 0.05$) between fish fed these diets in

Table 2.1.4: Mineral concentration in whole fish carcass of initial fish and fish fed dietary treatments 1, 2, 3 and 6 for seventy days (g or mg/kg,wet weight)

	Initial fish	Dietary treatment 1	2	3	6	± SE[1]
Macro-elements (g/kg)						
Ca	3.85	3.94[a]	3.36[a]	3.90[a]	3.43[a]	0.035
K	3.70	3.60[a]	2.90[a]	2.30[a]	2.80[a]	0.039
Na	1.03	1.15[b]	0.98[a]	1.00[ab]	1.09[b]	0.003
Mg	0.26	0.25[b]	0.22[ab]	0.20[a]	0.23[ab]	0.001
Micro-elements (mg/kg)						
Fe	11.07	89.95[c]	89.31[c]	41.12[b]	21.51[a]	6.080
Zn	20.75	29.45[b]	28.32[b]	19.93[a]	21.32[a]	1.743
Mn	2.74	5.48[d]	4.20[c]	2.25[a]	2.95[b]	0.195
Pb	1.94	2.61[b]	2.04[a]	1.68[a]	1.81[a]	0.160
Cu	1.95	1.62[ab]	1.42[a]	1.48[a]	1.83[b]	0.084
Cr	0.79	0.97[b]	0.92[b]	0.66[a]	0.70[a]	0.026
Ni	0.44	1.03[a]	1.58[b]	1.31[ab]	1.50[ab]	0.145
Co	0.50	0.61[b]	0.54[b]	0.44[a]	0.46[a]	0.030
Cd	<0.01	<0.01	<0.01	<0.01	<0.01	-

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

abc Mean values for components with the same superscripts are not significantly different ($p < 0.05$)

Table 2.1.5: Mineral composition of fish livers of fish fed dietary treatments 1, 2, 3 and 6 over a seventy day experimental period (g or mg/kg,wet weight)

	Dietary treatment 1	2	3	6	± SE[1]
Macro-elements (g/kg)					
Ca	0.10[a]	0.08[a]	0.23[a]	0.07[a]	0.004
K	3.80[a]	3.42[a]	3.81[a]	3.40[a]	0.022
Na	1.33[a]	1.29[a]	1.60[a]	1.33[a]	0.011
Mg	0.08[a]	0.09[a]	0.07[a]	0.10[a]	0.001
Micro-elements (mg/kg)					
Fe	51.30[a]	61.11[ab]	79.48[b]	78.01[b]	5.748
Zn	33.87[a]	31.22[a]	58.25[a]	34.75[a]	7.010
Mn	1.96[a]	0.17[a]	2.40[a]	1.69[a]	0.432
Pb	2.12[ab]	1.87[ab]	3.73[b]	1.42[a]	0.481
Cu	74.94[a]	67.19[a]	158.42[c]	133.13[b]	2.197
Cr	1.37[a]	1.01[a]	2.57[a]	1.00[a]	0.528
Ni	1.79[a]	2.30[a]	3.77[a]	1.21[a]	0.884
Co	0.78[a]	0.57[a]	1.40[a]	0.57[a]	0.270
Cd	<0.01	<0.01	<0.01	<0.01	-

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

abc Mean values for components with the same superscripts are not significantly different ($p < 0.05$)

terms of Ca, and K concentration. However, a significant increase ($p < 0.05$) in the carcass concentration of several micro-elements was evident in fish fed the earthworm rations:- Fish fed L.terrestris had significantly higher ($p < 0.05$) levels of Fe, Zn, Mn, Pb, Cr and Co than fish fed the control commercial trout diet. Fish fed A.longa had significantly higher ($p < 0.05$) levels of Fe, Zn, Mn, Cr and Co and fish fed E.foetida had significantly higher ($p < 0.05$) levels of Fe than fish fed the control commercial trout diet (Table 2.1.4). A significant increase ($p < 0.05$) was noted in the Cu and Fe concentration in livers from fish fed E.foetida (diet 3) and the trout pellet (diet 6), similarly there was a significant increase ($p < 0.05$) in the liver Pb concentration of fish fed frozen E.foetida compared with the control trout pellet treatments.

Examination of the mineral uptake by fish fed the different diets based on the mineral composition of the fish carcass is limited by the variation arising between treatments in the feed intake and growth of fish during the experimental period. In order to facilitate a comparison between dietary treatments the following parameters have been calculated and are presented in Table 2.1.6:-

$$\underline{\text{Intake}} \text{ (Total intake of each mineral element)} = \text{concentration of element in the diet} \times \text{intake of diet (per fish) during exptl. period}$$

$$\underline{\text{Retention}} = \{\text{conc.of element in final fish} \times \text{mean wt.of final fish}\} - \{\text{conc.of element in initial fish} \times \text{mean wt.of initial fish}\}$$

$$\underline{\text{Ret./gm}} = \frac{\text{Retention}}{\text{Increase in wt. of fish during experiment (g)}}$$

Table 2.1.6: Total dietary intake, retention and retention per g. fish wet weight gained of mineral elements over the 70 day experimental period

Element		Dietary treatments 1	2	3	6
Macro-elements (mg)					
Ca	Intake	89.35	178.51	49.34	770.13
	Retention	78.21	54.81	1.89	46.57
	Ret/g	4.09	2.63	21.01	2.69
K	Intake	184.74	226.47	52.28	246.20
	Retention	65.79	35.66	-ve	21.08
	Ret/g	3.44	1.71	-	1.22
Na	Intake	88.82	91.56	34.10	196.91
	Retention	25.75	18.86	1.64	20.68
	Ret/g	1.35	0.91	18.22	1.20
Mg	Intake	19.18	21.03	4.75	59.40
	Retention	4.47	3.34	-ve	3.07
	Ret/g	0.23	0.16	-	0.18
Micro-elements					
Fe	Intake µg	138.77	102.95	9.92	3.16
	Retention µg	4174.81	4277.05	929.24	689.50
	Ret/g	218.12	205.43	10,324.89	39.86
Zn	Intake mg	7.19	7.81	2.04	2.63
	Retention µg	834.24	823.53	-ve	386.16
	Ret/g	43.59	39.55	-	22.32
Mn	Intake mg	7.43	6.38	0.15	1.90
	Retention µg	190.11	132.55	-ve	57.42
	Ret/g	9.93	6.37	-	3.32
Pb	Intake mg	0.65	0.60	1.68	0.25
	Retention µg	70.80	45.56	-ve	27.36
	Ret/g	3.70	2.19	-	1.58
Cu	Intake mg	0.32	0.34	0.21	0.30
	Retention µg	20.74	13.18	-ve	87.29
	Ret/g	1.08	0.63	-	5.05
Cr	Intake mg	0.27	0.22	0.06	0.09
	Retention µg	24.16	23.17	-ve	9.37
	Ret/g	1.26	1.11	-	0.54
Ni	Intake mg	0.20	0.22	0.11	0.10
	Retention µg	38.07	68.12	26.92	58.17
	Ret/g	1.99	3.27	299.11	3.36
Co	Intake mg	0.20	0.22	0.03	0.11
	Retention µg	15.10	12.48	-ve	6.74
	Ret/g	0.79	0.60	-	0.39
Cd	Intake mg	0.11	0.08	0.04	-
	Retention µg	-	-	-	-
	Ret/g	-	-	-	-

Fish fed L.terrestris and A.longa exhibited higher retention per g fish weight gained for each micro-element except Ni, Cu and Cd compared with fish fed the commercial trout pellet. Since fish fed E.foetida gained virtually no weight over the 70 day period these results are not comparable.

2.1.3.3 Histological examination

Histological examination of the liver, spleen, gill and kidney tissues revealed no apparent differences between fish fed the various experimental diets.

2.1.4 DISCUSSION

The results of this experiment suggest that for a period of 70 days the earthworm species L.terrestris and A.longa adequately replaced the commercial trout pellet as the sole source of food for rainbow trout without adverse affect on the growth rate or health of the fish. However the earthworm E.foetida did not support fish growth and without some form of pre-treatment this species can serve little or no purpose as a complete diet or feed supplement for rainbow trout.

This result was surprising in view of the recommended use of E.foetida as a feeding stimulant for fish (Nightingale, 1980) and its sale for use as fish bait. Previous research using E.foetida in formulated dietary rations for mice, pigs and poultry has suggested that dried E.foetida meal adequately replaces other dietary proteins without a significant decrease in growth rate (McInroy, 1971; Sabine, 1978). Aston et al. (1982) fed various oligochaete worms (including E.foetida) and a commercial eel food to eels (A.anguilla) and reported poor growth rates with eels fed E.foetida. Similar results were

observed when frozen E.foetida was fed to Oreochromis niloticus (pers.obs.).

Several authors suggest that levels of the essential amino acids; methionine, cystine and glycine (glycine only being essential for growing chicks) in E.foetida may be limiting (Yoshida and Hoshii, 1975; Hori et al., 1983). Although the levels of essential amino acids were consistently lower in E.foetida compared with A.longa and L.terrestris both methionine and cystine were present at higher levels in E.foetida than either A.longa or L.terrestris (Table 2.1.2). It is unlikely that an amino-acid imbalance would cause the severe reduction in feed intake observed during this experiment when fish were offered frozen E.foetida.

The banded or striped appearance of E.foetida distinguishes it from the majority of other terrestrial lumbricids. This may be a warning to predators of its unpalatable nature. As a defense mechanism E.foetida ejects a foul smelling yellow fluid from the dorsal pores in response to unfavourable stimulii (Gerard, 1964; Edwards and Lofty, 1977). This is shown in Plate 2.1.1. Taxonomically E.foetida is so named because of its fetid or garlic smell/taste (Edwards and Lofty, 1977).

The coelomic fluid is believed to be responsible for this malodour and Cuenot (1898) suggested that the eleocytes within the coelomic fluid cause the foul smell. Eleocytes are derived from phagositic amoebocytes once these cells have encapsulated unfavourable substances present in the coelomic fluid (Stephenson, 1930).

Plate 2.1.1: E.foetida showing the yellow coelomic fluid expelled in response to unfavourable stimulii

The chloragogenous tissue with a liver-like function and the ability to store glycogen and lipid (Roots, 1960) has also been shown to accumulate toxins from within the coelom, in particular heavy metals e.g. lead (Ireland and Richards, 1977) and to play a part in nitrogen metabolism (Cohen and Lewis, 1949, 1950; Needham, 1957). Chloragocytes, broken away from the chloragogenous tissue around the gut, are also present within the coelomic fluid.

In addition, the coelomic fluid of E.foetida has also been reported to contain a heat labile haemolytic factor (Andrews and Kukulinsky, 1975; Roch et al., 1981). This haemolysin caused haemolysis of various vertebrate erythrocytes, including frogs, newts, sheep and humans (Roch et al., 1981).

Of all the earthworm species tested, E.foetida is one of the most likely candidates for commercial culture. Therefore further investigation into the factors responsible for its unpalatable nature and various simple and inexpensive processing techniques which may improve the acceptability of E.foetida to rainbow trout are included in Chapter 4.

If earthworm species which are palatable to fish are considered the results of this experiment are extremely encouraging.

Since no artificial inert marker was added to the diets nutrient digestibility co-efficients were not determined. However, the nutritive constituents of A.longa and L.terrestris are likely to be readily digested and utilized by trout if FCR's of 1.37 and 1.23 could be achieved despite the acid insoluble ash contents of 25.97% and 19.18% respectively in these diets

(Table 2.1.1). Nose and Mamiya (1963) have demonstrated a negative correlation between digestibility co-efficients and increasing ash content of diets for rainbow trout.

Assuming that acid insoluble ash is nutritionally unavailable to the fish this proportion could be subtracted from the total feed consumed by the fish and the FCR recalculated as 1.01 and 0.99 for fish fed A.longa and L.terrestris respectively. This compares favourably with the FCR of the commercial pellet (1.48) and suggests that the unidentified fraction of the earthworm termed NFE in Table 2.1.1 may be available for utilization by the fish.

This fraction (NFE) within the earthworm, partially consists of glycogen reserves which vary according to the nutritional status of the worm (Waraska et al., 1980). These workers determined the glycogen content of feeding L.terrestris to be 1.4% (wet weight). Durchon and Lafon (1951) also analysing L.terrestris reported a glycogen content of 11% (dry weight). The glycogen present within the earthworm tissue may have been utilised more efficiently by the fish than the carbohydrate component of the commercial trout pellet; and this may in part explain the better FCR observed.

In terms of PER and Apparent NPU there was no difference between the earthworm diets and the commercial pellet (Table 2.1.3). The earthworm diets supplied protein to the fish in excess of their basic dietary requirements, and this excess is almost certainly utilized by the fish as an energy source (Lee and Putnam, 1973). Rainbow trout require approximately 40-50% crude protein in their diet (depending on their developmental status) for optimum growth and protein utilization (Satia, 1974;

Zeitoun et al., 1976; Tiews, Gropp and Koops, 1976). The earthworms (on a dry weight basis) supplied 50.43% (A.longa) and 56.10% (L.terrestris) crude protein, while the commercial pellet comprised only 46.30% crude protein.

During this experiment fish offered diets with very different moisture levels apparently adjusted their feed intake over the 70 day feeding period so that they consumed the same amount of feed on a dry weight basis. Previous investigation with rainbow trout (Lee and Putnam, 1973) and channel catfish Ictalurus punctatus (Page and Andrews, 1973) have suggested that the feed intake of fish is governed by the dietary energy intake. It was evident from calculations of gross energy and digestible energy intake (Table 2.1.3) that fish maintained on the commercial pellet had a higher energy intake over the experimental period than fish maintained on diets 1 and 2. As mentioned previously the nature of the carbohydrate fraction within these diets is likely to differ and gross and digestible energy conversion values would therefore need to be adjusted.

As a result of being fed diets of frozen earthworms the final carcass composition of rainbow trout was significantly altered (Table 2.1.3). A significantly lower ($p < 0.05$) carcass lipid content was evident when fish were fed earthworms compared with fish fed the commercial pellet. The earthworm species A.longa and L.terrestris comprised 1.44% and 2.13% crude lipid (dry weight, Table 2.1.1) these levels fall well below the levels usually included in pelleted trout feeds (c.10%, Cowey and Sargent, 1972). This may explain the low carcass lipid content of fish fed diets 1 and 2. Although E.foetida

contained 9.04% crude lipid, fish fed diets 3 and 5 (E.foetida dietary combinations) were consuming little or no feed and their low carcass lipid composition would be consistent with starvation and the utilization of body lipid reserves. In addition, fish fed diets 3 and 5 also displayed the lowest liver:body weight ratio (LSI) consistent with a starving condition (Takeuchi and Watanabe, 1982), while the LSI of fish fed A.longa and L.terrestris was not significantly different ($p < 0.05$) from fish fed the commercial trout pellet (Table 2.1.3).

Mineral Composition:

The concentrations of macro-elements were lower in the earthworm diets compared with the commercial trout ration (Table 2.1.1). Although rainbow trout have been shown to be able to utilize Ca directly from their feed (Podoliak and Holden, 1965; Ichikawa, 1960) absorption of Ca through the gills from the surrounding water has also been demonstrated (Ichikawa et al., 1962) and the dietary Ca requirement has been determined as approximately 0.2% for salmonids (Rumsey, 1977). In the diets employed for this study an adequate supply of Ca was present (Table 2.1.1).

The major source of P to fish is from the feed (Lall, 1979) and consequently fish generally have a higher dietary requirement for P than for Ca (Ogino and Takeda, 1978). Experimental evidence suggests that a level of 0.65% to 0.80% P in the diet is sufficient to meet the dietary requirements for rainbow trout, the absolute amount depending on the availability and chemical form of the P present (Ogino and Takeda, 1978; Lovell, 1979). In general, P from animal sources is believed to be

more available to fish than P contained within plant feedstuffs (Lovell, 1979; Nakamura, 1982).

The ratio of Ca:P also has important implications in the uptake of P, excessive Ca in the diet inhibiting P uptake. A dietary Ca:P ratio of 1:2 has therefore been suggested as optimum for salmonids (Lall, 1979). Fish fed diets deficient in dietary P have generally exhibited poor growth and skeletal deformation (Andrews et al., 1973; Ogino and Takeda, 1976,1978). In terms of absolute quantity and Ca:P ratio, the earthworm diets used during the present study supplied P at adequate levels and no symptoms consistent with P deficiency were evident in fish maintained on these diets.

Diets deficient in Mg normally result in decreased feed intake, poor FCR and renal calcification in salmonids (Cowey et al., 1977). There was no histological evidence of renal calcification during the present study, and despite very low levels of Mg present in A.longa, L.terrestris and E.foetida compared with the commercial pellet, the Mg levels present were above the minimum dietary requirement for rainbow trout; 0.5-0.7 g/Kg (Ogino et al., 1978b; Knox et al., 1981). However the Mg concentration of the carcass of fish fed frozen E.foetida (diet 3) was significantly lower ($p < 0.05$) than fish fed the other dietary treatments (Table 2.1.4).

Earthworm tissue concentrations, for the majority of microelements monitored in the present study, fell within the range of values reported in previous research (Gish and Christensen, 1973; Van Hook, 1974; Van Rhee, 1976; Ireland and Richards, 1977; Ireland, 1979; Mori and Kurihara, 1979;

Ash and Lee, 1980; Hartenstein et al., 1980a & b; Aston et al., 1982b). However, concentrations of Fe and Mn in the species L.terrestris and A.longa were greater than previously reported (Ireland, 1979; Ash and Lee, 1980) possibly due to the presence of mineral soil within the gut lumen.

Evidence has been given in numerous publications, involving several different species of earthworm that certain micro-elements, in particular Cd, Pb and Zn may accumulate in the tissues of earthworms to levels above those in their surrounding substrate (Gish and Christensen, 1973; Van Hook, 1974; Mori and Kurihara, 1979; Ash and Lee, 1980). Cd and Pb, having no known essential function in living organisms (Vallee and Ulmer, 1972), may pose a substantial problem if present in diets at relatively high concentrations.

High dietary concentrations of Ca generally reduce the uptake of these elements into animal tissues (Hsu et al., 1975; Six and Goyer, 1970). However, the levels of Ca within the diets consisting of frozen earthworm were particularly low (Table 2.1.1) and would presumably exert only a minor role in reducing the uptake of these heavy metals present into the fish tissue. Of the potentially toxic elements investigated, Cd showed no evidence of accumulation in fish fed frozen earthworm diets but there was some evidence of uptake of Zn and Pb into the fish carcass, and Pb within the livers of fish fed frozen earthworm diets. Similarly, Ireland (1977) reported an increase in tissue Pb concentrations in toads (Xenopus laevis) fed diets consisting of earthworms with high Pb content. Conversely, eels (Anguilla anguilla) fed solely on E.foetida for a period of 75 days

exhibited similar whole carcass concentrations of the elements Cd, Cu, Pb, Ni and Zn at the start and end of the experimental period (Aston et al., 1982b). However, the levels of Zn (14.9 ppm, dry wt) and Pb (5.0 ppm, dry wt) within the E.foetida employed as an eel diet by Aston et al. (1982b) were lower than those present in earthworms used as trout diets in this experiment (Table 2.1.1).

Levels of micro-elements within the earthworm tissue represent the maximum mineral concentration since earthworms had not been previously starved to void their gut lumen of substrate matter. For this reason the mineral profile of the earthworms may represent their gut contents rather than tissue concentrations. Furthermore, fish were starved for 24 hours before being sacrificed for mineral analysis. Evidence suggests that 24 hours is an insufficient period for total evacuation of the gut contents (Kionka and Windell, 1972). Therefore in future experiments the further precaution has been undertaken of washing the alimentary tract free of undigested feed with distilled water prior to carcass analysis.

Although the liver tissue could be used to more accurately represent mineral uptake into the fish tissue which was not affected by the gut contents, tissues other than liver may serve as sites of accumulation for some of the micro-elements studied (Underwood, 1977). Livers of fish fed frozen slices of E.foetida exhibited a significantly higher ($p < 0.05$) uptake of Cu and Pb despite the low feed intake of fish maintained on this diet (diet 3).

In conclusion, this feeding trial has demonstrated that certain species of earthworm (A.longa and L.terrestris) can

adequately replace a commercial trout pellet as the sole source of feed for rainbow trout over a 70 day feeding period. However, at present neither of these two earthworm species is suitable for commercial culture. E.foetida, which can be commercially cultured in organic rich substrates was apparently unpalatable to rainbow trout. Some form of pre-treatment would be necessary to improve the acceptability of this species to the fish, before it could be used as a complete or supplementary diet in fish feeding. In addition, there was some evidence to suggest the uptake and accumulation of potentially toxic heavy metals into the tissues of fish consuming earthworm diets and this must be closely monitored especially if earthworms are grown on contaminated substrates.

EXPERIMENT 2.2

THE NUTRITIONAL EVALUATION OF THE EARTHWORM SPECIES DENDRODRILUS SUBRUBICUNDUS AND DENDROBAENA VENETA AS A FEED FOR RAINBOW TROUT

2.2.1 INTRODUCTION

Experiment 2.1 assessed the nutritional value of three species of earthworm as a feed for rainbow trout. Two of the species evaluated A.longa and L.terrestris were highly palatable to the fish but are generally considered unsuitable for commercial culture. The third species evaluated E.foetida, while highly amenable to commercial culture, was apparently unpalatable to rainbow trout.

The aim of the present experiment was to evaluate a further two species of earthworm: Dendrobaena veneta and Dendrodrilus subrubicundus as a complete feed for rainbow trout over a sixty day feeding trial. Both of these species appear to thrive in organic waste matter and neither species has previously been nutritionally evaluated in livestock feed.

A 'worm meal' derived from dried D.veneta was also evaluated as a fish meal replacer in trout diets. The performance of fish fed these experimental diets was compared with fish fed two control diets: a commercially available trout pellet and a semi-synthetic trout ration in which all the dietary protein was supplied by herring meal.

As previously discussed (Section 2.1.4) when earthworms which have been grown on substrates contaminated with heavy metals are used in livestock feed potentially toxic mineral elements may be transmitted through the food chain. Therefore levels of Ca, P, K, Na, Mg, Fe, Zn, Mn, Pb, Cu, Co and Cd were monitored in diets and fish fed these diets over the experimental period.

2.2.2 MATERIALS AND METHODS

2.2.2.1 Diets

Five experimental diets were evaluated over the sixty day experimental feeding period:-

Diet 1	Frozen *D.veneta*
Diet 2	Frozen *D.subrubicundus*
Diet 3	Commercial trout pellet (control)
Diet 4	Semi-synthetic pellet; 50:50 dried *D.veneta* meal protein: 'herring' meal protein
Diet 5	Semi-synthetic pellet; 100% herring meal protein (control)

Diet 1: *Dendrobaena veneta (var zebra)*, supplied by British Groundbaits, Bardfield Saling, Essex, U.K. were separated from the inert peat packing material, rinsed with tepid water and stored at -20°C until required. These earthworms have a striped appearance similar to *E.foetida* and exude a pale yellow coelomic fluid in response to unfavourable stimuli, although this has a less noxious odour than the coelomic fluid of *E.foetida*. Due to a similarity in appearance, *D.veneta var zebra* may be confused with *E.foetida*. In a random sub-sample of one hundred individually identified earthworms nine *E.foetida* were present among the *D.veneta*. Due to the smaller mean weight of *E.foetida* (0.7g) compared with *D.veneta* (1.4g), in terms of gross weight the contamination amounted to 4.5% and was considered to have negligible effect on the feeding response of the fish.

Diet 2: *Dendrodrilus subrubicundus* were collected from the trickling filter beds of a local sewage works (Dunblane Sewage Works, Nr. Stirling, Scotland) and stored at -20°C until required. The earthworms were identified by Dr. J. E. Satchell, Institute of Terrestrial Ecology, Merlewood Research Station, Grange-over-Sands, Cumbria. (Until recently these worms were classified as

Table 2.2.1: Ingredient composition of the semi-synthetic test diets (% by weight)

Ingredient (%)	Diet 4	Diet 5
Herring meal	30	60
Dried *D.veneta* meal	35	-
Cod liver oil	2.2	2.5
Corn oil	4.4	6.0
Corn starch	12.9	15.0
Dextrin	6.5	7.5
Vitamin mix[1]	2	2
Mineral mix[2]	4	4
Carboxymethyl cellulose binder	2	2
Chromic oxide	1	1

1. Supplying per Kg diet: Thiamine HCl 50mg; Riboflavin 50mg; Calcium pantothenate 100mg; Niacin 200mg; Pyridoxine HCl 40mg; Biotin 6mg; Folic acid 15mg; Cyanocobalamin 0.1mg; Inositol 2000mg; Ascorbic acid 1000mg; Choline chloride 4000mg; Menadione 40mg; Alpha-tocopherol acetate 400mg; Para-amino benzoic acid 50mg; Vitamin A acetate 2000 IU; Vitamin D_3 1000mg.
2. Supplying per Kg diet: $CaHPO_4.2H_2O$ 29.00g; $MgSO_4.7H_2O$ 5.10g; NaCl 2.40g; KCl 2.00g; $FeSO_4.7H_2O$ 1.00g; $ZnSO_4.7H_2O$ 0.22g; $CuSO_4.5H_2O$ 0.0314g; $MnSO_4.4H_2O$ 0.1015g; $CoSO_4.7H_2O$ 0.0191g; $CaIO_3.6H_2O$ 0.0118g; $CrCl_3.6H_2O$ 0.0051g.

Dendrobaena subrubicunda). D.subrubicundus naturally occur in large quantities in the trickling filter beds of sewage treatment plants (Solbé, 1975) and can therefore be assumed to thrive on substrates high in organic matter.

Diet 3: The performance of fish fed these two species of earthworm as a complete diet was compared with fish fed a commercially available trout pellet (Edward Baker Ltd., Sudbury, Suffolk), employed as a control diet.

Diet 4: For dietary evaluation D.veneta was dried at 60°C for 24 hours and the resulting dried product ground to a free flowing powder. This 'worm meal' comprised 56.98% crude protein and 19.20% crude lipid and was used to replace 50% of the protein supplied by herring meal (which comprised 66.53% crude protein and 9.18% crude lipid) in a semi-synthetic trout ration.

Diet 5: A semi-synthetic trout ration in which all the dietary protein was supplied by herring meal was employed as a second control diet.

Diet 4 and Diet 5 were formulated to contain 45% crude protein, 14% lipid and the ingredient composition of these two diets is given in Table 2.2.1. Chromic oxide was included in both diets at a level of 1% for the determination of digestibility coefficients.

The dry ingredients for each diet were combined with the required quantity of oil in a Hobart A400 feed mixer. Mineral compounds supplying the elements Mg, Fe, Zn, Cu, Mn, Co, I and Cr were dissolved in distilled water and mixed with the other ingredients. Sufficient distilled water was then added to bind the

ingredients together. The mixture was extruded through a 3mm die and the resulting spaghetti-like strands dried under a stream of warm air (35°C) until the moisture content was reduced to c. 10%. The diets were stored in airtight containers at 4°C until required.

The proximate and mineral composition of the diets is given in Table 2.2.2 and the amino acid and fatty acid composition of the earthworms is given in Tables 2.2.3 and 2.2.4 respectively.

An accurate record was kept of the feed consumed by fish fed each dietary treatment during successive bi-weekly periods of the experiment. The frozen earthworm diets were stored at -20°C in thermos flasks until required (Section 2.1.2.1). Fish were fed "to appetite" twice daily at 1000 and 1700 hours (as described in Section 2.1.2.1) for the first six weeks of the experiment and subsequently once daily, mid-morning until the end of the 60 day experimental feeding period.

2.2.2.2. Animals and Tanks

Ninety rainbow trout, of mean weight 24g, were supplied by Mr. D. Brien, Almondbank Fish Farm, Nr. Perth, Scotland and divided into six groups of fifteen fish, ensuring that the mean weight of each group was approximately the same. Fish from one group were killed, by a sharp blow to the head, dissected and the alimentary tract washed free of undigested feed with distilled water. These fish were then stored at -20°C for subsequent proximate and mineral analysis. The remaining five groups of fish were anaesthetized, weighed individually and each group housed in a 60ℓ tank as described in Section 2.1.2.2. Each tank of fish was allocated one of the five dietary treatments.

The water temperature during the experimental period

Table 2.2.2: Proximate and mineral composition of the experimental diets

Component	Dietary treatment 1 D.veneta	2 D.subrubicundus	3*1 Commercial pellet	4 50% D.veneta pellet	5 Herring meal pellet
Moisture (%)	81.67	84.26	9.24	10.88	8.13
Nutrient content (% dry wt)					
Crude protein	56.98	67.79	52.12	44.86	45.49
Lipid	19.15	12.41	13.25	23.13	13.83
Ash	4.24	6.66	12.24	11.50	13.34
NFE*2	19.63	13.14	22.39	20.51	27.34
Energy content (kcal/100g dry wt)					
Gross energy*3	585.24	556.86	512.52	557.48	500.04
Digestible energy*4	496.51	476.92	424.63	473.49	406.60
Protein:gross energy (g:kcal)	0.097	0.122	0.102	0.080	0.091
Mineral composition					
Macro-elements (g/Kg,dry wt)					
Ca	1.48	1.94	17.27	12.69	24.40
P	16.45	14.15	17.96	24.88	23.65
K	7.45	9.43	10.91	7.13	8.73
Na	4.43	4.58	4.12	6.69	9.26
Mg	0.57	0.68	2.13	1.07	1.41
Micro-elements (mg/Kg,dry wt)					
Fe	320.33	370.43	301.73	428.75	327.04
Zn	111.31	204.87	183.79	114.04	104.95
Mn	9.81	18.25	56.14	48.90	32.96
Pb	7.85	15.28	4.62	7.61	4.96
Cu	16.59	21.28	15.31	15.97	11.88
Co	0.53	1.24	1.11	2.32	1.68
Cd	3.35	1.07	0.52	1.82	0.54

*1 Edward Baker Ltd., Sudbury, Suffolk

*2 Nitrogen Free Extract = (100-H_2O + crude protein + lipid + ash)

*3 Calculated on an estimated 5.7 kcal/g protein; 9.5 kcal/g lipid; 4.0 kcal/g carbohydrate

*4 Calculated on an estimated 5.0 kcal/g protein; 9.0 kcal/g lipid; 2.0 kcal/g carbohydrate (Cowey et al., 1972)

Table 2.2.3: Amino acid composition of the two earthworm species used as experimental diets (expressed as g amino acid/100g recovered amino acid)

Amino acid	D.subrubicundus	D.veneta
Aspartic acid	10.37 (11.36) *1	9.58 (11.68)
Alanine	5.48 (6.00)	5.62 (6.85)
Cystine	1.95 (2.13)	3.15 (3.85)
Cysteic acid	0.20 (0.22)	0.33 (0.40)
Glutamic acid	13.67 (14.97)	15.07 (18.37)
Glycine	5.20 (5.69)	6.04 (7.36)
Proline	3.73 (4.08)	3.30 (4.03)
Serine	5.11 (5.60)	5.15 (6.28)
Taurine	0.11 (0.12)	0.10 (0.12)
Arginine	6.56 (7.19)	6.31 (7.69)
Histidine	2.93 (3.21)	2.68 (3.27)
Isoleucine	4.48 (4.91)	4.34 (5.29)
Leucine	7.90 (8.65)	7.46 (9.09)
Lysine	7.45 (8.16)	6.77 (8.26)
Methionine	2.58 (2.83)	2.78 (3.39)
Phenylalanine	5.04 (5.52)	4.54 (5.53)
Threonine	5.14 (5.63)	4.67 (5.70)
Tyrosine	3.81 (4.18)	4.54 (4.42)
Valine	6.51 (7.13)	6.59 (8.03)

*1 Values in parentheses indicate amino acid content of the earthworms expressed as g amino acid/100g protein (16gN)

Table 2.2.4: Fatty acid composition of earthworms (diets 1 and 2) and the semi-synthetic pellet (diet 5) (values expressed as area per cent of total fatty acids)

Fatty acids[1]		D.veneta	D.subrubicundus	Semi-synthetic pellet
	10:0	0.10	-	0.04
[2]. iso	12:0	1.41	0.59	-
[3]. n	12:0	10.28	9.93	0.10
[2]. anteiso	13:0	4.34	5.20	-
n	13:0	3.47	0.41	-
iso	14:0	1.13	1.01	-
n	14:0	10.10	3.61	3.23
	14:1	0.14	2.23	0.02
anteiso	15:0	3.97	2.37	0.05
n	15:0	2.11	2.34	-
iso	16:0	0.77	-	-
n	16:0	9.67	5.41	13.18
	16:1	0.63	1.85	5.75
anteiso	17:0	0.79	-	-
n	17:0	1.09	1.24	0.33
iso	18:0	-	-	0.35
n	18:0	5.01	5.61	2.61
	18:1	8.61	12.34	21.77
	18:2	5.46	4.96	23.98
	18:3	0.12	0.14	0.21
n	20:0	3.22	6.02	8.24
	20:2	1.45	2.23	0.51
	20:4	0.92	3.00	0.31
	20:5	3.91	7.50	3.90
	20:6 (?)[4]	0.12	0.20	0.11
n	22:0 (?)	1.87	1.88	0.13
	22:1	7.85	6.88	7.19
	22:5	0.35	0.56	0.72
	22:6	0.20	0.34	5.54
n	24:0	0.11	-	0.57
	24:1	0.81	0.51	0.66
Total saturated fatty acids (%)		59.44	45.62	28.83
Total mono-unsaturated fatty acids (%)		19.49	26.04	35.90
Total polyunsaturated fatty acids (%)		11.08	16.70	34.77
Short chain fatty acids (<16C,%)		37.05	27.69	3.44
Long chain fatty acids (>16C,%)		52.96	60.67	96.06

1. Fatty acids designated by chain length:number of double bonds

2 and 3. Variations in fatty acids dependent on the configuration of the C atoms. n = normal/iso, anteiso = branched

4. Identification tentative - not detected.

was 14.5°C ± 3.5°C and fish were subject to natural photoperiod.

Fish were weighed at bi-weekly intervals and at the end of the experiment as described in Section 2.1.2.2. In addition, faecal samples were obtained by hand stripping (Nose, 1967; Austreng, 1978) during the final week of the experiment from fish fed diets containing the inert marker (Cr_2O_3).

On the final day of the experiment the fifteen fish from each dietary treatment were killed by a sharp blow on the head. From five fish in each group the liver was removed and liver somatic index determined (Section 2.1.2.1). The gut lumen of these and five additional fish was washed free of undigested feed and the ten whole fish (including livers) stored at -20°C for subsequent proximate and mineral analysis. From the remaining five fish in each group slices of liver, spleen, kidney and gill were removed and stored in fixative (10% buffered formalin) for subsequent histological examination.

The liver and samples of white dorsal muscle tissue from the latter five fish (only from those fish maintained on frozen earthworm diets and the semi-synthetic control ration, diets 1, 2 and 5) were stored at -20°C for subsequent fatty acid analysis. The semi-synthetic control diet (diet 5) was analysed in preference to the commercial trout pellet (diet 3) as the complete ingredient composition of this diet was available.

2.2.2.3 Chemical Methods

Moisture, ash, crude lipid, crude protein and mineral composition were determined in replicate samples of whole fish carcass and experimental diets as described in Section 2.1.2.3. For digestibility determination faecal samples (dried to constant weight at 105°C) and diets were also analysed for total nitrogen

(Munro and Fleck, 1969) and chromic oxide content (Furukawa and Tsukahara, 1966). Apparent digestibility coefficients were calculated by the method of Maynard and Loosli (1969):

$$\text{Apparent protein digestibility (\%)} = 100 - \left(\frac{\%Cr_2O_3 \text{ in diet}}{\%Cr_2O_3 \text{ in faeces}}\right) \times \left(\frac{\text{\% crude protein in faeces}}{\text{\% crude protein in diet}}\right)$$

$$\text{Apparent dry matter digestibility (\%)} = 100\left(1 - \frac{C}{B} \times \frac{B}{C}\right)$$

where C = Cr_2O_3 in diet (g/100g); B = Cr_2O_3 in faeces (g/100g).

Frozen earthworm tissue was prepared for amino acid analysis by hydrolysis of the tissue in 6NHCl for 16 hours (Roach et al., 1967). The amino acid composition of the resulting hydrolysate was determined at the Institute of Marine Biochemistry, Aberdeen, Scotland using a JEOL JLO-6AH auto amino acid analyser (JEOL (U.K.) Ltd., Jeol House, Grove Park, Collingdale, London). The amino acid tryptophan was not determined in these samples due to its destruction during acid hydrolysis.

