THE ASSESSMENT OF PROTEIN QUALITY OF CARP (*Cyprinus carpio*) DIETS

by

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THESIS

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SUMMARY

Protein quality of carp diets was assessed by five methods:

1. True digestibility, true NPU, BV (as percentage) and PER were determined for approximately iso-energetic diets containing ca.38% protein from 4 different sources. Fish meal gave values of 94.Ø, 72.5, 77.Ø, and 1.21 respectively; egg 93.Ø, 65.4, 7Ø.3, 1.26; Pruteen 68.4, 63.6, 68.4Ø, 1.36; and Casein 91.Ø, 56.9Ø, 62.5, 1.33.

2. Blood urea was dtermined and found to be significantly increased with increasing protein concentration in the diet.

3. Ammonia excretion rate was determined; it increased with a decline in protein quality, being greater on groundnut, rapeseed meal, and sunflower diets than on fishmeal, cottonseed meal, and pruteen.

4. Protein sources were incubated in vitro with digestive fluids of fish. Protein digestibilities for fishmeal diets containing 14 and 27% protein were 9Ø.2 and 93.Ø% respectively; casein (18 and 36%), 91.5 and 93.2%; soybean (1Ø and 2Ø%), 84.2 and 85.3%; sunflower(8 and 16%), 64.2 and 66.1%; and fish meal plus soybean meal (ca. 18.2%) 86.5.

5. Plasma free amino acids were individually determined at Ø, 6, 24 and 48 h after force-feeding diets containing 15 and 3Ø% protein from six different sources. Total free AA were highest at 24 h for casein and fishmeal, and at 48 h for egg, soybean, rapeseed and sunflower. The 24 h essential amino acid indices (EAAI) for the six diets at 15% protein were, in the same order, 93.Ø, 1ØØ, 1ØØ, 86.4, 62.4, and 97.2. At 3Ø% protein, the 24 h EAAI were 78.5, 84.3, 1ØØ, and 83.8 for casein , fishmeal, egg, and rapeseed respectively.

Key Words: Carp Protein-quality Amino-acid Digestibility.

To my wife ETIDAL for sacrifice, patience and her tremendous enthusiasm which gave me enough confidence to complete the study, even when the going was tough and the finish no more than a hazy dream.

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GLOSSARY

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

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

NPU Net Protein Utilisatiion

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

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

ENE Endogenous nitrogen excretion

FCR Food Conversion

PER Protein Efficiency Ratio = $\frac{\text{Weight gained}}{\text{Protein fed}}$

NB Nitrogen Balance

SGR Specific Growth Rate

NER Nitrogen Efficiency Ratio

RNU Relative Nitrogen Utilization

NGI Nitrogen Growth Index

RPV Relative Protein Value

NPR Net Protein ratio

PPV Productive Protein Value

SCP Single Cell Protein

AA Amino Acid

EAA Essential Amino Acid

EAAI Essential Amino Acid Index

NEAA Non Essential Amino Acid

FAA Free Amino Acid

GLOSSARY (cont.)

TEAA	Total Essential Amino Acid
PEAAI	Plasma Essential Amino Acid Index
FNEAA	Free Non Essential Amino Acid
TNEAA	Total Non Essential Amino Acid
NRC	National Research Council
RE	Reabsorption Efficiency
NE	Non Essential
Ala	Alanine
Arg	Arginine
Asp	Aspartic acid
Glu	Glutamic acid
Gly	Glycine
His	Histidine
Ile	Isoleucine
Leu	Leucine
Lys	Lysine
Met	Methionine
Phe	Phenylalanine
Pro	Proline
Ser	Serine
Thr	Threonine
Tyr	Tyrosine
Val	Valine

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CHAPTER 1.

Chapter 1.

1. Introduction.

The quality of a protein is dependent on its AA profile and its AA availability relative to the requirements for EAA and non-protein nitrogen by the target species. The purpose of measuring protein quality is essentially to determine the usefulness of protein for supporting production in a given animal entity. However, the interactions between AA composition, AA availability and AA requirements for various types of production make necessary accurate protein quality assessments.

The AA composition of a protein may not be known. It may not be feasible, or equipment may not be available to analyze all samples for AA. Furthermore, reliance on tabulated AA composition values may lead to erroneous conclusions about the sample on hand. Payne (1972) showed that considerable deviations are evident in AA values reported, even for the more common foodstuffs. Compositional analyses may not be deemed necessary for the evaluation of a particular protein, even though these analyses could aid in the interpretation of results.

The primary AA sequence of a protein influences its chemical, physical and biological properties. Proteins have manifold functions. As enzymes they catalyze chemical reactions and structural proteins participate in cellular architecture. In body fluids they transport substrates. Several hormones are proteins. Not all of

the AA which compose the proteins are synthesized in the body; and must be supplied with the food as dietary essentials. During passage through the digestive tract proteins are hydrolyzed to AAs by proteolytic enzymes (Fauconneau & Michel, 1970). AAs are absorbed by the intestinal cells and enter circulation to be utilized by the various organs for the synthesis of proteins. When present in excess, they are deaminated and serve as substrates for carbohydrate and fatty acid metabolism (Kaplan & Pitat, 1970).

Alteration in the concentration of one essential or indispensable AA will have serious consequences on the balance of protein synthesis in the body and will therefore also affect growth. Thus, the nutritional value of a protein is dependent on its AA composition and availability relative to requirement. The availability of AA in a particular protein may also not be known. Overall AA nitrogen digestibility may differ from the total nitrogen digestibility, because the non- AA nitrogen may be absorbed at a different rate than that of the AA nitrogen. Furthermore, it has been shown (Eggum & Jacobsen, 1976) that some AA may be located predominantly in the less digestible fractions of a food, rendering them less available than the total true protein nitrogen. Preparation procedures may alter the AA availability of a protein. Heat treatment, for example, has been shown to lower the availability of reactive AA (Carpenter 1960; Lakin, 1973) and to enhance

the availability of other AAs by destroying enzyme inhibitors (Olomucki and Barnstein, 196Ø).

Numerous techniques for the evaluation of the nutritive quality of proteins have been developed. Reviews have been published on the subject of protein quality evaluation (Bodwell, 1977; Satterlee et al., 1977, 1979, Pellett, 1978, Evans & Witty, 1978). The advantages and disadvantages of the various methods have been discussed in detail. The techniques involved in selecting a method to evaluate the quality of proteins range from a chemical and biological ranking of protein with respect to nutritional quality to the requirement of the food industries for a rapid, accurate, widely-applicable, low cost method to identify the nutritional quality of proteins in commercially processed foods (Nesheim, 1977; Bodwell, 1977; Lachance et al., 1977).

Continuous efforts are being made to modify and/or computerize results of available techniques to establish methods which are easily applied and at the same time officially acceptable (Nesheim, 1977, Gross, 198Ø). A critical survey of the results has been presented (Bodwell, 1977). One problem in describing the nutritional value of a protein is that neither animals nor humans consume a single protein but a mixture of sources. It is, therefore, important to know the relative quality of a protein when processed and consumed together with other proteins or in combination with energy sources, minerals, vitamins and fibre (Beaton,

1977; Pellett, 1978 ; Ebihara et al., 1979, Krajcovicova & Dibak, 198Ø).

Classification of proteins with respect to AA composition and capacity to stimulate growth with minimum loss of nitrogen gives little information on cellular activities which are regulated by the AA content in the body tissues. AAs become available to the body after they are transported across the intestinal membranes. Biological availability of AAs constitute an important variable when the nutritional quality of a protein is analysed (Harper, 1981; Kies, 1981).

Considerable information is available on the nutritional properties of proteins when measured by chemical analysis and metabolic studies. The efficiency of usage of absorbed AA at the cellular level is another important consideration. As more basic information becomes available, a more precise understanding of the role of protein quality in metabolic functions will be achieved. A number of studies have been made in mammals and man to determine protein quality but little work has been done in fish.

The present study was, therefore, undertaken to establish methods for assessing protein quality, rather than to develop a specific method. However, it is considered important to compare in vitro methods with in vivo growth studies of protein utilization, therefore, it is proposed that this is also investigated.

The nutritive value of dietary protein depends on its AA content and on the extent of absorption of the constituent AAs into the blood stream. In terrestrial animals, a large amount of research has therefore been directed toward the correlation of plasma FAA with the adequacy of the test diet (Leathem, 1968). However, only a few publications have dealt with the subject in fish (Nose, 1972). Fish require a high percentage of protein in their diet for optimum growth and it is of particular importance to employ some biochemical method such as a plasma AA index in addition to growth studies in order to evaluate the quality of test protein. While AA composition of a test protein has been mainly used for evaluating its quality, it is evident that other important factors, such as the rate of release and absorption of constituent AAs from a protein during digestion will influence the quality of dietary protein. Hence, to use only AA composition of a test protein appears inadequate in evaluating its quality.

1.1. Background.

Dietary protein is required for three basic functions; (1) maintenance- the making good of tissue wear and tear; (2) repletion of depleted tissues; (3) growth or formation of new additional protein.

AAs divide into two basic categories, those that are essential and cannot be synthesised by the fish and those that are NE and can be synthesised provided that

an alternative source of nitrogen is available. There is also a third vague category of semi EAA which can be synthesised but not at a rate fast enough to satisfy metabolic demands; such AA are usually classed as essential.

Protein utilization by fish depends essentially on species and size, environmental factors, protein quality , quantity of dietary protein and of utilizable dietary energy, the kind of energy source and amount of feed (Mertz, 1972). The AA profile of protein describes the relative proportion of the EAA within that protein. A protein with a " good " profile is one whose composition, in terms of proportions of EAA, approximates to the quantitative requirements of the fish.

1.2. <u>Qualitative AA Requirements.</u>

Many animals have been found to require the same ten AA as essential dietary constituents (EAA), namely arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophane, valine. The same is true in various species of fish, as shown in Table 1.1.

Table 1.1. Species Requiring The Ten EAA.

Species		Reference
Chinook salmon	Oncorhynchus tschawytscha	Halver, (1957)
Rainbow trout	Salmo gairdneri	Shanks et al., (1962)
Sockeye salmon	Oncorhynchus nerka	Halver & Shanks (196Ø)
Channel catfish	Ictalurus punctatus	Dupree & Halver (197Ø)
Japanese eel	Anguilla japonica	Nose (197Ø)
European eel	Anguilla anguilla	Arai et al., (1972)
Common carp	Cyprinus carpio	Nose (1979)
Red sea bream	Chrysophrys major	Yone (1975)
Red belly tilapia	Tilapia zilli	Mazid et al., (1978)
Plaice	Pleuronectes platessa	Cowey et al., (197Ø)
Sole	Solea solea	Cowey et al., (197Ø)
Sea bass	Dicentrachus labrax	Metailler et al., (1973)
Carp	Cyprinus carpio	Nose et al., (1974)

1.3. Quantitative EAA Requirements.

For many years the only quantitative data on EAA requirements of fish were those of Halver and his colleagues (Mertz, 1972) relating to chinook salmon (Oncorhynchus tschawytscha). Other workers have attempted to adapt such a diet to other species with varying degree of success.

Quantitative AA requirements determined for several fish species are generally based on weight gain, feed efficiency, and sometimes FAA in the plasma of individuals fed graded concentrations of particular AAs (Table 1.2).

Diets consisting entirely of AAs or a mixture of protein and AAs were used to establish quantitatively the indispensable AA requirements and the sparing action on some of these by dispensable AAs in several fishes, such as the salmonids (Delong et al., 1962; Chance et al., 1964; Klein and Halver, 197Ø), the channel catfish (Harding et al., 1977; Wilson et al., 1977), tilapia (Mazid, 1979) and the carp (Nose, 1978). The AA test diet has thus been shown to support normal growth comparable to a protein diet in the salmonids and, to a certain extent, in the channel catfish. However, in the carp, the nutritive values of AA protein hydrolysates, or mixtures of FAA and protein as nitrogen sources, have been found to be inferior to standard protein diets (Aoe et al., 197Ø; Aoe et al., 1974; Nose et al., 1974, Nose, 1978). Mazid et al., (1978) showed that tilapia also grew poorly on an AA diet. Neverthless, the growth of these two fishes fed the AA test diet was believed sufficient to

1.2. The EAA Requirements of Four Species of Fish.d.

AA	Chinook Salmon a	Japnese eel b	Carp c	Rainbow trout c
Arg	2.4(6.Ø/4Ø)	1.7(4.Ø/42)	1.52(3.8/4Ø)	1.4(3.5/4Ø)
His	Ø.7(1.8/4Ø)	Ø.8(1.9/42)	Ø.56(1.4/4Ø)	Ø.64(1.6/4Ø)
Ilu	Ø.9(2.2/41)	1.5(3.6/42)	Ø.92(2.3/4Ø)	Ø.96(2.4/4Ø)
Leu	1.6(3.9/41)	2.Ø(4.8/42)	1.64(4.1/4Ø)	1.76(4.4/4Ø)
Lys	2.Ø(5.Ø/4Ø)	2.Ø(4.8/42)	2.12(5.3/4Ø)	2.12(5.3/4Ø)
Met	Ø.6(1.5/4Ø)	1.2(2.9/42)	Ø.64(1.6/4Ø)	Ø.72(1.8/4Ø)
	Cys = 1 %	Cys = Ø % Ø.9Ø(2.9/42)	Cys = +	Cys = +
	Cys = 1 %	Cys=2%		
Phe	1.7(4.1/41) Tyr= Ø.4%	2.2(5.2/42) Tyr = Ø% 1.2 (2.9/42) Tyr= 2 %	1.16(2.9/4Ø) Tyr= +	1.24(3.1/4Ø) Tyr= +
Thr	Ø.9(2.2/4Ø)	1.5(3.6/42)	1.32(3.3/4Ø)	1.36(3.4/4Ø)
Try	Ø.2(Ø.5/4Ø)	Ø.4(1.Ø/42)	o.42(Ø.6/4Ø)	Ø.2Ø(Ø.5/4Ø)
Val	1.3(3.29/4Ø)	1.5(3.6/42)	1.16(2.9/4Ø)	1.24(3.1/4Ø)
Total	12.3(3Ø.49)	14.8(35.4)	11.28(28.2Ø)	11.64(29.64)

Notes : a. Data for Chinook salmon from Mertz (1969).
b. Data for Japanese eel from Nose (1978).
c. Data for rainbow trout and Carp from Ogino (198Ø)
d. Values are expressed as g /1ØØ g of dry diet. In parentheses the numerators are g / 1ØØ g of protein and denominators are the percent total dietary protein.

establish AA requirements.

Ogino (198Ø) has employed a novel approach to elucidation of the quantitative EAA requirements of carp and rainbow trout. The method depends on the assumption that increase in the EAA content of fish is supplied from the dietary protein. Hence, analysis of the fish tissue will enable calculation of the EAA requirements. The results drived from feeding carp and rainbow trout a diet containing 4Ø% protein, that is 8Ø% digestible, at 3% of the body weight per day are presented in Table 1.2. If the feeding rate, dietary protein and protein digestibility are known, the required dietary AA profile may be calculated assuming that all of the AAs are equally digestible.

Various alternative methods of determining quantitative AA requirement have been proposed to replace the classical growth response experiments. Work on the chick and pig (Maynard et al., 1979) has revealed that dietary AA deficiency resulted in lowering the plasma content of the AA under study. Increasing the dietary AA to produce maximum gain and feed efficiency, however, resulted in increased plasma FAA.

The relationship between EAA pattern of the diet and concentrations of FAA in fish tissues have been studied not only with regard to AA metabolism but also in order to determine AA requirements of fish.

Kaushik (1978) determined the arginine requirement of rainbow trout from a convential dose/response (growth)

curve and also by measuring the tissue (blood and muscle) concentrations of free arginine. After the dietary requirement of the trout for arginine had been met, any further increase in arginine intake led to an increase in the concentration of free arginine in blood and muscle. Good agreement was obtained between the two methods. However, Wilson et al.,(1977, 1978) also used this technique to evaluate the lysine, threonine and methionine requirements of channel catfish by feeding graded amount of the AA in casein-gelatin based diets; values obtained were in close accordance with equivalent values deduced from growth and feed efficiency experiments.

Tanaka et al., (1977a) increased somewhat the growth rate of carp by addition of phosphate salts, algae and indigestible materials to an AA diet and postulated that the much shorter intestinal retention time of the AA diet compared to the protein (casein) diet was the reason for its low nutritive value, although Aoe et al., (197Ø) found no such improvement on growth with an elongated retention time of an AA test diet in the carp intestine. In a subsequent study, Tanaka et al., (1977b) determined that the single addition of some AAs to a casein diet improved the the growth rate of carp, while the addition of other FAAs reduced it. This phenomenon was not a function of the intestinal retention time. Hokazono (1979) found the addition of L-Lysine improved the nutritive value to a casein diet for carp, while the combination of L-lysine with other AAs lowered it. An

extreme case was the addition of L-lysine plus L-arginine to the control casein diet, which reduced growth by more than 60%. This may have been due to an excess of an individual AA or the creation of an imbalance .

Muramatsu et al., (1971), in the rat found varying degrees of growth depression when individual AAs were in excess to a casein diet. Andrews and Page (1974) discussed the possibilities of the underutilization of FAA or disruption of AA balance occurring in supplementation of the most limiting AA to diets containing soybean meal for channel catfish. Andrews et al., (1977) later substantiated that channel catfish were unable to utilize supplemental FAA to a protein diet. However, Harding et al., (1977) and Wilson et al., (1977) were able to obtain a growth response in channel catfish to graded amounts of FAA when added to basal diets consisting of mixtures of purified proteins and FAAs. Wilson (1984) reported that the EAA composition of the whole body protein of a fish appears to be a good index of the proper dietary AA balance required by that species. The EAA requirement values for channel catfish and common carp were found to be highly correlated with the EAA composition of the whole body protein of the fish. The EAA composition of the whole body protein of the channel catfish remained relatively constant for fish weighing from 30 g to 860 g, thus, indicating that the AA requirement, when expressed as a percentage of

dietary protein, should not change with fish size. Because of this direct relationship, the whole body EAA composition should serve as excellent index in formulating test diets for fish. AA composition of egg and larval stages for AA has been shown to be a good guideline for estimating quantitative AA requirements of fry and fingerling stages.

Rainbow trout have been shown capable of utilizing supplemental AA when added to diets in which proteins of the fish meal component were replaced by the sulphur AA deficient soybean meal (Dabrowska and Wojno, 1978). Rumsey and Ketola (1975) also found rainbow trout to grow significantly faster when certain FAA were supplemented to a soybean meal diet than without supplementation. Rainbow trout have been shown to respond to dietary methionine when added to an AA diet, providing that sufficient vitamin A was also present (Eckhert and Kemmerer, 1974). Yamada et al., (1981) studied plasma AA changes in rainbow trout force-fed casein and a corresponding AA mixture, and reported that the EAA are directly increased after 12 hours and 24-36 hours for AA and casein diets. However, the concentrations and pattern of plasma NEAA appear to be influenced by both dietary NEAA and also by interconversion reactions which they undergo.

Plakas et al., (198Ø) compared the plasma AA patterns of carp after feeding casein and corresponding AA mixture diets when investigating the reason for the inability

of FAA to serve as a dietary nitrogen source for the normal growth of carp. All EAA in the plasma of carp were maximal 4 hours after ingestion of the casein diet, whereas with the AA diet, arginine and lysine peaked at 2 hours, then declined to prefeeding values at 4 h, while leucine, isoleucine and valine reached a maximum at 4h.

It is widely believed that all the EAAs must be available to the tissues almost simultaneausly for protein synthesis to occur, and AA from incomplete mixtures are not stored in the body but are irreversibly further metabolized (Geiger, 1947). Thus it may be that variation in the rate of absorption of AA from synthetic AA diets is the reason for the poor growth of non-salmonid fish on such diets.

Murai et al., (1981) reported that fingerling carp fed casein diet coated with EAA (including tyrosine) showed a comparable growth to fish fed a casein-gelatin diet. These fish achieved more than four-fold faster growth and almost two-fold better feed efficiency than fish fed a gelatin diet with the same AA but uncoated. It is reported in man that the rates at which individual AA are absorbed from an AA mixture are not equal to those absorbed through the normal digestion process of intact protein (Silk et al., 1973). Because simultaneous presentation of AA in the tissues is necessary for optimal protein synthesis (Matthews, 1977), Murai et al., (1981) proposed a hypothesis that coating

AA with casein minimized variation in the absorption rates of individual AA .

The methionine requirement of channel catfish was investigated by Andrews (1977) by simply varying the dietary methionine in practical rations on soybean meal and corn gluten meal. Quantitative dietary methionine requirements for several fish species have been shown to depend on dietary cystine concentration, since cystine can substitute for a portion of methionine requirement. This is the result of conversion of methionine to cystine being a common pathway of intermediary metabolism in many terrestrial animals as well as fish (National Research council 1973). Also Andrews et al., (1977) investigated the arginine requirements for catfish by altering the amount of gelatin in a casein- based diet. A significant increase in growth rate was achieved by raising protein-bound arginine from 1.1 to 1.7%. However, synthetic arginine supplemented to the casein diet failed to elicit the same response. However, care should taken when formulating diets and interpreting results from such experiments since the amounts of AA in the diet, and not just the one under investigation, will vary when the dietary protein sources are adjusted.

Quantitative AA requirements determined for several fish species are generally based on weight gain, feed efficiency, and sometimes plasma FAA of individuals fed graded concentrations of particular AAs (Table 1.3). In addition to those values listed in Table 1.3, coho salmon

have shown to require 2.4% arginine in the dry diet (6.Ø% dietary protein) and Ø.7% (1.7%) histidine (Klein and Halver 197Ø), and also Ø.2 (Ø.5%) tryptophan (Halver 1965).

The quantitative AA requirements listed in Table 1.2 and 1.3 can be affected by AA interactions. Thus the NEAA tyrosine and cystine are derived metabolically from the EAA phenylalanine and methionine respectively. With a dietary deficiency of either tyrosine or cystine the EAA will have to be used to synthesise them, thereby elevating the quantitative requirements for the EAA. The conversion efficiency of phenylalanine to tyrosine has been reported as 6Ø% in carp (Nose, 1978). Conversely, excess tyrosine or cystine in the diet spares these EAAs and reduces the dietary requirement. Harding et al., (1977) found the cystine replacement value for methionine on a m mole sulphur basis was 6Ø%.

In certain cases it has been shown that one AA can affect the requirement of another by directly interfering with its metabolism. Nose (1978) found a dietary balance of isoleucine with regard to leucine to cause marked growth depression in carp. Another well- documented example is the lysine-arginine antagonism where elevated dietary lysine increases the requirement for arginine. However, Robinson et al., (1981) failed to elicit such a response in channel catfish.

Few AA deficiences are known to characterise pathological symptoms. Rather, nearly all give rise to a loss of

Table.1.3. Quantitative Dietary AA Requirements for Several Fish Species. [1]

AA	Channel catfish[2]	Chinook salmon[3]	Japanese eel[4]	Common carp[5]
Arg	4.3(1.Ø)	6.Ø(2.4)	4.5(1.7)	4.2(1.6)
His	1.5(Ø.4)	1.8(Ø.7)	2.1(Ø.8)	2.1(Ø.8)
Ilu	2.6(Ø.6)	2.2(Ø.9)[6]	4.Ø(1.5)	2.3(Ø.9)
Leu	3.5(Ø.8)	3.9(1.6)[6]	5.3(2.Ø)	3.4(1.3)
Lys	5.1(1.2)	5.Ø(2.Ø)	5.3(2.Ø)	5.7(2.2)
Met	2.3(Ø.6)[7]	4.Ø(1.6)[8]	5.Ø(1.9)[8]	3.1(1.2)[9]
Phe	5.Ø(1.2)[10]	5.1(2.1)[6,11]	5.8(2.2)[11]	6.5(2.5)[1]
Thr	2.3(Ø.5)	2.2(Ø.9)	4.Ø(1.5)	3.9(1.5)
Trp	Ø.5(Ø.1)	Ø.5(Ø.2)	1.1(Ø.4)	Ø.8(Ø.3)
Val	3.Ø(Ø.7)	3.2(1.3)	4.Ø(1.5)	3.6(1.4)

1. Expressed as percentage of dietary protein with requirement as percentage of dry diet in parentheses.
2. Based on 24% dietary protein (Robinson et al., 198Ø).
3. Based on 4Ø% dietary protein unless otherwise noted (NRC, 1973).
4. based on 37.7% dietary protein (Nose and Arai unpubl. data, cited in Cowey and Sargent 1979).
5. based on 38.5% dietary protein (Nose,1979).
6. based on 41% dietary protein (NRC, 1973).
7. In the absence of cystine which can replace 5Ø to 6Ø% of methionine requirement (Harding et al., 1977).
8. methionine + Cystine.
9. In the absence of cystine.
10. Phe + Tyr Requirement . Tyr can replace Ca. 5Ø % of Phe (Robinson et al., 198Ø).
11. In the absence of Tyr.

Source : Millikin , 1982 .

appetite, resulting in low food intake with a consequent fall in growth rate and activity (Cowey and Sargent, 1979). However, more specific symptoms have been described in rainbow trout given diets lacking tryptophan (Kloppel and Post, 1975). The fish showed transient scoliosis, and the vertebral column of all scoliotic fish was affected by a protrusion of fibrous material between the cartilaginous processes of the vertebrae. Tyrosine was reported to be dispensible for growth in carp (Nose et al., 1974). However, diets low in both phenylalanine and tyrosine caused growth depression. Thus the tyrosine requirement for a low (1%) phenylalanine diet was determined and was found to be Ø.8% of the diet and 2.1% of the dietary protein.

The variation in EAA requirements for the fish species so far studied is sufficiently great as to result in a need to formulate the EAAs in diets on a species basis. For those species whose quantitative requirements are, as yet, undetermined it would be advisable to formulate diets to contain the highest amounts of EAA required by any species so far evaluated .

There are some incomplete data to suggest that the EAA requirements for carp in extensive culture may, in part, be provided from intestinal microflora. Syvokiene et al., (1974, 1975) and Lesauskiene et al., (1974, 1975) cited by Dabrowski (1979) found in carp fry as well as in two-and three-year-old fish that some EAAs and NEAAs were being derived from the gut bacteria. The EAAs

valine, lysine, threonine, leucine, methionine and histidine were synthesised by the gut bacteria at varying rates and were concluded to be contributing to the requirement of carp for these AA (Jauncey, 1982).

1.4. AA Availability.

It is necessary not only to know the AA profile of feed proteins but also to determine the availabilities of the AA they contain. In particular two of the EAAs, lysine and methionine, readily undergo changes during the processing of feedstuffs that may render them unavailable to the fish.

Lysine is a basic AA which contains a second -amino group which must be free and reactive or the lysine, although chemically measured, will not be biologically available (Cowey and Sargent, 1972, Cowey, 1978). Methods for measuring " available " lysine have been published (Carpenter and Ellinger, 1955) and determinations in fish have been found to correlate well with experimentally determined biological values in birds and mammals (Cowey, 1978).

Methionine is difficult to measure in feedstuffs as it readily undergoes oxidation during processing to form a sulphoxide or sulphone. Methionine is usually determined by performate oxidation to methionine sulphone, but this does not reveal what proportion of the sulphone was originally present. In addition methionine sulphoxide may have some biological value to fish if they are able to reduce it to methionine. Njaa (1977) has proposed a

method that measures both methionine and methionine sulphoxide in the feed and Ellinger and Duncan (1976) have described a specific methionine method. These remain to be evaluated in a practical context. As remarked by Cowey (1978) " measurement of sulphur AA in protein feeds is identified as a need in assessment of nutrients for fish. The biological value of methionine sulphoxide (if any) also ought to be evaluated ".

A significant contribution to formulation of diets for channel catfish recently came from an extensive investigation of true AA availability (corrected for metabolic fecal AA) and apparent AA availability (Digestibility) values of various feedstuffs commonly incorporated into commercial catfish diet (Wilson et al., 1981). Results generally suggested that reasonable agreement occurs between average apparent AA availability and protein digestibility values of any one specific protein source. However, individual AA availabilities were quite variable within and among various feed ingredients tested. Also, apparent AA availabilities were considerably less than the true values for feed ingredients containing relatively low protein contents (e.g., 9 to 19%), such as rice bran, rice mill feed, wheat middlings and corn.