Muscle, liver and diet samples were prepared for fatty acid analysis by an "in situ" method developed at the Lord Rank Research Centre, High Wycombe, Bucks. The results of this "in situ" method were compared with results obtained for duplicates of the same samples in which the lipid was first removed by the method of Bligh and Dyer (1959). No difference emerged between the results obtained by the two methods.

Replicate samples, estimated to contain 30mg lipid, were ground with sufficient anhydrous Na_2SO_4 to absorb all the moisture present. Sufficient internal standard (5.26mg/ml

Tripentadecanoin in chloroform; equivalent to 5.00mg/ml Pentadecanoic Acid) was added to allow accurate calculation of the majority of the fatty acids present. Earthworm samples were also prepared without internal standard.

The fatty acid methyl esters were obtained by addition of BF_3-methanol after saponification of the fatty acids with methanolic 1NNaOH (Morrison and Smith, 1964). The fatty acid methyl esters were collected in a layer of hexane and aliquots from this layer removed for gas chromatographic analysis.

The gas chromatograph was equipped with a hydrogen flame ionization detector, connected to a 2m x 2mm glass column packed with 10% SP2340 acid washed chromasorb W. A flow rate of 10ml/min of N_2 gas was adopted, the injection temperature set at 280°C; detector at 250°C and the column temperature increasing from 80°C-250°C at a rate of 4°C/minute.

Identification of the resulting methyl esters was achieved by comparison of retention times with those of the following standards prepared using mixtures of authentic methyl esters.

(i) Fatty acid methyl esters standard (FAMES) which includes most of the saturated fatty acid methyl esters of chain length 8-24 and several unsaturated fatty acid methyl esters (18:1, 18:2, 18:3, 22:1, 24:1)*1.

(ii) Polyunsaturated fatty acids (PUFA), the fatty acid profile of a fish oil including most of the fatty acids likely to occur in these samples.

*1 Nomenclature of fatty acids X:Y = number of carbon atoms: number of double bonds.

(iii) Methyl palmitate and the methyl ester of 20:4.

(iv) Methyl palmitate and the methyl esters of 18:1, 20:1, 20:2, 24:1.

(v) A mixture containing iso*1 14:0, n*1 14:0, anteiso*1 15:0, n 15:0, iso 16:0, n 16:0 and anteiso 17:0.

(vi) A mixture containing iso 18:0, n 18:0, anteiso 19:0, iso 20:0, n 20:0 and anteiso 21:0.

Despite the extensive use of standards and due to the complexity of the earthworm lipids, certain fatty acids remained unidentified. Samples were therefore analysed using a non-polar column (chromosorb G) so that each fatty acid emerged in order of ascending carbon chain length. Unsaturated fatty acids emerge first followed by the saturated fatty acids of that carbon chain length. By calculation of the size ratios of these peaks and comparing these with values calculated from previously obtained chromatograms of the same sample (which included the unknown peaks) the identification of unknown fatty acids could be deduced.

2.2.2.4 Histological Methods

Fixed tissues were prepared for histological examination as described in Section 2.1.2.4.

2.2.2.5 Statistical Analysis

Statistical analysis was carried out as described in Section 2.1.2.5.

*1 Normal (n) and branched (iso and anteiso) C atom configuration.

2.2.3 RESULTS

Fish fed the two control diets (diets 3 and 5) and frozen D.subrubicundus (diet 2) fed aggressively and grew well throughout the experimental period. Fish fed frozen D.veneta (diet 1) and dried D.veneta meal within a semi-synthetic ration consumed less diet and exhibited slower growth rates.

2.2.3.1 Fish Growth and Feed Utilization Efficiency

Fish growth and feed utilization efficiency of fish maintained on the five dietary treatments for 60 days is given in Table 2.2.5 and fish growth is presented graphically in Figure 2.2.1.

No significant difference ($p < 0.05$) emerged in the mean final body weight of fish fed diets 1, 2, 3 and 5 (the frozen earthworm diets and the control diets) after sixty days. The mean final body weight of fish fed diet 4 (semi-synthetic ration containing dried D.veneta meal) was significantly lower ($p < 0.05$) than fish fed diet 5 (the semi-synthetic control ration) but not significantly different ($p < 0.05$) from fish fed the remaining three diets (diets 1, 2 and 3).

Fish fed frozen D.subrubicundus and frozen D.veneta apparently achieved a more rapid growth rate during the first six weeks of the experiment compared with the growth rate observed during the final 18 days of the experimental period (Figure 2.2.1).

Table 2.2.6 shows the mean feed intake (dry weight and wet weight) and percentage weight gain of fish fed the five experimental diets during each bi-weekly interval of the experimental period. From week 2 to week 6 fish were fed twice daily "to appetite". During weeks 2-4 fish fed frozen D.subrubicundus and frozen D.veneta had a higher feed intake and percentage weight

Table 2.2.5: Growth, feed utilization, liver somatic index and carcass composition of rainbow trout fed the experimental diets for 60 days

Mean values	Dietary treatments 1	2	3	4	5	± SE*1
Mean initial weight (g)	24.43[a]	24.51[a]	24.16[a]	24.47[a]	24.55[a]	0.602
Mean final weight (g)	38.37[ab]	45.02[ab]	43.81[ab]	36.68[b]	46.00[a]	2.812
Weight gain (%)	57.06	83.68	81.33	49.90	87.37	
Specific growth rate (%)	0.75	1.01	0.99	0.68	1.05	
Total food intake (g,wet weight)	1493.82	1959.06	337.99	260.70	334.97	
Food intake (mg/day,dry wt)	354.53	367.09	365.19	276.59	366.35	
Weight gain (mg/day)	232.33	341.83	327.50	203.50	357.50	
Food conversion ratio	1.53	1.07	1.12	1.36	1.02	
Protein efficiency ratio	1.15	1.37	1.72	1.64	2.15	
Nitrogen intake (mg/day)	201.93	248.85	190.32	124.08	166.65	
Nitrogen deposition (mg/day)	41.61	60.61	54.69	32.76	57.77	
Apparent N utilization (%)	24.78	27.12	30.22	26.72	35.06	
Apparent dry matter digestibility (%)	-	-	-	55.32	59.32	
Apparent nitrogen digestibility (%)	-	-	-	85.48	86.97	
Gross energy intake (kcal/day/fish)*2	2.075	2.044	1.872	1.540	1.832	
Digestible energy intake (kcal/day/fish)*3	1.760	1.751	1.551	1.310	1.490	
Liver somatic index (%)	1.42[b]	1.55[b]	1.18[a]	1.11[a]	1.16[a]	0.072
Carcass composition (% wet wt)	**Initial**					
Moisture	74.28	75.29[a]	73.94[a]	73.94[a]	74.67[a]	73.42[a]	0.765
Crude protein	15.95	17.91[b]	17.70[b]	16.70[a]	16.10[a]	16.16[a]	0.212
Lipid	7.25	5.05[a]	6.24[b]	7.41[c]	6.88[b]	7.69[c]	0.318
Ash	2.45	2.79[b]	2.45[a]	2.32[a]	2.40[a]	2.42[a]	0.065

*1 Standard error - calculated from residual mean square in the analysis of variance.
abc Mean values for components with the same superscripts are not significantly different ($p < 0.05$)
*2) *3) for method of calculation see Table 2.1.3

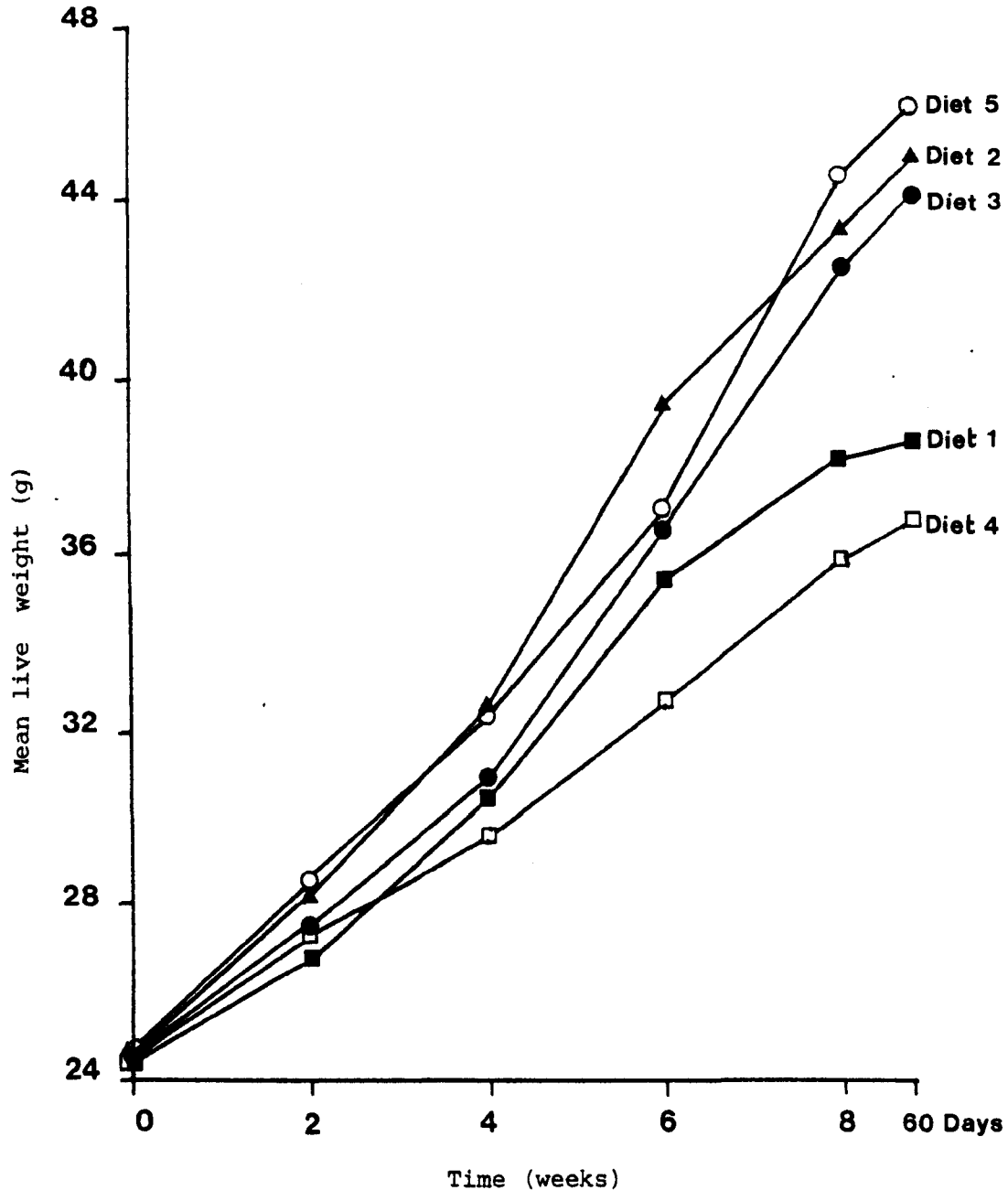


Figure 2.2.1: The growth response of rainbow trout fed the five dietary treatments

Table 2.2.6: The feed intake of fish fed a wet and dry diet (g/fish/day) at varying feeding frequencies and the percentage weight increase in fish body weight (%/fish/day) at successive bi-weekly intervals

Dietary treatment		Week number 0-2	2-4	4-6	6-8	8-60 days
			2*		1*	
	a*	1.41	1.93	1.80	2.48	1.84
D.veneta	b*	0.26	0.35	0.33	0.46	0.34
(Diet 1)	c*	0.66	1.00	1.15	0.55	0.24
	a	1.67	2.27	2.69	2.47	3.13
D.subrubicundus	b	0.26	0.36	0.42	0.39	0.49
(Diet 2)	c	1.03	1.13	1.52	0.72	0.96
Commercial trout	a	0.30	0.34	0.41	0.51	0.59
pellet control	b	0.28	0.31	0.37	0.46	0.53
(Diet 3)	c	0.93	0.88	1.34	1.14	0.92
50% dried D.veneta protein in pellet	a	0.29	0.30	0.28	0.37	0.37
	b	0.25	0.26	0.25	0.33	0.33
(Diet 4)	c	0.86	0.56	0.73	0.68	0.62
Herring meal protein	a	0.32	0.33	0.34	0.57	0.55
control pellet	b	0.29	0.31	0.31	0.52	0.50
(Diet 5)	c	1.16	0.96	1.01	1.46	0.84

1* Fish fed once daily

2* Fish fed twice daily

a* Feed intake, g/fish/day expressed on a wet weight basis

b* Feed intake, g/fish/day expressed on a dry weight basis

c* Percentage weight increase/fish/day (live weight)

gain compared with fish fed the control diets (diets 3 and 5). During weeks 4-6 fish fed frozen D.subrubicundus had a higher feed intake and percentage weight gain than fish fed the two control diets and similarly fish fed frozen D.veneta had a higher feed intake and percentage weight gain than fish fed the control semi-synthetic trout pellet (diet 5). However from week 6 to week 8, when fish were fed only once daily, those fish fed the control diets had the higher feed intake and percentage weight gain compared with fish fed frozen earthworms. Possibly, due to the high moisture content of frozen earthworm diets fish fed these diets were unable to satisfy their daily requirements in one feed per day.

The moisture content of the experimental diets varied considerably (Table 2.2.2) and therefore feed intake by the fish has also been expressed on a dry weight basis and the FCR calculated using these dry weight values (Table 2.2.5).

Fish fed frozen D.subrubicundus and the two control diets exhibited more efficient FCR compared with fish fed frozen D.veneta and the semi-synthetic pellet containing dried D.veneta meal. A higher PER was achieved by fish fed the pelleted diets (diets 3, 4 and 5) compared with fish fed frozen earthworm diets (diets 1 and 2) and in terms of Apparent NPU fish fed diets 1, 2 and 4 utilized the dietary protein less efficiently than fish fed the control diets (diets 3 and 5).

Apparent digestibility coefficients for total dry matter and nitrogenous components of the diet were marginally higher in the semi-synthetic control pellet compared with the semi-synthetic pellet containing dried D.veneta meal (Table 2.2.5).

Despite differences in the moisture content of the diets, fish exhibiting similar growth rates achieved the same dry matter feed intake (mg/day/fish) over the experimental period (Table 2.2.5). In terms of energy intake (kcal/day/fish) those fish fed frozen earthworms had the same energy intake and fish fed the two control diets had the same energy intake, but lower than fish fed frozen earthworms (Table 2.2.5).

2.2.3.2 Carcass Composition and Liver Somatic Index

The carcass composition of rainbow trout at the start and end of the sixty day feeding trial and the liver somatic index is given in Table 2.2.5.

Fish fed frozen D.veneta and frozen D.subrubicundus (diets 1 and 2) and dried D.veneta meal within a semi-synthetic pellet (diet 4) exhibited significantly lower ($p < 0.05$) carcass crude lipid content compared with fish fed the control diets (diets 3 and 5). A significantly higher carcass crude protein content and LSI was evident in fish fed only on frozen earthworms (diets 1 and 2) compared with fish fed the remaining three diets.

Fatty Acid Composition: The fatty acid profiles of the two earthworm species were complex (Figure 2.2.2a) especially when compared with the fatty acid profiles of either corn oil or fish oil (Figure 2.2.2b; the lipids used in the formulation of the semi-synthetic control diet). The fatty acid composition of the diets consisting of frozen D.veneta and D.subrubicundus and the semi-synthetic control diet is given in Table 2.2.4. The major differences between the fatty acid profile of the earthworms and the semi-synthetic diet were:-

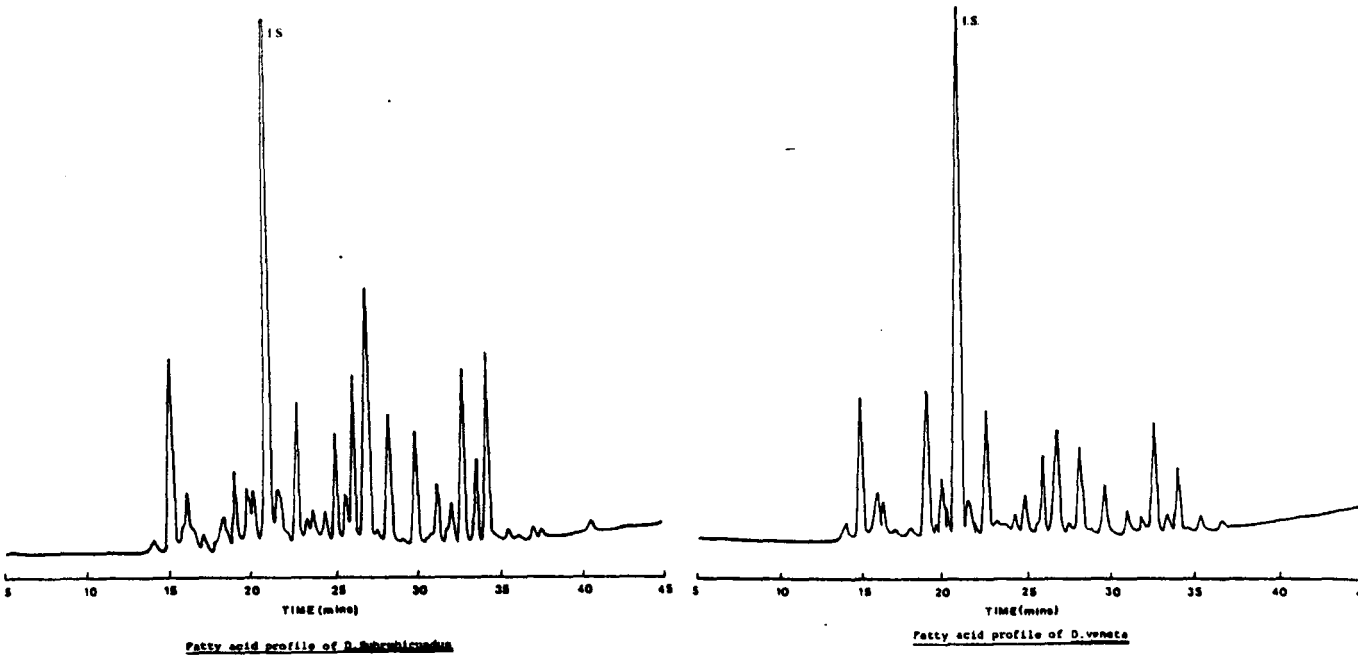


Figure 2.2a: The fatty acid profile of D.subrubicundus and D.veneta

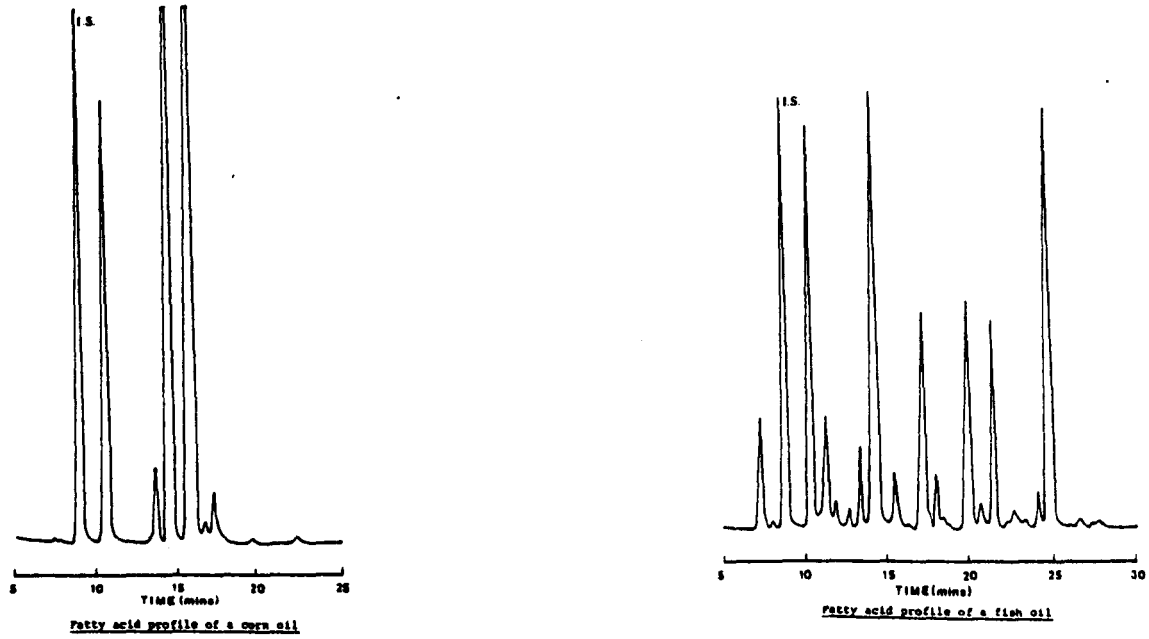


Figure 2.2b: Diagrammatic representation of
The fatty acid profile of a corn oil and a fish oil

I.s = Internal Standard

For GC operating conditions see text

(i) The predominance of saturated fatty acids in the earthworm lipids and the far greater percentage of polyunsaturated fatty acids (PUFA's) and mono-unsaturated fatty acids in the semi-synthetic diet.

(ii) The higher percentage of short chain fatty acids (carbon chain length < 16C) in the earthworm lipids compared with the semi-synthetic pellet (comprised almost entirely of fatty acids with carbon chain length > 16C, Table 2.2.4).

These differences were to some extent reflected in the fatty acid profiles of the muscle and liver tissue of fish fed these diets (Table 2.2.7a,b). The muscle tissue was apparently affected slightly more than the liver tissue and in both tissues a difference in fatty acid composition between fish fed frozen earthworm diets and fish fed the semi-synthetic control diet was evident. Tissues of fish fed frozen earthworm diets comprised a marginally smaller proportion of unsaturated fatty acids and a higher proportion of short chain fatty acids (< 16C) compared with the tissues of fish fed the semi-synthetic control diet.

Mineral Composition: Diets. The mineral composition of the experimental diets is given in Table 2.2.2. The macro-elements Ca and Mg were present at lower concentrations in the frozen earthworm diets compared with the pelleted diets (the latter being supplemented with a mineral mix). The micro-elements Pb, Cu and Cd were present at consistently higher concentrations in the diets containing earthworms (diets 1, 2 and 4) compared with the control diets (diets 3 and 5).

Fish carcass. The mineral composition of the whole fish carcass at the start and end of the experimental period is given in Table 2.2.8. The carcass of fish maintained

Table 2.2.7a: Fatty acid composition of the liver of rainbow trout fed on dietary treatments 1, 2 and 5 for the experimental period of 60 days (values expressed as area % of total fatty acids)

Fatty acids[1]		Dietary treatment 1	Dietary treatment 2	Dietary treatment 5
	10:0	-	-	0.04
[2]. iso	12:0	-	-	-
[3]. n	12:0	0.37	0.29	0.05
[2]. anteiso	13:0	0.14	0.16	-
	13:0	0.11	0.07	-
iso	14:0	0.10	0.05	-
n	14:0	2.28	1.24	1.17
	14:1	-	-	-
anteiso	15:0	0.99	0.55	-
n	15:0	-	-	-
iso	16:0	0.21	0.07	-
n	16:0	16.45	17.22	14.46
	16:1	3.95	3.69	1.54
anteiso	17:0	-	-	-
n	17:0	1.04	0.46	0.56
iso	18:0	0.59	0.31	0.21
n	18:0	6.85	6.88	6.39
	18:1	15.60	21.13	15.08
	18:2	5.12	2.69	10.37
	18:3	0.12	0.10	0.20
n	20:0	1.88	2.60	4.87
	20:2	1.56	1.16	2.44
	20:4	0.05	-	0.20
	20:5	1.17	2.15	2.64
	20:6 (?)[4]	6.75	4.54	0.44
n	22:0 (?)	0.35	0.67	-
	22:1	10.75	9.82	5.19
	22:5	1.14	1.81	0.84
	22:6	18.20	17.61	28.92
n	24:0	0.14	0.09	0.86
	24:1	0.92	1.09	0.66
Total saturated fatty acids (%)		31.50	30.66	28.61
Total monounsat. fatty acids (%)		32.78	36.89	24.91
Total polyunsat. fatty acids (%)		32.55	28.90	43.61
Short chain fatty acids (<16C,%)		3.99	2.36	1.26
Long chain fatty acids (>16C,%)		92.84	94.09	95.87

1,2,3 and 4 = For explanation of terms see Table 2.2.4

Table 2.2.7b: Fatty acid composition of the muscle tissue of rainbow trout fed dietary treatments 1, 2 and 5 for the experimental period of 60 days (values expressed as area % of total fatty acids)

Fatty acids[1]		Dietary treatment 1	Dietary treatment 2	Dietary treatment 5
	10:0	-	-	-
2. iso	12:0	0.13	0.10	-
3. n	12:0	2.37	1.98	0.13
2. anteiso	13:0	0.31	0.67	-
	13:0	0.32	0.11	-
iso	14:0	0.21	0.26	-
n	14:0	4.06	2.58	2.08
	14:1	1.50	1.32	-
anteiso	15:0	-	-	-
n	15:0	-	-	-
iso	16:0	0.18	0.08	-
n	16:0	17.38	15.15	18.71
	16:1	3.59	7.48	2.77
anteiso	17:0	-	-	-
n	17:0	0.79	0.61	0.29
iso	18:0	0.24	0.32	0.16
n	18:0	5.86	6.09	4.22
	18:1	19.13	25.56	19.76
	18:2	10.84	9.87	17.46
	18:3	0.18	0.23	0.21
n	20:0	3.75	5.08	5.22
	20:2	1.06	1.25	0.94
	20:4	0.28	0.27	0.42
	20:5	2.06	2.31	2.66
	20:6 (?)[4]	1.45	0.81	0.26
n	22:0 (?)	-	0.23	-
	22:1	5.36	4.69	3.94
	22:5	0.80	1.10	0.80
	22:6	14.02	7.66	16.83
n	24:0	0.05	0.04	-
	24:1	0.65	0.75	0.54
Total saturated fatty acids (%)		35.65	33.30	30.81
Total monosat. fatty acids (%)		31.29	41.05	27.95
Total polyunsat. fatty acids (%)		29.63	22.25	38.64
Short chain fatty acids (<16C,%)		8.90	7.02	2.21
Long chain fatty acids (>16C,%)		87.67	89.58	95.19

1,2,3 and 4 = For explanation of terms see Table 2.2.4

Table 2.2.8: Mineral concentration in whole fish carcass of initial fish and fish fed the experimental diets for 60 days (values expressed as g or mg/kg wet weight)

Mineral	Initial fish	Dietary treatments 1	2	3	4	5	± SE[1]
Macro-elements (g/kg)							
Ca	3.27	3.75^{a}	3.08^{a}	2.94^{a}	3.28^{a}	2.93^{a}	0.305
P	2.16	2.50^{e}	1.92^{ab}	2.25^{d}	2.00^{bc}	1.77^{a}	0.069
K	2.54	3.61^{b}	3.20^{ab}	3.07^{ab}	3.06^{ab}	2.84^{a}	0.196
Na	0.70	0.86^{a}	0.77^{a}	0.80^{a}	0.81^{a}	0.82^{a}	0.072
Mg	0.15	0.23^{a}	0.21^{a}	0.21^{a}	0.21^{a}	0.21^{a}	0.009
Micro-elements (mg£kg)							
Fe	12.11	16.18^{ab}	15.39^{ab}	15.52^{ab}	17.11^{b}	10.49^{a}	1.627
Zn	14.81	26.61^{b}	23.47^{ab}	18.54^{a}	21.96^{ab}	21.96^{ab}	1.826
Mn	1.36	1.45^{ab}	1.02^{a}	1.91^{b}	1.79^{b}	1.76^{b}	0.164
Pb	1.14	0.96^{ab}	1.33^{b}	0.83^{a}	0.95^{ab}	0.74^{a}	0.161
Cu	1.02	0.88^{a}	1.28^{bc}	1.14^{ab}	1.59^{c}	1.25^{b}	0.114
Co	0.39	0.13^{a}	0.11^{a}	0.12^{a}	0.11^{a}	0.15^{a}	0.015
Cd	0.11	0.17^{a}	0.12^{a}	0.15^{a}	0.13^{a}	0.12^{a}	0.018

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

abcde Mean values for components with the same superscripts are not significantly different ($p < 0.05$)

Table 2.2.9: Total dietary intake, retention and retention per g live fish weight gain of mineral elements over the sixty day experimental period

Element		Dietary treatments 1	2	3	4	5
Macro-elements (mg)						
Ca	Intake	31.48	42.73	378.41	210.60	536.34
	Retention	64.04	58.51	49.63	40.22	54.50
	Ret/g	4.59	2.85	2.53	3.29	2.54
P	Intake	349.92	311.66	393.53	412.89	519.85
	Retention	43.01	33.37	46.36	20.50	28.27
	Ret/g	3.09	1.63	2.36	1.68	1.32
K	Intake	158.47	207.70	239.05	118.33	191.89
	Retention	76.43	81.62	72.95	50.02	68.46
	Ret/g	5.48	3.98	3.71	4.10	3.19
Na	Intake	94.23	100.88	90.27	111.02	203.54
	Retention	16.04	17.47	18.27	12.79	20.68
	Ret/g	1.15	0.85	0.93	1.05	0.96
Mg	Intake	12.12	14.98	46.67	17.76	30.99
	Retention	5.08	5.72	5.35	3.84	5.98
	Ret/g	0.36	0.28	0.27	0.31	0.28
Micro-elements (µg)						
Fe	Intake	6814.00	8156.01	6611.33	7115.28	7188.67
	Retention	311.30	382.32	373.82	317.56	171.49
	Ret/g	22.33	18.64	19.02	26.01	7.99
Zn	Intake	2367.76	4512.34	4027.10	1892.54	2306.91
	Retention	644.08	678.43	439.45	427.92	648.37
	Ret/g	46.20	33.08	22.36	35.05	30.23
Mn	Intake	208.68	401.96	1230.11	811.52	724.49
	Retention	20.71	10.87	49.13	30.67	45.85
	Ret/g	1.49	0.53	2.50	2.51	2.14
Pb	Intake	166.98	336.55	101.23	126.29	109.03
	Retention	7.77	30.71	7.61	5.73	4.83
	Ret/g	0.56	1.50	0.39	0.47	0.23
Cu	Intake	352.90	468.70	335.46	265.03	261.13
	Retention	7.63	31.40	24.09	32.14	31.23
	Ret/g	0.55	1.53	1.23	2.63	1.46
Co	Intake	11.27	27.31	24.32	38.50	36.93
	Retention	-ve	-ve	-ve	-ve	-ve
	Ret/g	-	-	-	-	-
Cd	Intake	71.26	23.57	11.39	30.20	11.87
	Retention	3.83	2.70	3.91	1.71	3.28
	Ret/g	0.27	0.13	0.20	0.14	0.15

on frozen D.veneta had significantly higher ($p < 0.05$) P and Zn concentrations compared with fish fed the commercial trout pellet (diet 3). The Pb concentration was significantly higher ($p < 0.05$) and the Mn concentration significantly lower ($p < 0.05$) in the carcass of fish fed frozen D.subrubicundus compared with fish fed the control diets (diets 3 and 5).

To facilitate comparison between dietary treatments the mineral intake, retention and retention per g fish weight gain were calculated as described in Section 2.1.3.2 and these results are given in Table 2.2.9. Higher mineral retention per g fish weight gain was recorded for the elements Fe, Zn and Pb when fish were fed diets containing earthworms (diets 1, 2 and 4).

2.2.3.3 Histological Examination

No apparent difference emerged in the liver, gill, kidney and spleen tissue between fish fed the five dietary treatments.

2.2.4 DISCUSSION

Maintaining rainbow trout on diets comprised solely of frozen D.subrubicundus or frozen D.veneta caused no significant decline in the growth rate or health of the fish over an experimental period of 60 days. However, a decline in the efficiency of feed utilization by fish fed frozen D.veneta compared with fish fed the control diets was evident.

Although there was little difference between the FCR of fish fed frozen D.subrubicundus and fish fed the control diets, fish fed frozen D.subrubicundus exhibited a lower PER and Apparent NPU compared with fish fed the control diets. A possible reason for this (discussed in Section 2.1.4) was that the high protein

content of the earthworm (on a dry weight basis) may have supplied protein in excess of the dietary requirement of rainbow trout with the consequent utilization of excess protein by the fish as an energy source.

Feeding earthworms to rainbow trout over a period of 60 days had a significant effect on the carcass composition of the fish (Table 2.2.5). A similar result was reported in experiment 2.1 (Table 2.1.3). In contrast to the earthworm species used in experiment 2.1, in which the lipid content of the earthworms tested was exceptionally low, the species of earthworm employed in the present experiment had lipid contents similar to those of the control pellets (c. 13%, Table 2.2.2). Furthermore the results of Hansen and Czochanska (1975) and results obtained during this study (Table 2.2.4) suggest that the lipids of earthworms are suitable for fish diets, containing polyunsaturated fatty acids including the essential fatty acids:linoleic and linolenic acid (Castell et al., 1978). Several possibilities arise which may explain the low carcass lipid content and high liver somatic index of fish maintained on earthworm diets:

(i) The carbohydrate fraction of the diets. Unlike most animals, fish have a limited capability to utilize the carbohydrate fraction of their diet due to poor control over plasma glucose levels (Walton and Cowey, 1982). The carbohydrate fraction of the various diets employed in this study was likely to have been supplied by considerably different compounds. The carbohydrate fraction within the pelleted diets tested was predominantly supplied in the form of raw corn starch and dextrin (Table 2.2.1) whereas the carbohydrate store within the earthworm is principally believed to be glycogen (Durchon and Lafon, 1951; Waraska et al., 1980)

and to a lesser extent mucco-polysaccarides (Laverack, 1963).

Both raw starch and dextrin have limited digestibility when included in diets for rainbow trout. Apparent digestibility of the carbohydrate generally ranges from 40% (raw starch) to 75% (dextrin) and depends on the level of inclusion in the diet (Phillips and Brockway, 1959; Cowey and Sargent, 1972; Bergot, 1979). However, simple sugars such as glucose are utilized well and attain almost 100% digestibility in trout (Bergot, 1979). Although no reference can be found regarding the digestibility of glycogen, since it is formed within the metabolic pathways involved in carbohydrate utilization (Freedland and Briggs, 1977), for the purposes of this discussion the assumption has been made that the digestibility and utilization of glycogen in trout is comparable to glucose.

In terms of absolute amounts, the carbohydrate content of the pelleted diets fed was higher when compared to the frozen earthworm diets (Table 2.2.2). However, the digestibility and therefore metabolizable energy varies according to the type of carbohydrate present (1.6 kcal/g for raw starch; 4.1 kcal/g glucose, Bergot, 1979) and therefore the frozen earthworm diets may have provided a higher percentage metabolizable energy from the carbohydrate present compared with the control pelleted rations.

Various authors have reported that increasing levels of metabolizable energy from carbohydrate sources (between 15-49% of the diet, Phillips et al., 1966; Austreng et al., 1977; Reftsie and Austreng, 1981) in iso-nitrogenous and iso-energetic diets for rainbow trout resulted in decreased carcass lipid content (Austreng et al., 1977; Reftsie and Austreng, 1981; Hilton and Atkinson, 1982) and increased liver somatic

index and liver glycogen content (Phillips et al., 1948,1966; Lee and Putnam, 1973; Austreng et al., 1977; Pieper and Pfeffer, 1980; Hilton and Atkinson, 1982). The results were similar to those reported here when earthworms were fed as a complete diet for rainbow trout.

(ii) The incorporation of various novel protein sources, in particular single cell proteins, into the diets of fish has often resulted in a hitherto unexplained reduction in carcass lipid content of fish maintained on these diets. Appler and Jauncey (1983) reported decreasing lipid and protein content in the carcass of Sarotherodon niloticus fingerlings fed increasing levels of the filamentous green algae Cladophora glomerata in the diet. Tacon and Ferns (1976) reported a similar decrease in carcass lipid content with increasing levels of activated sludge single cell protein in diets for rainbow trout, and Atack and Matty (1978) reported a low fat content of fish fed methanophilic bacterial protein despite similar dietary lipid levels and feed intake by the fish. Earthworms have been shown to feed, to some extent, on the single cell protein in their ingesta (Miles, 1963) and their body lipid composition reflects this (Hansen and Czachanska, 1975).

(iii) Anabolic steroids such as testosterone and growth hormone have been shown to be present in sewage sludges (Dorfman and Ungar, 1965) and may therefore also be present within earthworms grown on such substrates. The presence of low levels of anabolic steroids in diets has been associated with reduced carcass lipid and increased carcass crude protein content in mammals (Bell et al., 1972) and also with enhanced rates of growth (Higgs et al., 197[illegible]; Yamazaki, 1976).