1.5. Dietary protein.

The past fifty years have seen the understanding of the food requirements of man and his food animals advance to the stage where the study of comparative nutrition is a recognised activity. This has been greatly enhanced by increased understanding of the underlying physiological and biochemical processes involved. In so far as terrestrial animals are concerned, advances such as the discovery of vitamins, definition of the trace elements and elucidation of EAA and fatty acids have allowed the virtual establishment of the nutrient requirements for many species. With the increasing food demands of an expanding population man has a growing need to understand the nutrition of those species upon which he feeds. As interest in fish culture, as a means of producing high quality protein for human consumption, has increased, this has stimulated great interest in the nutrition of fish. (Jauncey, 1982b)

The rearing of large numbers of animals in relatively confined conditions, whether terrestrial or aquatic, necessitates a detailed understanding of their nutrition in order to provide a diet that is adequate for their growth and well being. In the past, large quantities of fish, enough to satisfy demand, were available from natural sources, but more recently the scientific intensive farming of fish has become economically available due to growing demand coupled with a depletion of natural resources. Practical, economic fish husbandry

has become essential in those areas of the world with a low dietary protein intake. Incomplete or incompletely applied knowledge of fish nutrition has often meant that the potential of streams and impoundments suitable for fish culture in these areas has not always been reached. The success or failure of fish culture projects depends on the nutritional status of the fish. Until recently the emphasis was on natural feedstuffs and, consequently, little or no data existed on the nutrient requirements of fish. Fish farming on a global scale still largely depends on natural food with some supplementation with the by-products of other forms of agriculture or industry, such as slaughter-house wastes, grain wastes, silkworm pupae, night soil, etc. At low culture densities these diets are adequate as most of the nutrient requirements of the fish are satisfied from natural sources. However, at high densities fish are dependent on artificial feeds, only benefitting slightly, if at all, from natural food, and thus inadequate supplementary feeding leads to poor growth, nutritional disease and due to poor fish condition, increased susceptibility to parasitic and bacterial infestations.

Due to the generally more intensive culture practices used with these species, research in fish nutrition has tended to center on salmonids. However, more recently, intensification of fish culture systems for other species has led to diversification in fish studied. In this review, research into carp nutrition will be the

main subject of discussion, but where there are omissions in this field or where analogies may be drawn from research on other species, these will also be discussed. In order to evaluate nutritionally balanced diets properly it is necessary to understand the biochemical roles and interactions of the various food components. The biochemistry of fish nutrition is a field too vast and detailed to be covered here and reference should be made to other workers on the subject (Cowey and Sargent, 1972, 1979; Halver, 1972, Wilson, 1984).

1.1. Dietary protein Requirements.

Muscle is, anatomically, by far the major component of the fish body and protein usually accounts for 65- 85% of the dry matter. The amount of dietary protein producing maximum growth of fish depends on its AA profile plus AA balance; AA availability, a delicate balance of dietary protein to energy ratio, and non protein energy source (i.e. amount of fat in relation to carbohydrate). Several studies are available regarding protein nutrition in carp. Ogino and Saito (197Ø) found optimum dietary protein utilization in young carp to occur at 38% with a rearing temperature of 23 °C. The weight gain of the fish was proportional to the protein content in the diet up to the maximum used (55%). In a subsequent study by Ogino et al., (1976), the optimum dietary protein for young carp was reported to be approximately 35% on the basis of growth at 2Ø °C, and no further increase in

growth rates resulted from protein above this amount. It was also determined that carp can utilize dietary carbohydrates effectively as energy sources, as opposed to rainbow trout, and while the whole body lipid content was positively correlated with dietary lipid in trout, it is correlated with carbohydrate in carp. A study by Sen et al., (1978) to ascertain optimum protein and carbohydrate for young carp revealed 45% casein and 26% dextrin, as providing the best growth. An insignificant or slight decline in gain was found at protein above 45% at the experimental water temperature of 24 - 32 °C. The differences in the dietary protein requirements of carp established in the above three studies were probably due to the rearing temperatures involved. Increasing the rearing temperature within certain limits results in an increase in optimum dietary protein as reported for chinook salmon (Delong et al., 1958).

The dietary protein requirements of a number of fish determined to date are given in Table 1. 4. The values are uniformly high, hence the protein requirements of fish appear to be two to four times higher than in warm blooded animals (Mertz, 1972). Protein treatments showed that the apparent protein requirement was about 50% of dry diet ingredients for fry, and that this percentage decreased as fish size increased (NAS- NRC 1973). Trout and salmon had the same apparent protein requirement when the animals were raised at identical

Table 1.4. Quantitative Protein Requirements of Several Fish Species (Millikan, 1982).

Species	Requirements % dry Diet	Reference
Salmo gairdneri	40	Saita (1974)
	45	Halver et al. (1964)
	42	Austreng & Refstie (1979)
	40-45(1)	Zeitoun et al. (1973)
Oncorhynchus kisutch	40	Zeitoun et al. (1974)
O. tshawytcha	55	Delong et al. (1958)
O. nerka	45	Halver et al. (1964)
Micropterus salmoides	40	Anderson et al. (1981)
M. dolomieui	45	Anderson et al. (1981)
Moron saxatilis	55(2)	Millikan (1982)
Pleuronectes platessa	50	Cowey et al. (1972)
Fugu rubripes	50	Kanazawa et al. (1980)
Anguilla japonica	44.5	Nose & Arai (1972)
Tilapia zilli	35	Maized et al. (1979)
Tilapia aurea	36(2)	Davis & Stickney (1978)
Ictalurus punctatus	35	Lovell (1972)
	35	Murray et al. (1977)
Chanos shonos	40	Lim et al. (1979)
Ctenopharyngodon idella	41-43	Dabrowski (1977)
Cyprinus carpio	38	Ogino & Saito (1970)
Chrysophys aurata	38	Sabaut & Luquet (1973)

(1) Protein requirement increased from 40 to 45% as salinity increased from 10 to 20%.
(2) Highest protein concentration examined.

temperatures.

For example, salmonids require about 50% protein during the initial feeding stage of fry, decreasing to 40% protein after 6 to 8 weeks, with a further reduction to 35% protein for yearlings (NRC 1973). Channel catfish fry require a minimum of 40 % protein, decreasing to 30 to 36% for fingerlings and 25 to 30% protein for subadults weighing > 114 g (NRC 1977, Andrews 1977). The higher protein concentrations in these two ranges produce better growth of channel catfish fingerlings and subadults, whereas the lower protein concentrations provide better protein conversion (weight protein fed/ weight gain; Andrews 1977).

Some experiments have shown that the protein requirement increased in salmonids as the salinity increased; the rainbow trout requirement of 40% of the diet as protein at 1% salinity increased to 45% at 2% salinity (Zeitoun et al., 1973). Since a salinity of 1% is almost isotonic with internal fluids (0.9%) of rainbow trout fingerlings, the higher dietary protein requirement for rainbow trout reared in a salinity of 2% suggests that this may be to the increased demands of osmoregulation in a hypertonic external environments for this species (Zeitoun et al., 1973). Coho salmon, O. kisutch, smolts require 40% protein in 1% and 2 %. Although maximum weight gain occurred at 40% protein in both salinities, maximum protein retention occurred at 40% protein in 1% and at 50% protein in 2% (Zeitoun et al.,

1974). Millikan (1982) concluded that the hyperosmotic environment (2%) did not stress coho salmon smolts in the same manner as in rainbow trout fingerlings.
Several marine fish appear to require more than half the diet ingredients as protein components and thus are more carnivorous than terrestrial carnivores (Yone et al., 1974).

1.5.2. Measures of protein utilization.

The efficiency with which fish are able to utilise dietary protein is usually determined by measurement of one or other of the following parameters :
PER which is defined as the gain in wet weight of the animal per gram of crude protein consumed (Osborne et al., 1919) thus :

PER = weight gain, g wet fish / g crude protein fed

Although PER values give a somewhat better indication of the nutritional status of the fish, with respect to dietary protein, than FCRs (grams of diet fed per gram of weight gained), they do not take into account the proportion of ingested protein used for maintenance, and are based on the assumption that the growth of the fish consists of tissues with identical composition in all groups. An improved assessment of the nutritional status of the fish with respect to dietary protein utilization is apparent efficiency of deposition of dietary protein as body tissue. The NPU in fish is generally determined

by the carcass analysis method of Bender and Miller (1953) and Miller & Bender (1955). If no corrections are made for endogenous nitrogen losses the results are expressed as Apparent NPU :

Apparent NPU (%) = Nb - Na / Ni X 1ØØ

Where Nb is the body nitrogen at the end of the test, Na the body nitrogen at the begining of the test, and Ni the amount of nitrogen ingested.

Estimation of endogenous nitrogen losses will permit the calculation of True NPU. These can measured by finding the body nitrogen loss on a zero protein diet. However, this poses acceptability (Palatability) problems that are usually overcome by the feeding of a low protein diet as proposed by Cowey et al., (1974). Hence :

True NPU (%) = B- (Bk - Ik) / I X 1ØØ

Where B is the total body nitrogen of fish fed the test diet and Bk the total nitrogen of fish fed the low protein diet, with nitrogen intakes of I and Ik respectively.

Estimates of endogenous nitrogen excretion (ENE, nitrogen excreted by fish fed a zero protein diet) have been made for carp. Ogino et al., (1973) reported the ENE of carp to be 7.2 mg N/1ØØ g of fish/day at 2Ø °C and 8.6 mg N at 27°C. A more recent publication (Ogino et al., 198Ø) records the ENE of carp as 14 mg/1ØØ g body

weight/day at 22°C, while Kaushik (1980) found that the daily nitrogen excretion rates were directly related to nitrogen consumption in carp as well as in trout. Also, Tatrai (1981) reported that the ammonia, urea and total nitrogen excretion of young bream (Abramis brama) was about 50-60% of the nitrogen consumed with the food. Murai et al., (1984) fed carp with four diets; (1) 38.4% AA mixture; (2) 30% AA plus 7.65% casein; (3) 25% casein plus 10% gelatin; (4) 5% casein plus 30% gelatin. He found that the sum of the FAA (as nitrogen) reached 36% of total nitrogenous substances excreted by fish fed diet 1, but dropped to 12.8% in fish fed diet 2. On the other hand, the fish fed the casein-gelatin diets (3 and 4) excreted only a minor quantity of FAA (less than 1% of total nitrogen in both cases), and no remarkable differences in AA composition between the fish fed diets 3 and 4 were noted, although there was more than a ten-fold difference in growth rate and feed efficiency between the fish fed these two diets. Obtaining a value for true NPU in this way, together with the determination of true protein digestibility, permits calculation of the BV of the protein which is the measure of the percentage of absorbed nitrogen retained as body tissue :

BV (%) = (True NPU / True Digestibility) X 100

Definition of the minimum dietary protein giving optimum weight gain of a species of fish was first investigated

in chinook salmon in 1958 by DeLong et al., and this type of experiment has since been reported on many fish species.

Ogino and Saito (197Ø) were the first to attempt definition of the optimum dietary protein for carp using diets containing varying proportions of casein to give Ø.4 - 55% crude protein. Over the whole range they found that weight gain was directly proportional to dietary protein. However, the accumulation of body protein was found to reach a maximum at 38% dietary protein. This study was conducted at 23 °C with diets containing a metabolizable energy content of approximately 3.7 Kcal/ g. These authors also found that both PER and NPU decreased linearly with increasing dietary protein. These findings were supported by Cowey et al., (1972) working with plaice. In contrast Hegested and Chang (1965) reported that NPU and PER in rats declined only when protein adequate to achieve maximum growth was exceeded. Similar conclusions were drawn by Zeitoun et al., (1973) working on rainbow trout, who found PER and NPU increased with dietary protein up to 4Ø% inclusion, which produced maximum growth; beyond this both parameters declined. That this should be so is contrary to the results of Cowey et al., (1972) with plaice and Dabrowski (1977) with grass carp.

The situation with regard to dietary protein and NPU & PER obviously requires further investigation. However it is clear that as the dietary protein increases beyond a

certain point, its utilization for growth decreases. The practical importance of this may be that for economic resons the protein in practical rations will be below that producing maximum growth to ensure more efficient protein utilization and to reduce feed costs.

Juvenile rainbow trout fed with semisynthetic diets containing casein as a sole source of protein have attained a PER of more than 3 and an apparent NPU of over 5Ø. This means that less than 2 kg of dietary protein produces 1 kg of body protein of trout. Similar values have been obtained with carp. These values compare well with other farm animals (Table 1.5).

Table 1.5. Comparison of Body and Edible Protein Produced From 1ØØØ g Crude protein .

Species	Production From 1ØØØ g Crude Protein	
	Body protein	Edible protein
Rainbow trout	282-345 g	2Ø1-248 g
Calf	272-345 g	187-225 g
Pig	25Ø-277 g	169-2Ø4 g
Chicken	322-36Ø g	182-193 g

Source ; Steffens (1981).

Mazid et al., (1979) and Teshima et al., (1978) investigated the protein requirements of T.zilli by varying the inclusion of casein and dextrin in the diet. Growth rate was reported to increase with increasing dietary protein up to about 35%, after which growth decreased in both experiments. Diets were reported to be isocaloric. However, high proportions of dextrin are unlikely to be well absorbed, hence more variation in digestible energy may have occurred.

Davis and Stickney (1978) using T.aurea obtained increases in growth rate up to 36% protein provided as a combination of fish meal and soybean meal, but greater amounts than 36% were, however, not investigated. Also using a fishmeal-based diet, Jauncey (1982) reported that maximum growth in S. mossambicus occurred with 40% protein as compared with 36% reported by Cruz and Laudencia (1977). The latter however reported good growth with between 29 and 38% protein. Jackson and Capper (1982) found the growth of S. mossambicus fingerlings fed 20% protein resulted in growth of 80% of that on a 40% protein ration. Similarly Mazid et al., (1979) reported the growth of T.zilli fingerlings fed a 21% protein ration to be 96% of that at the optimum protein content. Jauncey and Ross (1982) reported that the optimum dietary protein in terms of growth is not necessarily optimum in economic terms. If high-protein feeds are expensive then economics may be improved at sub-optimal dietary protein content, increasing the time taken for fish to reach

market size but incurring lower feed costs. However, in extremely intensive tilapia farms with high capital investments, it may be more economically acceptable to maximise growth rates, reducing the time taken for fish to reach market size; even this may incur high feed costs. Thus the optimum economic content of dietary protein is determined, to some extent, by the relationship between feed costs, capital investment, the nature of the farming operation, and the level and stability of market prices.

One of the most important factors affecting dietary protein requirements in tilapia is age, the dietary protein producing maximum growth decreasing with increasing fish age. Therefore, diets for first feeding of fry have the highest protein and, concomitantly, the greatest cost. Even so it is generally advisable to feed fry at the optimal protein content as it is important to produce healthy fry with a high survival rate. A small amount of high-cost, high-quality feed produces a large number of valuable fry. Balarin and Haller (1982) have proposed the following protein contents as optimal for tilapia for optimum growth :

Below 1 g	35 - 50%
1- 5 g	30 - 40%
5 - 25 g	25 - 30%
Above 25 g	20 - 25%

1.6. Nutritional Value of Protein.

Biological tests of nutritional quality of proteins for birds and mammals were designed to compare how different food proteins rate in their ability to meet AA requirements of the animal; how the chemically assesed "score" of a protein compares with its actual BV. Such tests are necessary to define the limiting AA in food proteins and are useful in determining the availability of AA, especially in proteins that have been subject to extensive processing (Cowey, 1979). The tests are non-specific in that they attempt to measure the qualitative and quantitative adequacy of ten different compounds at one time. In addition the limiting AAs will differ in different proteins and even in a given protein at different ranges of intake. Measurement of nutritional value ought to be made at or near the maintenance ration of protein intake where the AA composition is the limiting feature (Cowey, 1979). Nevertheless, there are marked differences in the nutritive value of different types of proteins of similar AA content, and wide variation in the nutritive quality of different samples of the same protein. Variations of this kind, which arise from differences in strains or variety, from variation in raw material sources, and changes induced by processing, impose severe limitations on the choice of a single figure which can be representative of nutritive value.

Many reviews of nutritional quality of proteins in mammals are available and the subject has been discussed

with reference to fish by Cowey and Sargent (1972), Cowey (1979) and Bender (1982).

1.7. The Biological Value of Food Protein.

It will be clear from the foregoing discussion that the utilization of dietary protein is greatly dependent on the pattern of EAAs which it provides. The more closely a food protein meets the needs of the animal for EAA the greater will be its utilization. As accurate chemical methods are available for analysing proteins, a first step in evaluating their nutritional value would be the determination of their AA composition. It would seem then a straightforward step to supplying the required AA pattern from a mixture of proteins. Unfortunately the processing of proteins frequently interferes with the availability of certain AA to the body. While AAs remain chemically measurable, a proportion of some of them become biologically unavailable; in particular many proteins are considerably altered by heat processing, during which terminal groups of certain AA are prone to combine with other compounds (carbohydrate and lipid) or groups (Cowey and Sargent, 1972). Other AAs such as methionine or tryptophan may be oxidized. Consequently the proteins become resistant to proteolytic enzymes.

The best known example relates to the EAA lysine. In the intact protein the ϵ-amino group of lysine residues in the protein will react under conditions of moist heat with active groups of non-protein materials to form chemical linkages which cannot be broken by

proteolytic enzymes. Carpenter and Ellinger (1955) have shown that such substituted lysine residues are biologically unavailable; only lysine residues with reacting ϵ-amino groups are apparently available. Carpenter (196Ø) has described a method of measuring the biologically available lysine in a food protein by reacting ϵ-amino groups with 1-fluoro-2, 4-dinitrobenzene and estimating the total ϵ-dinitrophenyl lysine (DNP-lysine) after acid hydrolysis.

Thus variations will occur both in the nutritive quality of different samples of the protein and in the nutritive value of different types of proteins of similar AA content. Although a knowledge of the chemically determined AA composition is extremely useful, the nutritive value of proteins and of mixed protein diets can only finally be assessed by testing them on the animal species for which they are intended, and under the conditions in which they will be used.

It is well known that animal proteins are superior as foods to plant protein. Eggs have the highest nutritional value, followed by milk and muscle meats, followed by plant proteins. The chemical score, the ratio of EAA in the finished feed compared to the ratio in whole egg protein is only a partial measure of what fish actully get from the protein fed (Hastings, 1969). The AA which would be limiting, as far as the animal's requirement for that particular ration is concerned, can be calculated, but more definite predictions can be obtained when the

digestibility coefficient of the protein component is measured by pepsin or papain digestion or by pancreatic digestion techniques. A better approximation of BV of the protein component can thus be obtained .

Cowey (1975) reported that, in contrast to the omnivorous mammals, differences in the nutritional values of food proteins for fish are evident at high protein intake. This was already evident from the data of Ogino and Chen (1973a) on carp and of Cowey et al., (1974) on plaice, Pleuronectes platessa . Similar differences (Table 1.6) in nutritional quality are evident when casein and isolated fish protein were fed to rainbow trout, Salmo gairdneri (Rumsey and Ketola, 1975). These differences in nutritional value between different proteins given in high amount in the diet must be due to high requirements of fish for EAA. In general, however, measurements of nutritional quality of likely feed proteins show a fair measure of agreement in that fish meal is appreciably superior to other sources such as cereal proteins and single cell proteins (Nose, 1971; Cowey et al., 1971; Orme and Lemm, 1973; Ogino and Chen, 1973a). As implied above, the absolute values obtained for different proteins by the methods applied vary considerably, but fish meal has given consistently higher values than other feedstuff proteins by virtually all the methods applied. The higher BV of fish meal for many has led to a heavy commercial depedence on its use in diets used for high-density fish production. However, in recent

Table 1.6. Nutritional Value of Proteins for Different Species of Fish.

Species	Protein	Dietary protein level g/ 100 g	BV %	Reference
Salmo gairdneri	Casein	up to 53.5	50.8	Nose (1971)
	White fish meal	up tp 37.2	44.5	Nose (1971)
	Soybean meal	up to 35.3	25.0	Nose (1971)
Cyprinus carpio	Casein	43	63.0	Ogino & Chen (1973b)
	White fish meal	43	64	Ogino & Chen (1973b)
	Corn gluten meal	43	39	Ogino & Chen (1973b)

years the supply of fish meal has become increasingly uncertain and the price has risen rapidly. The replacement or reduction of fish meal to a minimum in commercial feeds has therefore received much attention.

1.8. Alternative Protein Sources.

1.8.1. Possibility of Replacing Fish Meal.

Much of the research into the possibility of using unconventional protein sources as replacements for fish meal in compounded fish feeds has centred on salmonids, as this family includes the species most commonly reared on completely artificial diets. Many of the results obtained so far have proved encouraging. Replacement of fish meal in the diets of warm-water fish species has received less attention since complete compounded rations have only recently been employed in the commercial culture of such species. The use of plant proteins would seem to be the most appropriate and significant, as such proteins are likely to be more constantly available, and cheaper to produce, than fish meal (Jauncey, 1982)

1.8.2. Possibility of Using Soybean Meal.

Soybean meal is an obvious choice as it is the dominant oilseed protein on a worldwide basis and is readily available at a cost per tonne half that of Peruvian or menhaden fish meal (Anonymous, 1978). Assuming a protein content for fish meal of 70% and 50% for defatted

soybean meal, the relative cost per unit of protein from soybean meal is 70% of that of fish meal.

Cowey et al., (1971) replaced approximately half of the protein, in a 40% codmeal protein diet, with soybean meal and found that this depressed both growth and protein utilization of plaice. Similar results have been reported for channel catfish (Andrew and Page, 1974) where the isonitrogenous replacement of dietary menhaden meal with soybean meal depressed growth and food utilization even when the soybean meal was supplemented with methionine, cystine and lysine to that of the concentrations found in the fish meal control. These three AA are generally considered to be the most limiting in soybean (NAC, 1973). However, Krishnandhi and Shell (1967) found that channel catfish grew as well on a 30% protein diet containing a 50 : 50 mixture of soybean and casein proteins as they did on a diet containing casein alone.

Koops et al., (1976) evaluated isonitrogenous replacement of fish meal at 39% and 47% with either soybean meal or soybean protein in diets of rainbow trout. They found that 25% of dietary fish meal could be replaced by soybean protein, but that greater replacement resulted in depressed growth. Rumsey and Ketola (1975) found that the growth of rainbow trout fingerlings fed diets containing 49% protein with soybean as the sole protein source was significantly improved by supplementation with AAs in certain combinations. A recent comprehensive study of the replacement of fish meal with soybean meal in diets of

Tilapia aurea at various protein contents found that at 36% protein, tilapia grew as well on an all-soybean protein diet as they did on all-fish meal protein diet (Davis and Stickney, 1978). All of the diets in this study were supplemented with methionine to bring the total in the diet to 1.1%.

Information on the effects of substitution of fish meal with soybean meal in carp diets is scarce. Kaneko (1969) reported from unpublished Japanese data that one-third of the white fish meal in carp diets could be replaced by soybean oil meal with no depression of growth. Hepher et al., (1971) reported that pond carp feeds containing fish meal produced better growth than those containing soybean meal. Viola (1975) isonitrogenously reduced the fish meal content of 25% protein carp feed to 5-15% by replacement with soybean meal supplemented with AAs, vitamins and minerals. The group fed the soybean diet did not perform as well as the controls despite these supplementations. Atack et al., (1979) reported that a soybean protein concentrate was poorly utilised as the sole protein source in 30% protein rations for carp. Jauncey (1979) determined the effect of isonitrogenous substitution of fish meal in a 30% protein carp diet with soybean protein concentrate. Substitution of only one-third of the dietary protein with soybean protein caused a significant decrease in growth rate and food utilisation. This corresponded to a decrease in the calculated methionine content of the diets from 2.9

to 2.4% where the diets contained 1.5% cystine. These figures correspond to the EAA requirements reported by Nose (1978) who found the requirement for methionine to be 2.1% in the presence of 2% cystine and 3.1% in its absence. Reinitz (198Ø) reported that fish meal could be reduced from 2Ø to 5% of the ration for rainbow trout by isonitrogenous replacement with soybean meal without any reduction in weight gain of the fish.

Attempts to raise the nutritional value of soybean meal by supplementing crystalline AA has been evaluated in the salmonids by several workers (Rumsey and Ketola, 1975). AA supplemetation should, theoretically, improve the utilisation of proteins deficient in EAAs such as soybean. However, supplementation of diets for warm-water fish has generally proved unable to elicit a growth response (Hepher et al., 1971; Andrews and Page 1974; Viola, 1975; Hepher, 1978) possibly due to an inability of these fish to utilise FAA or peptides (Aoe et al., 197Ø, 1974; Page 1974). Ogino and Chen (1973a) determined the BV of several different protein sources to carp. The BV of proteins from animal sources such as egg yolk (89), casein (8Ø) and white fish meal (76) were found to the higher than those from vegetable sources such as soybean meal (74) and gluten meal (55). Meske and Pfeffer (1977) reported on trials to produce a fish meal-free dry food for carp. The diet developed consisted of a mixture of soybean meal, acid whey powder and fat in a ratio of 4: 5 : 1 plus vitamins and trace elements. This feed

(designated SM 3) was found to be superior, in terms of growth and food utilisation, to a commercial fish meal based trout diet. Meske et al., (1977) continued this research, investigating the incorporation of s single-celled alga (Scenedesmus obliquus) in the fish meal-free ration. They found that a diet containing soybean meal(26.3%), acid whey powder (34%), fat (6.7%) and Scenedesmus (32%) produced growth equivalent to that of a commercial, fish meal-based, trout feed and a very similar food conversion (1.3 as compared to 1.28).

1.8.3. Single Cell Protein.

In addition to soybean meal, several other potential dietary protein sources have been examined in carp feeds. Atack et al., (1979) fed a variety of novel proteins to carp, in addition to soybean protein, as the sole protein sources in 3Ø% protein feeds. The results of this expirement are presented in Table 1.7. Slight variations in dietary protein in this experiment make absolute comparisons impossible. However, the results indicate that carp, like salmonids, require diets with high protein quality for optimal growth in tanks. A major portion of this could be provided by bacterial and yeast protein, but the plant proteins tested were poorly utilised and it is unlikely that they could be used to contribute significant dietary protein in intensive culture systems.

Table 1.7. Utilisation of Some Novel Protein in Carp Diets (Atack et al., 1979).

Protein source	FC	PER	NPU%	Digestibility%	BV
Herring meal	1.42$_{c}$	2.82$_{f}$	64$_{j}$	8Ø.3	79$_{n}$
Methanophilic bacterium (ICI"pruteen")	1.14$_{d}$	2.54$_{g}$	49$_{k}$	93.Ø	52$_{p}$
Casein	1.55$_{c}$	2.Ø8$_{h}$	47$_{k}$	96.6	49$_{g}$
Petroleum yeast	2.86$_{a}$	1.35$_{i}$	42$_{i}$	83.7	51$_{g}$
Soybean protein					

Note : Figures in each column with different superscripts are significantly different ($p < Ø.Ø5$).

1.8.4. Plant protein.

Unorthodox sources of dietary protein worthy of investigation for fish include algal meals, the commonest of which are Chlorella, Scenedesmus and Spirulina. Hepher et al., (1978) have discussed this possibility in some detail pointing out that as methods of algal culture and harvesting are improved the economic provision of algal meals as dietary protein sources becomes more probable. Growth trials were performed using algal meal (an unspecified mixture of Chlorella and Euglena) isonitrogenously to replace soybean meal in a soybean/ fish meal diet. It was found that diets containing the algal meal produced a better growth response than soybean meal.

Ogino et al., (1978) determined the nutritional quality of another novel plant protein, leaf protein concentrate (LPC) mechanically extracted from rye grass. Casein, as the reference protein source, was partially and wholly replaced by LPC in diets for carp and rainbow trout. Values of PER and NPU for LPC were relatively high and improved by combination with casein. PER and NPU were higher in carp fed 43% LPC plus 57% casein (of total protein) than when either protein source was fed singly. The LPC evaluated contained 57% protein which makes feasible its incorporation into relatively high protein fish diets. In both LPC and casein, methionine might be assumed to be the first limiting AA, but it appears that the overall balance of AA in the two proteins together is superior to that of casein alone. Murray and Marchant

(1984) studed the effect of mixed microbial bimass in rainbow trout diets. In semi-purified diets of 40% crude protein content, up to 50% of the dietary casein N could be isonitrogenously replaced by SCP without any loss in growth rate (SGR), dietary N utilization (NER), or nitrogen productive value (NPV) in fingerling trout. With total replacement of casein N by SCP, however, NER and NPV were reduced to 50 - 65% compared with trout fed casein alone.

1.8.5. Other sources.

Other feedstuffs considered as possible dietary protein replacements for fish are non-protein nitrogen (NPN) compounds, which are well utilised by ruminants. Cowey and Sargent (1972) quote Vallet who obtained good growth of grey mullet when replacing half the dietary protein with urea. Dabrowski and Wojno (1978) reported growth of carp on 30% protein diets equalling that on a 42 % diet when supplemented with 2.5% urea or 9.3% ammonium citrate. On the other hand, Hepher et al., (1978) concluded that carp cannot utilise urea as a non-protein nitrogen source.