(iv) The level of dietary phosphorus has been shown to affect the lipid content of the fish. Takeuchi and Nakazoe (1981) have demonstrated significantly increased carcass lipid content of carp maintained on a low P diet. Their results suggested that the deficiency of P in the diet was inhibiting β oxidation of fatty acids. Prior to this work Murakami (1970) observed that the addition of P in the diet of carp decreased the lipid content of muscle and viscera tissue and increased the protein content of muscle tissue. Similar findings have been reported in red sea bream, Chrysophrys major by Sakamoto and Yone (1973) and their conclusions were similar to those of Takeuchi and Nakazoe (1981): P deficient fish were unable to effectively utilize lipids absorbed from the diet and therefore lipid accumulated in the fish.

The fourth explanation is believed least likely to explain the low carcass lipid content of fish fed earthworm diets. All levels of dietary P in diets employed for this experiment should have been adequate (Lall, 1979) and fish exhibiting higher carcass lipid content had been fed the highest level of dietary P. Furthermore reduced LSI as well as increased carcass lipid were also reported as a result of P deficiency (Sakamoto and Yone, 1973) which is contrary to the results of this experiment.

(v) Increased LSI has been significantly correlated with increasing levels of dietary protein (Lee and Putnam, 1973). Additionally, carcass lipid content significantly increased as the ratio of protein:energy increased in diets containing 8 and 16% (but not 24%) dietary lipid. The protein:gross energy ratio of the five experimental diets employed in this feeding trial is given in Table 2.2.2. Although those fish fed the highest level

of dietary protein also exhibited significantly higher LSI no direct relationship was evident between dietary protein:gross energy ratio and the carcass lipid content of fish during this experiment.

Fatty Acid Composition: The results of the present study with D.veneta and D.subrubicundus and previous research into the composition of earthworm lipids (Cerbulis, 1967; McLaughlin, 1971; Hansen and Czochanska, 1975) indicate a complex fatty acid profile of earthworm lipids exhibiting characteristics of both marine and terrestrial animals. Generally, iso, anteiso and isoprenoid branched chain fatty acids are believed to be of microbial origin suggesting that the diet of earthworms consists to some extent of living and dead micro-organisms from the ingested soil (Hansen and Czochanska, 1975).

Compared with the semi-synthetic control diet containing fish oil, earthworm lipids comprised proportionately more saturated fatty acids and less monounsaturated and polyunsaturated fatty acids. In addition, the fatty acids of earthworms had a greater preponderance of shorter chain (< 16C) fatty acid than the semi-synthetic control diet.

Early studies suggested that a high proportion of saturated fatty acids in the diet was detrimental to fish (Phillips and Po doliak, 1948; Phillips et al., 1966). However, more recent research in which animal fats (comprising predominantly saturated fatty acids) were included almost exclusively in fish diets, with only sufficient marine oil to supply the fatty acid requirements of the fish, have shown no detrimental effect in terms of growth rates, feed utilization and general health of

the fish, implying that saturated fatty acids may be assimilated by rainbow trout (Yu et al., 1977a,b; Cowey et al., 1979; Reinitz et al., 1981) and chinook salmon Oncorhynchus tshawytscha (Mugridichian, 1981).

To some extent the fatty acid profile of the fish liver and muscle tissue reflected that of the diet on which the fish were maintained (Tables 2.2.4 and 2.2.7). A slightly higher proportion of shorter chain fatty acids were evident in fish fed frozen D.subrubicundus (D.s.) and D.veneta (D.v.) compared with fish fed the semi-synthetic control diet (s.s.d.) where some of these fatty acids were undetected. The fish appear to have achieved a balance in the proportion of unsaturated to saturated fatty acids in their tissues. In the diets, the ratio of saturated to unsaturated fatty acids was 1.94 (D.v.); 1.07 (D.s.) and 0.41 (s.s.d.). In the fish liver tissue this ratio has apparently been adjusted to 0.48 (D.v.); 0.47 (D.s.) and 0.42 (s.s.d.) and in the muscle tissue the ratios were 0.59 (D.v.); 0.52 (D.s.) and 0.46 (s.s.d.).

This may suggest that the saturated fatty acids were preferentially oxidised to supply energy in order to maintain an approximately constant level of saturated fatty acids in the fish tissue. A similar adjustment by fish fed diets containing a substantially higher proportion of saturated fatty acids than those encountered in this experiment was observed by Yu et al. (1977b) and Mugridichian (1981).

Fish muscle tissue tended to reflect the diet of the fish to a greater extent than the liver tissue, the latter containing a higher percentage of polyunsaturated fatty acids (PUFA). Liver tissue contains more membraneous organelles of which PUFA are an essential constituent for the maintenance of membraneous fluidity (Cowey et al., 1979).

Only trace quantities of 22:6 (docosohexanoic acid) were present in the frozen earthworm diets, however, the tissues of fish fed these diets contained an appreciable amount of this fatty acid (Table 2.2.7) suggesting that fish have been able to elongate and desaturate fatty acid precursors in the diet to form this fatty acid (Kayama, 1963; Castell et al., 1972). An increase in the 20:3w9 (eicosatrienoic acid) has been suggested as evidence of fatty acid deficiency in several animals and Castell et al. (1972) proposed the ratio of 20:3w9/22:6w3 as an index of fatty acid deficiency in rainbow trout. The fatty acid profile of fish fed frozen earthworms contained no evidence of 20:3 fatty acid although 22:6 fatty acid was present at levels comparable to the control.

It therefore appears that fish fed frozen D.veneta and frozen D.subrubicundus as a complete diet for 60 days suffered no detrimental effect to the tissue fatty acid composition. Furthermore these diets contained a smaller proportion of unsaturated fatty acids making them less prone to oxidative rancidity during storage (Cowey et al., 1979).

Feed Intake: It has been generally accepted that fish regulate the amount of feed consumed according to their energy intake (Lee and Putnam, 1973; Page and Andrews, 1973; Cho et al., 1976). In this experiment fish exhibiting similar growth rates achieved the same dry matter feed intake (mg/day/fish) over the 60 day feeding trial despite the different moisture content of the diets (Table 2.2.5). A similar result was reported in experiment 2.1 (Table 2.1.3). As discussed in section 2.1.4 an overall energy conversion figure of 4 kcal/g (gross energy) and 2 kcal/g (digestible energy) for the carbohydrate fraction of the diet

has been based on the assumption that this fraction consists of starch. Where simple and compound sugars have been included in the diets the value should be adjusted according to their digestibility. In experiment 2.1 fish fed frozen earthworms had a lower energy intake than fish fed the commercial trout ration however in this experiment the reverse occurred (Tables 2.1.3 and 2.2.5).

Mineral Composition: Possibly as a result of removing undigested feed from the gut of fish sacrificed for mineral analysis the carcass mineral composition of fish fed these five experimental diets tended to be lower than that observed in the carcass of fish in experiment 2.1 (Tables 2.1.4 and 2.2.7). However, the absolute concentrations of many of the mineral elements in D.veneta and D.subrubicundus was also lower compared with earthworm species used as diets in experiment 2.1, presumably a reflection of their lower ash content (Table 2.1.1 and 2.2.2). The high level of Pb in frozen D.subrubicundus may be a reflection of the proximity of Dunblane Sewage Works to a busy road, collecting run-off which may contain lead tetra-ethyl antiknock petrol additive. The level of Mn in frozen D.veneta was below the dietary requirement of rainbow trout, 12-13mg/Kg Mn (Ogino and Yang, 1980). This may explain the reduced rate of weight increase towards the end of the experiment where fish were fed solely on frozen D.veneta. Rainbow trout were apparently able to compensate for the low levels of dietary Ca present in the frozen earthworm diets (diets 1 and 2) by absorbing Ca from the surrounding water through the gills and fins. Consequently the retention of Ca by fish fed the frozen earthworm diets exceeded the dietary intake of this element (Table 2.2.9).

Some evidence emerged that Zn and Pb may have accumulated in the carcass of fish fed diets containing earthworms (diets 1, 2 and 4; Tables 2.2.8 and 2.2.9). Both Zn and Pb have been shown to accumulate in the tissues of earthworms (Czarnowska and Jopkiewicz, 1978; Hartenstein et al., 1980a,b) and since both these elements were also recorded at higher levels in the carcass of fish fed earthworm diets in experiment 2.1 their passage through the food chain should be closely monitored if earthworms are used in animal feeds.

Cd has also been shown to accumulate in earthworm tissues more than any other element (Van Hook, 1974; Hartenstein et al., 1980a). However, although Cd levels were higher in the earthworm diets compared with the control diets, there was no significant accumulation of this element in the fish tissues (Table 2.2.8). In absolute terms, those fish fed frozen D.veneta exhibited the highest Cd retention/g and final carcass Cd concentration (Tables 2.2.8 and 2.2.9).

To conclude: The earthworm D.subrubicundus proved highly palatable to rainbow trout and when fed frozen as the sole diet for 60 days caused no reduction in the growth response of these fish compared with fish fed the control diets. Frozen D.veneta caused a slightly reduced rate of fish growth, although this was not significant over the 60 day feeding period. In both instances the earthworm diets significantly affected the carcass composition of the fish. Fish fed the frozen earthworm diets exhibited lower carcass lipid and higher carcass crude protein contents compared with fish fed the other three diets. However,

the fatty acid composition of the fish liver and muscle tissue was only marginally affected by the diets of frozen earthworms compared with the semi-synthetic control diet. The inclusion of D.veneta (dried as a meal) within a semi-synthetic ration caused a significant reduction in fish growth and feed utilization efficiency compared with the semi-synthetic control diet. There was some evidence of Zn and Pb uptake by fish fed diets containing earthworms.

CHAPTER 3

THE NUTRITIONAL VALUE OF DRIED EARTHWORM MEAL IN A PELLETED FEED FOR RAINBOW TROUT

EXPERIMENT 3.1

THE NUTRITIONAL VALUE OF DRIED EISENIA FOETIDA MEAL IN A PELLETED FEED FOR RAINBOW TROUT

3.1.1 INTRODUCTION

The cost of using frozen earthworms as diets for rainbow trout involves the initial cost of freezing (c. £40/tonne) and subsequent cost of storing the frozen product (c.£1/tonne/week). Unless the earthworms were exceptionally cheap to produce, such costs would make their use in fish diets an uneconomic proposition. The production of a dried meal from earthworms for use in fish feed formulation presents the following advantages:-

(i) Although the initial cost of drying a product comprising approximately 80% moisture would be high, subsequent storage and transport costs would be minimised.

(ii) The analysis of 'batches' of dried earthworm meal and subsequent blending would facilitate the production of a nutritionally consistent product. Differences in the nutritional composition of earthworms resulting from variation in season, age or substrate from which worms were harvested would thus be minimised.

(iii) Assuming that heat would be employed to drive off excess moisture many parasites and pathogens originally present in the earthworms could be destroyed.

(iv) Protein is one of the most expensive ingredients used in fish feeds and research has aimed to "spare" dietary protein in fish feeds by replacing a percentage of the protein with lipid (Watanabe, 1977) and/or carbohydrate (Pieper and Pfeffer, 1979). Providing sufficient protein is present to supply the essential amino acid requirement, lipid and carbohydrate in the diet may be utilised by the fish as an alternative energy source and fish may therefore achieve higher PER and Apparent NPU. Due to the high levels of dietary protein supplied by frozen earthworm diets in

experiments 2.1 and 2.2 it has been suggested that protein was being utilized as an energy source (Sections 2.1.4 and 2.2.3.1). Earthworm meal incorporated into a diet which has been formulated to contain the minimum dietary protein essential for rainbow trout and supplemented with carbohydrate and lipid may result in more efficient protein utilization by the fish.

Eisenia foetida was chosen as the earthworm species for evaluation in this feeding trial due to its availability in sufficient quantity. In this experiment 50% and 100% of the herring meal protein within a semi-synthetic diet was replaced by E.foetida (dried and ground to a meal). These diets were compared with a semi-synthetic control diet in which all the dietary protein was supplied in the form of herring meal and all three diets were fed to rainbow trout over a 50 day feeding period.

3.1.2 MATERIALS AND METHODS

3.1.2.1 Diets

Commercial drying operations are designed to reduce damage caused to the nutritional constituents of the product by controlling heating conditions and the level of moisture present (Bender, 1970). In dealing with the quantities of meal required for use in the feeding trials described in this chapter, commercial methods were not feasible and the dried worm meal evaluated in these experiments was prepared by freeze drying.

Freeze dried E.foetida was supplied by Dr. C. A. Edwards and J. R. Lofty, Rothamsted Experimental Station, Harpenden, Herts. The worms were collected from a local sewage works and after being freeze dried were ground to produce a homogenous meal.

The resulting 'worm meal' comprised 61.71% crude protein, 9.33% lipid and 4.89% ash.

A semi-synthetic diet comprising 100% herring meal as the protein source was employed as the control diet, 50% and 100% of the herring meal protein within the semi-synthetic diet was replaced by the 'worm meal' protein and all rations formulated to contain 45% protein and 14% lipid. The dietary formulation and proximate composition of the following three dietary treatments is shown in Table 3.1.1.

Diet 1: Control, 100% herring meal protein
Diet 2: 50% herring meal protein + 50% E.foetida meal protein
Diet 3: 100% E.foetida meal protein

All dietary ingredients were mixed and pelleted as described in Section 2.2.2.1. For the measurement of digestibility coefficients, an indicator mix Cr_2O_3:polyethylene (1:1), was included in the diets.

All fish were fed twice daily "to appetite" as described in Section 2.1.2.1 and an accurate record of feed intake by fish fed each diet was kept during the 50 day experimental period.

3.1.2.2 Animals and Tanks

Sixty rainbow trout, of mean weight 30g. were supplied by Howietoun Fish Farm, Bannockburn, Nr. Stirling, Scotland and divided equally between three 60ℓ circular tanks ensuring that the total weight of fish in each tank was approximately the same. At the start of the experiment, the twenty fish in each tank were lightly anaesthetized and their individual weights recorded as described in Section 2.1.2.2. This experiment and experiment 2.1

Table 3.1.1: Formulation and proximate analysis of the experimental diets (values expressed as % by weight)

Component	Dietary treatment 1	2	3
Ingredient analysis (%)			
Herring meal	68	34	0
Dried E.foetida meal	0	34	68
Cod liver oil	1.5	1.5	1.5
Corn oil	3	4	5
Corn starch	12	11	10
Dextrin	7	7	7
Vitamin mix[1]	2	2	2
Mineral mix[1]	4	4	4
Carboxymethyl cellulose binder	1.5	1.5	1.5
Indicator mix[2]	1	1	1
Nutrient content (%, moisture free)			
Crude protein	50.01	47.64	46.00
Lipid	11.25	12.02	12.68
Ash	15.99	12.58	9.48
Nitrogen free extractive[3]	38.74	40.34	41.32
Energy content (kcal/100g)			
Gross energy[3]	546.89	547.10	547.94
Digestible energy[3]	428.78	427.06	426.76

1. Vitamin and Mineral mix given in Table 2.2.1
2. 1:1 Cr_2O_3:polyethylene
3. Calculated as in Table 2.2.2

were conducted simultaneously and the same initial fish sample served for both experiments (Section 2.1.2.2).

Fish were housed in tanks similar to that shown in Figure 2.1.1. Water was supplied in a throughflow system at a temperature of 7.5°C ± 1°C during the experimental period. Fish were subject to natural photoperiod.

The fish in each of the tanks were allocated one of the experimental diets. Procedures employed for the regular bi-weekly weighing of fish and the collection of faecal samples during the final week of the experiment are described in Section 2.1.2.2.

On the final day of the experiment all fish were weighed individually and killed by a sharp blow on the head. Ten fish from each dietary treatment were stored at -20°C for subsequent whole carcass proximate analysis. From five fish the liver was removed and weighed for the calculation of liver somatic index, and from the remaining five fish in each group small slices of liver, spleen, kidney and gill were removed and stored in fixative (10% buffered formalin) for subsequent histological examination.

3.1.2.3 Chemical Methods

Proximate analysis of fish samples and experimental diets was carried out as described in Section 2.1.2.3. Nitrogen free extractive and gross and digestible energy content were determined by calculation (Section 2.1.2.3). In addition, replicate sub-samples of diets and faeces were analysed for chromic oxide and total nitrogen content and the apparent dry matter and nitrogen digestibility coefficients calculated (Section 2.2.2.3).

3.1.2.4 Histological Methods

Tissue samples were prepared and examined as described in Section 2.1.2.4.

3.1.2.5 Statistical Method

Statistical analysis was carried out as described in Section 2.1.2.5.

3.1.3 RESULTS

3.1.3.1 Fish Growth and Feed Utilization Efficiency

All fish fed aggressively throughout the experiment except fish fed the highest dietary inclusion of E.foetida (diet 3). Fish fed this ration found the diet to be unpalatable, consuming only small quantities of the feed offered. The growth response and feed utilization efficiency of fish fed the three dietary treatments is given in Table 3.1.2 and the growth response presented graphically in Figure 3.1.1.

Fish fed the control diet exhibited the best growth response achieving a significantly higher ($p < 0.05$) mean final body weight than fish fed diets containing worm meal. Fish fed the diet containing the highest inclusion of worm meal (diet 3) exhibited a poor growth response and had a significantly lower ($p < 0.05$) mean final body weight than fish fed the other two diets. Although fish fed the 50% worm meal protein replacement level (diet 2) exhibited a good growth response, the mean final body weight of these fish was significantly lower ($p < 0.05$) than fish fed the control diet (diet 1).

The reduced growth rate of fish, corresponding with increasing dietary inclusion of worm meal, was apparently a reflection of feed intake; the efficiency of feed utilization

Table 3.1.2: Growth, feed utilization, liver somatic index and carcass composition of rainbow trout fed the experimental diets for 50 days

Mean values	Dietary treatment 1	2	3	± SE[1]
Mean initial weight (g)	29.80[a]	28.80[a]	28.60[a]	0.714
Mean final weight (g)	49.39[c]	45.04[b]	31.64[a]	1.200
Weight gain (%)	65.74	56.40	10.63	
Specific growth rate (%)	1.01	0.89	0.20	
Food intake (mg/day)	424	381	158	
Weight gain (mg/day)	302	325	61	
Food conversion ratio	1.08	1.17	2.59	
Protein efficiency ratio	1.99	1.96	0.92	
Nitrogen intake (mg/day)	31.52	26.56	10.56	
Nitrogen deposition (mg/day)	9.92	9.76	1.92	
Apparent N utilization (%)	31.47	36.75	18.18	
Apparent dry matter digestibility (%)	89.82	66.09	74.47	
Apparent N digestibility (%)	79.89	83.99	91.16	
Liver somatic index (%)	1.62[b]	1.44[ab]	1.28[a]	0.071

Carcass composition (% wet weight)

	Initial	Final			
Moisture	74.03	74.84[a]	74.36[a]	76.54[b]	0.431
Crude protein	15.37	15.55[a]	15.95[a]	15.46[a]	0.519
Lipid	7.19	6.53[b]	6.85[b]	5.11[a]	0.203
Ash	2.37	2.54[a]	2.49[a]	2.56[a]	0.040

1a,b,c = For explanation see Table 2.1.3

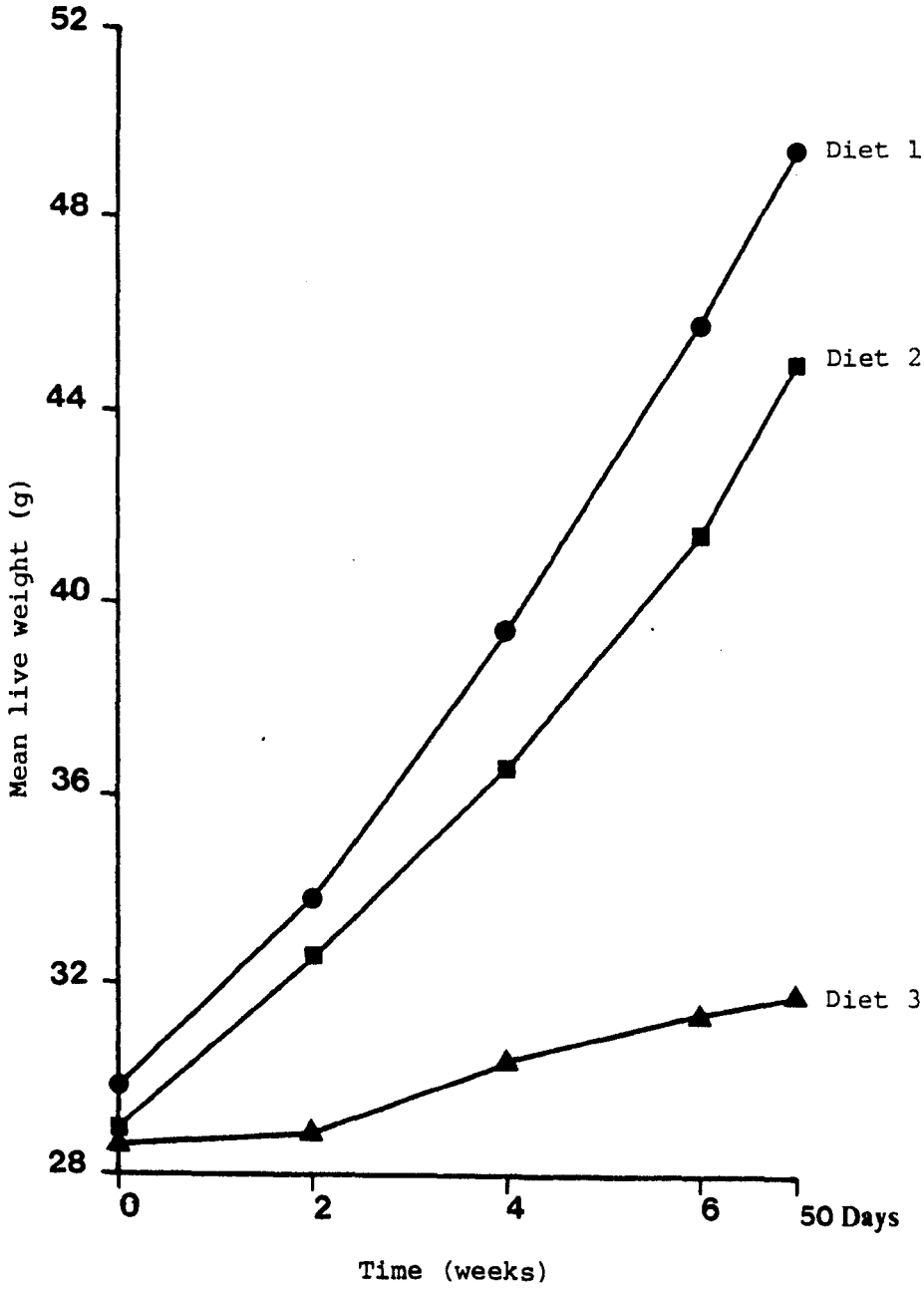


Figure 3.1.1: The growth response of rainbow trout fed the three experimental diets

being very similar between fish fed diets 1 and 2 in terms of FCR, PER and Apparent NPU (Table 3.1.2). The efficiency of feed utilization, as measured by these parameters, decreased substantially when fish were fed diet 3 (Table 3.1.2). Surprisingly, there was a progressive increase in apparent nitrogen digestibility with increasing inclusion of worm meal in the diet.

3.1.3.2 Carcass Composition and Liver Somatic Index

The carcass composition of fish sacrificed at the start (initial fish) and end (final fish) of the experiment together with the liver somatic index (LSI) data is given in Table 3.1.2.

There was no significant difference ($p < 0.05$) in the carcass moisture, crude protein, lipid and ash content between fish fed diets 1 and 2. However, fish fed the highest dietary inclusion of worm meal (diet 3) exhibited significantly higher ($p < 0.05$) carcass moisture content and significantly lower ($p < 0.05$) carcass lipid content compared with fish fed the remaining dietary treatments.

The LSI decreased with increasing dietary inclusion of worm meal. Fish fed diet 1 exhibited a significantly higher ($p < 0.05$) LSI than fish fed diet 3. The LSI of fish fed diet 2 was not significantly different ($p < 0.05$) from fish fed diets 1 and 3.

3.1.3.3 Histological Examination

Histological examination of the liver, spleen, gill and kidney tissue revealed no apparent differences between fish fed the various diets.

3.1.4 DISCUSSION

The growth response and feed utilization efficiency of rainbow trout fed diets in which half the herring meal protein was substituted by worm meal were encouraging. However, diets comprising 100% worm meal protein resulted in poor fish growth and feed utilization efficiency. This result was not surprising in view of the results of experiment 2.1 in which rainbow trout maintained on diets consisting solely of frozen E.foetida exhibited very little growth over the experimental period. Fish fed diet 3 were consuming very little feed and the reduced carcass lipid and LSI and increased carcass moisture content would be consistent with a starving condition and the utilization of body lipid reserves. Lower carcass moisture and correspondingly high carcass lipid content has been associated with well fed fish and the reverse condition associated with starved fish (Brett et al., 1969; Pandian and Raghuraman, 1972; Lee and Putnam, 1973).

No previous reports can be traced regarding the use of dried E.foetida meal in fish diets. However "worm meal" derived from this earthworm species has been evaluated in feeds for various other livestock and although the worm meal has been used to provide all the dietary protein, growth achieved by the experimental animals has usually been comparable to that of animals fed a control diet (McInroy, 1971; Sabine, 1978). The dietary protein requirement of these experimental animals (mice, pigs and poultry) was much lower than the dietary protein requirement of fish and therefore the absolute level of E.foetida meal included within diets evaluated in this feeding trial (34g and 68g/100g of feed) presented a larger proportion of the diet than in diets tested

with other livestock. This may account for the unpalatable nature of E.foetida not having previously arisen.

Hilton (1983) reported a similar decline in the growth rate of rainbow trout fed increasing levels of dietary inclusion of 'earthworm meal' derived from the species E.eugeniae.

The reduced growth rate of fish fed diets 2 and 3 may also have been caused by the nutritional content of these diets compared with the control diet. Diets containing worm meal comprised a smaller proportion of crude protein compared with the control ration (Table 3.1.1). However, the level of dietary protein in all diets was sufficient to meet the dietary protein requirements of these fish and the gross and digestible energy contents of all diets was similar (Table 3.1.1).

The type of lipid supplied in the experimental diets may also have influenced fish growth. Castell et al. (1972) reported that 1% linolenic acid (18:3w3)*1 was essential in the diet of rainbow trout to support optimum fish growth. Yu and Sinnhuber (1972) demonstrated that docosahexanoic acid (22:6w3) could also satisfy the essential fatty acid requirement of rainbow trout. Generally, marine fish oils (such as cod-liver oil used in these diets) are rich in w3 fatty acids and low in w6 fatty acids therefore these are suitable for inclusion in fish diets to furnish the essential fatty acid requirement (Cowey and Sargent, 1979). The majority of vegetable oils (such as corn oil, used in these experimental diets) contain high levels of w6 fatty acids and less w3 fatty acids and are therefore more suitable for inclusion in diets as an energy source for rainbow trout. Furthermore, high levels of corn oil within fish diets should be

*1 Fatty acids denoted by - No. of C atoms:no. of double bonds: position of first double bond.

avoided since high levels of w6 fatty acids (2.5% and 5% of the diet) have resulted in inferior growth in trials with rainbow trout (Yu and Sinnhuber, 1976).

Earthworms comprise a proportion of w3 fatty acids (Hansen and Czochanska, 1975) but this is lower than the proportion expected in marine fish oil. The higher corn oil inclusion in diets 2 and 3 may have led to an unfavourable w3:w6 fatty acid ratio in the diets, possibly resulting in reduced fish growth.

In conclusion: 50% of the dietary herring meal protein was replaced by dried E.foetida meal without affecting feed utilization efficiency or carcass composition of rainbow trout although a significant decline in fish growth rate was evident compared with fish fed the control ration. Fish fed diets containing 100% E.foetida meal exhibited poor growth rates and feed utilization efficiency and the carcass composition of these fish was significantly affected compared with fish fed the remaining two dietary treatments.

It is unlikely that E.foetida (or any earthworm species) will be cultured in quantities sufficient to provide 50 or 100% of the protein in commercially produced fish feeds. It is more feasible that earthworm meal would be available for inclusion in fish diets at levels of 5-20% in conjunction with other alternative protein sources.

By extrapolating the results of this experimental feeding trial, the tentative suggestion could be made that E.foetida meal could be included in trout rations at levels of 5-20% without significantly reducing the growth rate of the fish.

EXPERIMENT 3.2

THE NUTRITIONAL VALUE OF DRIED EISENIA FOETIDA MEAL AT LOW LEVELS OF INCLUSION IN 'PRODUCTION DIETS' FOR RAINBOW TROUT

3.2.1 INTRODUCTION

Commercially available trout rations, currently devised by 'least-cost' formulation methods, rely upon information regarding the cost and nutritional value of the range of ingredients available so as to determine the optimum level of inclusion of each product in the diet in order to provide a nutritionally balanced diet at an economically competitive price. As a result, the dietary protein requirement within a commercial trout ration is generally supplied using several ingredients each present at a relatively low level in the diet. Any commercially available dietary ingredient would be subject to such analyses and it is unlikely that any single product, for example dried worm meal, would be used exclusively to supply the dietary protein component in a production salmonid ration.

Rainbow trout, fed a semi-synthetic diet in which dried E.foetida meal was used exclusively to supply dietary protein, exhibited significantly reduced growth rates, feed utilization efficiency and altered carcass composition when compared with fish fed diets containing only herring meal as the protein source over a period of 50 days (Experiment 3.1). However, the significant improvement observed in Experiment 3.1 in the growth and feed utilization efficiency of fish fed semi-synthetic diets containing 50% dried E.foetida meal protein and 50% herring meal protein, tentatively implied that this 'worm meal' may be used to replace fish meal at lower levels of dietary inclusion without significantly affecting the growth and performance of rainbow trout.

In the present experiment, compound diets (simulating

commercially available trout rations) were formulated to include dried E.foetida meal at levels of 0%, 5%, 10%, 20% and 30% of the diet, directly replacing the herring meal component. All five diets were nutritionally evaluated, on the basis of fish growth, feed utilization efficiency and carcass composition, in a 84 day feeding trial with rainbow trout.

Earthworms, including E.foetida, have been shown to accumulate certain potentially toxic elements such as heavy metals for example Zn, Pb, Cu, Cd from their surrounding substrate (Czarnowska and Jopkiewicz, 1978; Ash and Lee, 1980; Hartenstein et al., 1980b). The results of previously reported experiments (Experiments 2.1 and 2.2) have demonstrated a significant increase in levels of Pb and Zn in the carcass of rainbow trout maintained on diets consisting solely of frozen earthworms. The possibility that these potentially toxic elements may accumulate in the fish as a result of including E.foetida in trout rations at low levels of dietary inclusion was investigated and concentrations of Fe, Zn, Pb, Cu and Cd were monitored in the experimental diets and whole fish carcass.

3.2.2 MATERIALS AND METHODS

3.2.2.1 Diets

Eisenia foetida, supplied by British Groundbaits, were freeze dried and ground to a free flowing meal. This 'worm meal', which comprised 64.48% crude protein and 11.17% lipid was included in the experimental diets at levels of 0% (control diet), 5%, 10%, 20% and 30%, directly replacing herring meal (68.03% crude protein and 7.59% lipid). All five diets were formulated to contain 45% crude protein and 12% lipid. The dietary formulation and the

nutritional and mineral composition of the diets is shown in Table 3.2.1. The range of ingredients used to supply dietary protein also included soyabean meal, meat and bone meal and blood meal.

All dietary ingredients were mixed and pelleted as described in Section 2.2.2.1.

Each of the five diets was fed twice daily "to appetite" to duplicate groups of rainbow trout for 84 days as described in Section 2.1.2.1. The bi-weekly feed intake of each group of fish was recorded.

3.2.2.2 Animals and Tanks

One hundred and seventy rainbow trout, of mean weight 7g, were obtained from Mr. D. Brien, Almondbank Fish Farm, near Perth, Scotland. One hundred and fifty fish were anaesthetized, weighed individually (by the procedure outlined in Section 2.1.2.2) and divided between ten tanks (15 fish/tank) ensuring that the mean weight of fish in each tank was not significantly different. The experimental fish holding tanks were similar to that illustrated in Figure 2.1.1 each supplied with fresh water (2ℓ/min/tank) in a throughflow system.

The twenty fish not included in an experimental group were killed by a sharp blow on the head and stored at -20°C for subsequent whole carcass proximate analysis.

The water temperature during the experimental period was 13°C ± 3.5°C and fish were subject to natural photoperiod.

All fish were weighed individually at bi-weekly periods and at the end of the experiment. During the final week of the experiment faecal samples were obtained (following the procedure outlined in Section 2.2.2.2) for the determination of

Table 3.2.1: Ingredient composition and nutrient and mineral content of the five experimental diets containing low dietary inclusion levels of E.foetida meal

Diet No: % inclusion of E.foetida meal	1 0%	2 5%	3 10%	4 20%	5 30%
Ingredient (% by weight)					
Herring meal	40	35	30	20	10
E.foetida meal	-	5	10	20	30
Soyabean meal	10	10	10	10	10
Meat and bone meal	15	15	15	15	15
Blood meal	5	5	5	5	5
Wheat meal	15	15	15	15	15
Corn oil	3	3	3	2.5	2
Cod liver oil	7	7	7	7.5	8
Carboxymethylcellulose	0.5	0.5	0.5	0.5	0.5
Vitamin mix*1	2	2	2	2	2
Mineral mix*1	2	2	2	2	2
Chromic oxide	0.5	0.5	0.5	0.5	0.5
Nutrient content (% by weight)					
Moisture	7.56	7.33	7.92	7.90	8.15
Crude protein	44.86	45.24	44.88	43.89	43.46
Lipid	12.24	12.97	12.25	10.93	10.37
Ash	15.40	15.14	14.04	14.85	12.75
Nitrogen free extractive*2	19.94	19.32	20.91	22.43	25.27
Mineral content (mg/Kg,dry weight)					
Fe	221.39	317.35	207.34	119.92	175.06
Zn	125.08	119.84	134.09	145.39	147.81
Pb	12.26	11.23	11.67	11.54	10.37
Cu	17.28	16.56	18.34	23.59	20.63
Cd	1.14	1.00	1.12	1.12	1.01

*1 Vitamin and mineral mix given in Table 2.2.1

*2 Defined in Table 2.2.2

digestibility coefficients.

At the end of the 84 day feeding period all fish were killed by a sharp blow on the head. The livers of six fish from each group were removed and weighed for the determination of liver somatic index (LSI). The whole fish carcass of nine fish from each group was also stored at -20°C for subsequent proximate and mineral analysis. All fish selected for mineral analysis were dissected and the gastro-intestinal tract washed free of undigested feed with de-ionized water.

3.2.2.3 Chemical Methods

Proximate and mineral analysis of whole fish carcass and experimental diets was determined using the methods referred to in Section 2.1.2.3. Nitrogen free extractive and energy content of the diets was determined by calculation (Section 2.2.2.3) and digestibility coefficients calculated after determination of the total N and Cr_2O_3 content of diets and faecal samples (Section 2.2.2.3).

3.2.2.4 Statistical Methods

Statistical comparison of the means was carried out as described in Section 2.1.2.5. Regression analyses were calculated using the "Minitab" computer package, Pennsylvania State University and the significance of linear regression ascertained by F-test.