Schlumberger and Labat (1978) fed carp fry on moist diets prepared from industrial wastes. Paper mill waste (56% fiber, 44% minerals) and two different powder wheys (70% and 13% protein respectively) were mixed with fish meal, cod liver oil, starch and vitamins. The test diets were compared to a 51% protein commercial dry pelleted feed. Both whey proteins were very poorly assimilated and

the incorporation of urea and other additives in the feed did not improve this. The high mineral and fiber content of the paper mill waste also resulted in extremely poor growth and food conversion.

Lukowicz (1978) investigated the replacement of fish meal in carp diets with meal of Antarctic ocean krill (Euphausia superba). Diets were prepared containing 42% crude protein :

A. Krill as sole protein source .

B. 50% krill, 50% miscellaneous animal proteins.

C. 50% fish meal, 50% miscellaneous animal proteins. Diet A was found to produce growth rates, FCR and NPU equal to diets B and C, demonstrating that in compounded commercial feed all of the animal protein sources could be replaced by krill with concomitants in the feed.

1.8.5. Silage.

Although a number of novel animal proteins have been investigated as fish meal replacers(for example, silk worm pupae, blow fly larvae, earthworms and krill), none as yet have proved to have any commercial impact. However, the silage of animal wastes is a technique that is making headway for the feeding of cultured species, particularly salmonids. This method of preservation of feed materials has been practised for several years. The waste animal materials are liquefied by the action of endogenous enzymes in the presence of added mineral or organic acids such as sulphuric, phosphoric, formic, or propionic acids. The mixture is heated to inactivate enzymes,

water is removed, and it is then incorporated into diets as a protein source. Although it appears to be effective as a growth promoter for fish, there are a number of problems yet to be resolved. For example, there is much unsaturated lipid in the oil of fish silage, making it susceptible to oxidation and formation of toxic break-down products. There are problems in the economics of drying the silage and collection and storage of the raw waste material can present problems. Nevertheless, because of the immense amount of waste products produced by the fishery industry, an economical way of converting inedible protein into acceptable protein for the animal feed industry, including fish feed, is much required. Presnt developments are encouraging.

1.9. Protein Digestibility.

The use of chromic oxide as an inert indicator method for the determination of various nutrient digestibilities in fish has a number of advantages over other methods, as summarised by Austreng (1978). Some of those are that quantitative feeding and collection of faeces are not essential, stress to the fish in the culture environment is minimised, the method is simple, and fish do not have to be killed.

Nose (1967) utilized this method for the determination of true digestibility of various proteins in rainbow trout. By correction for endogenous metabolic faecal nitrogen, it was determined that the true digestibility of a dietary protein is constant and not affected by

protein content of the diet, as would be for the apparent digestibility without correction. Page and Andrews (1973) in their study of the effect of protein/energy ratios on protein utilization by channel catfish, obtained higher apparent digestibilities with diets containing 35% protein (89-90% digestible) than at 25% (80 and 84%). Ogino and Chen (1973b) determined the true digestibilities of a range of dietary proteins in carp. Casein, white fish meal, dried egg yolk, gelatin, corn gluten meal, soybean meal and wheat germ proteins all had digestibilities in excess of 92%. Kim (1974) reported on the digestibility of a whole range of proteins by carp although giving no growth or food utilization data (Table 1.8).

Using the total faecal collection method Bondi et al., (1957) examined the digestible protein contents of a number of feeds for carp (Table 1.9). It was claimed, though without supporting evidence, that the digestion coefficients of fiber in these feeds ranged from 0 to 89%.

Cho et al., (1976), in a study with rainbow trout, found all diets to have high apparent digestibility for protein and fat (over 90 %) which was not a factor in regard to differences in growth and feed efficiency of the various diets. Windell et al., (1978) examined methods of faecal collection and determined that absorption of nutrients was complete in samples removed from the lower 2.5 cm of the intestine of rainbow trout. It was also found that

Table 1.8. The Digestibility of Various Protein sources by Carp. (Kim, 1974).

Protein sources	Protein in Test diet %	Apparent Digestibility	True Digestibility
White fish meal	7.8Ø	83.5Ø	9Ø.3Ø
White fish meal	24.6Ø	86.9Ø	89.2Ø
White fish meal	42.4Ø	88.8Ø	9Ø.2Ø
Silkworm pupae	2Ø.9Ø	63.9Ø	66.3Ø
Wheat germ	1Ø.3Ø	85.ØØ	9Ø.2Ø
Brown fish meal	13.9Ø	73.9Ø	77.6Ø
Purified soybean meal	12.3Ø	86.8Ø	91.6Ø

Table 1.9. The Digestible Protein Contents of Carp Feeds.
(Bondi et al., 1957)

Food	% Digestible	% Total Digestible Nutrients
Peanut meal	41.ØØ	73.5Ø
Soybean oil meal	34.ØØ	74.4Ø
Cottonseed oil meal (grade A)	38.7Ø	73.6Ø
Cottonseed oil meal (grade B)	32.3Ø	58.7Ø
Sweet lupin seed	3Ø.4Ø	75.2Ø
Condemned peas	23.ØØ	71.8Ø
Rice meal	9.6Ø	83.ØØ
Wheat bran	13.7Ø	61.4Ø
Barley feed	1Ø.2Ø	43.6Ø
Barley grain	6.2Ø	6Ø.9Ø
Corn grain	7.4Ø	74.ØØ
Alfalfa meal	13.6Ø	49.1Ø

with faeces collected from the water, nutrient leaching would occur, largely within the first hour after excretion, and that as much as 10% difference in apparent protein digestibility could occur. Austreng (1978) determined apparent digestibility for crude protein, fat, carbohydrate, ash and gross energy in a diet by different methods of manual stripping of faeces and dissection of different parts of the intestinal tract of rainbow trout. Absorption of protein was found to occur as far back as in the rectum and that manual stripping of samples should therefore take place as close to the anus as possible. Austreng analyzed faeces for water-soluble nitrogen and found digestion coefficients around 9% higher, agreeing closely with Windell (above). Inaba et al., (1962) also found digestibility of proteins in rainbow trout to be higher in faeces excreted in pond water than with those collected by stripping. It is interesting to note that in carp, Aoe et al., (1970) found higher nitrogen digestibility with an AA diet of low efficiency than with a casein-gelatin diet (90.9% as opposed to 81.9 %); however, no details on the method of faecal collection were provided .

Plakas and Katayama (1981) studied apparent digestibilities of individual AAs in three regions of the gut of carp, determined by the chromic oxide method with a protein and an AA diet. The absorption of AA from the AA diet was more rapid than from the casein diet; total AA absorption was similarly high with both diets, in all

three regions. Generally EAAs were absorbed more rapidly than NEAA, regardless of diet. Scherbina & Sorvacev (1969) fed carp with two plant protein sources in the diets; most of the dietary AAs were absorbed in the first 64% of the intestine length. A cottonseed-meal-based diet gave lower figures of AA absorption in both the first intestine segments and the whole digestive tract. Since a major quantitative function in dietary protein absorption was attributed to the anterior intestine this eliminated the pinocytotic uptake of whole protein molecules related to the second segment (Stroband & van der Veen, 198Ø) as playing an important function in stomachless fish. Other authors demonstrated that in fish with stomachs such as trout (Austreng, 1978), catfish (Pappas et al., 1973) and tilapia (Bowen, 1978), protein absorption takes place mainly in the anterior 5Ø % of the gut. Dabrowski & Dabrowska (1981) have indicated not only the disapperance of AAs along the gut of rainbow trout but have also measured the amount of FAAs in the digesta. Dabrowski (1983) reported that apparent absorption of AAs in common carp fed soybean meal was much lower than that in other fish species, namely rainbow trout, catfish and grass carp. Schmitz et al., (1984) reported the digestibility for eels fed different protein sources. The authors found the digestibilities of crude protein were casein Ø.99, gelatin Ø.94, fish meal Ø.94, bacterial protein Ø.89, concentrated soy protein Ø.96 and soybean meal Ø.94. Tacon and Rodrigues (1984) compared the use

of three external dietary markers (chromic oxide, polyethylene and acid-washed sand) and a natural dietary marker (crude fibre) for the estimation of apparent nutrient digestibility in rainbow trout fed a practical diet. They suggest that chromic oxide (< 1%) and crude fibre are reliable external and internal dietary markers for use with rainbow trout.

1.1Ø. Assessment of Methods Used to Determine protein Quality.

1.1Ø.1. Introduction.

As has been mentioned before the quality of a protein is dependent on its AA profile and its AA availability relative to the requirements for EAA and non-specific nitrogen by the target species. The purpose of measuring protein quality is to determine the usefulness of a protein for supporting life and production in a given animal. However, the interactions between AA composition, availability and requirements make difficulties for accurate assessment of protein quality. The AA composition of a protein may not be known, its determination may not be feasible, or equipment may not be available to analyze all samples for AAs. Also, reliance on tabulated AA compositions may lead to erroneous conclusions about the sample on hand. Payne (1972) showed that considerable deviations are evident in AA values reported, even for the more common foodstuffs compositional analyses may not be deemed necessary for the evaluation of a particular protein, even though these analyses could aid in the

interpretation of results.

Overall AA nitrogen digestibility may differ from the total nitrogen digestibility, because the non-AA nitrogen may be absorbed at a different rate from the AA N. Some AAs may be located predominantly in the less digestible fractions of a food rendering them less available than the total true protein nitrogen (Eggum and Jacobsen, 1976). Again, as has been already mentioned, preparation procedures may alter the AA availability of a protein. Heat treatment, for example, has been shown to lower the availability of reactive AA (Carpenter, 196Ø) and to enhance the availability of other AA by destroying enzyme inhibitors (Olomucki and Barnstein , 196Ø).

Requirements of fish for AA are not well established . The alterations in the nutrient requirements for forms of production other than growth are essentially unknown. Thus, the problems associated with protein quality determinations are easily recognized. The quantitation of protein quality cannot be readily defined, but protein quality assessments are necessary to define one or more of the following aspects of " quality ". (1) to measure the effectiveness by which a protein supplies AA to meet the requirements for a stated function. (2) To determine optimum quantities of a protein needed for a given function. (3) To compare proteins or changes in proteins that occur due to plant breeding or processing procedure. (4) To predict how a protein will function to supply the required AA in a complete diet.

1.1Ø.2. Methods Using Laboratory Animals.

1.1Ø.2.1. Feeding Methods.

Animal tests provide the standards by which laboratory procedures must be assessed, but those available do not all yield the same type of information, nor are they equally useful. Some of those frequently used are reviewed briefly (Table 1.1Ø).

The most common test animal is the rat, although attempts have been made to replace this with other monogastric animals such as mice, growing pigs and chicks (Waddel & Desai, 1981). Results from evaluation of proteins using different animal species vary due to digestive capacity, AA absorption and requirement of the species in question, although the general trend in results is similar (Bodwell, 1977). A low-quality protein is not converted into a high-quality protein by changing the test species.

Multipoint assays for evaluation of protein quality are used to compute the slope ratios between results obtained at varying protein contents in the food (Samonds & Hegstedt, 1977). The main discrepancy in results is caused by feeding a protein-free diet. AAs are recycled in the body to varying extents (Pellet, 1978) and obscure zero food-protein values (Beneditti et al.,1981). However, dose-response curves are not necessarily linear and may be affected by the AA composition of food proteins (McLaughlan, 1978; Phillips, 1981).

This must be taken into account in calculations of

Table 1.1Ø. Assessment of Protein Quality based on changes in Weight and Body Nitrogen. Bodwell (1977) and Evans & Witty (197Ø).

Method	Type of assay
PER	Weight gain per total protein intake
BV	Absorbed nitrogen retained in the body for maintenance and /or growth
NPU	Final carcass nitrogen of the test group minus that of the group on protein-free diet divided by nitrogen intake of the test group
RNU	Weight gain plus (Ø.1 times initial plus final body weight) divided by nitrogen intake
NGI	Slope of weight gain to nitrogen intake of the straight of the line
RPV	Slope of weight gain to protein intake relative to that of lactoalbumin

slope-ratios.

1.1Ø.3. Chemical methods.

1.1Ø.3.1. Amino Acid Analysis.

The determination of the AA composition of hydrolyzed proteins can yield utilizable information about the protein or nitrogen fractions of foods. As opposed to protein quality assessments using only growth as a criterion, AA analysis aids in understanding where deficiencies or excesses occur and in predicting responses with mixtures of proteins.

Discretion must be used with AA analysis. Errors may occur when the requirements for AA by the animal under study such as fish are not well established. Furthermore, the AA analysis cannot give any indication as to the availability of either the protein in general or of a specific AA.

1.1Ø.3.2. Chemical score.

Because of the difficulty in comparing the nutritional merits of proteins with differing AA composition, Mitchell and Block (1946) proposed a scoring system whereby proteins could be ranked. A chemical score is assigned to a protein by calculating the ratio of each EAA to that in the reference standard, and by using the lowest ratio as the chemical score. The AA profile of whole egg is commonly used as the reference standard, because the BV of this protein is high for both the rat and man.

Chemical scores are highly correlated to NPU; however,

NPU can not be readily predicted from the chemical score. It has been illustrated (Payne, 1972) that the regression of chemical score on NPU is linear, but that it does not pass through the origin. This is due to the fact that chemical scores assign values to proteins for growth, whereas NPU indicates the value of proteins for both growth and maintenance. As with AA analyses alone, it has been shown (Payne, 1972; Bender, 1973) that chemical scores do not reflect AA availability. Oser (1959) argued that although a protein may be devoid of a single EAA, its supplemental effect on a complete diet, as well as its maintenance contribution, should be calculable.

1.1Ø.3.3. Essential Amino Acid Index.

Based on the assumption that each EAA must make some contribution to the nutritional status of a protein. Oser (1951) described an integrated EAAI. The ratios of AA in the test protein to those in the standard protein are converted to a logarithmic scale, and the antilogarithm of the mean values gives the EAAI. A limit of 1ØØ% is set for AAs present in the test protein in excess of those in the standard protein. The author stated later (Oser, 1959) that the EAAI gives greater detail than the chemical score about the effects of protein or AA supplementations that affect AAs other than the critical one. The most objectionble feature of this method is the lengthy computation needed to arrive at the EAAI for each protein. Payne (1972) concluded that improvement in

predicted values over chemical score is not great enough to warrant using the EAAI.

1.1Ø.3.4. Amino Acid Availability.

There are few data on rapid chemical methods for determining the bioavailability of single AAs suspected of being limiting in a foodstuff or a diet. The difficulties in developing such techniques may well be because few AAs exhibit indentifying properties, and also that the nature of mechanisms which lower AA availability is not well known.

A few successful methods for chemically determining the availability of lysine have been devised. The best known and most frequently used technique is the dinitrofluorobenzene (DNFB) technique (See section. 1.7). Roach et al., (1967) noted that the DNFB test for available lysine is somewhat inaccurate for foodstuffs with high carbohydrate contents because the DNP-Lysine is destroyed by the carbohydrate during the assay. Booth (1971) suggested correction factors based on recoveries of DNP-Lysine added to foodstuffs for various groups of foods. Although values for total available lysine may be somewhat inaccurate, it has been shown that they agree very well with chick growth when lysine in the limiting AA (Carpenter and Woodham, 1974). Roach et al., (1967) also proposed a modification of the DNFB approach. Available lysine is determined by finding the difference between total lysine and lysine remaining in solution after treatment with DNFB. This alleviates the need for

correction factors for different types of food.

1.1Ø.3.5. In Vitro Digestion.

High-quality proteins are those that are well digested and contain all the EAAs necessary for protein synthesis in vivo, in a ratio that supports good growth. Since protein synthesis requires that all the AAs be available at the same time and as AAs are liberated from food in vivo only after a meal, a measure of the amount of EAAs released by digestive enzymes in vitro should provide an estimation of protein quality. Natural conditions should be copied as closely as possible in order to obtain the truest possible picture of protein digestion in vivo. Such an approach avoids the problem that AA analysis is unsatisfactory for low-digestibility foodstuffs (Bender, 1973). Such in vitro enzymatic digestion has been used for a long time (eg. estimation of AA availability in heat-processed foods (Riesen et al., 1947) and in combination with EAA profiles, has enabled protein quality to be predicted. Reviews are given by Satterlee et al., (1977, 1979). Such a technique has not been explored for fish.

In an attempt to relate the BV of food proteins to the pattern of AA released by the digestive enzymes, Sheffner et al., (1956) developed an integrated pepsin digest residue (PDR) AAI, which combined the pattern of EAAs released by in vitro pepsin digestion with that of the residue. The PDR index was reported to correlate closely with the NPU value of a variety of proteins, and its

subseqent application to heat processed proteins indicated that the method does predict changes in the NPU value of processed proteins (Sheffner, 1967).

A pepsin pancreatin digest index (PPDI) that involved measuring the AA released by digestion with pepsin followed by pancreatin and by acid hydrolysis was developed by Akeson and Stahmann (1964). The PPDI for twelve proteins calculated in the same manner as the PDRI was shown to correlate with their BV reported in the literature (Table 1.11).

Buchanan (1969) and Buchanan and Byers (1969) described an *in vitro* system for measuring protein digestibility of leaf protein concentrates with an enzymatic digestion utilizing papain. The parameters obtained gave satisfactory agreement with *in vivo* results from bioassays. Saunders *et al.*, (1973) developed a papain-trypsin system, the *in vitro* results of which correlate well with *in vivo* digestibility (r=Ø.91). Maga *et al.*, (1973) pointed out that initial rates of hydrolysis by trypsin on some commonly used protein sources were good indicators of their digestibility. Rhinehart (1975) modified Mega's procedure and investigated the correlation between *in vitro* and *in vivo* protein digestibility, examining several enzyme systems which included trypsin, pepsin-trypsin, trypsin-chymotrypsin, and trypsin-chymotrypsin-peptidase combinations. The results were encouraging, with correlation coefficients of Ø.77, Ø.72, Ø.8Ø and Ø.74 respectively. The enzymatic methods for protein

quality evaluation in foodstuffs have some marked advantages and some disadvantages as compared to animal assays. The advantages as illustrated by application of the pepsin pancreatin digest method are:

(1) It is less expensive to conduct and requires less time than assays based on rat feeding .

(2) Its results show less variation than PER assays with rats.

(3) A single in vitro assay will indicate the relative amounts of each EAA released by the enzymes from the protein under assay, thus giving a quantitative estimation of protein quality and also which EAA may be lacking or so low as to reduce the protein quality. In contrast, a single assay based on rat feeding may give only an indication of quality but no information on the reason for low quality.

(4) The digest method can be used to monitor the adverse effects of processing operations on proteins that may reduce their digestibility or the availability of lysine, or oxidized sulphur AAs.

An advantage of animal assays is that they may reveal the presence of toxins or the absence of other essential nutrients. However, repeated animal assays are required to identify the limiting AA, vitamin or mineral. Although in the final analysis the nutritional quality of the protein must be established by feeding, it is suggested that enzymatic methods will be very useful in preliminary evaluation of protein quality and in determining how to

process or blend protein sources for maximum protein utilization by animals, including fish.

Table 1.11. Comparison of Pepsin Pancreatin Digest Index (Calculated Without the Tryptophan Correction) With BVs Reported in the Literature for Growing Rat, the Chemical Score (1) and EAAI (2).

Food protein	BV	PPDI excluding tryptophan	PPDI including tryptophan	Chemical Score	EAAI
Whole egg	96(3),97(4)	100	100	100	100
Egg white	83(3),82,97(6)	89	90	100	97
Milk	90(3,5),84(3,4)	87	86	79	89
Lactalbumin	85(3),84(5)	85	87	79	88
Beef	76(3,5)	80	78	81	83
Casein	73(5),69(5),78(7)	78	79	66	89
Soybean raw	57(3),59(7)				
Heated	75(3,5),74(7)	68	67	51	82
Yeast	63(3), 69(5)	74	74	55	81
Wheat flour	52(3,5)	54	52	42	61
Gluten	40(6),61(7)	49	47	40	55
Zein	_____	42	27	17	33
Gelatin	25(5), 0(5)	29	18	17	23
	r=0.982 (0.995)(9)	0.99 (0.994)(9)	0.94		0.979
Regression equation(10)		1.097X-11.0	0.97X-0.54 (0.94X+1.98)(9)	0.83X-14.48	1.00X-9.46 (0.954X+0.65)(9)
SE of estimate		1.45	1.05	2.58	1.65

(1) Bender (1961).
(2) Oser (1959).
(3) Block & Mitchell (1946).
(4) Sommer (1938).
(5) Mitchell & Block (1946).
(6) Mitchell & Beadles (1950).
(7) Rippon (1959).
(8) Bender et al., (1953).
(9) Values in parentheses exclude gelatin.
(10) Y = Predicted BV ; X = PPDI.

1.1Ø.3.6. Plasma Urea Nitrogen.

Experiments of several authors have shown that the blood urea content of mammals reflects the protein quality of the diet. Punchal et al., (1962) demonstrated that the blood urea content of pigs increased with a decline in protein quality, measured as growth rate of the animals. Munchow and Bergner (1968) carried out more detailed investigations and found a very high negative correlation between BV and blood urea content in experiments with pigs and rats. The same relationship was obtained in experiments with rats by Eggum (197Ø). Nielsen (1973) also found a significant relationship between blood urea concentration and the quality of bacon pigs after slaughter, but the correlation was not as high as in the other experiments. At least three factors influence blood urea concentration, the quality and quantity of protein in the diet and the time of sampling after feeding. By standardising techniques it is possible to eliminate the effects of both protein quality and time after feeding(Eggum, 197Ø).

Kumta and Harper (1961) demonstrated in rat experiments, that a dietary AA imbalance increased blood urea considerably in comparison with that obtained with consumption of a balanced protein. Similarly, Schimke (1962) observed decreased activities of hepatic urea cycle enzymes in association with low-protein diets.

In studies with human subjects, Fomon et al., (1979) observed that children who received supplemented soyprotein

showed significantly higher blood urea than children who received soyprotein supplemented with methionine.

Schoeneberger and Gross (1982) and Gross et al., (1982) demonstrated in rat experiments that blood urea concentration correlates highly inversely (r=-Ø.95) with PER. This correlation may be influenced by protein digestibility.

Kirchgessner et al., (1977) found a high negative correlation between the calculated EAAI of a food protein and the blood urea content in rats (r= Ø.78). Thus, protein quality could become the decisive factor influencing blood urea concentration and thus become a good indirect measure of the protein adequancy of the diet of an individual. This should be investigated for fish.

1.1Ø.3.7. Ammonia Excretion Rate as Index for Comparing Efficiency of Utilization of Different Dietary Proteins in Carp.

The main end product of protein metabolism in fishes is ammonia which is largely formed in the liver and excreted passively by the gills (Forster & Goldstein, 1969). Ammonia weak acids and bases diffuse across biological membranes mainly in their lipid-soluble nonionized forms which accounts for their ready movement into and out of all cells. With fish gills and other excretory surfaces, the pH difference that exists between the internal and external environment of aquatic animals may be a significant factor in determining rates of ammonia elimination.

Alkaline seawater tends to retard and acidic freshwater accelerates diffusion of the free base into the external environment. Euryhaline fish that can withstand wide ranges of salinity, either by conformity or regulation, have the excretion of ammonia accelerated by transition from the alkaline seawater to neutral or acidic freshwater. Except for its relative toxicity, ammonia has many advantages over urea and uric acid as the chief end product of nitrogen metabolism; for example, no expenditure of energy is required for the conversion of protein nitrogen to ammonia, and indeed some of the reactions involved in its production, such as the deamination of glutamate, lead ultimately to the production and capture of free energy .

Maetz and Garcia Romeu (1964), followed up a suggestion made earlier by Krogh (1939) and demonstrated that NH_4^+ was able to exchange for Na^+ absorbed by the gills of freshwater fish. Excretion of N in this form thus has a useful dual purpose in helping Na^+ accumulation, whose active absorption is very important in maintaining ionic balance.

Measurements of ammonia and total nitrogen excretion may be important indicators of the effect of various environmental and nutritional factors on protein metabolism, and can give insight into the nitrogen balance of fish. Several workers have studied the effects of temperature, body weight and ammonia accumulation on rates of nitrogen excretion (Fromm & Gillette, 1968; Geuerin-Ancey, 1976)

but experiments relating to the effects of feeding are few (Savitz, 1971; Beamish and Thomas, 1984; Ming, 1985). After feeding, rates of nitrogenous excretion are high (Brett & Zala, 1975 ; Elliot, 1976), the majority of the nitrogen excreted being derived from the deamination of AA making up the food proteins. The excretion of these amino fractions (exogenous excretion) therefore represents an energy loss from the food, although the remaining carbon residues of the AAs are available for metabolic use. In addition, some excreted nitrogen is derived from catabolism and turnover of body proteins (endogenous excretion) and both exogenous and endogenous sources of nitrogen contribute to a common metabolic pool of AAs. In order to separate the relative contributions of these two sources, the endogenous nitrogen excretion has been defined as " the lowest rate of nitrogen excretion attained after an empirically defined time interval on a low nitrogen but otherwise complete diet" (Brody, 1945). This has been interpreted as being the rate of excretion of a fish in nitrogen balance i.e maintaining the status without sacrificing body protein (Brett & Groves, 1979), although Birkett (1969) uses the expression "endogenous nitrogen excretion" for the lowest rate of excretion monitored when the fish receives nitrogen from no external source. Efficient utilization of dietary protein for growth is not among the economically important traits which have been considered for genetic selection in fish. In this regard, the development of simple and

effective methodology for measurement of nutrient efficiency is urgently needed (Gjedrem, 1983). Brett and Zala (1975) reported that Sockeye salmon exhibit a sharp peak in ammonia excretion rate 4-4.5 h after a single daily meal. For trout fed diets of different composition, Garcia et al., (1981) used ammonia excretion to indicate relative amounts of ingested protein used for protein synthesis and as a source of energy. On the basis of these observations, ammonia excretion would appear to have potential as an index of dietary protein utilization applicable to genetic selection for efficiency of protein utilization or to assessment of protein quality.

Garcia et al., (1981) in comparing efficiency of protein utilization for different diets fed to groups of rainbow trout of the same strain, found agreement between PER and PPV ranking, and ranking based on total ammonia excreted in 24 h. Ming (1985) found significant differences among strains of rainbow trout for excretion of ammonia-N at various times after feeding. Protein efficiency ranking of the strains, determined as the inverse of peak NH_3-N excretion ranking, agreed with PER and NPU ranks obtained in a 3-week growth study using experimental animals from the original laboratory population (Ming, 1985). Ming (1985) therefore, found the peak values to be a better parameter than slope for expressing strain differences in ammonia excretion rate. In addition, post prandial times of peak ammonia excretion rate are compatible with the 6-h apptite cycle reported for rainbow trout (Fange

and Groves, 1979), and for sockeye salmon (Brett and Zala, 1975). Rankings based on maximum rate of excretion are therefore good indicators of the relative efficiency with which dietary protein is used for growth, by different strains of trout.

The proportion of total N excreted as ammonia appears to vary among species and feeding conditions from about 45-9Ø % (Fromm, 1963; and others). Beamish and Thomas (1984) found the proportion of NH_3-N excreted increased with dietary protein concentration but not meal size. In contrast, Kaushik (198Ø) reported an increase in the proportion of NH_3-N excreted with meal size for rainbow trout whereas the urea-N remained a significant and similar fraction of dietary protein irrespective of meal size. High proportions of urea-N have been reported in the excretions of other teleosts (Geurin-Ancey, 1976; Tatrai, 1981).

1.1Ø.3.8. Blood AA as Indicator of Protein Quality.

The products of digestion of dietary proteins are carried by the blood to the tissues mainly as FAAs, whose concentrations in the blood result from the difference between tissue uptake and their supply from the diet; the AA most abundant in the diet should thus be in greatest concentration in the blood. A good deal of detailed work on this question has been done in terrestrial animals, but rather less in fish.

There may be several reasons for plasma AAs not reflecting diet AAs, one being the difference in availability of the individual AAs within the same protein. However, such differences do not explain all. Part of the answer may lie in differences in the rates of absorption of the individual AA. Delhumeau et al., (1962) demonstrated that the rate of absorption of lysine was only 63% that of cystine. The AAs in fish meal were approximately 95% available whereas in barley the availability was only about 8Ø% (Eggum, 1966). This difference must necessarily affect the AA pattern in blood plasma, and make it difficult always to obtain a direct relationship between AAs in feed and plasma.

Zimmerman and Scott (1965) reported that there was a definite relationship between plasma AA in chicks and the AA adequacy of the diet. The first limiting AA remained at a very low concentration in blood irrespective of the severity of the AA deficiency, which explains why, in some instances, supplementing diets with the

limiting AA has failed to increase its concentration in plasma (Owing and Balloum, 1961).