3.2.3 RESULTS

3.2.3.1 Fish Growth and Feed Utilization Efficiency

All fish fed aggressively on their respective diets for the duration of the experiment and achieved similar growth rates (Figure 3.2.1). There was no significant difference ($p < 0.05$) in the mean final body weights of fish fed each of the five

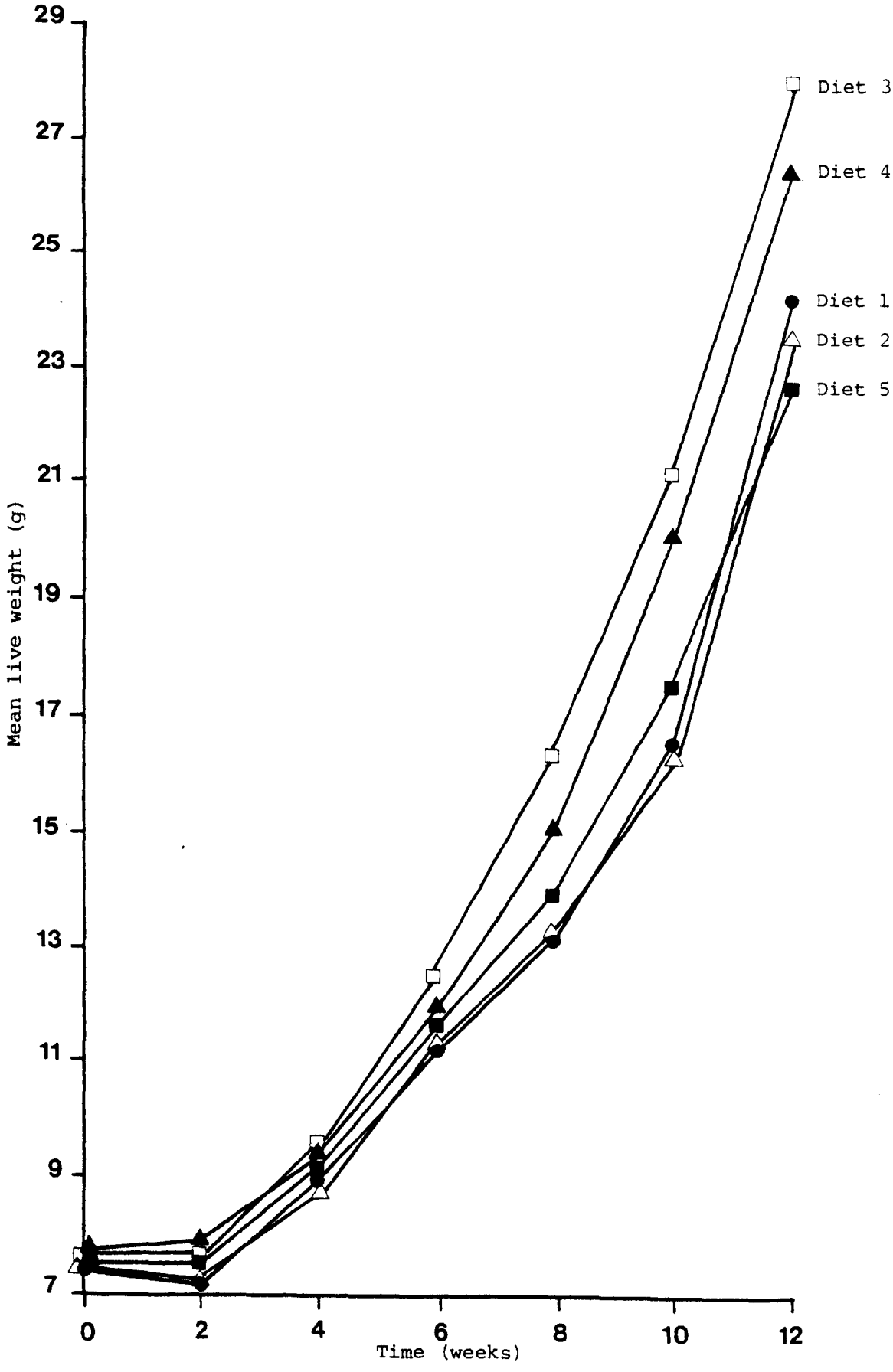


Figure 3.2.1: The growth response of rainbow trout fed 'production diets' containing low levels of E.foetida meal (mean live weight of duplicate groups of fish)

experimental diets at the end of the 84 day feeding period (Table 3.2.2). Feed utilization efficiency measured in terms of FCR, PER and Apparent NPU (all terms defined in Section 2.1.3.1) showed little difference between fish fed the five experimental diets, although in absolute terms those fish fed diet 3 (10% E.foetida meal) achieved the highest growth and efficiency of feed utilization (Table 3.2.2.2). Surprisingly, apparent N digestibility of the diets increased with increasing dietary inclusion of dried E.foetida meal. Similarly, despite the low apparent dry matter digestibility of the control ration, dry matter digestibility increased with increasing worm meal inclusion in the diets (Table 3.2.2.2).

3.3.3.2 Carcass Composition and Liver Somatic Index

On the basis of LSI, carcass ash content and carcass crude protein content no significant difference emerged between fish fed the five diets (Table 3.2.2) and no significant difference ($p < 0.05$) was evident in the carcass moisture and lipid content between fish fed the control diet and fish fed 30% dietary inclusion level of E.foetida meal. However, the inclusion of 5%, 10% and 20% dried E.foetida meal in the diet resulted in a significant alteration in the carcass moisture and lipid content of fish fed these diets compared with fish fed the remaining two diets (Table 3.2.2). Those fish fed 5% dietary inclusion of E.foetida meal exhibited a significantly lower ($p < 0.05$) carcass moisture content compared with fish fed the control diet and fish fed the 30% dietary inclusion level of E.foetida meal. Fish fed diets containing 5%, 10% and 20% dried E.foetida meal exhibited significantly increased ($p < 0.05$) carcass crude lipid content compared with fish fed the control diet.

Table 3.2.2: Growth, feed utilization efficiency and carcass composition (proximate and mineral) of rainbow trout fed experimental diets containing low levels of dried E.foetida meal for 84 days (all values are mean values for duplicate groups of fish)

Diet No:	1	2	3	4	5	± SE *1
Mean initial wt (g)	7.25	7.28	7.28	7.69	7.46	
Mean final wt (g)	24.19[a]	23.57[a]	27.97[a]	26.34[a]	22.81[a]	1.774
Mean weight gain (%)	233.16	223.76	284.20	242.52	205.76	
Mean specific growth rate (%)	1.434	1.399	1.603	1.537	1.330	
Mean food intake (mg/day/fish)	312.73	311.90	360.47	351.54	303.03	
Mean weight gain (mg/day/fish)	201.67	193.93	262.63	222.26	186.10	
Mean food conversion ratio	1.55	1.61	1.37	1.58	1.63	
Mean protein efficiency ratio	1.45	1.38	1.63	1.45	1.42	
Mean apparent N utilization (%)	24.94	24.08	25.82	23.88	23.29	
Mean apparent dry matter digestibility (%)	45.79	53.24	50.94	59.51	59.03	
Mean apparent N digestibility (%)	93.05	92.65	94.51	94.31	95.26	
Mean liver somatic index (%)	1.69[a]	1.63[a]	1.55[a]	1.58[a]	1.69[a]	0.074

Carcass composition Proximate composition (% wet weight)

	Initial fish	After 84 days					
Moisture	77.96	73.34[b]	72.50[a]	72.66[ab]	72.66[ab]	73.79[b]	0.241
Crude protein	15.07	16.60[a]	16.70[a]	16.39[a]	16.08[a]	16.15[a]	0.207
Lipid	4.68	7.83[a]	8.64[bc]	8.97[c]	8.95[c]	8.10[ab]	0.207
Ash	2.38	2.58[a]	2.52[a]	2.43[a]	2.42[a]	2.48[a]	0.056
		Mineral composition (mg/Kg,wet weight)					
Fe		10.21	12.26	11.27	11.13	11.48	
Zn		25.19	30.86	29.12	22.19	27.35	
Pb		1.29	1.41	1.29	1.59	1.40	
Cu		1.37	1.26	1.09	1.50	1.32	
Cd		0.17	0.18	0.10	0.17	0.16	

*1 } For explanation of terms see Table 2.2.5.
abc }

Table 3.2.3: Regression equations relating the level of each element in the fish carcass (Y) to the level of dietary inclusion of dried *E.foetida* meal (X)

Fe:	$Y = 11.1 + 0.0146X$	$r^2 = 5.7\%$	n.s.*1
Zn:	$Y = 27.9 - 0.0710X$	$r^2 = 6.4\%$	n.s.
Pb:	$Y = 1.33 - 0.0052X$	$r^2 = 25.9\%$	n.s.
Cu:	$Y = 1.27 + 0.0031X$	$r^2 = 6.0\%$	n.s.
Cd:	$Y = 0.16 - 0.0001X$	$r^2 = 0.1\%$	n.s.

*1 n.s. = not significantly related ($p < 0.05$).

3.2.3.3 Mineral Composition

Of the five micro-elements monitored during this experiment the absolute levels of only Zn and Cu in the diets increased with increasing dietary inclusion of dried E.foetida meal (Table 3.2.1).

The relationship between the level of micro-elements in the fish carcass (Y) shown in Table 3.2.2, and the dietary inclusion level of E.foetida meal (X) was examined by regression analysis. The regression equations for each element are shown in Table 3.2.3. No significant accumulation ($p < 0.05$) of the micro-elements Fe, Zn, Pb, Cu and Cd was evident in the carcass of rainbow trout as the level of inclusion of E.foetida meal increased from 0-30% of the diet.

3.2.4 DISCUSSION

Dried E.foetida meal adequately replaced the herring meal component of a 'production diet' (at levels of 5-30% dietary inclusion) without adversely affecting the growth performance and feed utilization efficiency of rainbow trout over an 84 day feeding period. Thus the general trend which emerged from the results of Experiment 3.1, in which the growth performance of rainbow trout increased with decreasing dietary inclusion of E.foetida, continued so that at the lower levels of dietary inclusion investigated in this feeding trial no significant difference ($p < 0.05$) was observed in the growth of fish fed the five experimental diets.

Similar results have been reported when low levels of dried E.foetida meal were included in diets for other livestock including mice, pigs and poultry (McInroy, 1975; Sabine, 1978). Worm meal derived from species other than E.foetida has been

evaluated as a fish meal replacer in diets for fish: Dried P.excavatus in diets for O.niloticus (Guerro, 1981) and dried E.eugeniae in diets for rainbow trout (Hilton, 1983) were successfully fed at low levels in the diet (15% and 23% by weight, respectively) without adverse effect on fish performance. However, an increase in the level of dietary inclusion of E.eugeniae from 23% to 34.4% and 48% (by weight) resulted in a significant decline in the growth rate of rainbow trout (Hilton, 1983).

The inclusion of 30% by weight of E.foetida meal in rainbow trout diets caused no significant alteration in the fish carcass composition and LSI and this was similar to the result reported in Experiment 3.1 in which the carcass composition of fish fed diet 2 (34% E.foetida meal, by weight in the diet) was not significantly different ($p < 0.05$) from the control fish. The significant increase ($p < 0.05$) observed in the carcass lipid content of fish fed diets 2, 3 and 4 (5, 10 and 20% by weight of E.foetida meal in the diet, respectively) was unexpected in view of the significant decrease ($p < 0.05$) in the carcass lipid content of fish fed 7-71% by weight of dried D.subrubicundus meal in the diet compared with the control fish in Experiment 3.3. However, no significant alteration in the carcass composition of rainbow trout fed 23-48% by weight of E.eugeniae in the diet (Hilton, 1983)

was observed. The level of lipid reported in the carcass of rainbow trout in this experiment (7.83-8.97% wet weight) remained within the acceptable limits for rainbow trout: 3.7%-14.2% wet weight (Pieper and Pfeffer, 1979).

Until recently some proportion of fish meal in the diet

has been considered essential to maintain good growth and feed utilization efficiency by rainbow trout and sources of protein secondary to fish meal have generally resulted in reduced fish performance when included at levels above 5-20% by weight in trout diets (Tacon, 1981). These secondary sources include plant proteins (soyabean meal); novel protein products (insect larvae, single cell protein); agricultural wastes and by-products (live-stock excreta); by-products from food processing industry and industrial by-products (paper-pulp waste). Many of these products have been successfully used to partially replace fish meal in salmonid diets, for example 14-28.5% defatted poultry by product meal (Higgs et al., 1978); 7-21% methanophilic bacterial protein "Pruteen", without methionine supplementation (Kaushik and Luquet, 1980); 12-17% dried sludge from paper processing wastes (Orme and Lemm, 1973) and 30% leaf protein concentrate (Ogino et al., 1978). However, at higher levels of dietary inclusion loss of fish performance generally results.

Restriction in the level of dietary inclusion of these products has usually been due to the presence of anti-nutritional factors, for example protease inhibitors and lectins in plant products (Liener, 1980) or to amino acid deficiency/imbalances such as the excessive amounts of leucine and tryptophan in blood meal (Tacon, 1981), excess cystine in day old chick meal (Tacon, 1982) and possibly in the case of earthworm meal a deficiency of the sulphur amino-acids:cystine and methionine (Yoshida and Hoshii, 1975; Amerio, 1983; Hilton, 1983).

By balancing the essential nutritional constituents supplied by each source of protein, so that excess amino acids from one source complement a deficiency of those amino acids in

another source, a mixture of protein products can be used to totally replace or to replace a higher percentage of fish meal in the diet (Gropp et al., 1979; Chvapil, 1980). Certain novel sources of animal protein such as krill meal have been successfully used alone to totally replace the fish meal component of rainbow trout diets (Koops et al., 1979).

The possibility that E.foetida may contain anti-nutritional factors which may limit the level of dietary inclusion is further discussed in Chapter 4. Amino acid supplementation may also further increase the nutritional value of this novel protein source. However, fish fed amino acid supplemented E.eugeniae meal at high levels of dietary inclusion exhibited significantly lower ($p < 0.05$) mean final body weight than fish fed a control diet containing only herring meal protein (Hilton, 1983).

Concentrations of trace elements measured in the experimental diets (Table 3.2.1) fell below the maximum levels permitted in salmonid feedstuffs (Edwards and Densem, 1980) and also fell within the range (except Pb) of concentrations measured in commercially available feedstuffs in Europe (Tacon and de Silva, 1983). Over the 84 day feeding period no significant accumulation ($p < 0.05$) of Fe, Zn, Pb, Cu or Cd in the carcass of rainbow trout was evident as the dietary inclusion level of E.foetida meal increased from 0-30%.

The high dietary Ca level present in these diets (due to the meat and bone meal inclusion) but not present in the frozen earthworm diets in Experiments 2.1 and 2.2 may afford additional protection against heavy metal uptake by the fish (Hsu et al., 1975; Six and Goyer, 1970). Furthermore evidence exists that fish may be able to regulate tissue levels and excrete excess

amounts of several heavy metals including non-essential elements such as Cd (Cearly and Coleman, 1974; Miettinen, 1975).

In conclusion: over an 84 day feeding period dried E.foetida meal adequately replaced the fish meal component of a 'production diet' for rainbow trout at dietary inclusion levels up to 30% without adversely affecting the growth rate, feed utilization efficiency and carcass composition of the fish. There was no evidence of accumulation of the elements Fe, Zn, Pb, Cu and Cd in the fish carcass as the dietary inclusion level of dried E.foetida meal increased from 0% to 30%.

EXPERIMENT 3.3

THE NUTRITIONAL VALUE OF DRIED DENDRODRILUS SUBRUBICUNDUS MEAL IN A PELLETED FEED FOR RAINBOW TROUT

3.3.1 INTRODUCTION

Experiments 2.1 and 3.1 have demonstrated that at high levels of dietary inclusion E.foetida (the earthworm species commonly employed in commercial vermiculture) was apparently unpalatable to rainbow trout. In the present experiment therefore, another species of earthworm: Dendrodrilus subrubicundus was evaluated in diets for rainbow trout. This earthworm occurs in abundance in sewage purification plants (Solbé, 1975) and therefore may feasibly be commercially produced from organic wastes. Information available regarding the life cycle and fecundity of D.subrubicundus suggests that under ideal conditions this species may reproduce as rapidly as E.foetida (Evans and Guild, 1948).

D.subrubicundus was freeze dried and used to replace 0, 10, 50 and 100% of the fish meal protein in a semi-synthetic trout ration. These diets were nutritionally evaluated over a 50 day feeding period with rainbow trout.

Experiments 2.1 and 2.2 suggested that the use of earthworms of various species (including D.subrubicundus) in diets for rainbow trout resulted in elevated levels of certain potentially toxic elements in the carcass of fish at the end of the experimental period. In the present experiment the concentrations of Ca, K, Na, Mg and the trace elements Fe, Zn, Mn, Cu, Pb, Co and Cd were monitored in the whole fish carcass at bi-weekly intervals throughout the experimental period and in selected fish tissues at the start and end of the experiment.

3.3.2 MATERIALS AND METHODS

3.3.2.1 Diets

D.subrubicundus was collected from the trickling filter beds of a local domestic sewage works (Dunblane Sewage Works, Nr. Stirling, Scotland). On harvesting, the earthworms were freeze dried, ground to a free flowing powder, and used to replace herring meal protein in a semi-synthetic trout ration.

Four experimental diets were formulated: a control diet, in which herring meal supplied the sole source of dietary protein fed and three diets in which D.subrubicundus meal protein replaced 10, 50 and 100% of the herring meal protein:

Diet 1: Control - 100% herring meal protein
Diet 2: 10% worm meal protein: 90% herring meal protein
Diet 3: 50% worm meal protein: 50% herring meal protein
Diet 4: 100% worm meal protein.

All diets were formulated to contain 45% crude protein and 14% lipid. The ingredient composition of the diets is given in Table 3.3.1 and the nutrient, energy and mineral composition of the 'worm meal' and the four experimental diets is given in Table 3.3.2. For comparison the mineral composition of the sewage substrate in which the earthworms had grown is also given. The amino acid composition of the 'worm meal' and the experimental diets is given in Table 3.3.3.

All dietary ingredients were mixed and pelleted as described in Section 2.2.2.1.

Fish were fed twice daily "to appetite" as described in Section 2.1.2.1 and an accurate record of their bi-weekly feed intake was kept.

Table 3.3.1: The dietary formulation of the four experimental diets (% by weight)

Ingredient composition %	Diet No. 1	2	3	4
Herring meal	61	55	31	-
Dried *D.subrubicundus* meal	-	7	36	71
Cod liver oil	3	3	2	2
Corn oil	6	5	4	4
Corn starch	16	16	14	11
Dextrin	8	8	7	6
Vitamin mix[1]	2	2	2	2
Mineral mix[2]	1	1	1	1
Carboxymethylcellulose	2	2	2	2
Chromic oxide	1	1	1	1

1. Vitamin mix given in Table 2.2.1

2. Mineral mix given in Table 2.2.1 omitting 29.00g/kg $CaHPO_4.2H_2O$

Table 3.3.2: Nutrient, energy and mineral composition of worm meal and experimental diets, and mineral composition of sewage substrate (all values expressed on a dry weight basis)

Component	Diet No. 1	2	3	4	Worm meal[1]	Sewage substrate
Moisture (%)	7.31	7.17	11.56	10.31	9.07	-
Nutrient content (%)						
Crude protein	46.81	45.99	47.31	45.20	65.13	-
Lipid	18.20	14.96	15.71	17.28	9.62	-
Ash	11.47	10.99	10.49	11.34	13.05	-
Nitrogen free extract[2]	23.52	28.06	26.49	26.18	12.20	-
Energy content (kcal/100g)						
Gross energy[2]	533.80	516.50	524.87	526.52	511.43	-
Digestible energy[2]	444.89	420.71	430.92	433.88	436.63	-
Mineral content						
Macro-elements (g/kg)						
Ca	3.27	11.00	14.60	3.19	1.82	7.72
K	7.24	8.32	6.48	7.25	8.27	10.91
Na	6.54	6.40	4.78	4.66	4.48	3.05
Mg	0.19	0.29	0.38	0.20	0.60	0.62
Micro-elements (mg/kg)						
Fe	355.70	336.07	316.34	321.05	356.90	1792.32
Zn	78.51	80.02	102.30	131.41	197.91	183.63
Mn	26.76	27.21	28.17	32.44	18.32	98.29
Cu	8.97	11.06	16.21	21.13	28.72	136.55
Pb	5.13	6.98	10.70	13.64	22.15	256.06
Co	4.33	3.64	4.88	4.82	0.33	2.56
Cd	0.64	0.73	1.10	1.33	1.63	1.95

1. Earthworm species Dendrodrilus subrubicundus
2. Method of calculation given in Table 2.2.2

Table 3.3.3: Amino acid composition of the four experimental diets, the freeze dried D.subrubicundus meal and the essential amino acid requirements of rainbow trout

Amino acid (g/100g meal)	Dietary treatment 1	2	3	4	Freeze dried D.subrubicundus
Aspartic acid	4.07	4.00	3.80	3.67	5.36
Alanine	2.56	2.70	2.17	2.11	2.86
Cystine	0.15	0.29	0.20	0.14	0.45
Cysteic acid	0.20	0.28	0.22	0.32	0.30
Glutamic acid	5.82	5.81	5.40	5.11	7.17
Glycine	2.52	2.56	2.27	1.81	2.48
Proline	1.75	1.81	1.70	1.49	2.04
Serine	1.83	1.92	1.89	1.83	2.66
Taurine	0.46	0.52	0.33	0.11	0.11
Arginine	2.21	2.29	2.52	2.40	3.50
Histidine	1.31	1.44	1.49	1.32	1.93
Isoleucine	1.76	1.81	1.72	1.58	2.21
Leucine	2.99	3.40	3.14	2.97	4.35
Lysine	4.32	4.48	3.98	3.44	4.95
Methionine	0.35	0.36	0.21	0.33	0.42
Phenylalanine	1.39	1.64	1.54	1.46	2.17
Threonine	2.11	2.01	1.90	1.91	2.77
Tyrosine	1.04	1.41	1.32	1.11	1.83
Valine	1.93	1.96	1.71	1.64	2.29
Essential amino acids (E.A.A.) (g amino acid/100g protein)					**E.A.A. requirement of rainbow trout[1]**
Arginine	4.72	4.98	5.33	5.31	3.5
Histidine	2.80	3.13	3.15	2.92	1.6
Isoleucine	3.76	3.94	3.64	3.50	2.4
Leucine	6.39	7.39	6.64	6.57	4.4
Lysine	9.23	9.74	8.41	7.61	5.3
Methionine	0.75 (0.32)[*2]	0.78 (0.63)	0.44 (0.42)	0.73 (0.31)	1.8 (0.9)
Phenylalanine	2.97	3.57	3.26	3.23	3.1
Threonine	4.51	4.37	4.02	4.23	3.4
Tyrosine	2.22	3.07	2.79	2.46	2.1
Valine	4.12	4.26	3.61	3.63	3.1

1. From Ogino (1980), expressed as g/100g of dietary protein

*2. (x) requirement for cystine

3.3.2.2 Animals and Tanks

One hundred and sixty rainbow trout of mean weight 17g, were obtained from Mr. D. Brien, Almondbank Fish Farm, Nr. Perth, Scotland. One hundred and forty fish were anaethetized and weighed individually (Section 2.1.2.2) and divided between four 60ℓ tanks (35 fish per tank), supplied with fresh water (2ℓ/tank/min) in a through flow system as illustrated in Figure 2.1.1. Each of the four tanks was allocated one of the experimental diets.

The twenty fish not included in an experimental tank were sacrificed at the start of the feeding trial. Ten fish were stored at -20°C for subsequent whole carcass proximate and mineral analysis. The remaining ten fish were dissected and the following tissues removed for mineral analysis: liver, kidney, bone (vertebrae), muscle (white dorsal muscle), eye, operculum, gill, intestine, skin and fins. The gastro-intestinal tract of fish used for mineral analysis throughout the experiment was washed free of undigested feed with distilled water.

The temperature of the water during the experimental period was 16°C ± 2°C and fish were subject to natural photoperiod.

At bi-weekly intervals during the 50 day experimental period all fish were weighed (Section 2.1.2.2) and a batch of six fish removed, ensuring that the mean weight for each tank remained approximately the same as before their removal. These fish were killed by a sharp blow on the head and stored at -20°C for subsequent whole carcass mineral analysis.

During the final week of the experiment, faecal samples were collected (Section 2.2.2.2) and dried at 105°C to constant

weight prior to determination of total nitrogen and chromic oxide content.

At the end of the 50 day experimental period all fish were killed by a sharp blow on the head. The fish maintained on each diet were divided up for analysis as follows: six fish were dissected and tissue samples (listed previously) removed for mineral analysis. The liver weight of each of these fish was recorded for calculation of LSI. From the remaining five fish in each group, blood samples were collected, from the severed caudal vein for haematocrit and erythrocyte fragility determination. These fish were then stored at -20°C for whole carcass proximate and mineral determination.

3.3.2.3 Chemical Methods

Proximate analysis of whole fish carcass and experimental diets was determined by the methods described in Section 2.1.2.3. Nitrogen free extractive and energy content of the diets was determined by calculation (Section 2.2.2.3). Digestibility coefficients were calculated after determination of the total nitrogen and chromic oxide content in diet and faecal samples (Section 2.2.2.3).

Moisture free samples of fish tissue and whole fish carcass were prepared for mineral analysis by the method described in Section 2.1.2.3 and the concentration of Ca, K, Na, Mg, Fe, Zn, Mn, Cu, Pb, Co and Cd measured using a Perkin Elmer 373 Atomic Absorption Spectrophotometer according to the manufacturer's instructions. Prior to the determination of Ca and Mg concentrations, Lanthanum chloride was added to give a final concentration of 0.75%.

Amino acid analysis of the experimental diets was conducted as described in Section 2.2.2.3.

Blood haematocrit was determined as described by Blaxhall and Daisley (1973) and erythrocyte fragility by the method of Cowey et al. (1981).

3.3.2.4 Statistical Method

Statistical analysis was carried out as described in Section 2.1.2.5.

3.3.3 RESULTS

Fish fed the control diet (diet 1) and diet 2 (in which 10% of the dietary herring meal protein was replaced by worm meal protein) consumed the pellets aggressively throughout the experiment. There was, however, a noticeable decrease in voracity of feeding by fish fed diets 3 and 4 (50 and 100% of the dietary herring meal protein replaced by worm meal protein, respectively). Since all fish were fed "to appetite", fish maintained on diets 3 and 4 consumed less food in total during the 50 day feeding period. This was surprising in view of the highly palatable nature of D.subrubicundus when fed frozen as a complete diet to rainbow trout (Experiment 2.2).

3.3.3.1 Growth Response and Feed Utilization Efficiency

The growth response and feed utilization efficiency of fish fed the four experimental diets is given in Table 3.3.4. The growth response of the fish is shown graphically in Figure 3.3.1.

A comparison of the growth performance of fish fed the four dietary treatments indicated that fish fed the lowest dietary inclusion of worm meal (diet 2) were comparable to fish fed the control ration, but as the dietary level of worm meal increased fish performance decreased proportionately. At the end of the

Table 3.3.4: Growth, feed utilization, liver somatic index, blood parameters and carcass composition of rainbow trout after a 50 day experimental period

Diet No:	1	2	3	4	± SE[1]
Mean initial weight (g)	17.51[a]	17.53[a]	17.36[a]	17.41[a]	0.611
Mean final weight (g)	36.04[bc]	38.43[c]	32.13[b]	27.60[a]	1.470
Weight gain (%)	105.83	119.22	85.08	58.53	
Specific growth rate (%/day)	1.44	1.57	1.23	0.92	
Food intake (mg/day/fish)	463	523	443	357	
Food conversion ratio (FCR)	1.31	1.28	1.54	1.73	
Protein efficiency ratio (PER)	1.76	1.82	1.55	1.43	
Apparent nitrogen utilization (ANU) (%)	30.08	32.24	28.23	24.99	
Apparent dry matter digestibility (%)	56.73	49.50	63.88	68.58	
Apparent nitrogen digestibility (%)	79.76	75.26	81.61	84.34	
Liver somatic index (g liver/100g body weight)	1.21[a]	1.14[a]	1.14[a]	1.57[b]	0.069
Blood parameters					
Blood haematocrit (%)	36.49[a]	41.04[a]	36.28[a]	36.25[a]	2.153
Erythrocyte fragility	63.68[a]	73.60[a]	72.87[a]	66.81[a]	4.654

Carcass composition (% wet weight)	Initial	After 50 days				
Moisture	74.28	70.67[a]	72.22[b]	71.71[ab]	72.59[b]	0.459
Crude protein	15.95	16.14[a]	16.64[b]	16.77[b]	16.61[b]	0.134
Lipid	7.25	10.30[b]	8.78[a]	8.74[a]	8.20[a]	0.217
Ash	2.45	2.18[a]	2.56[b]	2.56[b]	2.68[b]	0.051

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

abc Mean values for components with common superscripts are not significantly different ($p < 0.05$)

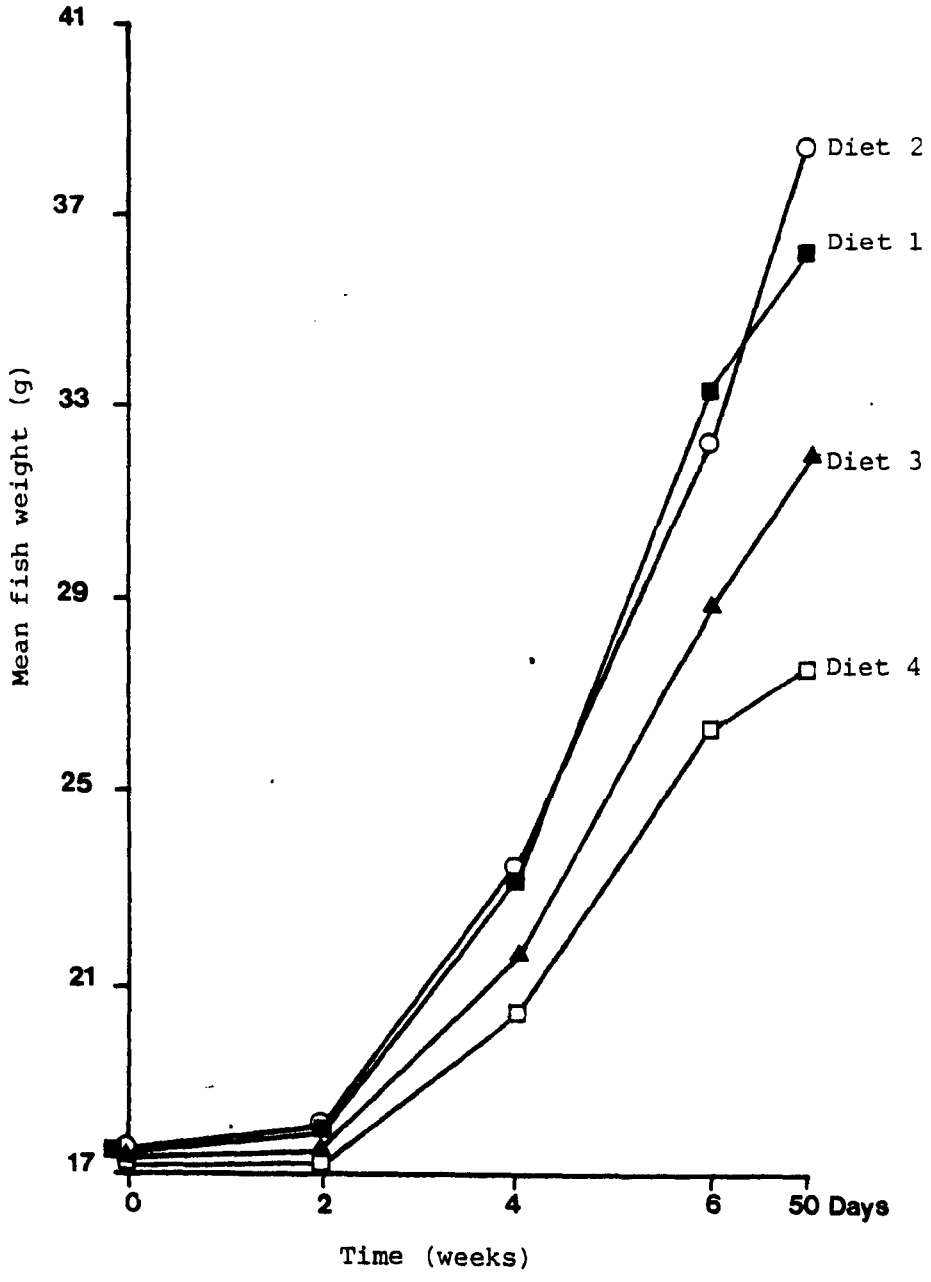


Figure 3.3.1: The growth response of rainbow trout fed four experimental diets

50 day feeding period the mean final body weight attained by fish fed diets 2 and 3 was not significantly different ($p < 0.05$) from fish fed the control diet. The growth response of fish fed the control diet, in terms of mean final body weight, percentage weight gain and specific growth rate lay between that of fish fed diet 2 and fish fed diet 3. Those fish maintained on diet 2 achieved the highest absolute increase in body weight gain and were significantly higher ($p < 0.05$) than fish fed diet 3. The mean final body weight of fish fed diet 4 was significantly lower ($p < 0.05$) than fish fed the other three diets. The variation in fish growth appeared to be a direct result of the different feed intakes of the fish.

Parameters measuring feed utilization efficiency (FCR, PER and Apparent NPU, Table 3.3.4, defined in Section 2.1.3) followed the same trend described above. It was surprising therefore that the dry matter and nitrogen digestibility coefficients displayed an opposite trend: those diets displaying the best results in terms of fish growth and feed utilization efficiency had the lower digestibility coefficients, whereas diets 3 and 4 showed higher digestibility coefficients as the proportion of worm meal in the diets increased.

3.3.3.2 Carcass Composition, Liver Somatic Index and Blood Parameters

The carcass composition of fish at the start of the experiment and after feeding the experimental diets for 50 days, the liver somatic index and blood parameters are given in Table 3.3.4.

The inclusion of freeze dried worm in the diet, at any level, significantly affected the gross carcass composition

of the fish. Moisture (with the exception of fish fed diet 3), crude protein and ash levels were all significantly higher ($p < 0.05$) and lipid significantly lower ($p < 0.05$) in the carcass of fish fed diets containing worm protein compared with fish fed the control diet. In addition, the LSI of fish fed diet 4 (containing the highest worm meal inclusion level) was significantly higher ($p < 0.05$) than fish fed the other dietary treatments (Table 3.2.4).

No significant difference ($p < 0.05$) was evident between fish fed the various dietary treatments in terms of blood haematocrit and erythrocyte fragility (Table 3.3.4).

3.3.3.3 Tissue Mineral Composition

Table 3.3.5 shows the mineral composition of the whole fish carcass of fish at the start of the experiment and at successive bi-weekly intervals during the experimental period. Levels of the macro-elements Ca, K, Na and Mg and the trace elements Mn and Co showed no significant alteration at any of the time intervals monitored. However, the concentration of Cu, Pb, Zn and Fe appeared to correspond with the dietary inclusion of worm meal. In particular the Pb concentration in the fish carcass increased both at successive bi-weekly intervals and with increasing levels of worm meal in the diet (Table 3.3.5).

Since higher levels of worm meal in the diet resulted in a lower feed intake over the experimental period, differences in total mineral intake between treatments were not as great as expected. For ease of comparison between diets for which fish exhibited different feed intakes the total mineral intake, retention and retention per g live fish weight gain during the experimental period have been calculated as described in Section

Table 3.3.5: Mineral concentration in whole fish carcass at bi-weekly intervals (mg or g/kg wet weight) (Values in parentheses indicate ± S.E.)

Element	Ca	K	Na	Mg	Zn	Fe	Mn	Pb	Cu	Co	Cd
Week 0	3.270	2.540	0.696	0.152	15.43	12.67	1.43	1.19	1.07	0.41	0.11
Week 2											
Diet 1	6.986^{a}	2.974^{a}	0.777^{a}	0.279^{a}	18.29^{a}	15.90^{a}	1.36^{a}	1.19^{a}	0.99^{a}	0.38^{a}	0.11^{a}
Diet 2	5.884^{a}	2.974^{a}	0.808^{a}	0.268^{a}	19.51^{a}	16.06^{a}	1.29^{a}	1.24^{a}	0.91^{a}	0.35^{a}	0.10^{a}
Diet 3	6.571^{a}	2.687^{a}	0.802^{a}	0.291^{a}	19.49^{a}	20.55^{a}	1.30^{a}	1.27^{a}	0.97^{a}	0.38^{a}	0.11^{a}
Diet 4	6.245^{a}	2.868^{a}	0.890^{a}	0.253^{a}	20.76^{a}	16.10^{a}	1.52^{a}	1.29^{a}	0.87^{a}	0.37^{a}	0.10^{a}
	(1.187)	(0.145)	(0.050)	(0.041)	(1.128)	(1.718)	(0.196)	(0.027)	(0.067)	(0.020)	(0.004)
Week 4											
Diet 1	4.360^{a}	2.516^{ab}	0.798^{a}	0.260^{a}	17.88^{a}	13.29^{a}	1.20^{a}	1.21^{a}	1.07^{c}	0.33^{a}	0.07^{b}
Diet 2	5.089^{a}	2.387^{a}	0.805^{a}	0.239^{a}	19.56^{a}	13.72^{a}	1.33^{a}	1.30^{a}	0.87^{a}	0.36^{a}	0.10^{c}
Diet 3	5.508^{a}	2.555^{b}	0.838^{a}	0.254^{a}	19.06^{a}	14.17^{a}	1.20^{a}	1.34^{a}	0.99^{bc}	0.41^{a}	0.10^{c}
Diet 4	4.150^{a}	2.406^{a}	0.853^{a}	0.243^{a}	19.04^{a}	13.38^{a}	1.06^{a}	1.21^{a}	0.82^{a}	0.30^{a}	0.04^{a}
	(1.042)	(0.044)	(0.094)	(0.047)	(2.243)	(0.679)	(0.174)	(0.121)	(0.036)	(0.032)	(0.005)
Week 6											
Diet 1	5.618^{a}	2.478^{a}	0.724^{a}	0.257^{a}	17.04^{a}	10.36^{a}	1.10^{a}	1.35^{a}	0.63^{a}	0.40^{a}	0.10^{a}
Diet 2	4.155^{a}	2.158^{a}	0.704^{a}	0.203^{a}	18.86^{a}	12.67^{ab}	1.33^{a}	1.28^{a}	0.93^{ab}	0.41^{a}	0.15^{b}
Diet 3	4.622^{a}	2.478^{a}	0.788^{a}	0.206^{a}	17.29^{a}	14.34^{b}	0.99^{a}	1.40^{a}	1.00^{b}	0.36^{a}	0.14^{b}
Diet 4	4.132^{a}	2.207^{a}	0.762^{a}	0.220^{a}	19.28^{a}	12.70^{ab}	1.21^{a}	1.48^{a}	1.37^{c}	0.40^{a}	0.13^{b}
	(0.638)	(0.096)	(0.031)	(0.018)	(1.286)	(0.737)	(0.123)	(0.063)	(0.096)	(0.031)	(0.007)
50 days											
Diet 1	3.903^{a}	2.307^{a}	0.680^{a}	0.221^{a}	17.91^{a}	6.92^{a}	1.21^{a}	1.37^{a}	0.92^{a}	0.35^{ab}	0.12^{a}
Diet 2	4.109^{a}	2.693^{a}	0.657^{a}	0.271^{a}	16.74^{a}	9.11^{a}	1.19^{a}	1.27^{a}	0.79^{a}	0.38^{b}	0.11^{a}
Diet 3	3.995^{a}	2.124^{a}	0.702^{a}	0.265^{a}	17.57^{a}	9.97^{b}	1.08^{a}	1.30^{b}	0.92^{a}	0.28^{a}	0.13^{a}
Diet 4	4.709^{a}	2.605^{a}	0.741^{a}	0.266^{a}	22.12^{b}	11.81^{b}	1.33^{a}	1.58^{b}	1.14^{b}	0.34^{ab}	0.12^{a}
	(0.258)	(0.183)	(0.030)	(0.023)	(1.286)	(0.585)	(0.091)	(0.047)	(0.068)	(0.021)	(0.009)

2.1.3 and are given in Table 3.3.6. Although levels of Mn and Co were higher in diets containing worm meal (Table 3.3.2) in terms of total mineral intake over the 50 day feeding period very little difference was evident between treatments (Table 3.3.6).

The absolute quantity of Fe ingested by the fish decreased with increasing levels of worm meal in the diet (Tables 3.3.2 and 3.3.6) but, the amount of Fe retained per g of live fish weight gained, increased as levels of worm meal in the diet also increased.

The concentrations of seven micro-elements measured in the individual tissues selected are shown in Table 3.3.7. Pb was found at higher levels in bone and bony tissue (fins and operculum) than in the other tissues measured, and the concentration increased with increasing inclusion of worm meal in the diet.

There was no positive accumulation of Cu in the liver of fish as increasing amounts of Cu were ingested in the diet; however, in the majority of other tissues measured, the concentration of Cu was higher after 50 days in fish which had been maintained on the diet comprising 100% dietary worm meal.

Levels of Zn in certain tissues (muscle, operculum, gills, fins and skin) tended to increase with increased herring meal in the diet. In other tissues (kidney, eye, bone and intestine) the increase in Zn concentration corresponded with an increasing dietary inclusion of worm meal.

Fe followed a similar pattern to Zn, except in the liver, gills and fins, where higher levels of worm meal in the diet resulted in increased Fe concentrations in these tissues.

Table 3.3.6: Total dietary intake, retention and retention per gm fish wet weight gained of mineral elements over the 50 day experimental period

Element		Diet No:	1	2	3	4
Ca	Intake (mg)		75.77	287.65	322.83	56.91
	Retention (mg)		83.40	100.59	71.59	73.04
	Ret/gm*		4.50	4.81	4.85	7.17
K	Intake (mg)		167.75	217.57	143.73	129.34
	Retention (mg)		38.66	58.96	24.15	27.68
	Ret/gm		2.09	2.82	1.64	2.72
Na	Intake (mg)		151.53	167.36	106.02	83.13
	Retention (mg)		12.32	13.17	10.48	8.33
	Ret/gm		0.66	0.63	0.71	0.82
Mg	Intake (mg)		4.40	7.58	8.43	3.57
	Retention (mg)		5.30	7.75	5.87	4.69
	Ret/gm		0.29	0.37	0.40	0.46
Fe	Intake (µg)		8241.57	8788.23	7016.42	5727.53
	Retention (µg)		27.55	127.99	100.39	105.37
	Ret/gm		1.49	6.12	6.80	10.34
Zn	Intake (µg)		1819.08	2092.52	2269.01	2344.35
	Retention (µg)		375.30	372.83	296.66	341.87
	Ret/gm		20.25	17.84	20.09	33.55
Mn	Intake (µg)		620.03	711.54	624.81	578.73
	Retention (µg)		18.57	20.66	9.88	11.81
	Ret/gm		1.00	0.99	0.67	1.16
Cu	Intake (µg)		207.83	289.22	359.54	376.96
	Retention (µg)		14.42	11.60	10.99	12.84
	Ret/gm		0.78	0.56	0.74	1.26
Pb	Intake (µg)		118.86	182.53	237.33	243.43
	Retention (µg)		28.54	27.95	21.11	22.89
	Ret/gm		1.54	1.34	1.43	2.25
Co	Intake (µg)		100.33	95.19	108.24	85.99
	Retention (µg)		5.44	7.41	1.88	2.24
	Ret/gm		0.29	0.36	0.13	0.22
Cd	Intake (µg)		14.83	19.09	24.40	23.73
	Retention (µg)		2.39	2.30	2.27	1.39
	Ret/gm		0.13	0.11	0.15	0.14

* Ret/gm = $\frac{\text{Absolute amount of the element retained per fish over 50 days}}{\text{Wet weight increase of fish during 50 days}}$

Table 3.3.7 Concentrations of micro-elements in selected fish tissues at the start and end of the 50 day feeding period(values are expressed as mg/kg on a dry weight basis)

	Liver	Kidney	Muscle	Eye	Operc.	Gill	Fins	Bone	Intes.	Skin
Zn Initial	22.74	n.d.*	1.49	28.83	298.04	n.d.	83.73	140.33	76.93	n.d.
Diet 1	168.13	113.24	75.77	302.93	344.50	365.64	281.49	85.49	169.57	169.77
Diet 2	174.11	227.33	40.71	514.45	171.54	164.94	202.87	126.27	120.17	141.30
Diet 3	151.84	160.96	23.90	366.75	225.99	251.05	147.02	101.58	263.80	102.45
Diet 4	151.56	307.89	27.47	324.50	153.94	119.68	135.72	159.42	356.67	131.38
Fe Initial	61.36	125.36	51.15	29.78	37.74	56.43	11.27	272.86	14.53	2.97
Diet 1	185.68	231.99	19.56	25.58	113.83	109.77	9.58	7.91	30.69	13.80
Diet 2	399.66	343.03	16.34	31.61	17.21	168.04	10.57	5.86	33.75	54.36
Diet 3	703.00	239.31	16.58	24.02	8.01	116.95	10.38	9.56	25.35	83.64
Diet 4	421.18	326.43	13.88	31.28	12.13	113.52	18.74	10.45	249.99	11.48
Mn Initial	4.84	45.98	0.95	9.49	2.24	1.43	14.37	15.58	5.56	n.d.
Diet 1	3.59	4.82	1.89	0.74	20.31	13.50	22.50	20.28	2.52	5.55
Diet 2	4.13	4.79	1.40	1.84	17.96	12.52	20.60	16.78	11.39	1.96
Diet 3	3.03	0.17	1.64	n.d.	11.92	6.50	12.37	13.69	7.34	1.03
Diet 4	3.85	4.60	1.30	n.d.	28.58	8.34	14.96	13.25	3.83	2.87
Pb Initial	2.56	3.19	1.39	1.21	14.37	3.86	12.23	9.48	n.d.	4.45
Diet 1	n.d.	n.d.	1.41	n.d.	21.05	2.36	11.45	17.91	6.57	6.58
Diet 2	8.64	17.95	2.67	9.65	22.66	9.46	20.36	21.20	11.39	1.31
Diet 3	n.d.	11.99	2.84	3.41	36.79	7.06	17.89	21.62	10.38	0.41
Diet 4	2.04	n.d.	2.31	n.d.	33.78	7.25	22.07	22.17	4.80	4.91
Cu Initial	4.61	2.92	24.18	1.21	0.62	1.20	2.40	2.65	1.07	n.d.
Diet 1	89.83	6.44	1.96	3.34	3.46	2.69	4.27	3.31	7.71	2.41
Diet 2	102.77	2.92	2.25	1.08	0.79	4.41	3.06	4.78	6.06	3.72
Diet 3	94.13	4.03	1.94	1.85	1.44	6.95	1.92	2.94	4.38	3.95
Diet 4	92.00	11.74	11.72	2.32	6.10	5.20	6.68	6.39	12.44	39.76
Co Initial	0.29	2.13	n.d.	1.78	n.d.	n.d.	0.60	0.76	n.d.	n.d.
Diet 1	n.d.	n.d.	0.08	n.d.	6.52	2.32	5.36	4.63	1.70	1.59
Diet 2	n.d.	n.d.	0.14	n.d.	1.56	1.32	2.45	3.24	1.44	0.66
Diet 3	1.69	8.52	0.30	n.d.	3.91	3.09	7.80	3.10	3.83	0.13
Diet 4	n.d.	9.21	0.87	2.32	11.30	3.03	7.91	6.63	2.09	1.23
Cd Initial	1.03	5.85	0.08	1.31	n.d.	n.d.	0.60	0.75	n.d.	n.d.
Diet 1	2.40	4.82	0.25	2.04	3.83	1.55	2.03	1.85	1.31	0.81
Diet 2	n.d.	6.50	0.14	3.75	4.67	1.32	2.45	3.24	1.44	0.66
Diet 3	3.38	8.05	0.45	3.56	6.48	2.61	3.13	2.94	1.56	0.35
Diet 4	1.13	4.60	0.29	1.16	4.10	1.69	2.19	2.13	1.60	0.82

n.d.* indicates that the concentration of the element in solution is below the detection limits of the machine.

For the remaining elements measured; Cd, Co and Mn, no trend was evident to suggest that increasing levels of worm meal in the diet resulted in higher concentrations of these trace elements in the individual tissues.

As may be expected, the results indicate that certain elements have a preference for particular tissues and are found there in higher concentrations.

3.3.4 DISCUSSION

At a level of 10% of the protein source D.subrubicundus worm meal adequately replaced the herring meal protein in the trout ration tested, incurring no loss in fish growth performance and feed utilization efficiency. As discussed in Section 3.1.4 previous research using low levels of earth worm meal (derived from E.foetida) in feeds for various livestock has proven successful. The fish Oreochromis niloticus fed diets containing 15% earthworm meal (derived from P.excavatus) achieved significantly higher ($p < 0.01$) final body weights than fish fed a control diet or diets containing 10 and 25% worm meal (Guerro, 1981). Hilton (1983) reported a poor feeding response by rainbow trout fed increasing levels of dried E.eugeniae meal in the diet, however, the mean final body weight of fish fed a diet containing 23% worm meal was not significantly different ($p < 0.05$) from fish fed the control diet.

Higher levels of inclusion of D.subrubicundus worm meal in trout diets (diets 3 and 4) resulted in reduced feed intake and feed utilization efficiency, and a consequent reduction in the growth of fish maintained on these diets during the experimental period. This was similar to the results reported

in Experiment 3.1 although the growth achieved by fish fed 100% D.subrubicundus meal as the protein source appeared better than that of fish fed 100% E.foetida meal protein in the diet.

Despite the evidence given in experiment 2.2 that frozen D.subrubicundus fed as a complete diet was highly acceptable to rainbow trout, freeze dried D.subrubicundus meal included within a pellet at high levels resulted in reduced feed intake by the fish. There are several possibilities which may explain this decline in fish performance:

(i) Unpalatable or taste repellent compounds may have been present in the freeze-dried worm, which could have reduced feed intake by the fish to below the level required for maximum fish growth and feed utilization efficiency. The high herring meal component of diet 2 may have effectively masked the presence of such compounds. Similarly, the high percentage of moisture in the frozen earthworm may have reduced the concentration of taste repellent compounds to levels which had little effect on the feed intake of the fish.

(ii) The high dietary inclusion of worm meal may have resulted in a nutritional deficiency or imbalance of the fatty acid or amino acid composition of the diets. The possibility of fatty acid imbalance in these dietary formulations has been discussed in Section 3.1.4. When D.subrubicundus was fed frozen as a complete diet for rainbow trout, the worms supplied 67.7% crude protein on a dry weight basis (Table 2.2.2). However when dried D.subrubicundus meal served as 100% of the protein source in a pelleted diet, the level of crude protein, on a dry weight basis was 45.20%. At this lower level in the diet one or more

essential amino acids may have become limiting. An amino acid deficiency within a protein may be compensated for by providing a higher quantity of the deficient protein in the diet (Gropp et al, 1979). The amino acid composition of the diets has been compared with the essential amino acid requirement of rainbow trout (Ogino, 1980) in Table 3.3.3. The four experimental diets apparently supplied essential amino acids at levels above the requirement of rainbow trout with the exception of methionine and cystine which may have been limiting in all four diets. It should be pointed out however that the low methionine concentrations present may have been due to oxidative losses during hydrolysis.

(iii) Heavy metals, pathogens, parasites, detergents and pesticide residues are commonly present in sewage substrates (Carrington, 1978; Edwards and Densem, 1980) and may have been transmitted through the worms into the fish diets causing impaired fish performance.

D.subrubicundus meal also significantly affected the gross carcass composition of the fish. The reduced carcass lipid content of fish fed diets 2, 3 and 4 may have been due to the lower lipid content of the experimental diets on which these fish were maintained, as compared with the control diet (Table 3.3.2). Other possible factors which may explain the reduced carcass lipid content of fish fed diets containing worm meal have been discussed in Section 2.2.4.

Despite the relatively short duration of this feeding trial and the nature of the sewage substrate on which the D.subrubicundus were grown (domestic origin), the carcass and

tissues of fish fed diets containing worm meal showed some evidence of uptake and accumulation of Fe, Zn, Cu, Pb and Cd (Tables 3.3.5, 3.3.6 and 3.3.7). The higher Pb concentration in fish carcass and bone tissue (Tables 3.3.5 and 3.3.7) may be attributed to the absorption of the increasing amounts of Pb ingested as the worm meal component of the diet increased. The cumulative and bone seeking nature of Pb has been well documented (Six and Goyer, 1970; Hsu et al., 1975; Underwood, 1977).

Rainbow trout suffered no deleterious effect when maintained on diets containing 178 mg/Kg Cu (as $CuSO_4.5H_2O$) and 638 mg/Kg Zn (as $ZnSO_4.7H_2O$) for 20 weeks, although elevated levels of Cu and Zn were recorded in the livers of these fish (Knox et al., 1982). In the present study, dietary concentrations of both Cu and Zn were well below these levels, however after 50 days Cu and Zn concentrations were significantly higher ($p < 0.05$) in the carcass of fish fed the highest dietary inclusion of worm meal, although no significant accumulation of these elements occurred in the livers of these fish (diet 4, Tables 3.3.5 and 3.3.7). The retention of Cu and Zn per g live fish weight gained was also greater in fish fed this diet than fish fed the control diet (Table 3.3.6).

The concentration of all micro-elements measured in the experimental diets was not greatly in excess of those reported by Tacon and de Silva (1983) for commercial fish feeds available in Europe. Levels of trace elements Fe, Zn, Mn, Cu and Co fall below the maximum permitted concentration in trout feedstuffs (Edwards and Densem, 1980). However, the chemical form of the element and developmental status of the fish determines its biological availability to the animal and thus uptake may be

independent of the absolute amount of the element in the diet (Fritz, 1973). For example, the chemical form of Fe in worm meal may have been biologically more available for uptake than that present in herring meal as indicated by the increase in uptake and retention of Fe into the carcass of fish fed diets 2, 3 and 4 compared with fish fed the control diet, even though absolute amounts of Fe ingested decreased in these diets (Tables 3.3.2, 3.3.5 and 3.3.7). However, as the experiment proceeded the tissue concentration of Fe in the whole fish carcass declined until, at the termination of the experiment, Fe concentrations were below those recorded in the fish carcass at the start of the experiment.

An antagonistic reaction may have arisen between Fe and other mineral elements in the diet. For example high dietary intakes of Zn, Mn, Cu, Co and Cd have been reported to interfere with Fe uptake and metabolism in other animals (Underwood, 1977). Ogino and Yang (1978) have reported that increasing the Zn concentration in diets for rainbow trout resulted in a decrease in both Cu and Fe concentration in the whole carcass and tissues (eye, vertebrae, liver and intestine) of the fish.

Antagonisms and interactions between mineral elements would also have varied from diet to diet as levels of the individual elements varied. These factors would have masked the clear pattern of uptake for any one element as the concentration of that particular element increased in the diet. In particular, the level of Ca was greater in diets 2 and 3 than diets 1 and 4. Considering the protective role that Ca plays in the uptake and metabolism of the majority of the elements studied (Underwood, 1977) the level of elements in the fish carcass and tissue may

not be strictly comparable between treatments. This may serve to explain why the retention of Zn, Cu, Pb and Cd per g fish weight gained was low in fish fed diet 1 and higher in fish fed diet 4, but values for fish fed diets 2 and 3 did not lie in the intermediate range (Table 3.3.7). The higher concentration of Ca in diets 2 and 3 may reduce the uptake and retention of these elements into the fish carcass.

Of the elements which displayed some evidence of accumulation in fish carcass and tissue, all except Fe (Ash and Lee, 1980), have been shown in previous studies to accumulate in earthworm tissue to levels above those of the surrounding media (Czarnowska and Jopkiewicz, 1978; Hartenstein et al., 1980b). The results of experiment 2.2 also gave some evidence for the accumulation of Zn and Pb in the carcass of fish fed frozen D.subrubicundus.

Concentrations of the mineral elements in both freeze dried earthworm and the sewage substrate are given in Table 3.3.2. Although mineral concentrations were higher in the sewage substrate, this term denotes the whole medium from which the worms were collected, including the bacterial slime, vegetation and sewage effluent, and not necessarily the fraction on which the D.subrubicundus were feeding. This may explain why worm tissue concentrations of certain elements were lower than in the surrounding media which was in contrast to previously reported results.

The D.subrubicundus used in this study were frozen directly after collection from the sewage substrate and neither washed nor stored on an inert medium to void their gut lumen.

Purging the worms in this way has been shown to reduce the concentrations of heavy metals in the earthworm tissue (Ash and Lee, 1980; Hartenstein et al., 1980b). Ireland and Richards (1981) have demonstrated that a percentage of the metal accumulated by earthworms is not internalised but adsorped onto the mucus coat covering the body surface. Heavy metals may also be bound to the chloragocytes in the coelom of the earthworm (Ireland, 1978). Any post harvest treatment, either through purging, washing or blanching which would result in the removal or diminution of the potentially noxious heavy metal burden within earthworms used in livestock diets would also serve to lessen the accumulative toxicity of these elements when fed to fish or other livestock.

In conclusion, dried D.subrubicundus meal adequately replaced herring meal in the diet of rainbow trout, providing 10% of the protein source without loss in fish growth and feed utilization efficiency. At levels of 50 and 100% protein replacement by worm meal protein, fish growth and feed utilization efficiency were decreased. At all levels of dietary inclusion of dried D.subrubicundus meal the carcass composition of the fish was significantly altered compared with fish fed the control diet. Furthermore there was evidence to show that the concentrations of Fe, Zn, Cu and Pb increased in the whole carcass and tissues of fish fed high levels of worm meal in the diet.

CHAPTER 4

AN INVESTIGATION INTO THE EFFECT OF SIMPLE PRE-TREATMENT METHODS DESIGNED TO IMPROVE THE ACCEPTABILITY OF EISENIA FOETIDA TO RAINBOW TROUT

4.1 INTRODUCTION

At present Eisenia foetida is the earthworm species most commonly employed to process organic wastes in commercial vermiculture enterprises operating in North America, Italy, Britain and the Philippines and 'earthworm meal' derived from this species is most likely to become available for use in livestock diets. Dried E.foetida meal may be satisfactorily used to provide low levels of dietary protein in livestock feeds including fish feeds (Sabine, 1978; Section 3.2.4). However, at high levels of dietary inclusion and when fed frozen either as the sole feed or as a supplementary feed, E.foetida did not support fish growth (Aston et al., 1982; Sections 2.1.4 and 3.1.4). Mortalities arising during fish feeding trials could not be directly associated with the dietary inclusion of E.foetida and histopathological examination revealed no evidence of toxic effects of the diets to the fish. Apparent digestibility coefficients of the nutritional components within the E.foetida were high (Tables 3.1.2 and 3.2.2) and it was therefore assumed that E.foetida was simply unpalatable to trout. The experiments described in this chapter aimed to achieve a simple processing technique which effectively eliminated such unpalatable factors as were present in E.foetida and so improve the acceptability of this species of earthworm to rainbow trout.

A short term screening test was devised which provided a tentative assessment of the value of any treatment process applied to E.foetida (for example: mechanical, chemical or heat treatment) more rapidly than could be achieved by conducting a feeding trial. Those processing techniques which apparently improved the acceptability of E.foetida to rainbow trout were then evaluated in

a longer term feeding trial.

Several possible explanations regarding the unpalatable nature of E.foetida to rainbow trout were briefly discussed in Section 2.1.4. L.terrestris and A.longa, also fed to rainbow trout in Experiment 2.1 were highly palatable to the fish and fish fed these earthworms achieved growth rates comparable to fish fed the commercial trout pellet (Section 2.1.3). Reasons for the unpalatable nature of E.foetida were sought on the basis of obvious differences between the unpalatable species: E.foetida and the palatable species: L.terrestris and A.longa.

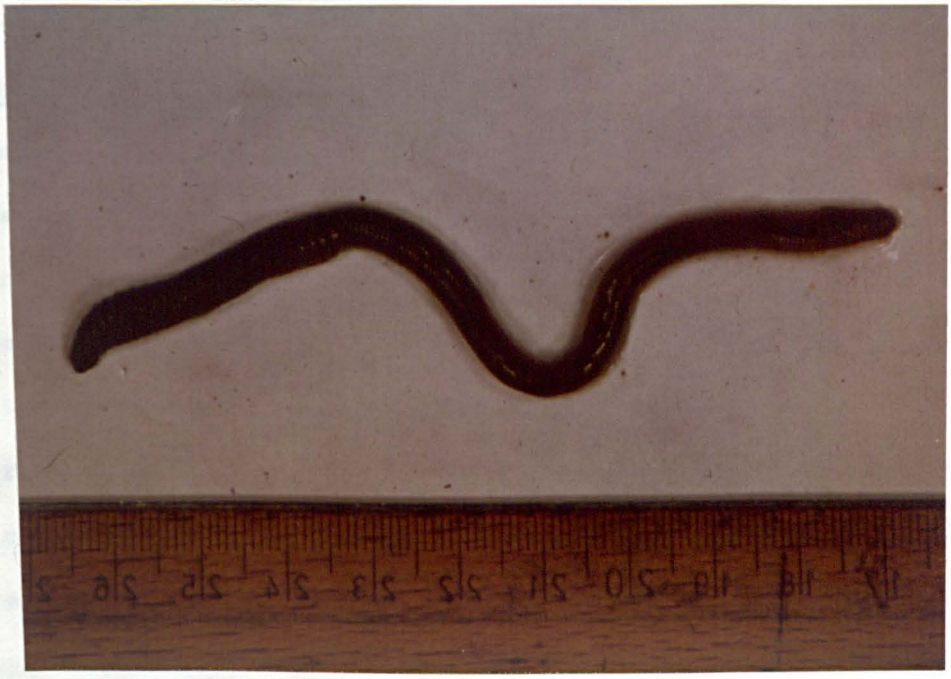
The substrate from which these earthworms were collected differed considerably: L.terrestris and A.longa were collected from rough pasture and E.foetida collected from a sewage treatment works. Contaminants within the sewage sludge such as pesticides, antibiotics, heavy metals and detergents may be present in the gut and tissues of earthworms collected from this substrate. However, D.subrubicundus, also collected from a sewage substrate, proved highly palatable to rainbow trout (Section 2.2.3). Maintaining the earthworm E.foetida on an inert substrate for a short period in order to 'scour' or 'cleanse' the worms apparently improved their quality as fish bait (Barrett, 1949; Gaddie and Douglas, 1975). Whether this process was intended to clear the earthworm gut lumen or surface mucoid coat of unfavourable substances or to prevent the production of secondary metabolites by effectively starving the earthworms was not specified. However, similar procedures in which livestock are fed high quality diets and fish maintained in ponds of high water quality for short periods have been employed to eliminate offensive tainting of the flesh prior to slaughter. In this context the effect of holding

E.foetida on various substrates for ten days before feeding the worms to rainbow trout was investigated.

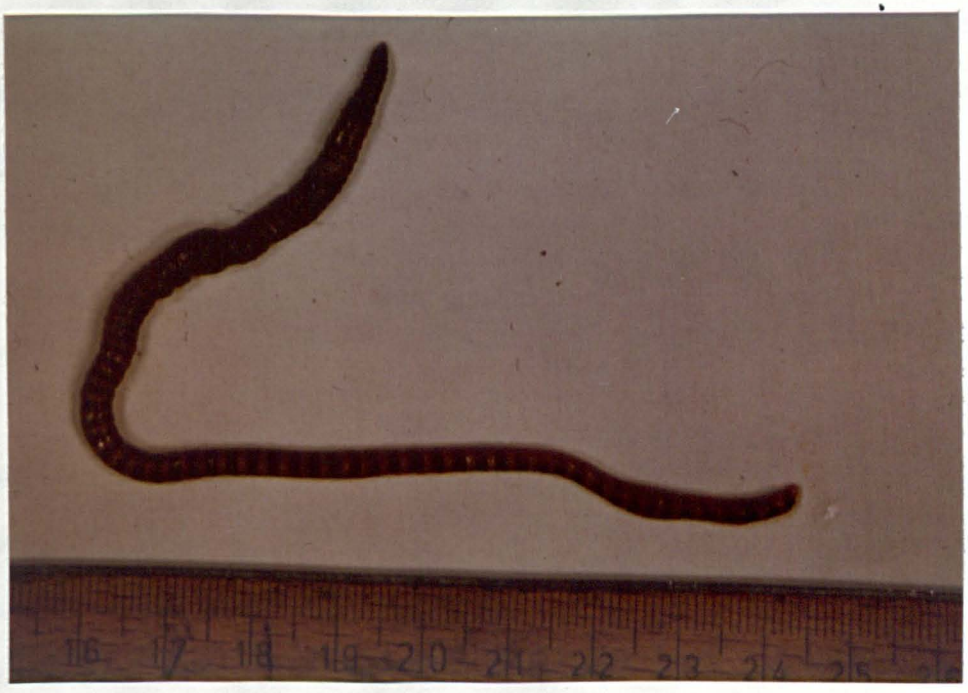
The striped or banded appearance of E.foetida readily distinguishes this species from the uniformly pigmented A.longa and L.terrestris (Plate 4.1). However two possible sub-species of E.foetida have been described (André, 1963; Bouché, 1972); a uniformly pigmented form given the sub-specific name Eisenia fetida andrei by Bouché (1972) and a striped form: Eisenia fetida fetida (Bouché, 1972). The possible reclassification of the species E.foetida into two sub-species is currently under debate and for the purposes of the major part of this thesis both forms have been referred to as E.foetida. It was considered pertinent to establish the relative palatability of the two forms of E.foetida to rainbow trout.

Generally, earthworm coelomic fluid has a milky white colour but in certain species (D.subrubicunda, D.veneta and E.foetida) the coelomic fluid is coloured yellow due to the presence of fatty droplets associated with the eleocytes (Edwards and Lofty, 1977). In addition the coelomic fluid of E.foetida has a noxious garlic odour and hence this species derives its name. Coelomic fluid exuded through the earthworms dorsal pores affords protection against dessication and harmful micro-organisms often present in the substrate (Vallembois et al., 1982). The noxious odour of the coelomic fluid of E.foetida may additionally serve as a defense mechanism against predators (Edwards and Lofty, 1977) and in this context it is likely to be unpalatable.

A colourless or yellowish fluid with a noxious fetid odour has also been reported in the hypobronchial gland of muricid



L.terrestris



E.foetida

Plate 4.1: L.terrestris, uniformly pigmented and E.foetida having a striped or banded appearance

gastropods (Halstead, 1963). The volatile compounds responsible for this odour were investigated in the species Reisha (Thais) clavigera and R(T)bronni (Shiomi et al., 1982a). The sulphur (S) compounds methyl mercaptan and dimethyldisulphide, detected in high quantities, were judged responsible for the fetid odour. These volatile S compounds were also considered responsible for the offensive odour of the flat-head fish Calliurichthys doryssus (Shiomi et al., 1982b). The presence of certain S compounds is considered essential to impart the flavour associated with some foods such as onion and cabbage (Bailey et al., 1961), however, excessive amounts of these compounds is also generally associated with food spoilage (Persson and von Sydow, 1973). The presence of potentially noxious volatile S compounds in the earthworm E.foetida was therefore investigated in a preliminary manner and compared with the earthworm L.terrestris. The effect of various treatment processes on the qualitative nature of the volatile S compounds in E.foetida was also assessed.

Also present within the coelomic fluid of earthworms are the chloragogen cells broken away from the chloragogenous tissue surrounding the gut epithelium and involved in nutrient storage (Roots, 1960) and nitrogen metabolism (Cohen and Lewis, 1949,1950; Needham, 1957). The chloragogen cells have also been implicated in iron metabolism, in the synthesis of the earthworm respiratory pigment (Linder, 1955) and in the elimination of potentially toxic elements, for example Pb (Ireland, 1978). In all these functions the chloragogenous tissue resembles the vertebrate liver. Furthermore, Roch et al. (1981) and Vallembois et al (1982) have suggested that the chloragogen cells of

E.fetida andrei and E.f.fetida also produce a secretion with bacteriostatic activity which acts as an anti-infection defense. This secretion also exhibited haemolytic activity and caused haemolysis of various vertebrate erythrocytes (Roch et al., 1981). Haemolysins within earthworm extracts have also been reported by Andrews and Kukulinsky (1975) in E.foetida and by Cooper et al. (1974) in L.terrestris. Earthworms have also been demonstrated to possess natural agguitinins within the coelomic fluid and some of these agglutinins can be induced against different types of vertebrate erythrocytes (Cooper et al., 1974; Wojdani et al., 1982).

It is evident from the above discussion that several factors within the coelomic fluid of E.foetida may have been responsible for the poor feeding response of rainbow trout. Experiments were therefore conducted in which the coelomic fluid of E.foetida was extracted and added to an otherwise highly palatable trout ration and the feeding response of the fish monitored.

In order to ascertain in which fraction the unpalatable component of E.foetida was present, dried E.foetida was separated into a lipid and lipid soluble fraction and a lipid-free fraction. Each fraction was then added to two separate palatable semi-synthetic dietary formulations which were then fed to rainbow trout.

Finally E.foetida was subjected to a combination of treatment processes each of which had proved partially successful and the combined effect of these processes (achieved in a single step by 'blanching' the earthworms) was evaluated by using treated

earthworms as a diet for rainbow trout in a six week feeding trial.

4.2 PRELIMINARY INVESTIGATIONS

4.2.1 Short term Screening Test

4.2.1.1 Experimental Procedure: Rainbow trout (25-30g in weight) were randomly divided between five 60ℓ tanks at a density of three fish per tank. The fish holding tanks, similar to that shown in Figure 2.1, were supplied with fresh water at a rate of 2ℓ/min/tank in a throughflow system.

Earthworms of the species L.terrestris and E.foetida were collected locally and D.veneta were supplied by British Groundbaits. At the start of each feeding test fish were offered pieces (1 cm lengths) of fresh L.terrestris in order to demonstrate a positive feeding response before the fish feeding response to pieces of untreated/treated frozen E.foetida was observed and recorded. Rainbow trout rarely ignored L.terrestris when offered but under these circumstances the feeding test was discontinued. The procedure adopted for feeding rainbow trout with pieces of earthworm is shown in Figure 4.1. Fish were fed thrice daily alternately following the procedure depicted by the continuous or dotted line in Figure 4.1. The different feeding responses exhibited by the fish to the pieces of earthworm fed were ranked on an intensity scale with a score of 0-3 as follows:-

Score	Nature of the feeding response
0	Food item totally ignored
1	Food item initially held in the mouth but then rejected
2	Food item ingested but after some initial hesitation (either initially held in the mouth prior to ingestion or ingested without having been actively attacked)
3	Food item aggressively attacked and ingested.

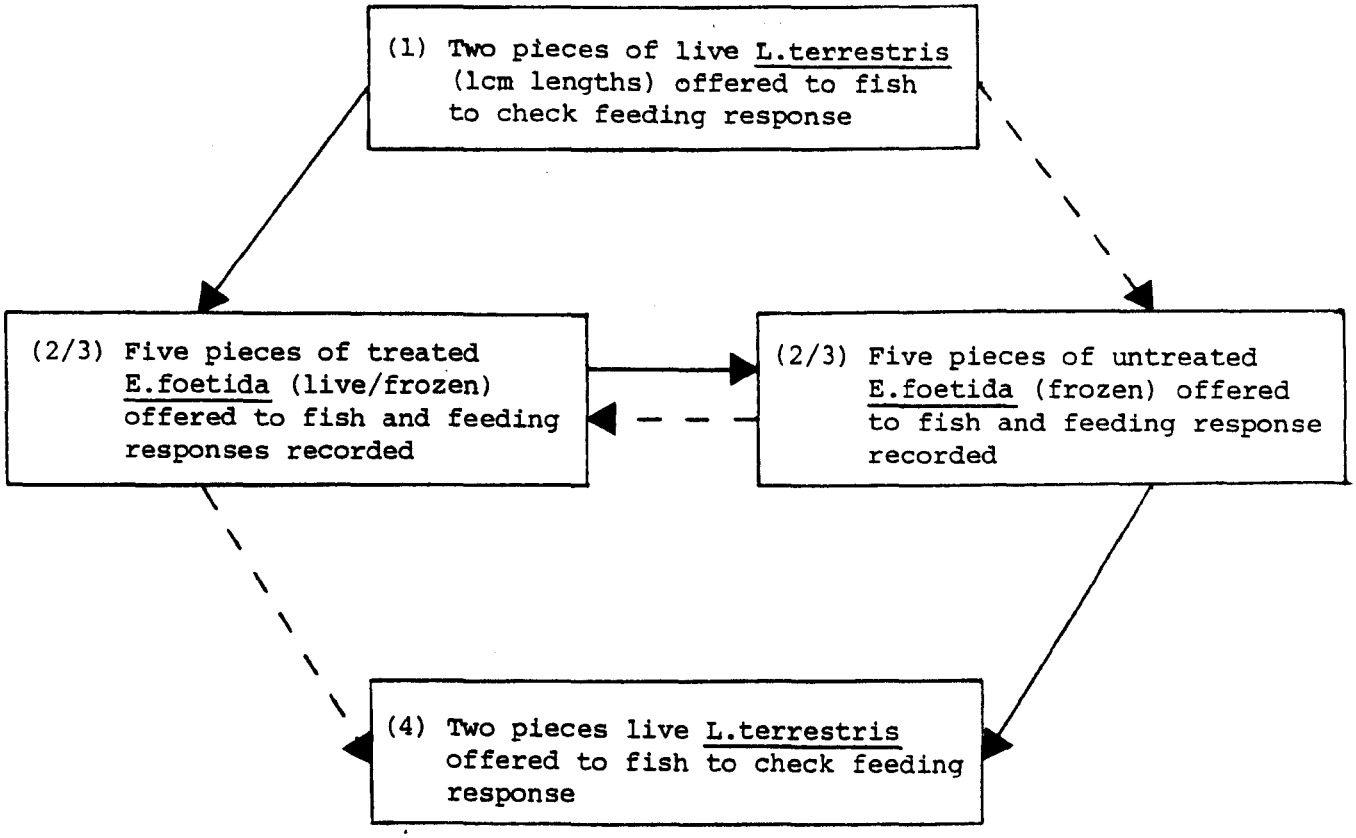


Figure 4.1 Diagrammatic representation of the short-term feeding test.