Smith and Scott (1965) showed that supplementing intact proteins with their first limiting AA tended to reduce the concentration of other AA in the plasma. This effect probably reflects an improved utilization of plasma AA for protein synthesis. However, if a dietary protein is too low in a particular AA, catabolism of body tissue proteins will be necessary. In spite of this, Dean and Scott (1966) used free AA concentration in plasma of chick to detect deficiences and excesses of dietary AA. Giving diets containing suboptimal amounts of either lysine or valine resulted in a marked lowering of the limiting AA and an increase in most of the other AAs in the plasma. A dietary excess of lysine resulted in a striking increase in plasma lysine and relatively small changes in other AAs. Similar observations were made by Stockland et al., (197Ø) with pigs. Supplementing the limiting AAs in meat and bone meal diets with crystalline AAs reduced the plasma concentrations of AAs that were not included in the supplement and increased those of AA that were supplemented, but to a relatively smaller extent than might have been expected from the dietary increase. These results were probably due to the same cause as that discussed by Smith and Scott (1965) and Dean and Scott (1966), i.e. improved AA balance with AA supplementation which stimulated rate of weight gain and removal of AAs from the plasma for protein synthesis.

In the field of AA metabolism, many researchers have reported on the muscle FAA content of fishes, but only a few have examined the animal in a comparative sense in regard to its various tissues or to the influence of its metabolic state. Gras et al., (1978) examined the FAA content of the muscle and skin of rainbow trout. The total pool of FAA was found to comprise two-thirds of the total measured ninhydrin-positive substances.

Nose (1972) examined changes in the FAAs in the plasma of rainbow trout at fasting and at various intervals after feeding a commercial diet. Concentrations increased soon after feeding and became maximal between 12 and 24 h afterwards, returning to fasting values only after 3 days. A positive and significant correlaton was noted between the EAA 12 h after feeding and their content in the feed. This was not the case with the NEAAs. Schlisio and Nicolai (1978) performed a similar experiment with rainbow trout, with very similar results, using a diet containing pure AAs as the nitrogen source. An increase in the FAA concentration occurred at a maximum at 12 h after feeding, and a return to pre-feeding values after 48 h. Considering both EAA and NEAA together, no distinct relation was noted between the AAs in the plasma 12 h after feeding and in the diet. In a more comprehensive study with the prawn, Deshimaru (1976) examined the changes in the FAA contents of muscle, hepatopancreas, and blood after feeding a casein-albumin test diet, and compared these

results to those after feeding a poorly utilizable AA test diet of similar composition. Blood concentrations of AAs were maximal around 3 h after feeding both diets and largely reverted to pre-feeding values at 12 h. The maximum AA blood concentration after feeding the protein diet was about twice that of the AA diet. There were obvious differences between individual AAs in their time sequence of appearance and the maximum concentrations produced by the two diets. Wilson et al., (1977) confirmed the dietary requirement of channel catfish for lysine by measuring free lysine in the serum. A similar correlation between serum free methionine and the methionine requirement of channel catfish as determined on the basis of growth and feed conversion was noted by Harding et al., (1977).

Kaushik and Luquet (1977) found the FAAs reached their highest value in blood, 6 h after a meal. The EAA (total or individual) slowly reached the maximum between 3 and 6 h after feeding, then slowly dropped down in fresh water trout, while in sea water, total FAA and NEAA rose to maximal 3 h after meal. However, most of the EAAs gradually increased after a meal attaining maximal values after 12 h. The EAA/NEAA ratio reached a peak after 12 h and dropped down to a minimum value at 24 h. Ahokas and Duerr (1975) demonstrated no relationship between muscle FAA and salinity in teleosts .

Several researchers have noted that although certain protein tests are able to sustain the normal growth of

carp Cyprinus carpio, crystalline AAs as a source of protein failed to sustain their growth (Nose et al., 1974; Tanaka et al., 1977a). Plakas et al., (198Ø) examined plasma AA patterns after feeding casein and a corresponding AA diet in investigating the reason for the inability of the latter to support normal growth of carp. Rainbow trout can grow nearly as well when fed a crystalline AA diet as when fed a high-quality protein . Yamada et al., (1981) found most of free EAAs in the plasma of rainbow trout force-fed casein attained a maximum between 24 and 36 h after feeding, and then declined to the fasting value after 72 to 96 h. In contrast, most of the plasma FEAAs increased immediately after force-feeding the AA mixture and peaked at 12h, returning to their fasting concentrations at 48 h. Plakas and Katayama (198Ø) examined the plasma AA pattern of carp after feeding casein and corresponding AA mixture diets; all EAAs in the plasma of carp were maximal 4 h after ingestion of the casein diet, except for arginine which declined slightly from 2 to 4 h. After feeding the AA diet, plasma free arginine and lysine were at peak values at 2 h after feeding, while others peaked by 4 h. Murai et al., (1982) reported on the balance of exogenous AAs in the plasma as an index of diet quality for carp finding that it correlated significantly with PER values, fish growth and food utilization Dabrowska (1984) found it unsuitable as an index for trout.

Moreover, Murai et al ., (1984) found that the dietary AA balance may affect not only growth and efficiency of diet utilization but also suceptibility to certain diseases. Ogata et al., (1985) reported that total FEAA in juvenile european eel gradually rose as the protein intake increased with a positive and significant correlation between these two parameters. On the other hand, the contents of free NEAA and ninhydrin-reactive substance were more strongly correlated with the extent of protein deposition than with dietary protein or protein intake.

Ogata and Arai (1985) found that in carp, coho salmon and rainbow trout, the total amounts of FEAAs in the erythrocytes were similar or slightly higher than those found in plasma, while the total amounts of FEAA in the erythrocytes were two to three-fold higher found in the plasma. In channel catfish, however, both total amounts of FEAA and NEAA in the plasma were about one-fifth of those in the erythrocytes.

Itoh et al., (1974) fed a casein diet and a corresponding AA diet and found the concentrations of EAAs in the plasma of rats fed the latter diet to rise higher than those of rats fed the former at 1 and 2 h after feeding, and then to fall below pre-feeding values by 6 h. Clark et al., (1973) found that the plasma FAA of rats are high at 4 and 6 h after feeding an AA diet, and that a diet lacking EAAs produced a corresponding low amount in the plasma and liver. Maximum plasma FAAs in the pig

were reached at 2 h after feeding, declined at 4 h, and then increased again to peak values at 6 h (Stockland et al ., 1971). Limited AAs in the diet decreased to base-line values more rapidly in the plasma while those in excess were much higher in the plasma than those at the approximate requirement and they decreased more slowly. Longenecker and Hause (1959) developed the plasma AA ratio as a method for determining the limiting AA in proteins. The plasma AA ratio expresses relative changes in the concentrations of each plasma FAA after the consumption of a protein meal as a function of the AA requirement of the animal, under the assumption that the AAs were removed from the blood at rates proportional to the requirements. It has proved applicable to humans (Longenecker and Hause, 1961), the chick (Hill and Olsen, 1963) and dog (Longenecker and Hause, 1959). McLaughlan et al., (1963) found that AAs in human plasma increased rapidly after the consumption of protein and the duration of the increase was proportional to the amount of protein consumed and its AA composition. McLaughlan (1964) proposed the use of plasma AA score to predict the limiting AA in diets using rats, and it has been proved by McLaughlan et al., (1967) to be suitable for use with practical diets for human nutrition. Smith and Scott (1965) demonstrated the use of plasma FAA concentration in the evaluation of the adequacy of AAs in various proteins for chick diet. Clark et al., (1975) found no effect on nitrogen retention and plasma FAA

concentrations when a dietary protein was replaced by its constituent AA in free form, and concentrations of FEAA in the plasma were not altered significantly by the substitution of NEAA with non-protein nitrogen in the diets of men.

1.11. **The Aims of The Research.**

In the preceding sections of this chapter, the background of the methods used in evaluation of protein quality has been given. In recent years there has been much interest in the possibility of using various alternative cheaper protein sources as a replacement for the expensive fish meal component of fish feeds. Common carp (*Cyprinus carpio*) was chosen as the experimental animal because of its tolerance of a wide range of environmental conditions and its rising economic importance as a table fish in most parts of the world. Worldwide production of the common carp contributes more to the freshwater fishery yield than any other species. However, the increasing manufacture of diets for the culture of warmwater fish necessitates assurance regarding the nutritive quality of protein from various feedstuffs for the fish. There are a number of procedures commonly utilised with homeothermic terrestrial animals for evaluating the nutritive value of protein sources. The adaptation of these procedures for use with fish poses some problems: (1) the relatively slow growth of fish associated with the the low metabolic rate at low water temperature mediates against short-

term tests for control; (2) the facility with which the fish utilize protein as a source of energy, and (3) the generally poor utilization by fish of carbohydrates present in plant protein supplements and concentrates.

In experiment 1 (chapter 3) an endeavour was made to use a short-term bioaassay method for evaluating fish meal, casein, whole egg protein and Pruteen for young carp at circa 38% protein. Also, the experiment determined the effect of dietary protein on growth, food utilization, carcass composition and protein absorption from two regions of the intestine (anterior and posterior part) in young carp.

In mammals, experiments of several authors have shown that blood urea content of experimental animals reflects the protein quality of the diet. Experiment 2 (chapter 4) was designed to use low quantities and low qualities of protein and to compare these sources with good quality protein and high amounts and to determine the effect of these on plsma urea values.

From the results of experiment 2 it was decided to investigate other plant protein sources (Soybean meal, Maize, Rice bran) at low protein percentage, circa 9%, and also blood constituents were studied. This study was undertaken in experiment 3 (chapter 5).

From the results of experiment 3 it was decided to investigate the excretory products of feeding metabolism for carp (Cyprinus carpio) in relation to dietary protein quality. This study was undertaken in experiment 4

(chapter 6).

Experiment 5 (chapter 7) was designed to use an in vitro method for measuring apparent digestibility of casein, fish meal, sunflower, soybean meal and soybean meal plus fish meal diets.

Experiments 6 & 7 (chapter 8) were designed to examine the change in free AA concentration in the blood plasma at various intervals after force-feeding carp with different protein sources at different concentrations (ca 15 & 30% protein), and to use the peak value determined EAAI as an index for protein quality determination .

Questions of the adaptations of proteolytic enzymes of fish to the qualitative and quantitative composition of protein diet have been assessed in the past. Therefore, experiments 8, 9 & 10 (chapter 9) were designed to investigate the effect of protein quality on proteolytic activity of carp at different times after feeding and in different sections of the gut .

CHAPTER 2.

Chapter 2. GENERAL MATERIAL AND METHODS.

2.1. The Experimental Facilities.

2.1.1. " System 1 " The Principal Recirculation System.

Two types of aquarium systems were built in order to achieve two basic requirements for the experiments. The first was for maintenance of constant environmental conditions, in particular, temperature, ammonia and dissolved oxygen. The second was to maintain a healthy stock of fish. If the fish are held in a restricted volume of water, changes in water quality occur which must be corrected. These change include, increase in the concentration of dissolved ammonia, dissolved organic material, solid faecal matter, dissolved carbon dioxide and a decrease in the dissolved oxygen in the water. The first limiting change is the depletion of oxygen due to metabolic requirements of the fish. This situation is easily corrected by mechanical agitation and aeration of the water. Huisman (1969) found that for common carp (Cyprinus carpio), no growth depression or toxic effects occurred provided that dissolved oxygen concentrations was maintained in excess of 3 mg/L.

The second limiting change is the production of ammonia by the fish, which is their primary method of nitrogen elimination. Unless action is taken, potentially harmful concentrations are quickly reached. The " safe " value for mirror carp is reported by Huisman (1969), to be 2 mg/L at an undefined temperature, dissolved oxygen or pH. Smith (1972) found a value of 1 mg/L as " safe "

for rainbow trout (Salmo gairdneri) with oxygen in excess of 7 mg/L. As the dissolved oxygen of the water falls, so does the threshold of ammonia toxicity (Larmoyeaux & Piper, 1973).

It was found that the toxicity of the ammonia ion (NH_4^+) is low, but toxic effect of ammonia (NH_3), its effect in the equilibrium, is much greater. Thus, for example, an increase in pH value would increase the concentration of unionised ammonia (Lloyed, 1961).

Huisman (1969) reported that ammonia concentration increased with a rise in temperature.

The ammonia has no toxic or growth inhibitory effects on mirror carp, if its concentration remains below Ø.5 mg/L between temperatures of 2Ø & 35 °C at an approximate pH of 7 and dissolved oxygen in excess of 5 mg/L.

Many methods of recirculation and water purification for fish culture have been published (Spotte, 197Ø; Hirayama, 1974; Mesk, 1976; Parker & Broussard, 1977). These methods vary widely in principle, degree of complexity and capital cost. Many researchers in the University of Aston Fish Culture unit have successfully used, for rainbow trout, recirculation systems based on a downflow submerged gravel filter (Jauncey, 1979; Atack & Matty, 1979). The advantage of this system relatively low capital cost, reliability and simplicity of construction and operation. This method of biological filtration involves the passage of ammonia laden water down through a gravel substrate which provides a larger

surface area on which nitrifying bacteria may grow. Bacteria growing on the filter bed are chemosynthetic autotroughs which oxidise simple organic compounds to more complex carbohydrate, lipids and proteins, by using ammonia as their energy source. The oxidation of ammonia produces nitrate and nitrite ions, and a cation is required to combine with these ions. It was found that Birmingham tap water is extremely soft and, for this reason, crushed cockle shells were mixed with the gravel filter medium to provide the required cation. Absence of sufficient cation would cause a fall in the pH due to accumulation of nitrous and nitric acids. The calcium carbonate from the cockle shells reacts with the nitrate ions thus;

$$NH_4^+ + 1.5\ O_2 \xrightarrow{\text{Nitrosomonas}} 2\ H^+ + H_2O + NO_2^-$$

$$4\ NH_3 + 7\ O_2 \xrightarrow{\text{Nitrobacter}} 4\ NO_2^- + 6\ H_2O$$

$$2\ NO_2^- + O_2 \longrightarrow 2\ NO_3^-$$

$$2\ ca\ CO_3 + 4\ NO_4^- \longrightarrow 2\ ca\ (NO_3)_2 + 2\ CO_2 + O_2$$

The toxicity of nitrate, produced by biological oxidation of ammonia, to fish is fairly low. Huisman (1969) found that no growth depletion in mirror carp with total NO_2^- / NO_3^- concentration up to 3ØØ mg/L.

The header and filter tanks were supplied with double-skinned insulated lids. Each header tank contained a 3 KW immersion heater (Bunting Titanum Ltd., West Bromwich, UK). Warm, filtered, aerated water was directed into the three 23ØL experimental tanks (Fig 2.1 & 2.2)) at a rate of 5.Ø L/min and left at the same rate through a central standpipe.