Feeding tests were conducted over a period of four days and the total score, indicative of the intensity of the fish feeding response to each particular feed type, expressed as a percentage of the maximum possible score. In this way the relative acceptability to rainbow trout of different species of earthworm and of untreated and treated E.foetida could be compared. Frozen D.veneta was compared with frozen E.foetida; live E.foetida was compared with frozen E.foetida; salt-treated E.foetida (live earthworms dipped in 5% NaCl solution until immobile, rinsed in distilled water, blotted dry and frozen) was compared with frozen E.foetida (untreated) and blanched E.foetida (live earthworms immersed in boiling water for 5 minutes, blotted dry and frozen) was compared with frozen E.foetida (untreated).

4.2.1.2 Results: The results of comparisons made using this short term screening test are shown in Table 4.1.

Table 4.1 The relative acceptability of earthworms of different species and of untreated and treated E.foetida to rainbow trout.

Earthworm species or pre-treatment process applied to E.foetida	Score*1 (%)
Frozen D.veneta	89
Frozen E.foetida (untreated)	73
Live E.foetida	27
Frozen untreated E.foetida	39
Salt-treated frozen E.foetida	52
Frozen untreated E.foetida	45
Blanched frozen E.foetida	72
Frozen untreated E.foetida	59

*1 Total score expressed as a percentage of the maximum possible score

Initially a comparison was made between the species E.foetida and D.veneta and the results suggested that frozen D.veneta was more acceptable to rainbow trout than frozen E.foetida. A comparison of the feed intake and growth response of fish fed frozen E.foetida in Experiment 2.1 and frozen D.veneta in Experiment 2.2 appear to confirm the results of the short term test. Live E.foetida was less acceptable to the fish than frozen E.foetida and salt-treated worms only marginally more acceptable than untreated worms when offered to the fish (Table 4.1). A marked increase in the acceptability of blanched frozen E.foetida compared with untreated frozen E.foetida appears to have been achieved and earthworms subjected to this treatment process were therefore further evaluated in a longer term feeding trial.

4.2.2 Volatile sulphur compounds present in earthworms

4.2.2.1 Experimental Procedure: Qualitative analysis of the S-volatiles present in earthworms was carried out at the Lord Rank Research Centre, High Wycombe, Bucks. Two separate investigations were made:-

(a) An initial comparison was made between the S volatiles present in a species of earthworm known to be palatable to rainbow trout (L.terrestris) and a species of earthworm apparently unpalatable to rainbow trout (E.foetida). To detect the possible secretion of S volatiles in response to unfavourable stimulii live earthworms were either left undisturbed or subjected to mechanical irritation for a period of 30 minutes prior to analysis of the S volatiles. The E.foetida used for these investigations were supplied by Dr. C. A. Edwards, Rothamsted Experimental Station and the L.terrestris were collected locally.

(b) A further investigation was made into the effect of various treatment processes on the nature of S volatiles present in E.foetida. Freeze dried E.foetida; blanched E.foetida; E.foetida coelomic fluid (collected by chemical irritation of the worms using di-ethyl ether) and a commercially available feed attractant prepared using E.foetida (supplied by British Groundbaits and marketed under the trade name "ACE") were subject to the analysis of S volatiles and the resulting chromatograms compared. All earthworms used in these experiments were supplied by Dr. C. A. Edwards and maintained on a pig manure substrate. Treated E.foetida were analysed immediately or stored at -20°C for a minimum period prior to analysis.

The experimental apparatus employed for the collection and detection of S volatiles present in the earthworms is illustrated in Figure 4.2 (based on apparatus used by von Sydow et al., 1970).

An equivalent of 30g of earthworm (wet weight) was used and where necessary dried earthworm samples were re-constituted to their original moisture content using distilled water. Earthworms, sealed within the equilibrating vessel were held at room temperature for 30 minutes and adequate movement of S volatiles into the headspace during this period was ensured by continuous mixing using a magnetic stirrer.

Headspace vapour (usually 400ml) was flushed from the top of the equilibrating vessel and directed through the apparatus by introducing a stream of N_2 carrier gas (20ml/min) into the base of the equilibrating vessel. Headspace vapour was carried through a teflon trap, submerged in liquid N_2 and containing 10-15mg Tenax GC 680 mesh which effectively adsorbed the S volatiles. By

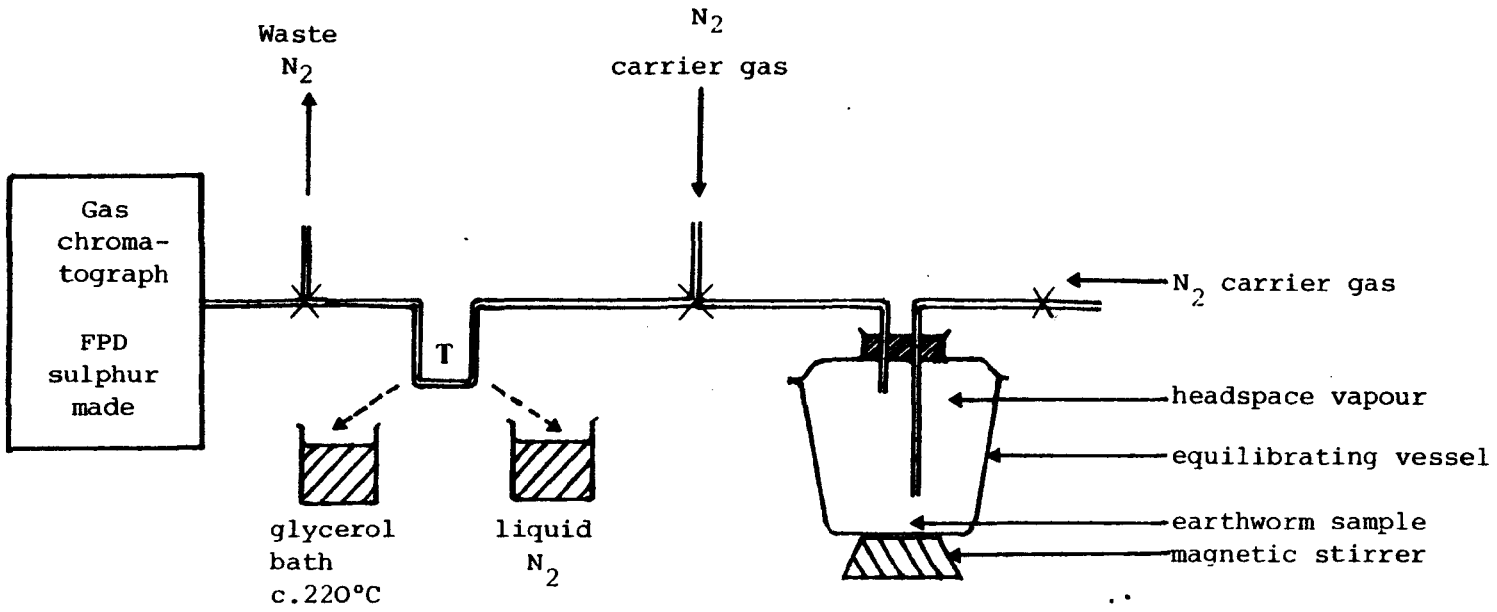


Figure 4.2: A simplified diagram showing the apparatus used for the collection of headspace vapour for the analysis of sulphur volatiles.

T = Teflon tube containing Tenax GC 680 mesh submerged in liquid N_2 for the collection of sulphur volatiles and subsequently in glycerol to drive sulphur volatiles on to the Gas Chromatograph (GC)

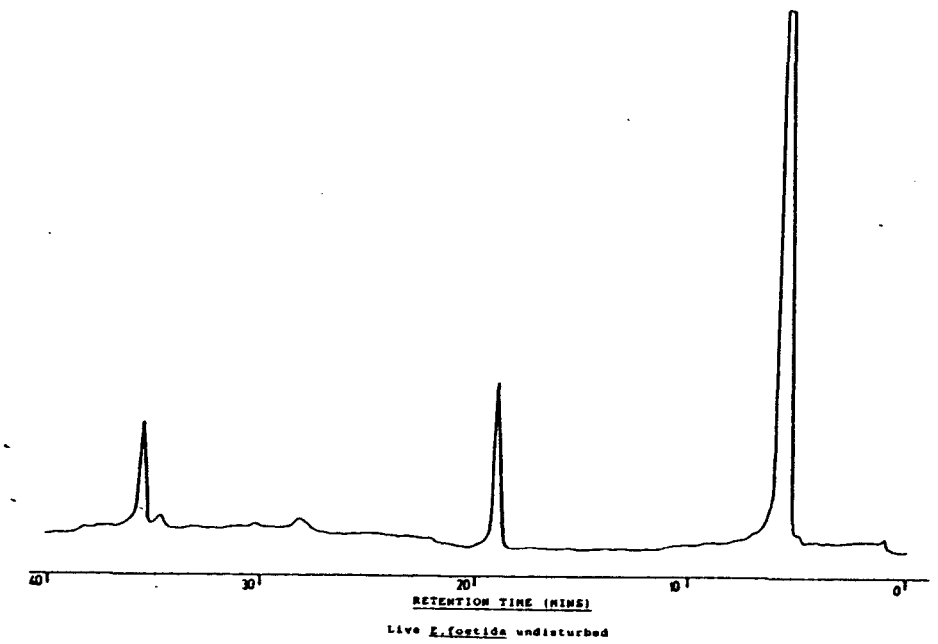
For GC operating conditions see text

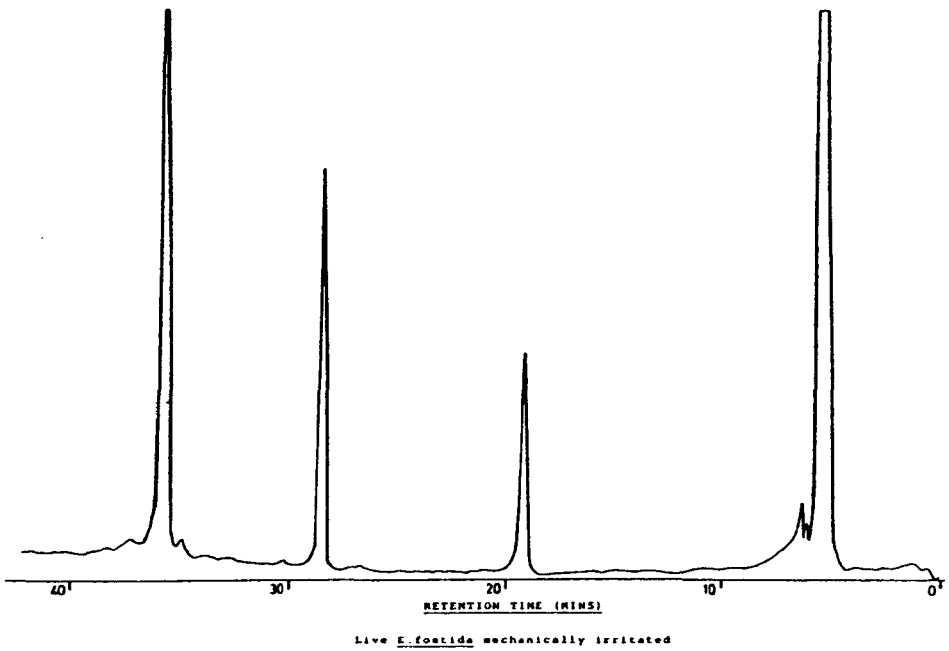
subsequently submerging the teflon trap in a glycerol bath (220°C) and redirecting the N_2 carrier gas flow, S volatiles which evolved were carried onto the glass capillary column (2m x 3mm, packed with 10% Carbowax 20M) of a gas chromatograph.

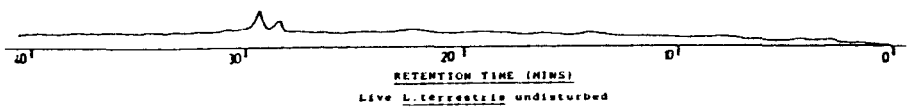
The gas chromatograph was connected to a flame photometric detector (FPD) in sulphur mode with flow rates of 20ml/min N_2 carrier gas; 75ml/min hydrogen and 50ml/min air. A detector temperature of 100°C was maintained and the column temperature was programmed 30°C-220°C at 4°C/min after an initial isothermal period of 5 mins.

The identification of emerging peaks on the gas chromatogram (GC) was carried out by comparing the retention times with retention times of the following known S volatiles: ethanethiol; dimethyl sulphide; dimethyl disulphide and diethyl disulphide. No attempt was made to quantify S volatiles present in the earthworm samples tested.

4.2.2.2 Results: (a) Comparison between the S volatiles present in E.foetida and L.terrestris. In an undisturbed state, the gas chromatogram (GC) pattern of S volatiles from E.foetida displayed more peaks and a larger quantity of volatile S compounds compared with L.terrestris (Figure 4.3). When earthworms of both species were subject to mechanical irritation the number of S volatiles from E.foetida increased compared with both undisturbed E.foetida and L.terrestris subject to mechanical irritation (Figure 4.3). The GC pattern of S volatiles from L.terrestris subjected to mechanical irritation displayed only one minor peak which was not present in the GC of undisturbed L.terrestris (Figure 4.3).







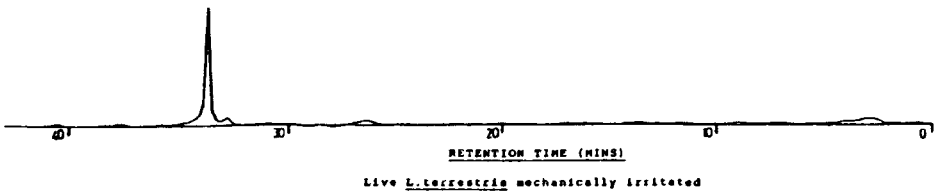


Figure 4.3: The sulphur volatiles of live E.foetida and L.terrestris.
FPD(sulphur) 400ml headspace, for GC operating conditions see text.

(b) Comparison between the S volatiles present in treated and untreated E.foetida. The GC pattern from a mixture of four authentic S volatiles is shown in Figure 4.4a. A comparison of the GC pattern from live, mechanically irritated E.foetida, freeze dried E.foetida and a coelomic fluid extract from E.foetida (Figure 4.4b,c & d respectively) revealed the presence of similar S volatiles (including dimethyl sulphide and diethyl disulphide) in each sample. However, three additional peaks (including ethanethiol and with very short retention times) were present in the freeze dried E.foetida sample (which were absent in live E.foetida). Unfortunately diethyl ether, used to extract the coelomic fluid sample repeatedly extinguished the hydrogen flame during this initial period (marked x, Figure 4.4d) and only ethanethiol could be identified as present.

The GC patterns from blanched E.foetida and the E.foetida based feed attractant (Figure 4.4e,f) revealed numerous peaks from S volatiles. Some of these S compounds (dimethyl sulphide and diethyl disulphide) were present in live E.foetida but many additional S compounds were also present in these samples which were not detected from live E.foetida.

4.3 FEEDING TRIALS - Experiments A,B,C,D & E.

Improvement in the acceptability of E.foetida to rainbow trout, achieved by pre-treatment of the earthworms was further investigated by conducting five consecutive feeding trials (Experiments A-E). Experiment A assessed the effect of holding live E.foetida on various substrates on the subsequent palatability of the earthworms to fish; Experiments B and C assessed the influence of E.foetida coelomic fluid on the palatability of

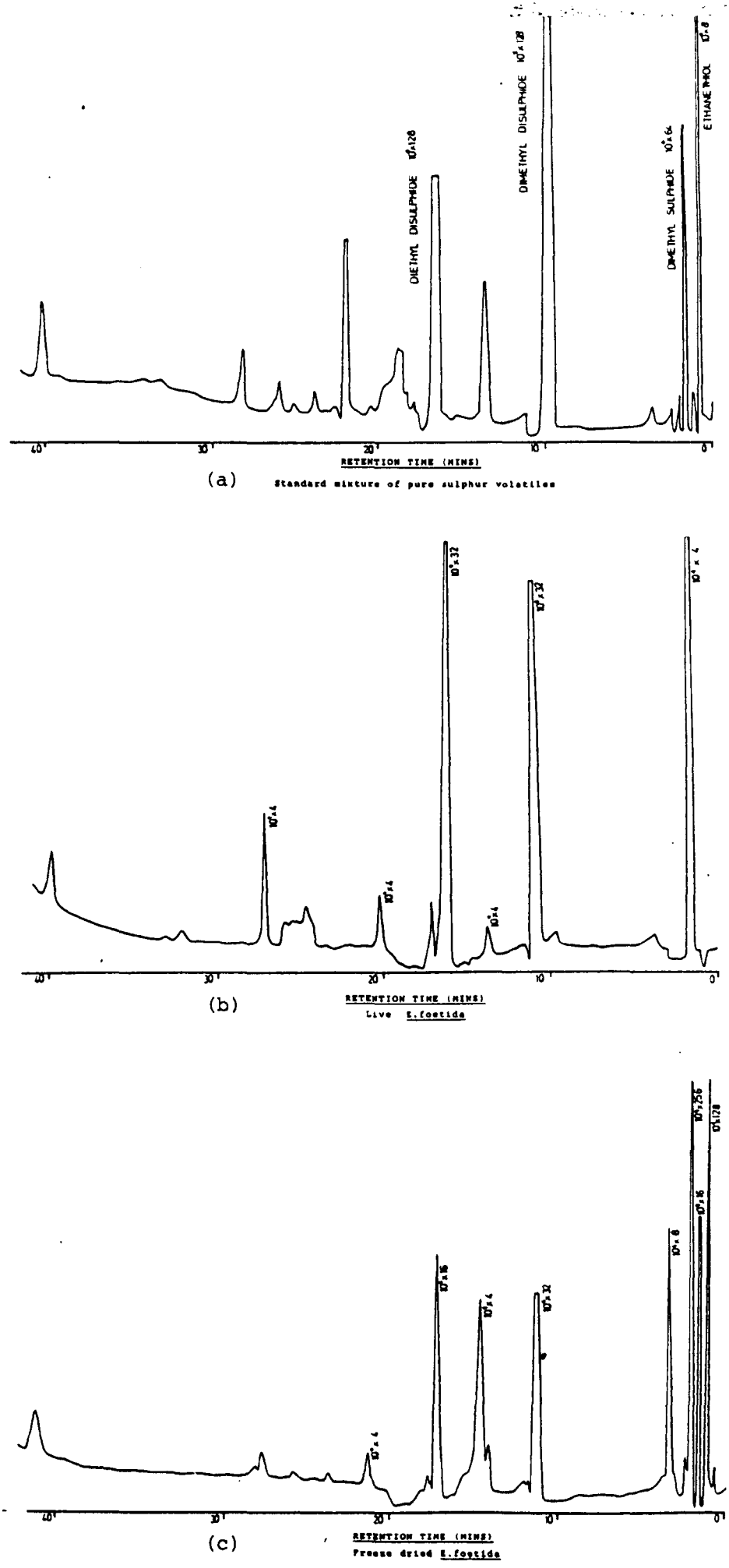
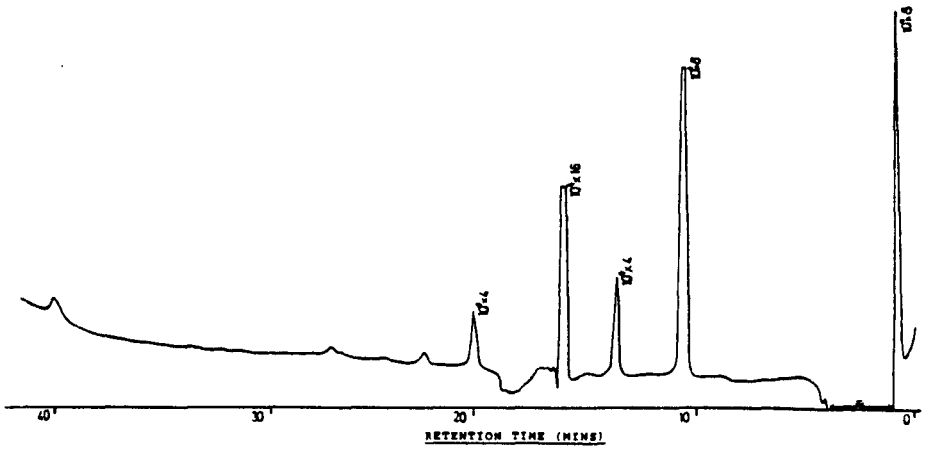
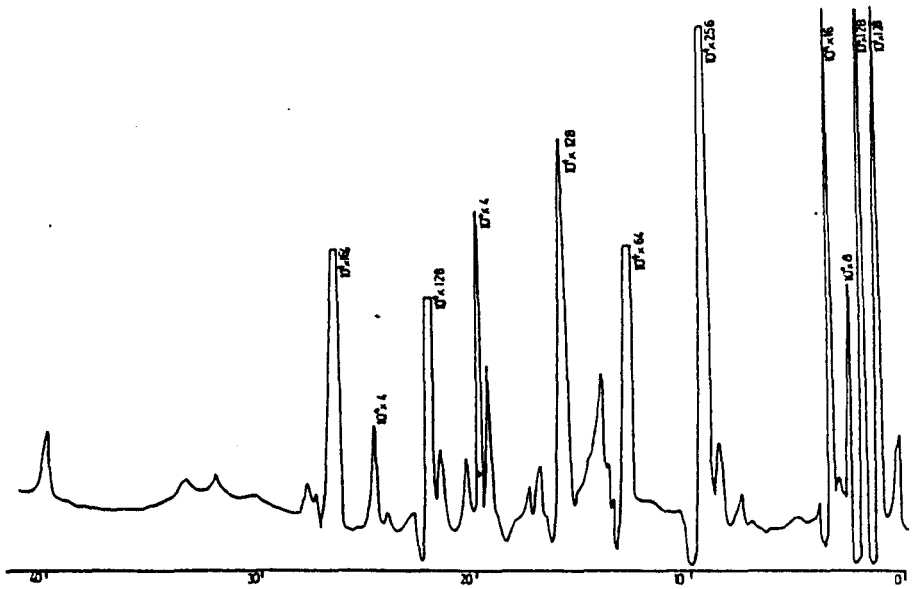


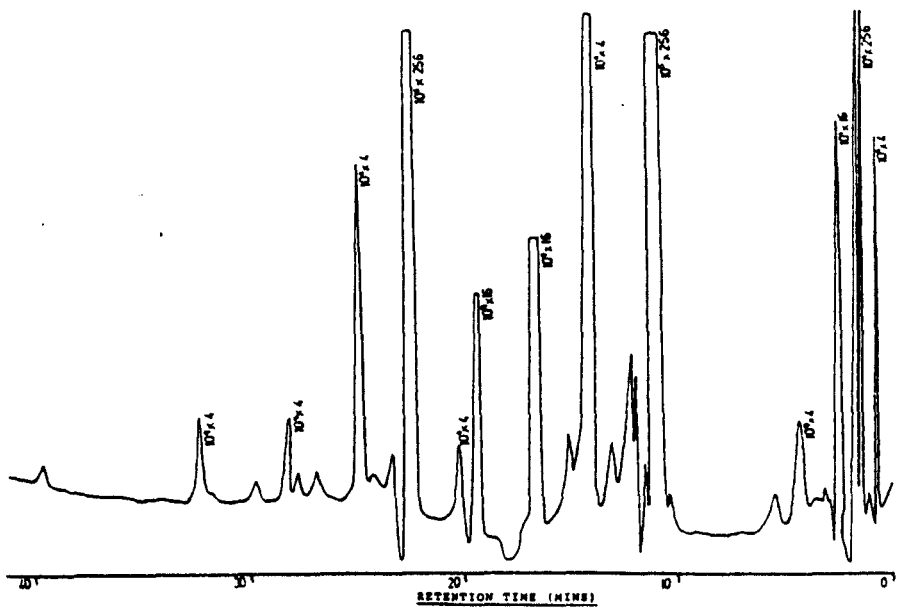
Figure 4.4: The sulphur volatiles of treated and untreated E.foetida FPD(sulphur) 20ml headspace for (a), 400ml headspace for (b,c,d & e) and 230ml headspace for (f). Volatiles collected over liquid N_2. For GC operating conditions see text.



(d) Coelomic fluid extract from E.foetida



(e) 'Blanched' E.foetida



(f) E.foetida based food attractant ("ACE")

Figure 4.4 (continued)

purified diets to rainbow trout; Experiment D investigated the fraction in which the unpalatable component of E.foetida was present, and Experiment E assessed the acceptability of 'blanched' E.foetida to rainbow trout.

Similar experimental methods were followed in conducting each feeding trial and these are outlined in Section 4.3.1 below. For clarity the dietary treatments and experimental results are subsequently reported together for each feeding experiment in turn.

4.3.1 General Experimental Methods, Experiments A-E.

4.3.1.1 Animals and Tanks

Rainbow trout used in Experiments A-E were supplied by D. Brien, Almondbank Fish Farm, Nr. Perth, Scotland and maintained in tanks of similar design to that illustrated in Figure 2.1, and supplied with fresh water at a rate of 2ℓ/min/tank in a through-flow system. The water temperature during each experimental period (A-E) is shown in Table 4.2. All fish were subjected to a natural photoperiod.

At the start and end of each experiment and at bi-weekly periods throughout the experiment all fish were lightly anaesthetized (Section 2.1.2.2) and weighed individually on a top-pan balance (±0.01g). The average size of fish selected for use in each experimental feeding trial is shown in Table 4.2. Experimental fish were divided between the required number of tanks ensuring that in each experiment, the overall weight of fish in each tank was approximately the same. The number of fish housed in each tank and allocated one of the experimental diets used in Experiments A-E is shown in Table 4.2. Unless specified otherwise all diets were fed to the fish twice daily "to appetite", following the procedures

Table 4.2: Experimental conditions employed during Experiments A-E

Experimental Feeding Trial	A	B	C	D	E
Mean fish weight (g)	1	23	25	8	32
No. fish/tank	15	10	15	20	15
Duration of feeding trials (days)	50	28	75	56	42
Mean water temperature (°C)	6.5 ± 1.5	6.5 ± 1	12.5 ± 4.5	11 ± 3	6.5 ± 2

outlined in Section 2.1.2.3 and an accurate record was kept of the bi-weekly feed intake of the fish.

Ten fish not included in an experimental group at the start of each feeding trial were killed by a sharp blow on the head and stored at -20°C for subsequent whole carcass proximate analysis. The duration of each feeding trial is given in Table 4.2. On the final day of each feeding trial all fish were killed by a sharp blow on the head. The livers from five fish in each group were removed and weighed for the calculation of liver somatic index (LSI). Ten whole fish (including livers) from each tank were stored at -20°C for subsequent whole carcass proximate analysis.

In addition, during the final week of Experiments C and D faecal samples were collected from the fish (as described in Section 2.2.2.2), dried at 105°C to constant weight and stored in airtight containers for subsequent total N and Cr_2O_3 analysis. On the final day of Experiment C, blood samples were also collected from the severed caudal peduncle of four fish fed each experimental diet. The blood samples, collected in heparinized vials, were shaken to aerate the blood and immediately used for the determination of erythrocyte fragility.

4.3.1.2 Chemical Methods

Proximate analysis of experimental diets and whole fish carcass was carried out using the methods described previously in Section 2.1.2.3. Apparent dry matter and N digestibility was calculated after determination of total N and Cr_2O_3 content of diet and faecal samples as described in Section 2.2.2.3.

Erythrocyte fragility was determined using an adaptation of the method described by Baker et al. (1966).

Samples (50µℓ) of aerated whole blood were dispensed into buffered saline concentrations of 0.85%, 0.65%, 0.60%, 0.55%, 0.50%, 0.46%, 0.43%, 0.40%, 0.36%, 0.33%, 0.30% and distilled water. Carefully mixed solutions were incubated at room temperature for 30 minutes, centrifuged and the absorbance of the supernatants read at 415nm against a buffered blank. From the resulting absorbtion curves the saline concentration at which 50% of the erythrocytes were lysed could be compared between fish fed the four dietary treatments.

4.3.1.3 Statistical Method

Statistical analysis of the data was carried out using the method described in Section 2.1.2.5.

4.3.2 Experiment A

4.3.2.1 Diets: E.foetida supplied by British Groundbaits, were separated from the substrate in which they had been cultured and approximately 3Kg of earthworms were introduced to each of three experimental substrates held in large plastic containers:-

(i) Horse manure collected from Drumbrae Farm Stables, Bridge of Allan, Scotland (the manure having been previously rid of earthworms by hand sorting). Horse manure supports rapid weight gain of E.foetida (Hartenstein, 1979b) and earthworms maintained on this substrate served as the control diet.

(ii) Sterilized peat (Fisons Ltd.) may be ingested by earthworms but provides little nutritive value (J. E. Satchell, pers. comm.) and earthworms maintained on this substrate were effectively starved.

(iii) Moist sand provided an abraisive substrate to 'scour' the earthworms.

The acceptability of each substrate to the earthworms was checked by introducing a few worms onto the substrate surface and observing their burrowing activity. Although moist sand had previously been used in earthworm culture media (Kale et al., 1982) earthworms failed to burrow into the moist sand substrate and therefore this was mixed with sterilized peat (1:1).

E.foetida were maintained on their respective substrates for ten days. After separation from the substrate earthworms were subjected to mechanical irritation by placing them in an Endecott test sieve shaker (Endecotts,London) for 10 minutes so as to elicit mucus and coelomic fluid secretion. One half of the earthworms collected from each substrate and subject to mechanical irritation were rinsed with tepid water, to remove mucus and coelomic fluid which had been secreted, before being frozen (-20°C).

Six experimental diets were fed to rainbow trout (Table 4.3) and each diet comprised a similar nutritional composition (Table 4.4).

Table 4.3: Trout diets employed during Experiment A.

Diet No.	Earthworm species	Substrate media	Mechanical irritation	Tepid water rinse
1	E.foetida	Horse manure	✓	✓
2	E.foetida	Horse manure	✓	-
3	E.foetida	Sterilized peat	✓	✓
4	E.foetida	Sterilized peat	✓	-
5	E.foetida	Sand/peat (1:1)	✓	✓
6	E.foetida	Sand/peat (1:1)	✓	-

Table 4.4: Experiment A: Nutritional composition of the experimental diets

Diet No:	1	2	3	4	5	6
Moisture (%)	82.37	82.77	84.35	83.64	85.17	83.98
Nutrient content (% dry wt)						
Crude protein	71.35	71.22	70.24	71.14	73.52	71.23
Lipid	10.90	9.24	10.81	12.15	11.66	12.03
Ash	5.11	4.98	5.29	5.12	5.69	6.31

Rainbow trout were offered slices of E.foetida as a sole diet for the duration of the feeding trial.

4.3.2.2 RESULTS: Fish growth, feed utilization efficiency, carcass composition and liver somatic index.

No significant improvement in the palatability of E.foetida to rainbow trout was achieved by maintaining the earthworms on inert substrates. All six diets were consumed in very small quantities by the fish during the feeding period.

Fish Growth and Feed Utilization Efficiency: Fish allocated diet 1 had a significantly lower mean body weight ($p < 0.05$) at the start of the experiment compared with fish fed diets 3 and 6 and this position was unchanged at the end of the 50 day feeding period (Table 4.5). The increase in body weight of fish fed the six diets was extremely poor (Figure 4.5) and the maximum fish weight gain observed over the 50 day feeding period was only 1.3g.

Parameters measuring feed utilization efficiency (defined in Section 2.1.3) also indicated that feed offered was poorly utilized (Table 4.5). This was predominantly a result of food wastage since fish were observed to take the frozen slices

Table 4.5: Experiment A: Growth, feed utilization, liver somatic index and carcass composition of rainbow trout fed experimental diets for 50 days

Diet No:		1	2	3	4	5	6	± SE[2]
Mean initial weight (g)		6.48[a]	7.29[ab]	7.88[b]	7.31[ab]	7.11[ab]	7.64[b]	0.354
Mean final weight (g)		6.67[a]	8.26[ab]	9.24[b]	8.61[b]	7.95[ab]	8.64[b]	0.550
Weight gain (%)		2.93	13.31	17.26	17.78	11.81	13.09	
Specific growth rate (%/day)		0.058	0.250	0.318	0.327	0.223	0.246	
Food intake (mg/day/fish)		374.70	341.33	392.63	387.67	382.56	390.75	
Food intake (mg/day/fish,dry wt)		66.06	58.81	61.44	63.43	56.73	62.60	
Weight gain (mg/day/fish)		3.80	19.40	27.20	26.00	16.80	20.00	
Food conversion ratio[*1]		17.38	3.03	2.26	2.44	3.38	3.13	
Protein efficiency ratio		0.014	0.080	0.098	0.022	0.060	0.072	
Nitrogen intake (mg/day/fish)		276.75	243.10	277.75	275.79	281.26	278.33	
Nitrogen deposition (mg/day/fish)		2.81	14.81	19.58	18.30	11.96	14.43	
Apparent N utilization (%)		3.39	9.09	13.94	10.15	7.96	8.39	
Liver Somatic Index (%)		1.49[a]	1.44[a]	1.31[a]	1.32[a]	1.46[a]	1.45[a]	0.122
Carcass composition (% wet wt)	Initial Fish	After 50 days						
Moisture	78.46	79.01[a]	78.94[a]	78.01[a]	78.45[a]	78.57[a]	78.95[a]	0.355
Crude protein	14.72	15.50[a]	15.29[a]	15.83[a]	15.16[a]	15.26[a]	15.19[a]	0.289
Lipid	5.48	3.38[ab]	3.18[a]	4.44[b]	3.97[ab]	3.67[ab]	3.66[ab]	0.310
Ash	2.23	2.08[a]	2.51[b]	2.39[ab]	2.38[ab]	2.50[b]	2.42[b]	0.095

*1 FCR = Food Intake (dry weight)/Weight gain (live weight)

2a,b,c = For explanation of terms see Table 2.2.5

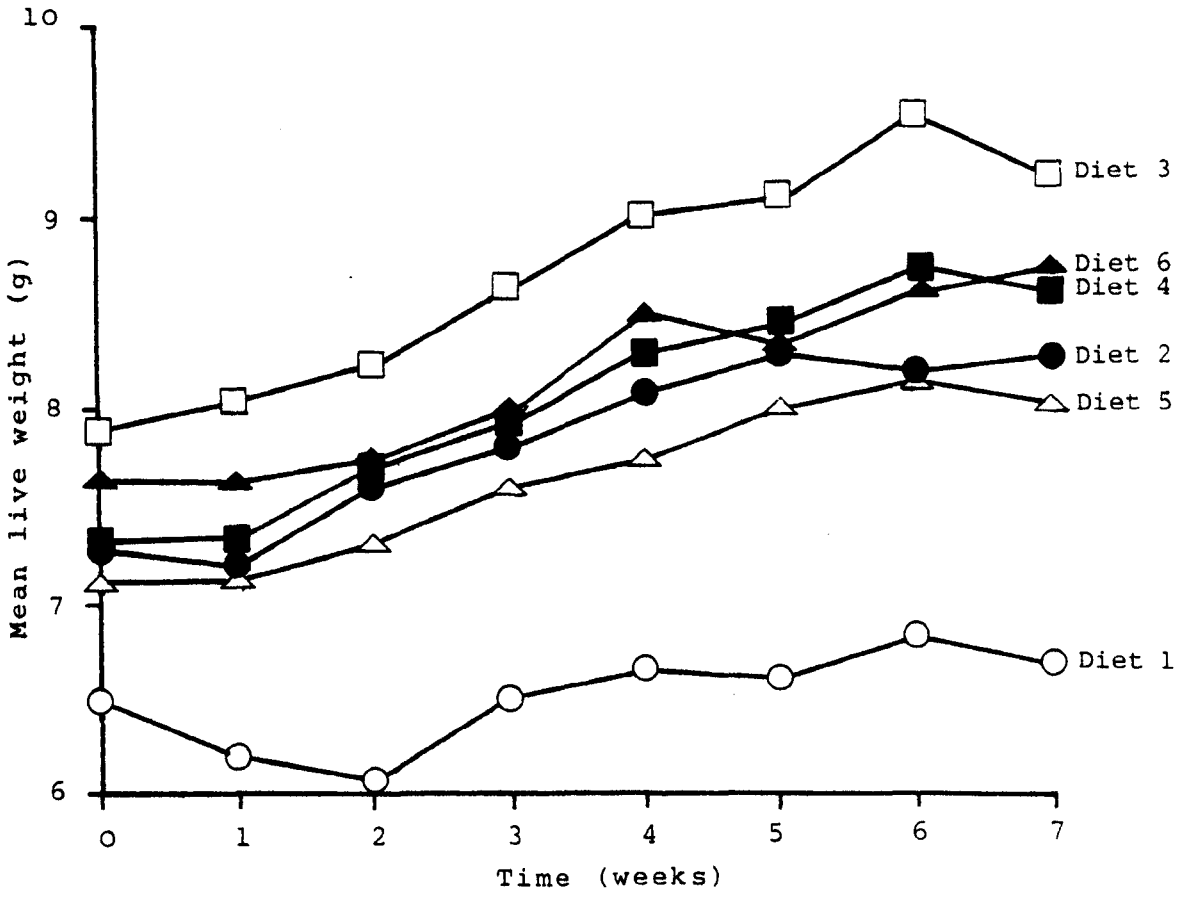


Figure 4.5: Experiment A: The growth response of rainbow trout fed the six experimental diets.

of worm initially but subsequently rejected them. Neither mechanical irritation nor rinsing in tepid water influenced the subsequent acceptability of E.foetida to rainbow trout.

Carcass composition and LSI: On the basis of LSI and whole carcass proximate composition no significant trends ($p < 0.05$) emerged between fish fed the various dietary treatments. Fish fed E.foetida for a period of 50 days exhibited a lower carcass lipid content and higher carcass crude protein content compared with fish at the start of the experiment.

4.3.3 Experiment B

4.3.3.1 Diets: Mechanical irritation employed in Experiment A to rid E.foetida of its coelomic fluid was not considered sufficiently effective. Chemical irritation using distilled water saturated with diethyl ether (Ireland, 1975b); formalin (Lakhani and Satchell, 1970) and 5% NaCl solution (pers.obs.) has also been employed to stimulate E.foetida to eject large quantities of coelomic fluid. The following procedure was therefore used to collect the coelomic fluid from 500g of live E.foetida for subsequent inclusion in rainbow trout diets:-

(i) Groups of 10-20 worms were immersed in 5ml distilled water saturated with ether.

(ii) Earthworms were held in 25ml distilled water to recover from the anaesthetic effect of the ether.

(iii) Earthworms were again immersed in 5ml distilled water saturated with ether.

(iv) Distilled water rinse: ether, a small amount of coelomic fluid and any adhering substrate was rinsed from the earthworms using excess distilled water.

(v) Alcohol rinse: earthworms were immersed in 100% alcohol to remove components soluble in alcohol and insoluble in water e.g. eleocytes (Cuenot, 1898).

(vi) Distilled water rinse: Finally earthworms were rinsed in excess distilled water, blotted dry and stored at -20°C until fed to the fish.

The large amounts of coelomic fluid expelled from the earthworms in steps (i) and (iii) and also collected in step (ii) were substituted for distilled water in the preparation of 400g of a semi-synthetic trout diet, formulated to contain 40% crude protein, 17% lipid and with the following ingredient composition (% by weight):- herring meal 59; cod-liver oil 3; corn oil 5.5; corn starch 15.5; dextrin 8; vitamin mix*1 2; mineral mix*1 4; carboxymethylcellulose 2; chromic oxide 1. A semi-synthetic diet identical to the above diet, but in which distilled water and not coelomic fluid was used to mix the dietary ingredients, served for comparison as the control diet.

In addition, during this experiment frozen slices of the two possible sub-species of E.foetida were offered to rainbow trout and the growth, feed utilization efficiency and carcass composition of fish fed each type of earthworm was compared. Similarly, the performance of fish fed these four diets was compared with fish fed frozen slices of E.foetida from which the coelomic fluid had been expelled.

The nutritional composition of the following five diets employed during this 28 day feeding trial with rainbow trout is given in Table 4.6:-

*1 The composition of the vitamin & mineral mix is given in Table 2.2.1.

Diet 1: Control-semi-synthetic pellet mixed using distilled water

Diet 2: Semi-synthetic pellet mixed using coelomic fluid expelled from 500g of live E.foetida

Diet 3: Frozen slices of E.foetida from which coelomic fluid had been expelled

Diet 4: Frozen slices of E.fetida fetida

Diet 5: Frozen slices of E.f.andrei.

Table 4.6: Experiment B: Nutritional composition of the experimental diets

Diet No:	1	2	3	4	5
Moisture (%)	6.91	9.18	85.06	83.40	81.87
Nutrient content (% dry wt)					
Crude protein	40.89	40.82	65.82	61.74	62.97
Lipid	18.04	17.06	7.16	10.76	6.56
Ash	11.13	12.01	5.36	7.11	7.59

4.3.3.2 RESULTS: Fish growth, feed utilization efficiency, carcass composition and liver somatic index

A clear division was evident between the performance of fish fed diets 1 and 2 and fish fed diets 3, 4 and 5 in terms of growth rate, feed utilization efficiency and whole carcass proximate composition (Table 4.7).

Fish Growth and Feed Utilization Efficiency: Fish fed the control diet (diet 1) achieved the highest absolute mean final body weight although this was not significantly higher ($p < 0.05$) than fish fed diets 2, 3 and 5. The feed utilization efficiency (all terms defined in Section 2.1.3) of fish fed diet 2 (the semi-synthetic

Table 4.7: Experiment B: Growth, feed utilization, liver somatic index and carcass composition of rainbow trout fed experimental diets for 28 days

Diet No:		1	2	3	4	5	± SE*2
Mean initial weight (g)		23.66[a]	23.31[a]	23.30[a]	23.16[a]	23.28[a]	2.198
Mean final weight (g)		33.53[b]	30.76[ab]	23.66[a]	24.57[ab]	25.79[ab]	2.930
Weight gain (%)		41.72	31.96	1.55	6.09	10.78	
Specific growth rate (%/day)		1.160	0.924	0.051	0.197	0.341	
Food intake (mg/day/fish)		380.67	363.67	837.67	674.67	782.00	
Food intake (mg/day/fish,dry wt)		354.37	330.29	151.87	100.80	129.81	
Weight gain (mg/day/fish)		329.00	248.33	12.00	47.00	83.67	
Food conversion ratio*1		1.08	1.33	12.66	2.15	1.55	
Protein efficiency ratio		2.27	1.84	0.125	0.708	1.04	
Apparent N utilization (%)		33.77	24.47	2.58	0.704	2.66	
Liver Somatic Index		1.81[b]	1.95[b]	1.31[a]	1.36[a]	1.19[a]	0.115
Carcass composition (% wet wt)	Initial Fish	After 30 days					
Moisture	78.38	76.44[a]	76.20[a]	78.81[b]	79.79[b]	80.06[b]	0.591
Crude protein	14.57	14.66[b]	14.26[b]	14.89[b]	14.63[b]	13.40[a]	0.215
Lipid	3.08	4.70[d]	5.88[e]	2.63[ab]	2.01[a]	3.49[c]	0.262
Ash	2.73	2.42[a]	2.42[a]	2.71[b]	2.63[b]	2.52[ab]	0.064

*1 FCR = Food intake (dry weight)/Weight gain (live weight)

*2a,b,c,d,e = For explanation of terms see Table 2.2.5

pellet mixed using E.foetida coelomic fluid) was noticeably reduced when compared with fish fed the control diet (diet 1, Table 4.7). Of the three frozen earthworm diets (diets 3, 4 and 5) fish fed frozen E.f.andrei (diet 5) achieved marginally higher fish growth and feed utilization efficiency (Table 4.7).

Carcass Composition and LSI: Fish maintained on diets 3, 4 and 5 (slices of frozen earthworms) exhibited significantly higher carcass moisture and ash content and significantly lower LSI and carcass lipid content compared with fish fed diets 1 and 2 (Table 4.7). Fish fed diet 2 (mixed using E.foetida coelomic fluid) had a significantly higher ($p < 0.05$) carcass lipid content than fish fed the control diet. Of the three frozen earthworm diets (diets 3, 4 and 5) fish fed frozen slices of E.f.andrei (diet 5) had a significantly higher ($p < 0.05$) carcass lipid content. Fish fed diet 5 also exhibited a significantly lower ($p < 0.05$) carcass crude protein content compared with fish fed the remaining diets.

4.3.4 Experiment C

4.3.4.1 Diets: This experiment also investigated the effect that E.foetida coelomic fluid had on the subsequent palatability of diets to rainbow trout. The experimental diets used in Experiment B were considered only partially satisfactory for the following reasons:-

(i) The coelomic fluid included in 400g of trout pellet was collected from only 500g of live E.foetida. 500g of E.foetida, dried to a meal and included within 400g of trout pellet would constitute approximately 20% of the diet. The results of Experiments 3.1 and 3.2 suggest that at this level an insignificant decline in fish growth would be expected.

(ii) Herring meal, supplying the dietary protein in Experiment B is strongly flavoured and may have effectively masked any unpalatable factor resulting from the dietary inclusion of E.foetida coelomic fluid.

The results of Experiment B have been included since they confirm the findings of this experiment and demonstrate the comparative acceptability of E.fetida fetida and E.f.andrei to rainbow trout.

In this experiment the control diet (diet 1) was formulated using caesin to supply the dietary protein and the dietary ingredients were mixed as described in Section 2.2.2.1 using distilled water. Coelomic fluid was collected from 1.5Kg of E.foetida by the procedure described previously (Section 4.3.3.1) and used in place of distilled water to mix the dietary ingredients of a synthetic diet (diet 2) identical in all other respects to the control diet. The quantity of coelomic fluid included within this diet was estimated to be equivalent to the amount that would have been present had the diet contained 100% E.foetida meal protein (c. 68g E.foetida meal/100g diet).

Earthworms from which the coelomic fluid had been extracted were freeze dried and ground to a homogenous 'worm meal'. This 'worm meal' (comprising 62.98% crude protein and 12.38% lipid) was used to supply 100% of the dietary protein in a semi-synthetic diet (diet 3). Untreated E.foetida were oven dried at 60°C, ground to a homogenous meal (comprising 53.60% crude protein and 19.00% lipid) which was used to supply 100% of the dietary protein in a semi-synthetic diet (diet 4).

Four experimental diets were fed to rainbow trout over a 75 day feeding period. The ingredient composition of the diets

is given in Table 4.8. All diets were formulated to contain 45% crude protein and 12% lipid and the nutritional content of the experimental diets is also given in Table 4.8.

Diet 1: Control - Synthetic diet mixed using distilled water

Diet 2: Synthetic diet mixed using coelomic fluid from 1.5Kg *E.foetida*

Diet 3: Semi-synthetic diet containing freeze dried *E.foetida* from which coelomic fluid had been expelled

Diet 4: Semi-synthetic diet containing oven dried *E.foetida*

Table 4.8: Experiment C: Ingredient composition and nutrient content of the experimental diets (% by weight)

Diet No:	1	2	3	4
Ingredients				
Caesin	50	50	-	-
E.foetida meal	-	-	75*1	64*2
Coelomic fluid extract	-	✓*3	-	-
Cod liver oil	4	4	2	1
Corn oil	8	8	3	1
Corn starch	18	18	7	16
Dextrin	12	12	4	9
Vitamin mix*4	2	2	2	2
Mineral mix*4	4	4	4	4
Carboxymethylcellulose	1	1	2	2
Chromic oxide	1	1	1	1
Nutrients				
Moisture	10.64	9.00	13.36	14.24
Crude protein	44.41	43.95	44.11	36.89
Lipid	11.49	12.33	11.89	11.89
Ash	5.34	8.37	8.32	7.23

*1 Freeze dried *E.foetida* meal

*2 Oven dried *E.foetida* meal

*3 Coelomic fluid extracted from 1.5Kg *E.foetida*

*4 Vitamin and Mineral mix given in Table 2.2.1.

4.3.4.2 RESULTS: Fish growth, feed utilization efficiency, carcass composition and liver somatic index.

The results of Experiment C, in terms of fish growth, feed utilization efficiency and proximate carcass composition (Table 4.9, Figure 4.6) showed similar trends to those reported for Experiment B.

Fish Growth and Feed Utilization Efficiency: Fish fed the synthetic control diet (diet 1) achieved a significantly higher ($p < 0.05$) mean final body weight than fish fed the synthetic diet containing E.foetida coelomic fluid (diet 2) and fish fed both diets had significantly higher ($p < 0.05$) mean final body weights than fish fed diets 3 and 4 (containing treated and untreated dried E.foetida meal, respectively). Weight gain, specific growth rate, FCR, PER and Apparent NPU (all terms are defined in Section 2.1.3) were all reduced as a result of including E.foetida coelomic fluid in the synthetic diet and the performance of fish fed diets 3 and 4 in terms of these parameters was very poor (Table 4.9). Apparent dry matter digestibility of nutritional components within the feed was lower for diets 2 and 4 compared with diets 1 and 3. Apparent N digestibility was high for diets 1, 2 and 3, but distinctly reduced in diet 4 (Table 4.9) possibly due to protein denaturation as a result of oven drying the earthworms (Bender, 1970).

Carcass Composition and LSI: There was no significant difference between fish fed the four diets in terms of LSI and carcass crude protein and ash content (Table 4.9). However, fish fed diets containing a high percentage of worm meal (diets 3 and 4) exhibited significantly higher ($p < 0.05$) carcass moisture content and significantly lower ($p < 0.05$) carcass lipid content compared with

Table 4.9: Experiment C: Growth, feed utilization, liver somatic index, blood parameters and carcass composition of rainbow trout fed experimental diets for 75 days

Diet No:		1	2	3	4	± SE*2
Mean initial weight (g)		25.10[a]	25.27[a]	25.22[a]	25.24[a]	2.453
Mean final weight (g)		52.72[c]	40.10[b]	23.07[a]	26.72[a]	4.158
Weight gain (%)		110.04	58.69	-ve	5.86	
Specific growth rate (%/day)		0.987	0.611	-	0.077	
Food intake (mg/day/fish)		382.29	349.87	94.91	261.77	
Weight gain (mg/day/fish)		368.27	197.73	-ve	19.73	
Food conversion ratio		1.04	1.77	-	13.27	
Protein efficiency ratio		2.17	1.29	-	0.20	
Apparent N utilization (%)		37.54	22.46	-	2.90	
Apparent dry matter digestibility (%)		71.03	67.37	72.50	65.07	
Apparent N digestibility (%)		98.24	97.61	91.54	86.38	
Liver somatic index (%)		1.19[a]	0.98[a]	1.10[a]	1.15[a]	0.085
Blood Parameters						
Erythrocyte fragility*1		0.403[a]	0.387[a]	0.380[a]	0.369[a]	0.019
Carcass composition (% wet wt)	Initial Fish	After 75 days				
Moisture	74.28	71.66[a]	73.15[a]	78.10[b]	76.85[b]	0.728
Crude Protein	15.95	16.65[a]	16.51[a]	15.07[a]	15.86[a]	0.602
Lipid	7.25	8.61[c]	7.07[b]	3.44[a]	3.78[a]	0.446
Ash	2.45	2.23[a]	2.48[a]	2.95[a]	2.72[a]	0.282

*1 Mean saline concentration at which 50% of the erythrocytes were lysed

*2a,b,c For explanation of terms see Table 2.2.5

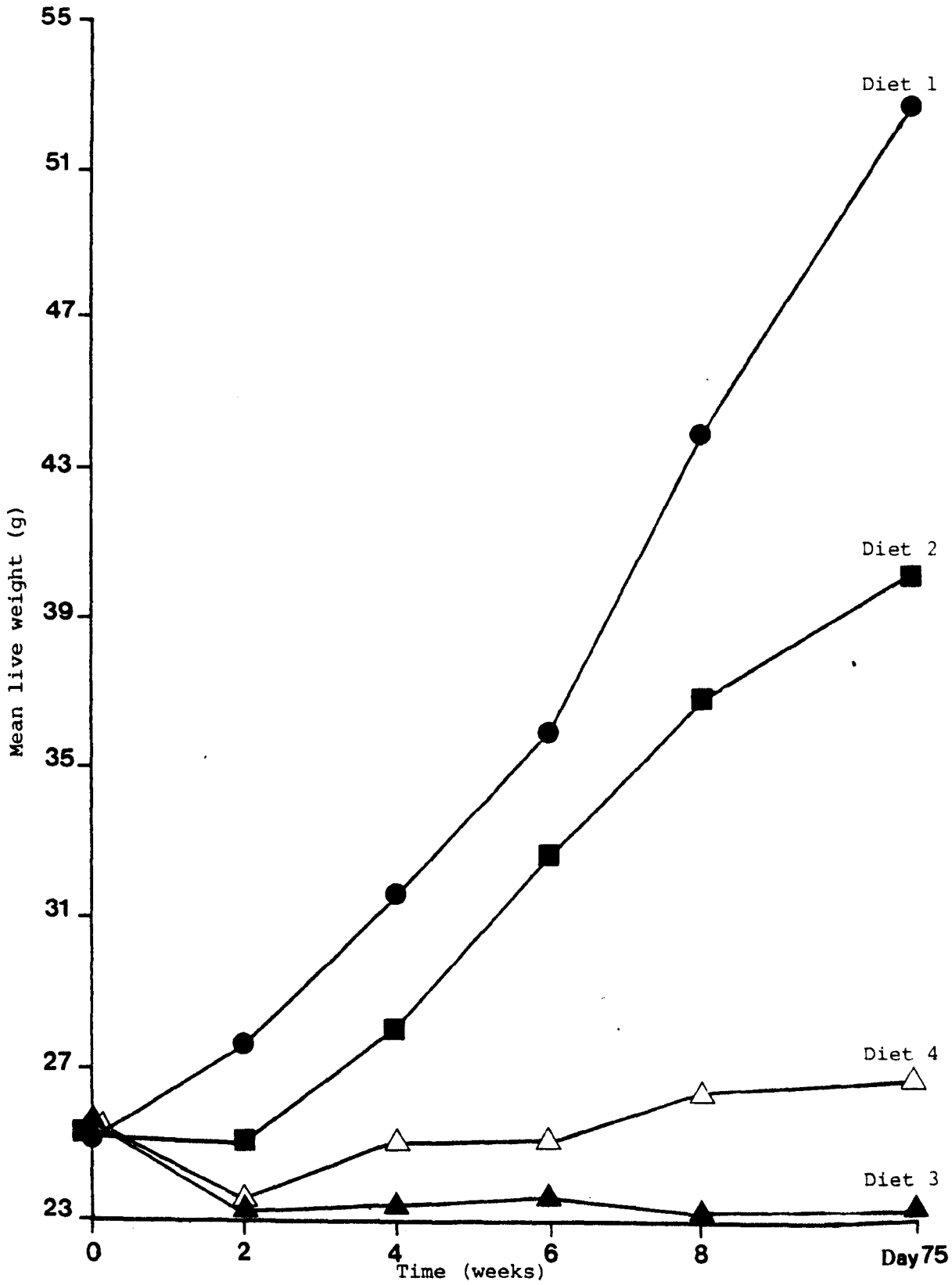


Figure 4.6: Experiment C - The growth response of rainbow trout fed four experimental diets

fish fed diets 1 and 2 (Table 4.9). Fish fed diet 2 (containing E.foetida coelomic fluid) exhibited a significantly lower ($p < 0.05$) carcass lipid content compared with fish fed the control diet (diet 1, Table 4.10).

Erythrocyte fragility: The salinity at which 50% erythrocyte lysis occurred in fish fed the four diets is shown in Table 4.9. No significant difference ($p < 0.05$) emerged between fish fed the four diets.

4.3.5 Experiment D

4.3.5.1 Diets: Freeze dried E.foetida meal was fractioned by Soxhlet extraction (AOAC, 1980) to provide a lipid and lipid soluble fraction and a lipid free fraction. Since this method involved refluxing dried worm meal with petroleum ether (boiling point 60°C) for 5 hours the untreated freeze dried E.foetida and the herring meal used to supply dietary protein in the control diets were also heated at 60°C for 5 hours. Four semi-synthetic diets were offered to rainbow trout in a 56 day feeding trial: Diet 1, in which herring meal supplied all the dietary protein and diet 2 in which untreated E.foetida meal supplied all the dietary protein served as the control fish and worm diet respectively.

The E.foetida lipid and lipid soluble fraction was included in a semi-synthetic diet (diet 3) at the level calculated to be present had all the dietary protein been supplied by E.foetida meal. The lipid free fraction of E.foetida supplied 100% of the dietary protein in a semi-synthetic diet (diet 4).

All diets were formulated to contain 40% crude protein and 14% lipid and the ingredient and nutritional composition of the diets is shown in Table 4.10.

Table 4.10: Experiment D: Ingredient and nutritional composition of the experimental diets

Diet No:	1	2	3	4
Ingredient (% by weight)				
Herring meal	57	-	57	-
Freeze dried *E.foetida* meal	-	67	-	-
Lipid & lipid soluble extract of *E.foetida*	-	-	6.25	-
Lipid-free freeze dried *E.foetida*	-	-	-	64
Cod liver oil	3	3	1	4
Corn oil	6	5	2	10
Corn starch	16	11	16.75	9
Dextrin	9	5	8	4
Vitamin mix*1	2	2	2	2
Mineral mix*1	4	4	4	4
Carboxymethylcellulose	2	2	2	2
Chromic oxide	1	1	1	1
Dietary composition Moisture (%)	7.16	11.60	9.57	11.74
Nutrient content (% dry wt)				
Crude protein	41.66	40.89	41.63	41.98
Lipid	15.00	13.97	14.19	14.17
Ash	10.61	9.14	10.94	8.76

*1 Vitamin and Mineral mix given in Table 2.2.1.

4.3.5.2 RESULTS: Fish growth, feed utilization efficiency, carcass composition and liver somatic index

Fish Growth and Feed Utilization Efficiency: Rainbow trout fed diet 1 (containing herring meal protein) achieved a significantly higher mean final body weight ($p < 0.05$) compared with fish fed diets containing a separated fraction of E.foetida (diets 3 and 4) or untreated freeze dried E.foetida (diet 2, Table 4.11). The percentage increase in weight of fish throughout the experimental period is shown in Figure 4.7. Highest feed utilization efficiency (FCR, PER, Apparent NPU) was achieved by fish fed diet 1 compared with fish fed diets 2, 3 and 4 (Table 4.11). Marginally higher fish growth and feed utilization efficiency was achieved by fish fed diet 4 (containing the lipid free fraction of E.foetida) compared with fish fed diet 3 (containing the lipid and lipid soluble fraction of E.foetida). The performance of fish fed diets 3 and 4 was consistently higher than fish fed diet 2 (containing untreated E.foetida meal, Table 4.11). However, apparent dry matter and nitrogen digestibility coefficients were higher for those diets containing E.foetida meal (diets 2 and 4) compared with diets containing herring meal (diets 1 and 3, Table 4.11).

Carcass Composition and LSI: Although only slight differences were evident between fish fed diets 2, 3 and 4 in terms of growth and feed utilization efficiency, the carcass composition of fish fed these diets was markedly different between treatments (Table 4.11). The carcass of fish fed diets 2 and 3 exhibited a significantly higher ($p < 0.05$) moisture content and a significantly lower ($p < 0.05$) lipid content compared with fish fed diet 1. Similarly, the carcass lipid content of fish fed diet 4 was significantly higher ($p < 0.05$) than fish fed diets 2 and 3,

Table 4.11: Experiment D: Growth, feed utilization, liver somatic index and carcass composition of rainbow trout fed experimental diets for 56 days

Diet No:		1	2	3	4	± SE*1
Mean initial weight (g)		8.54[a]	7.95[a]	9.06[a]	8.13[a]	0.985
Mean final weight (g)		28.50[b]	14.52[a]	18.56[a]	18.30[a]	1.550
Weight gain (%)		233.72	82.64	104.96	125.09	
Specific growth rate (%/day)		2.15	1.08	1.28	1.45	
Food intake (mg/day/fish)		433.27	197.08	260.63	269.22	
Weight gain (mg/day/fish)		348.46	117.28	169.55	181.69	
Food conversion ratio		1.24	1.68	1.54	1.48	
Protain efficiency ratio		1.93	1.45	1.56	1.61	
Apparent N utilization (%)		30.96	22.62	22.70	23.89	
Apparent dry matter digestibility (%)		52.26	65.01	57.34	67.92	
Apparent N digestibility (%)		78.12	86.48	80.74	88.90	
Liver somatic index (%)		1.71[a]	1.60[a]	1.46[a]	1.55[a]	0.081
Carcass composition (% wet wt)	Initial Fish	After 56 days				
Moisture	76.02	73.46[a]	77.38[b]	77.46[b]	74.42[ab]	0.521
Crude protein	15.27	15.53[a]	15.36[a]	14.89[a]	15.01[a]	0.230
Lipid	5.81	8.23[b]	4.98[a]	4.60[a]	7.88[b]	0.298
Ash	2.18	2.45[a]	2.55[a]	2.69[a]	2.37[a]	0.130

*1a,b,c For explanation of terms see Table 2.2.5

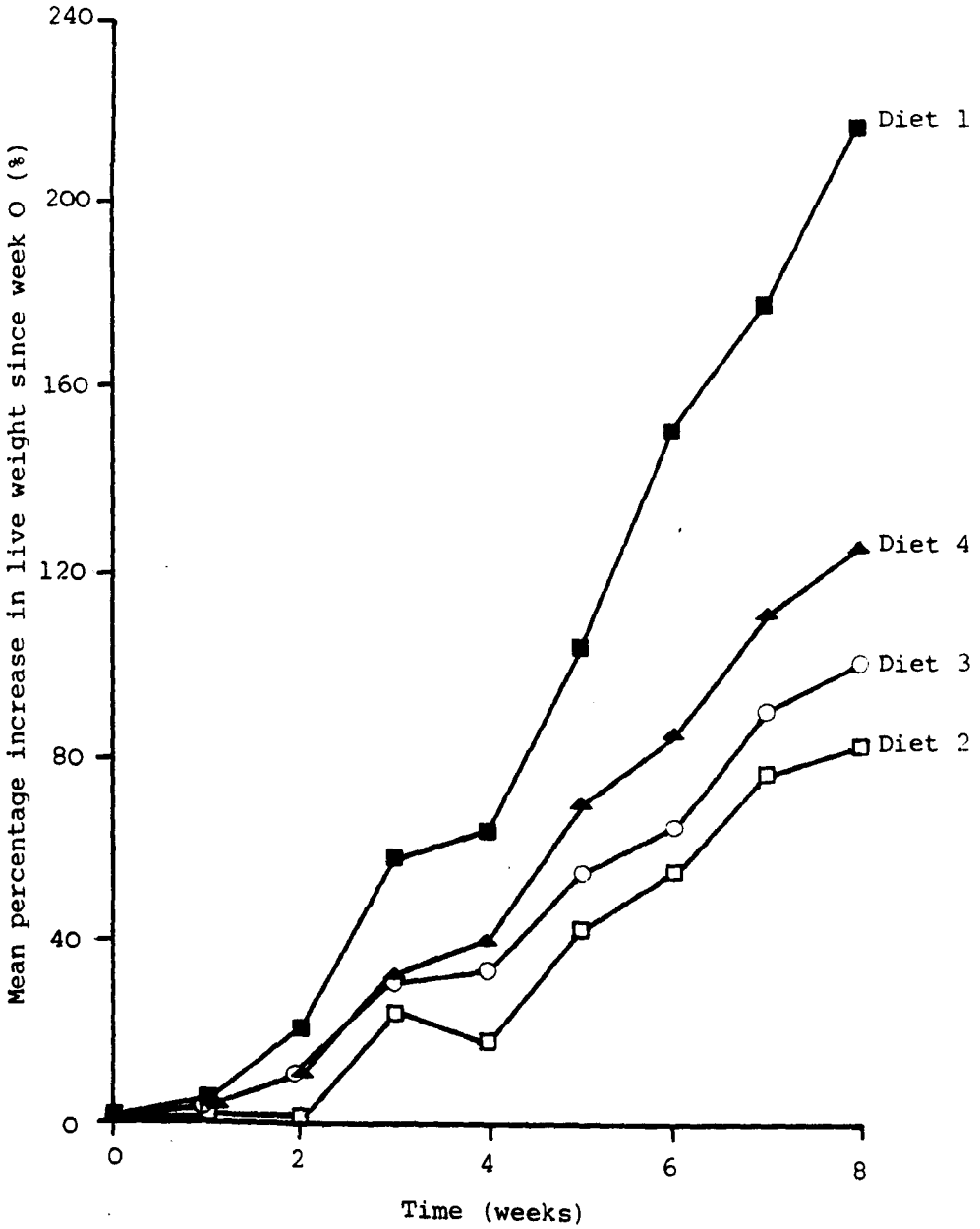


Figure 4.7: Experiment D: Mean percentage increase in weight since week 0 of rainbow trout fed the four experimental diets

although not significantly different ($p < 0.05$) from fish fed diet 1. No significant difference ($p < 0.05$) emerged between fish fed the four diets in terms of LSI and carcass crude protein and ash content.

4.3.6 Experiment E

4.3.6.1 Diets: In this experiment the effect of 'blanching' E.foetida was investigated. Live E.foetida were immersed in boiling water for 5 minutes, blotted dry using a paper towel and stored at -20°C until required. Blanched E.foetida were also freeze dried, ground to produce a homogenous meal (comprising 69.87% crude protein and 6.40% lipid) and used to supply 100% of the dietary protein in a semi-synthetic diet. The performance of fish fed these two diets was compared with fish fed frozen slices of untreated E.foetida and a semi-synthetic control diet in which herring meal supplied all the dietary protein.

The four experimental diets were fed to rainbow trout for a period of 42 days:-

Diet 1: Control - Semi-synthetic diet containing herring meal protein

Diet 2: Semi-synthetic diet containing 'blanched' E.foetida meal protein

Diet 3: Frozen 'blanched' E.foetida

Diet 4: Frozen untreated E.foetida

The dietary formulation of the pelleted feeds and the nutritional composition of the four experimental diets is given in Table 4.12. Due to the short day length during the experimental period fish were fed "to appetite" only once daily, mid-morning.

Table 4.12: Experiment E: Ingredient composition and nutrient content of the experimental diets

Diet No:	1	2	3	4
Ingredient (% by weight)				
Herring meal	60	-		
Freeze dried blanched *E.foetida* meal	-	57		
Cod liver oil	2.5	3.45		
Corn oil	6	6.9		
Corn starch	15	15.77		
Dextrin	7.5	7.88		
Vitamin mix*1	2	2		
Mineral mix*1	4	4		
Carboxymethylcellulose	2	2		
Chromic oxide	1	1		
Dietary composition Moisture (% by weight)	7.69	5.89	77.76	85.43
Nutrient content (%dry weight)				
Crude protein	45.99	40.11	65.91	63.03
Lipid	14.01	14.79	5.95	5.47
Ash	13.68	10.85	11.45	10.32

Frozen slices of blanched *E.foetida*

Frozen slices of untreated *E.foetida*

*1 Vitamin and Mineral mix given in Table 2.2.1.

RESULTS: Fish growth, feed utilization efficiency, carcass composition and liver somatic index

Fish Growth and Feed Utilization Efficiency: A distinct improvement in the feeding response and growth rate of fish fed frozen slices of blanched E.foetida (diet 3) compared with fish fed frozen slices of untreated E.foetida (diet 4) was evident (Figure 4.8). The mean final body weight of fish fed diet 3 was not significantly different ($p < 0.05$) from fish fed the control diet (diet 1, Table 4.13). However fish fed diet 2 (containing 100% dried, blanched E.foetida meal protein) achieved a significantly lower ($p < 0.05$) mean final body weight than fish fed diets 1 and 3 and was comparable to fish fed diet 4. Diet 1 was most efficiently utilized by the fish (measured in terms of FCR, PER and App NPU) and the frozen blanched E.foetida was also well utilized (Table 4.13). Poor feed utilization efficiency was achieved by fish fed diets 2 and 4.

After the initial two weeks of the experiment those fish fed frozen blanched E.foetida (diet 3) achieved similar rates of weight gain (% wt.gain/day/fish) as fish fed the control trout pellet (Table 4.14). However the daily feed intake (mg/day/fish, dry weight) of fish fed diet 3 remained consistently lower than fish fed the control pellet throughout the experimental period.

Table 4.14: Experiment E: Bi-weekly feed intake (mg/fish/day,dry wt) and % increase in weight (%/fish/day) of rainbow trout fed the four experimental diets

Week number:	0-2	2-4	4-6
Diet 1: Feed intake*1 (% weight gain*1)	506.43(1.76)	529.29(0.73)	392.14(0.30)
Diet 2: Feed intake (% weight gain)	286.43(0.40)	195.00(0.01)	146.43(0.05)
Diet 3: Feed intake (% weight gain)	285.00(0.76)	372.86(0.59)	267.14(0.47)
Diet 4: Feed intake (% weight gain)	210.00(0.38)	245.71(0.16)	167.86(-)

*1 Values given are the mean value for fish fed each diet.

Table 4.13: Experiment E: Growth, feed utilization, liver somatic index and carcass composition of rainbow trout fed experimental diets for 42 days

Diet No:		1	2	3	4	± SE*2
Mean initial weight (g)		31.38[a]	32.06[a]	31.33[a]	31.99[a]	1.603
Mean final weight (g)		44.87[b]	34.14[a]	39.99[b]	33.31[a]	2.051
Weight gain (%)		42.99	6.49	27.64	4.31	
Specific growth rate (%/day)		0.852	0.149	0.580	0.098	
Food intake (mg/day/fish)		475.95	209.29	1387.14	1425.71	
Food intake (mg/day/fish,dry wt)		439.29	196.90	308.57	207.52	
Weight gain (mg/day/fish)		1.37	3.98	1.50	6.60	
Protein efficiency ratio		1.58	0.63	1.01	0.24	
Apparent N utilization (%)		26.01	4.39	19.12	0.73	
Liver somatic index (%)		1.74[c]	1.34[ab]	1.57[bc]	1.27[a]	0.091
Carcass composition (% wet wt)	Initial Fish	After 42 days				
Moisture	73.91	74.04[a]	76.37[b]	74.87[a]	76.77[b]	0.419
Crude protein	16.82	16.79[ab]	16.31[a]	17.42[b]	16.38[a]	0.260
Lipid	6.10	7.22[c]	4.79[a]	5.69[b]	4.49[a]	0.276
Ash	2.24	2.39[a]	2.66[a]	2.36[a]	2.41[a]	0.189

*1 FCR = Food intake (dry wt)/Weight gain (live weight)

*2a,b,c = For explanation of terms see Table 2.2.5

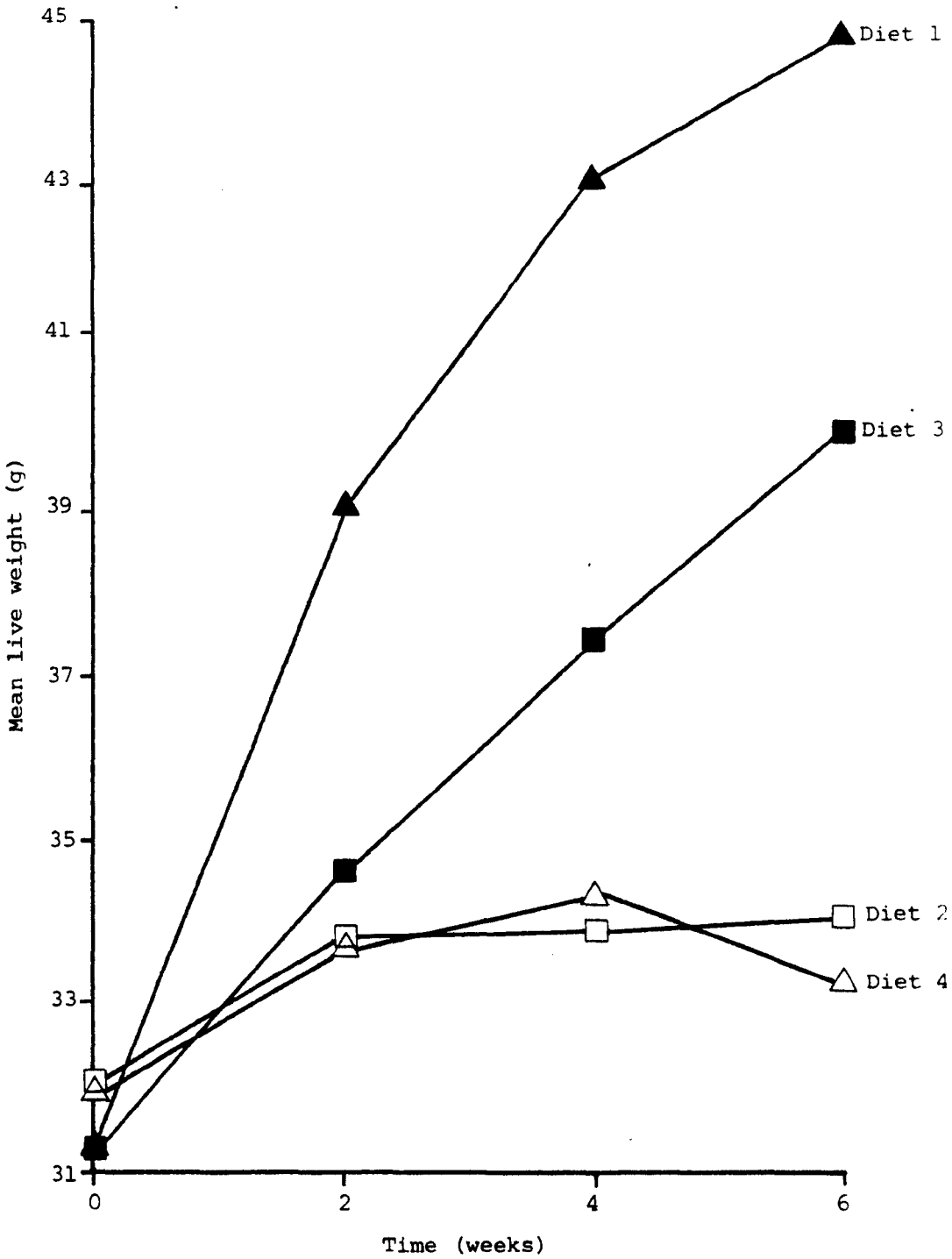


Figure 4.8: Experiment E: The growth response of rainbow trout fed the four experimental diets

4.4 GENERAL DISCUSSION

Rainbow trout maintained on diets containing a high proportion of E.foetida meal or consisting solely of frozen slices of untreated E.foetida generally exhibited reduced rates of growth and weight increase, poor feed utilization efficiency (as measured by FCR, PER and App NPU) and significantly altered carcass composition at the end of the feeding period when compared with fish fed a control trout pellet. This general trend, initially observed in Experiments 2.1 and 3.1 has been confirmed in the results of each of the present experiments (A-E), summarised in Tables 4.5, 4.7, 4.9, 4.11 and 4.13. The carcass composition of fish fed high levels of E.foetida in the diet in Experiments A-E generally had a significantly reduced lipid content and a significantly increased moisture content compared with fish fed the control, herring meal based trout pellet.

However, although feed utilization efficiency has usually been distinctly reduced as a result of including high levels of E.foetida in fish diets, the apparent digestibility coefficients of the dry matter and nitrogenous constituents of these diets remained high (Diets 3 & 4, Table 4.9, Diets 2 & 4, Table 4.11). This supports the suggestion that reduced feed intake and growth of fish fed diets containing E.foetida may be attributed to the unpalatable nature of the earthworm possibly as a result of the fetid odour/taste of its coelomic fluid.

An investigation into the relative acceptability of the two possible sub-species of E.foetida to rainbow trout in Experiment B indicated that while E.fetida andrei may be

marginally more acceptable to the fish, exhibiting slightly higher feed utilization efficiency and a less altered carcass composition compared with fish fed E.f.fetida (Table 4.7), the growth performance of fish fed E.f.andrei remained very poor and some method of pre-treatment would be essential before either of these earthworms could be satisfactorily used in trout diets.

The fetid smell/taste of E.foetida coelomic fluid may have been sufficient to reduce the feed intake of rainbow trout. For example, certain of the S volatiles present in E.foetida and possibly responsible for this malodour were tentatively identified as ethanethiol, dimethyl sulphide and diethyl disulphide (Figure 4.4) and similar S compounds: methanethiol and dimethyl disulphide were identified by Shiomi et al. (1982a,b) as responsible for the fetid odour of two species of muricid gastropod and the flat head Calliurichthys doryssus. The common occurrence of S volatiles in organic rich substrates in which earthworms are commonly cultured has also been reported (Mitchell et al., 1980; Waugh and Mitchell, 1981) and the presence of substrate within the gut of E.foetida may have contributed to the pattern of S volatiles emerging on the chromatogram (Figure 4.3 and 4.4). Since L.terrestris, used for comparative purposes, ingests predominantly mineral soil the absence of S volatiles might have been expected. However, the pattern of S volatiles from E.foetida coelomic fluid, uncontaminated by substrate suggested that the S volatiles present in E.foetida may be attributed to the earthworm and not directly to the substrate within the gut lumen. The results of Experiment A (Table 4.7) show that by maintaining E.foetida on various

substrates no significant improvement was achieved regarding the subsequent acceptability of the earthworms to rainbow trout.

E.foetida, subjected to the more severe treatment processes involved in blanching and in the production of the feed attractant ("ACE", British Groundbaits), contained similar S volatiles to those present in live E.foetida and several additional unidentified S compounds (Figure 4.4). The E.foetida based feed attractant has been shown to stimulate a feeding response in eels, when used at low concentrations (Delves-Broughton, pers.comm.) and the blanched, frozen E.foetida supported good fish growth in Experiment E. The presence of certain S compounds, for example those contributing to a meaty aroma, may enhance the palatability of feeds and these compounds may also effectively mask the presence of other compounds which may be responsible for the unpalatable nature of a feed.

The short-term screening test employed to assess the value of any treatment process applied to E.foetida in improving the palatability of these worms to rainbow trout indicated that blanched, frozen E.foetida was more acceptable than untreated frozen E.foetida (Table 4.1). This preliminary assessment was confirmed by the results of Experiment E. In all the short term feeding tests conducted, the fish apparently consumed a relatively high percentage of the untreated E.foetida offered (Table 4.1). This may be attributed to the fact that throughout the feeding tests rainbow trout were fed solely on the earthworms being tested and no additional feed was offered. As a result fish were underfed and may have more readily consumed an unpalatable feed.

A significant improvement was observed in Experiment E,

in the feed intake, growth response and feed utilization efficiency of fish fed frozen slices of untreated E.foetida. At the end of the 42 day experimental feeding period the mean final body weight of fish fed the frozen blanched E.foetida was not significantly different from fish fed the control trout pellet (Table 4.13).

In one single step the blanching process achieved the removal of a large proportion of the coelomic fluid from E.foetida and also subjected the earthworms to heat treatment of approximately 100°C for 5 minutes.

The coelomic fluid of E.foetida had previously been demonstrated in Experiments B & C to cause a reduction in the feed intake, rate of growth and feed utilization efficiency of fish fed pellets mixed with E.foetida coelomic fluid compared with fish fed identical pellets mixed with distilled water (Experiment B, diets 1 & 2, Table 4.7; Experiment C, diets 1 & 2, Table 4.9). However, the performance of fish fed diets containing E.foetida coelomic fluid was not reduced to the extent which might have been expected if 100% of the dietary protein were supplied by dried E.foetida meal (Experiment 3.1). Furthermore, diets comprised of E.foetida from which the coelomic fluid had been extracted (Experiment B, diet 3, Experiment C, diet 3) were consumed in very small amounts by the fish and fish fed these diets attained mean final body weights which were not significantly different from fish fed the diets containing untreated E.foetida (Table 4.7; 4.9). Therefore the coelomic fluid may only partially provide an explanation for the unpalatable nature of E.foetida to rainbow trout.

The coelomic fluid of E.foetida has been reported to

contain haemagluttinins and a haemolytic factor, which has been shown to cause haemolysis of various vertebrate erythrocytes (Andrews and Kukulinsky, 1975; Roch et al., 1981). The presence of phytohaemagluttinins (lectins) in various plant products make them unsuitable for use as food for higher animals unless they are properly cooked. Certain haemagluttinins are rapidly digested by pepsin and in order to exhibit a toxic action when ingested in the feed the haemagluttinins must resist digestion (Jaffe, 1980). Rats fed diets containing bean lectin have been shown to exhibit reduced nutrient absorption and therefore reduced growth rates (Jaffe, 1960).

It is believed unlikely that a haemolytic factor or haemagluttinin was responsible for the reduced feed intake of fish fed E.foetida since the apparent digestibility coefficients of pellets containing a high proportion of dried E.foetida meal were high (Tables 3.1.2, 3.2.2, 4.9, 4.11). The Eisenia foetida andrei factor, a bacteriostatic factor with haemolytic properties investigated by Roch et al. (1981) was considered to be two different molecular species with molecular weights of 40,000 and 45,000 (lippoprotein containing an associated glycoprotein). It was considered unlikely that large molecules such as these would be absorbed intact into the bloodstream of the fish without prior digestion. Haemolysins and haemagluttinins have also been reported to be present in earthworm extracts other than E.foetida. For example, L.terrestris (Cooper et al., 1974; Wojdani et al., 1982), an earthworm species which was readily consumed by rainbow trout and which supported rapid fish growth in Experiment 2.1. Moreover, the fragility of erythrocytes in the fish blood was not adversely affected by the dietary inclusion of E.foetida in Experiment C (Table 4.9).

Heat treatment of feeds may be used to beneficial effect for example in denaturing anti-nutritional factors such as the trypsin inhibitor present in soy beans (Liener, 1980). Furthermore, the activity of the haemolytic factor present in the coelomic fluid of E.fetida andrei has been shown to be destroyed by heating at 56°C for 15 min. (Roch et al., 1981) and the haemolysin described by Andrews and Kukulinsky (1975) in E.foetida was labile at 65°C for 20 mins. Haemagluttinins present in the coelomic fluid of L.terrestris were labile at 56°C for 30 mins (Cooper et al., 1974). However, heat treatment can also be detrimental, for example the amino acids, methionine, arginine, tryptophan and lysine have been shown to be readily damaged in overheated fish meal (Carpenter et al., 1962).

The effect of heat treatment depends upon the temperature involved, the duration of heating and the presence of moisture and reducing substances. Boiling in excess water, such as in the blanching process used to rid E.foetida of the coelomic fluid, causes no damage to the nutritive value of the feed (Bender, 19[illegible]). Carpenter et al. (1962) and Lea and Hannan (1949) have demonstrated that damage caused by heat is most severe in the presence of 5-14% moisture and this may explain the comparatively low apparent digestibility coefficients recorded for diet 4, Experiment C (Table 4.9) in which oven dried E.foetida meal supplied 100% of the dietary protein.

For the purposes of Experiment D, freeze dried E.foetida was subject to heat treatment of 60°C for 5 hours. Dry materials have been shown to be relatively resistant to damage by heat (M[illegible]ller, 1956). The feed intake, weight gain and feed utilization efficiency of rainbow trout fed the diet containing

100% heat treated E.foetida meal in Experiment D (diet 2, Table 4.11) appeared to have greatly improved when compared with the performance of rainbow trout fed diet 3 (100% freeze dried E.foetida meal protein in Experiment 3.1 (Table 3.1.2)). However, a direct comparison between the two experiments is not valid due to differences in fish size and experimental conditions and until further experimentation directly demonstrates the effect that heat treatment may have on the acceptability of E.foetida to rainbow trout it remains a matter of conjecture. Reformato et al. (1983) working with garter snakes, Thamnophis sirtalis have shown that the components in an extract from the earthworm L.terrestris which elicited a positive feeding response by the snakes retained their biological activity after heating at 100°C for 15 mins. This may also possibly apply to E.foetida.

Although fish fed frozen blanched E.foetida achieved a mean final body weight which was not significantly different ($p < 0.05$) from fish fed the control trout pellet in Experiment E, their overall weight gain during the feeding period was lower than fish fed the control diet (Table 4.13, Figure 4.8). Several factors may have been responsible for this:-

(1) Due to a short day length during Experiment E, fish were fed only once daily. It has been suggested previously (Section 2.2.4) that the high moisture content of frozen earthworm diets may physically restrict food intake in a single meal to the extent that fish fed frozen earthworms only once daily may be unable to satisfy their daily energy requirement for maximum growth.

(2) Prior to being selected for use as experimental fish the rainbow trout had been previously maintained on a standard commercial trout pellet. Therefore during the initial two weeks of the experimental period fish fed frozen earthworm diets had to become accustomed to a new type of diet. Table 4.14 clearly shows that the largest difference in terms of feed intake and weight gain between fish fed diet 1 (the control trout diet) and diet 3 (frozen slices of blanched E.foetida) occurred during weeks 0-2. Thereafter rainbow trout fed these diets achieved a similar rate of weight gain (Table 4.14).

(3) The factor/factors responsible for the unpalatable nature of E.foetida may not have been completely eliminated from the earthworm by the blanching process and further improvement may yet be achieved.

Fish fed frozen slices of blanched E.foetida in Experiment E achieved a FCR similar to fish fed the control diet (Table 4.13). However, their efficiency of feed utilization on the basis of PER and Apparent NPU was lower than for fish fed the control diet. As discussed in Section 2.1.4, diets consisting solely of frozen earthworms supply protein (on a dry weight basis) in excess of the dietary protein requirement of rainbow trout and under these circumstances protein may be catabolised as an energy source (Lee and Putnam, 1973).

Fish fed the frozen blanched E.foetida in Experiment E exhibited a significantly lower ($p < 0.05$) carcass lipid content compared with fish fed the control diet (Table 4.13). It is therefore possible that a factor within the earthworm responsible for the alteration in the fish carcass composition was not

eliminated in the process of blanching E.foetida. A similar alteration in the fish carcass composition was also reported when rainbow trout were fed frozen slices of A.longa and L.terrestris in Experiment 2.1, frozen slices of D.veneta and D.subrubicundus in Experiment 2.2 and free dried D.subrubicundus in Experiment 3.3.

The extraction of lipid and lipid soluble components from E.foetida and the inclusion of the resulting lipid and lipid soluble and lipid-free fractions of E.foetida in diets for rainbow trout in Experiment D produced a significant effect on the carcass composition of the fish (Table 4.11). Fish fed a diet containing the lipid-free fraction of E.foetida exhibited a carcass lipid content not significantly different ($p < 0.05$) from fish fed the control diet; while fish fed the diet containing the lipid and lipid soluble fraction of E.foetida exhibited a significantly reduced ($p < 0.05$) carcass lipid content compared with fish fed the control diet, but not significantly different ($p < 0.05$) from fish fed untreated E.foetida (Table 4.11). Among the factors within this lipid and lipid soluble fraction of E.foetida which may possibly account for this phenomena are the sterols, including the anabolic steroids: testosterone and growth hormone. These are likely to have been present within the sewage substrate on which E.foetida were cultured (Dorfman and Ungar, 1965) and may have been present within the earthworm. Testosterone and growth hormone have been shown to cause loss of body fat through oxidation in mammals (Bell et al., 1972). However, enhanced growth rates have also been commonly associated with the presence of anabolic steroids in the diet at low dosage

levels (Higgs et al., 1976; Yamazaki, 1976) which is contrary to the results reported in Experiment D.

Although the fat and fat soluble fraction of E.foetida constituted only a small proportion of the diet (6.25%, diet 3, Table 4.10), the major part of the diet being supplied by herring meal (strongly flavoured), a highly significant effect on the feed intake and feed utilization efficiency of fish fed this diet compared with fish fed the control diet (diet 1) was evident (Table 4.11).

In conclusion: the process of 'blanching' has been demonstrated to significantly improve the acceptability of E.foetida to rainbow trout, however the extent to which this improvement was due to the heat treatment involved or to the elimination of a large proportion of the earthworm coelomic fluid achieved by this process was not established. Certain S volatiles (ethanethiol, dimethyl sulphide and diethyl disulphide) within E.foetida and the coelomic fluid were tentatively identified as responsible for the offensive odour of this earthworm species. Rainbow trout fed frozen blanched E.foetida also exhibited a significantly reduced carcass lipid content compared with fish fed a control trout pellet, a trend commonly observed in fish fed untreated E.foetida. The removal of the lipid and lipid soluble fraction from E.foetida ensured that the carcass composition of the fish fed diets containing lipid free E.foetida meal was not significantly different from fish fed a control trout pellet.

CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

5. GENERAL DISCUSSION AND CONCLUSIONS

Five species of earthworm: Lumbricus terrestris; Allolobophora longa; Eisenia foetida; Dendrobaena veneta and Dendrodrilus subrubicundus, have been assessed in the course of experiments described in this thesis as a possible feed or feed supplement for rainbow trout.

Chemical evaluation of the nutritional composition of these earthworms suggested that earthworm tissue may represent a valuable high quality source of protein for use in trout diets having a well balanced essential amino acid profile suitable for salmonids (Tables 1.5, 2.1.2, 2.2.3 and 3.3.3). Some suggestion has been made that earthworm tissue may be deficient in the sulphur amino acids, methionine and cystine (Yoshida and Hoshii, 1978; Amerio, 1983; Hilton, 1983; Hori et al., 1983); for the majority of natural food organisms known to form part of the food chain of rainbow trout, methionine (plus cystine) is generally considered to be the first limiting amino acid present (Yurkowski and Tabachek, 1979).

The species of earthworm known to inhabit the mineral soil horizon: L.terrestris and A.longa generally had a low tissue lipid content and high ash content; in contrast those earthworm species which apparently thrive in organic rich substrates: E.foetida, D.veneta and D.subrubicundus had ash and lipid levels comparable to conventional fish feeds (Tables 2.1.1 and 2.2.1). The lipids of earthworms have been shown to consist of many polyunsaturated fatty acids including those essential in the nutrition of rainbow trout (Tables 2.1.2 and 2.2.4; Cerbulis, 1967; Hansen and Czochanska, 1975;

Castell, 1979). The ash content of earthworms is mainly derived from substrate within the gut and this can be readily eliminated by holding earthworms on an inert substrate for several hours.

Mineral elements within the earthworm tissue, for which an essential dietary requirement has been established for rainbow trout, were apparently present within the earthworms in sufficient concentration and the low Ca content of earthworm tissue (due to the lack of a bony skeleton) may be compensated for by the fish through absorption of Ca via the gills and fins from the surrounding water (Lall, 1979; Tables 2.1.6, 2.2.9 and 3.3.6). Micro-elements present within the earthworm tissue generally exceeded the dietary requirement of rainbow trout and it was therefore possible that these elements and other micro-elements present with no known biochemical function (Cd and Pb, Vallee and Ulmer, 1972) may accumulate within the fish tissues and pose a potential toxicological hazard.

The biological evaluation of earthworms as a feed for rainbow trout was initially investigated by offering the fish frozen slices of each of the five species of earthworm as the sole diet for the duration of the feeding trial. In this way L.terrestris, A.longa and E.foetida were evaluated in experiment 2.1 and D.veneta and D.subrubicundus in experiment 2.2. In each case the relative value of earthworms as a feed for rainbow trout was compared with a commercially available trout pellet and conclusions were based upon measurements of fish growth, feed utilization efficiency and final fish carcass composition.

The growth performance of fish fed frozen slices of the earthworms L.terrestris and A.longa in experiment 2.1 and D.veneta and D.subrubicundus in experiment 2.2 was comparable to fish fed the commercial trout pellet. The FCR achieved by fish fed the frozen earthworm diets indicated a satisfactory level of feed utilization especially in view of the elevated ash content of some of these diets (Tables 2.1.3 and 2.2.5). Although no direct measurement could be made of the coefficients of apparent dry matter and nitrogen digestibility the FCR achieved by fish fed only frozen earthworm inferred a high digestibility of dietary components.

In terms of dietary protein utilization, rainbow trout maintained solely on frozen earthworm achieved PER and apparent NPU values which were below those recorded for fish fed the commercial trout pellet. This was believed to be due to the earthworm diets supplying dietary protein in excess of the fishes daily requirement (on a dry weight basis; Cowey and Sargent, 1979) in which case the surplus protein may be utilized as an energy source (Lee and Putnam, 1973; Sections 2.1.4 and 2.2.4).

Fish achieving similar growth rates in experiments 2.1 and 2.2 were observed to exhibit the same dry matter feed intake over the experimental period, despite the ingestion of diets with very different moisture contents (Tables 2.1.3 and 2.2.5). It has generally been accepted that fish, like mammals, eat to meet their energy requirements (Rozin and Mayer, 1961; Lee and Putnam, 1973; Page and Andrews, 1973; Cho et al., 1976) and the results of these experiments appear to contradict this theory (Sections 2.1.4 and 2.2.4). However, difficulties arise in attributing an energy conversion factor to the carbohydrate

fraction of the different diets (frozen earthworm and commercial trout pellet) and until this matter is resolved no conclusions can be drawn.

Rainbow trout fed solely on frozen slices of the earthworm E.foetida found this diet to be unpalatable, consuming only small quantities of the feed offered and exhibiting virtually no growth over the experimental period (experiment 2.1, Table 2.1.3 and Figure 2.1.2).

Earthworms were subsequently dried and ground to produce a 'worm meal' and each of the species D.veneta, E.foetida and D.subrubicundus was thus evaluated as a potential fish meal replacer in formulated rations for rainbow trout in experiments 2.2, 3.1 and 3.3 respectively.

At low levels of dietary inclusion dried E.foetida meal (constituting 5, 10, 20 and 30% by weight of a production salmonid diet in experiment 3.2) and dried D.subrubicundus meal (replacing 10% of the fish meal protein in experiment 3.3) adequately replaced the fish meal component of a trout ration without loss of fish growth performance and feed utilization efficiency.

A significant decline in the feed intake and growth rate of fish fed high levels of dried E.foetida meal in the diet (replacing 50 and 100% of the fish meal protein) was evident compared with the performance of fish fed diets containing only fish meal protein (Table 3.1.2). This was believed to be due to the unpalatable nature of E.foetida to rainbow trout previously observed in experiment 2.1. However, a significantly reduced feed intake and growth response was also observed in rainbow trout fed

high dietary inclusion levels of dried D.veneta meal (replacing 50% of the dietary fish meal protein; experiment 2.2) and dried D.subrubicundus meal (replacing 50 and 100% of the dietary fish meal protein; experiment 3.3). This result was unexpected since these earthworm species were apparently highly palatable to rainbow trout when offered as frozen slices of whole worm. Although the factor responsible for the observed reduced growth of fish fed these rations was not identified, it was believed to have been due to a nutritional deficiency or imbalance in these diets (Sections 3.1.4 and 3.3.4).

Feed utilization efficiency measured in terms of FCR, PER and apparent NPU exhibited a tendency to decline with increasing dietary inclusion of worm meal. However, both dry matter and nitrogen digestibility coefficients increased with increasing levels of worm meal in the diet (Tables 3.1.2 and 3.3.4).

In the majority of feeding trials the dietary inclusion of worm meal resulted in a significantly altered carcass composition of the rainbow trout compared with fish fed a fish meal based ration. Generally, fish fed diets containing earthworms (fed as frozen slices or dried meal) exhibited a significantly decreased carcass lipid content which was associated with an increased carcass moisture content and/or increased carcass crude protein content (Tables 2.1.3, 2.2.5, 3.1.2 and 3.3.4).

Although earthworm lipids contained a higher proportion of saturated fatty acids and short chain fatty acids compared with the trout ration containing only fish meal protein (Table 2.2.4) this was demonstrated to have no significant effect on

the fatty acid composition of the muscle and liver tissue of fish fed diets containing frozen earthworms (Tables 2.2.6, 2.2.7; Section 2.2.3). The fish were apparently capable of preferentially oxidizing saturated fatty acids in order to maintain the required tissue fatty acid profile (Section 2.2.4; Yu et al., 1977; Mugridichian et al., 1981).

The causative agent responsible for the alteration in proximate composition of the fish carcass of fish fed diets containing earthworms has been fully discussed in Sections 2.1.4, 2.2.4 and 3.3.4. When E.foetida was offered to the fish at high levels in the diets the resulting altered carcass composition may have been due to an inadequate feed intake by the fish and subsequent utilization of body lipid reserves, which may also be associated with a decreased liver somatic index (LSI) and increased carcass moisture content (Brett et al., 1969; Pandian and Raghuraman, 1972). However, when diets were derived from species of earthworm apparently palatable to rainbow trout the effect may have been due to a component within the earthworm, for example the carbohydrate fraction of earthworm tissue or anabolic steroids derived from the sewage sludge from which some of the earthworm species originated. In the latter case the decreased lipid content may also be associated with normal LSI and increased nitrogen retention (Bell et al., 1972).

Fish fed diets containing earthworms (fed either as frozen slices or dried meal) exhibited no symptoms which could be attributed to a vitamin or mineral deficiency. All pelleted rations were formulated to contain a vitamin and mineral premix (Table 2.2.1) which ensured an adequate supply of these essential dietary constituents.

During several of the experimental feeding trials, levels of selected micro-elements were monitored in diets, fish carcass and fish tissue in order to investigate the possible accumulation of such elements within the fish which may have been correlated with the inclusion of earthworms in the diet.

The capacity of earthworms to accumulate certain micro-elements, in particular Cd, Pb, Zn and Cu, within their tissues from their surrounding substrate is well documented (Table 5.1). Certain discrepancies within Table 5.1 may arise from different interpretation of the term 'accumulate'. On the one hand accumulation is considered to occur if the concentration of the element in the earthworm tissue exceeds the concentration in the substrate. On the other hand, a positive correlation between the concentration of an element in the earthworm tissue and increasing concentrations of that element in the substrate is regarded as indicative of accumulation. It is also clear that interspecific differences occur in the quantity of heavy metals taken up by earthworms (Ireland and Richards, 1977).

The levels of micro-elements measured within the earthworm species employed as diets in the present feeding trials with rainbow trout generally fell within the range of values reported within earthworm tissue (Table 5.1).

Concentrations of these elements were, in general, below the maximum levels permitted in salmonid feedstuffs (Edwards and Densem, 1980). Of notable exception was the concentration of Fe, Zn and Mn in the earthworms L.terrestris and A.longa employed as diets in experiment 2.1, and this was presumed to be due to the high mineral soil content within the

Table 5.1: Evidence for the accumulation of heavy metals from the surrounding substrate into the earthworm tissue

Earthworm species studied	Elements investigated which accumulated within the earthworms	Elements investigated which did not accumulate within the earthworms	Reference
L.terrestris A.chlorotica Allolobophora trapezoides Allolobophora turgida	Cd,Zn,Ni	Pb	Gish & Christensen (1973)
Allolobophora spp. Lumbricus spp. Octolasium spp.	Cd,Zn	Pb	Van Hook (1974)
Dendrobaena rubida	Pb	-	Ireland (1975a)
L.rubellus D.rubida	Pb	Cu,Zn	Ireland & Richards (1977)
A.caliginosa A.chlorotica A.longa L.rubellus	Cu	-	Van Rhee (1977)
L.terrestris	Cd,Zn,Cu,Pb	-	Czarnowska & Jopkiewicz (1978)
Apporectodea tuberculata	Cd,Zn,Co,Hg	Hg,Sc,Tb	Helmke et al. (1979)
L.rubellus D.veneta E.tetrahedra	Cd,Pb	Cu,Zn,Mn	Ireland (1979)

(continued)

Table 5.1 (continued):

Earthworm species studied	Elements investigated which accumulated within the earthworms	Elements investigated which did not accumulate within the earthworms	Reference
E.foetida	Cd,Zn,Cr,Cu	-	Mori & Kurihara (1979)
L.terrestris L.rubellus A.chlorotica	Pb,Cd,Cu	Fe	Ash & Lee (1980)
E.foetida (4 weeks)	Cu,Zn,Pb	Cd,Cr,Ni	Hartenstein et al. (1980b)
E.foetida (longer term)	Cd,Zn,Ni,Pb,Cu	-	Hartenstein et al. (1980a)

earthworm gastro-intestinal tract. However, levels of certain non-essential elements, in particular Pb and Cd, within the earthworms were commonly observed to exceed the maximum values recorded for these elements within trout feeds commercially available in Europe (Tacon and de Silva, 1983).

Assessment of the maximum permitted concentration for elements within trout diets is complex since the absolute level of any element measured does not provide a direct indication of its biological availability. This may be dependent upon the chemical form of the element in question and the presence of numerous other elements with which antagonistic and synergistic reactions may occur. Of particular importance in this context is the macro-element Ca, which serves to protect organisms from the uptake of potentially toxic elements including Cd, Pb, Zn and Cu (Underwood, 1977). Since the level of Ca within the earthworm tissue is low compared with conventional fish feeds (Table 2.1.1 and 2.2.2) it presumably exerts only a minor protective role against micro-element uptake into the tissues of fish fed earthworm diets.

There was some evidence of micro-element uptake or accumulation within the tissues of fish fed earthworm containing rations (Tables 2.1.4, 2.2.8 and 3.3.5). In particular Pb was present at significantly higher concentrations in the carcass of fish fed diets containing high levels of the earthworm D.subrubicundus compared with fish fed a control trout ration. On closer examination levels of Pb in the fish carcass increased both with time and with increasing dietary inclusion of dried D.subrubicundus meal (and therefore increasing Pb concentration in the diet; Table 3.3.5) and the Pb was observed to concentrate

within the bone and bony tissue of the fish (Table 3.3.7).

When low levels of dried E.foetida meal (5-30% by weight) were included in a production salmonid diet there was no significant relationship between the level of Fe, Zn, Pb, Cu and Cd in the fish carcass, after a feeding period of 84 days, and the dietary inclusion level of worm meal.

The bio accumulation of Cd by earthworms is known to exceed that of any other element investigated (Helmke et al., 1979; Ireland, 1979; Mori and Kurihara, 1979; Wade et al., 1982). Furthermore, Cd may pose a potential threat if present in feedstuffs due to its toxicity, mobility, efficiency of deposition and long half life in animal tissues (Helmke et al., 1979; Wade et al., 1982). However, no significant accumulation of Cd within the carcass of fish fed diets comprised of earthworm was observed in the present feeding trials. Recent research suggests that fish may possess mechanisms by which to achieve the elimination and regulation of mineral elements, including non-essential elements such as Cd (Cross et al., 1973; Cearly and Coleman, 1974; Miettinen, 1975; Singh and Ferns, 1978). Alternatively, Cd within the earthworms may have been present in a form biologically unavailable to the fish.

During preparation of the experimental diets no attempt was made to reduce the heavy metal burden of diets containing earthworms either by elimination of the substrate matter within the earthworm gut or removal of the surface mucus coat to which heavy metals may be adsorped (Ireland and Richards, 1981).

At present E.foetida is the earthworm species most widely grown in vermiculture and research in earthworm culture has principally concentrated on this species. Consequently

further investigations were conducted with a view to improving the acceptability of E.foetida to rainbow trout. The foul smelling yellow coelomic fluid ejected by this earthworm species as a defense mechanism may have caused the reduced feed intake by rainbow trout and analysis of the volatile sulphur components within the E.foetida and a coelomic fluid extract from E.foetida revealed the presence of potentially noxious compounds possibly responsible for the low palatability of this earthworm species (Section 4.2.2.2).

A blanching process, designed to rid the E.foetida of this potentially noxious coelomic fluid fraction significantly improved the acceptability of this species of earthworm, fed in frozen slices of whole worm to rainbow trout and no significant difference in growth performance was observed between fish fed frozen slices of blanched E.foetida and fish fed the control, fish meal based diet. Heat treatment of the earthworms (100°C for 5 mins.) involved in the blanching process may also have been partially responsible for the improvement in the palatability of E.foetida to rainbow trout. This may have been achieved as a result of possibly denaturing unpalatable or anti-nutritional components within the earthworm; and further experimentation would be necessary to establish the relative value of removing the earthworm coelomic fluid and subjecting the earthworm to heat treatment in achieving the improved feeding response by rainbow trout. However, no significant improvement appeared to have been made in the feed intake and growth response of rainbow trout fed diets containing high levels of freeze dried blanched E.foetida meal (Table 4.13). Since a similar depressed feeding

response and growth rate was observed when rainbow trout were fed high dietary levels of dried worm meal derived from earthworm species other than E.foetida an additional factor, ubiquitous to all species of earthworm tested may have been responsible for these results. This was previously attributed to a nutritional imbalance or deficiency and further experimentation involving the supplementation of diets containing worm meal with synthetic amino acids may resolve this question.

An experiment designed to isolate the unpalatable fraction of E.foetida co-incidentally revealed that the factor responsible for the alteration in the proximate composition of the carcass of fish fed high levels of E.foetida in the diet lay within the lipid and lipid soluble components of E.foetida. Fish fed a lipid free extract of E.foetida exhibited no significant alteration in carcass composition compared with fish fed a fish meal based ration.

There are several possible hazards associated with the use of earthworms as an animal feed which must be closely monitored before earthworms can be incorporated into livestock feeds:-
(i) Faecal wastes, on which earthworms can be cultured are likely to contain pathogenic bacteria and/or viruses (Foot, 1977) and this is a major restraint in using these wastes in direct re-feeding and for the fertilization of fish ponds. Only scant attention has been paid to this aspect in vermiculture. Some evidence has been presented which suggests that the population of Salmonella enteritides ser typhimurium is reduced after passage through the gut of E.foetida (Mitchell et al., 1977; Brown and Mitchell, 1981). A major hazard which may therefore arise from

the use of the earthworms, grown on faecal wastes, as feed for fish is the transmission of viable pathogenic organisms to the fish and ultimately to man.

(ii) Earthworms, of various species, have been cited as intermediate hosts in the life cycles of various parasites, many of which invade domestic fowl and wild birds as their final hosts (Rysavy, 1969). The majority of the eggs and larvae of these parasitic worms pass through the earthworm gut without being damaged (Rysavy, 1969).

(iii) Biodegradable waste materials often contain organic residues of potentially toxic compounds such as pesticides, herbicides and antibiotics which may accumulate or be passed on through the food chain in which earthworms form a link. Earthworms can accumulate and concentrate heavy metals from their surrounding substrate into their body tissue (Table 5.1). Tacon and Ferns (1976) and Singh and Ferns (1978), feeding activated sewage sludge directly to rainbow trout as part of a formulated diet, gave evidence of the accumulation of certain potentially toxic mineral elements in the fish. Unless earthworms alter the chemical form of these elements, making them biologically unavailable, then similar problems may be encountered when earthworms grown on such wastes are used in diets for fish. Levels of heavy metals within the earthworm have been reported as potentially hazardous in natural food chains (Gish and Christensen, 1973; Helmke et al., 1979) and positive accumulation through the food chain has previously been demonstrated (Ireland, 1977).

At present the cost of using earthworms in fish

farming is virtually prohibitive: the largest earthworm farm in Britain, British Groundbaits, produces earthworms for sport fishing. In the fishing season demand for earthworms exceeds supply, but out of season surplus worms are available to fish farmers at a cost of £4,750/tonne, wet weight (Nightingale, 1980). On the basis of its nutritional composition feed compounders have attributed a value of £230/tonne, dry weight to the earthworm meal (Satchell, 1983). Obviously major changes must occur before the utilization of earthworms in fish farming becomes an economically feasible proposition:-

(i) Vermiculture as a method of waste management may expand either as a policy or within integrated agricultural/aquacultural systems. Since the primary objective would be the disposal of large quantities of organic wastes (producing earthworm biomass as a by-product) the earthworms may be produced on a sufficiently large scale and at a reduced cost so as to compete economically with other sources or protein currently used in commercial fish feeds.

(ii) Earthworm meal, included in small quantities within fish feeds may increase fish growth or feed palatability and possibly facilitate the use of less palatable/cheaper ingredients. This may be achieved by the production of an earthworm concentrate, serving as an 'attractant' in fish feeds. At present, British Groundbaits manufacture an earthworm concentrate which is believed to be highly effective at low concentrations as a feeding stimulant for eels (J. Delves-Broughton, pers.comm.). The minimal quantity required makes its use cost effective and does not preclude the production of large quantities of earthworms.

(iii) At present fish feed manufacturers are heavily reliant on a single commodity: fish meal, to meet their requirement for good quality protein (Tacon, 1981). The increasing emphasis on using the world's fishery resources as human food, coupled with the needs of other animal industries forecasts that fish meal supplies will decrease. Moreover, the majority of fish species cultured in Britain are carnivorous and consequently require rations containing a high level of good quality protein (40-60% protein) in order to achieve maximum production. As a result the cost of feed accounts for 40-60% of the total running costs of intensive fish farming. Alternative sources of protein are currently being sought to alleviate the dependence of the industry on a single commodity and to reduce the cost of intensive aquaculture through the use of less expensive feed ingredients.

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