Overflow water from the tanks drained into a common pipe which carried the water to a 68L conical solids trap (Fig. 2.3). The solid material was cleaned once per week during each experiment. Water passed from the trap to the surface of the biological filter (Fig 2.4). This filter consists of 6ØØL oblong tank containing 4ØØL of 1 to 1.5 cm broken gravel supported on a corrugated, perforated plate which was, in turn, supported on housebricks. Water was drawn through gravel beds by the action of a Beresford PV52 pump (James Beresford and Sons, Ltd., Birmingham, UK). Filter water was then pumped vertically upwards 3 m and sprayed into the header tank.

Make-up water was continuously added to the header tank both to compensate for losses through splashing and evaporation and to keep nitrate within acceptable limits. Two thirds of the total depth of the gravel in the filter was dug over once a month and the displaced detritus siphoned off. This was performed as the suction of the pump tended to pack the gravel down very tightly and , in addition prolific growth of micro-organisms on

the filter surface began to restrict the flow.

The flow-rates employed in " System 1 " are shown in Table 2.1.

Table 2.1. Flow-Rates Employed in " System 1 ".

Make-up water	Ø.6Ø L/min.
Header to Ring main	15.ØØ L/min.
Header to Filter	15.Ø1 L/min.
Inflow to each Experimental Tank	5.ØØ L/min.
Filter to header (via pump)	3Ø.Ø1 L/min.

Water quality parameters were measured and the values recorded (average of reading, twice a week) are shown in Table 2.2.

Table 2.2. Water Quality Criteria in " System 1".

Temperature	As preset ± Ø.5 C
Dissolved Oxygen	6 - 8 mg/L
Total Ammonia	< Ø.1 mg/L
Total Nitrate/Nitrite	Ø - 5 mg/L
pH	6.9 - 7.2

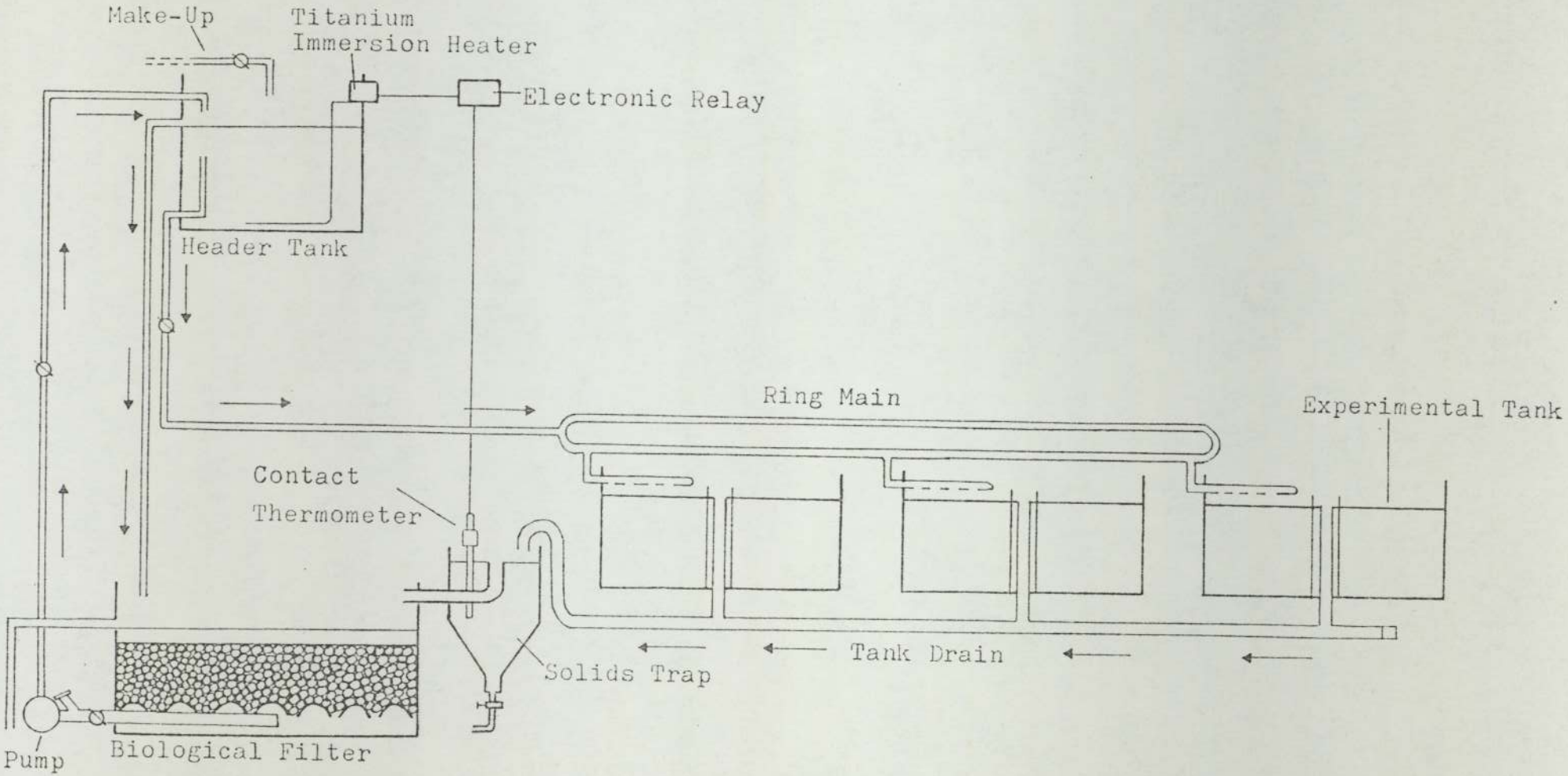

Fig.2.I. A diagrammatic representation of one three tank recycling unit of ' System I'.

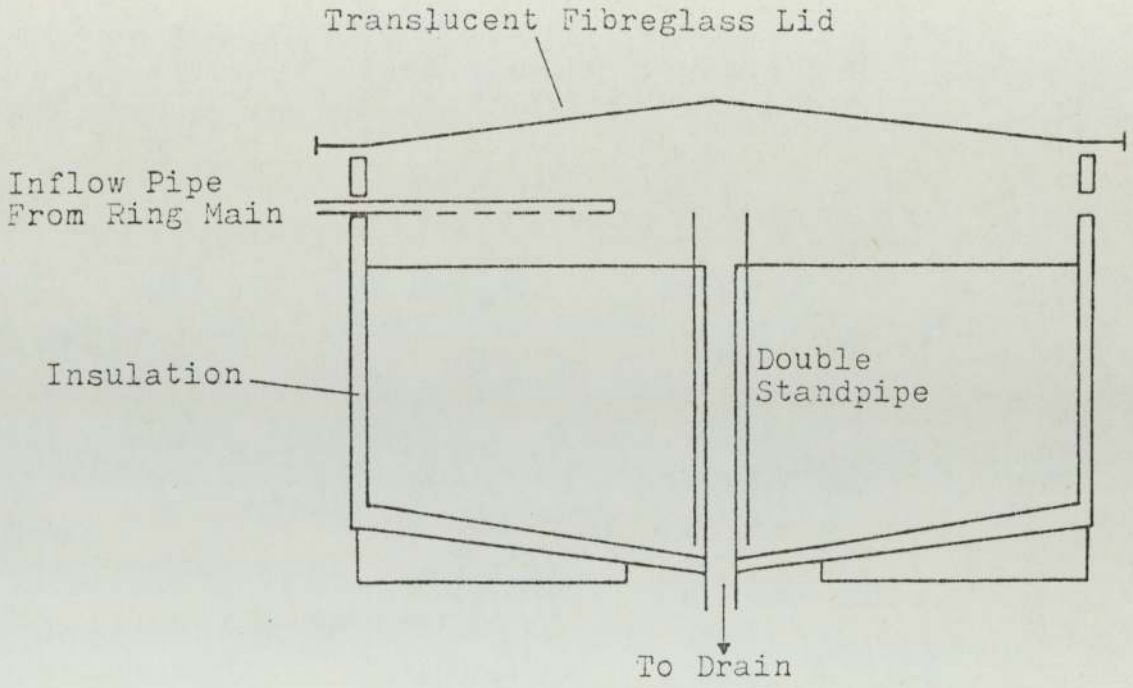

Fig. 2.2. A transverse section of an experimental tank of ' System I '.

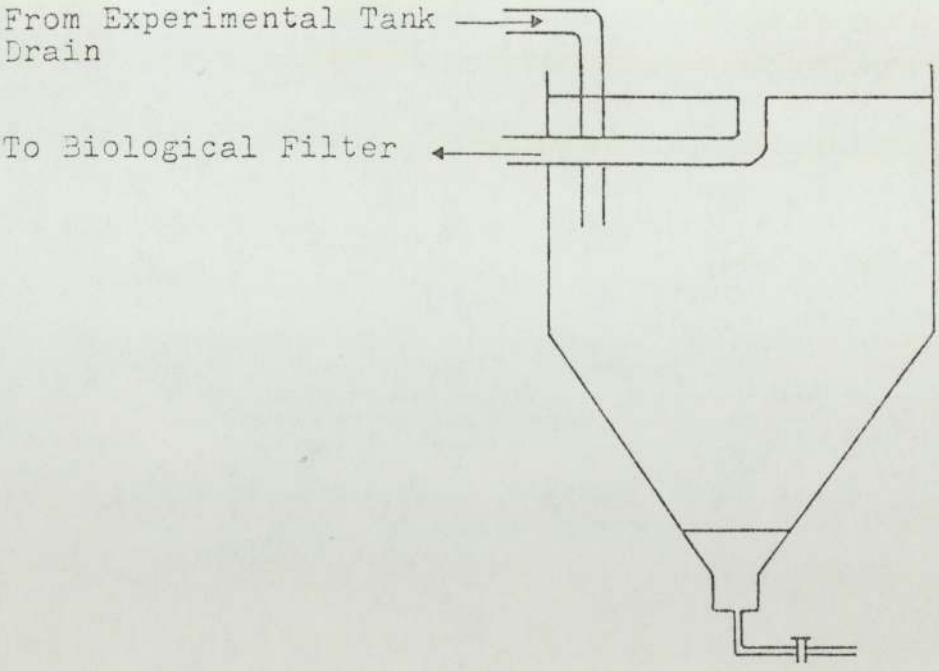

Fig 2.3. A transverse section of a solids trap of ' System I '.

2.1.2. Secondary Recycling Systems.

This system was used in Experiments 1 (Chapter 3); 4 (Chapter 6); 5 (Chapter 7); 8, 9, 1Ø (Chapter 9) during which water quality was measured twice weekly; the values obtained are presented in table 2.3.

2.3. Water Quality criteria in " System 2 ".

Temperature	as preset ± Ø.5 C
Dissolved oxygen	> 7 mg/L
Total Ammonia	< Ø.2 mg/L
Total Nitrate / Nitrite	< 1ØØ mg/L
pH	6.9 - 7.1

The arrangement of this system is shown in Fig 2.5. together with the flow rates and tank volumes. The principles employed in this recycling system are the same as those described for " System 1" (2.1.1.).

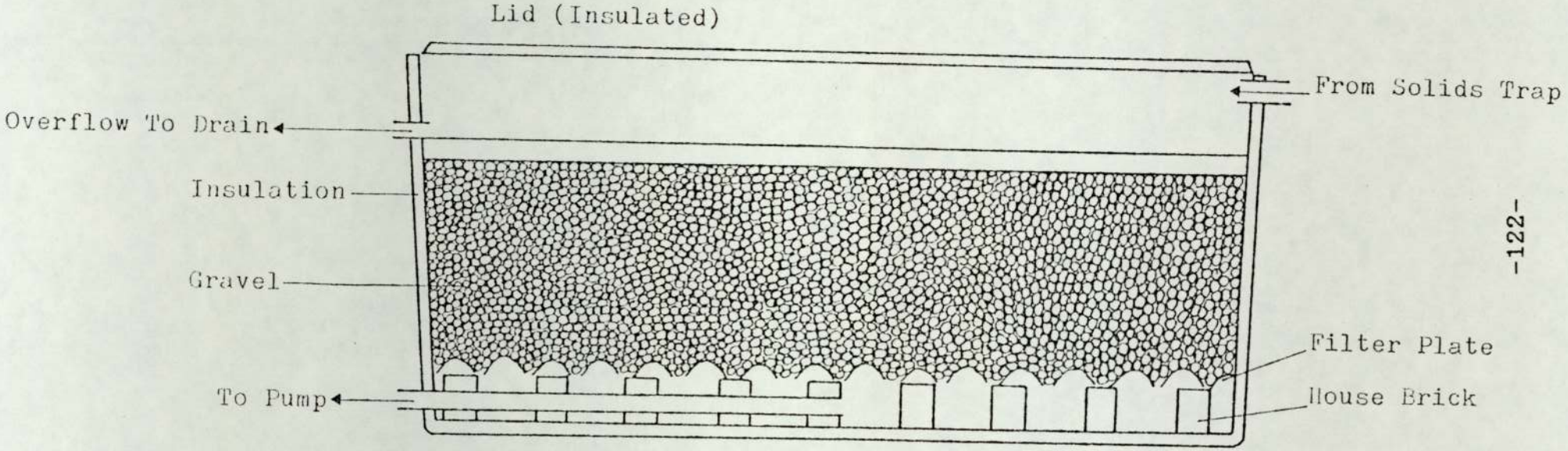

Fig 2.4. A transverse section of a biological filter of ' System I '.

2.2. Diet Formulation.

For Experiments 1 - 1Ø semipurified rations were prepared in the laboratory. All experimental diets were made by calculating the desired amount of protein and adding a mineral mixture (Table 2.4), vitamin premix (Table 2.5), binder (Carboxymethylcellulose, Sodium Salt, High viscosity) and 1% Chromium (III) Oxide (as an indicator). The remainder of the diets was filled with a mixture of dextrin, starch and cellulose to achieve the desired energy value.

2.3. Diet Preparation.

The amount of diet reqired for each experiment was estimated from the starting weight of the fish and expected maximum growth rate. An additional 1Ø% was added to this, to allow for losses during pelleting, drying and sieving.

All dietary ingredients were sieved to particle size of less than 2 mm prior to weighing to ensure that a homogenous mixture was obtained. The ingredients were then weighed out, according to the formulation, placed in bowl of Hobart A2ØØ (Hobart Ltd., London) food mixer and throughly blended for 4 minutes. To this mixture was added the weighed quantity of cod liver oil and blending continued for further 4 minutes. Water was added to make dough, which was extruded through a Hobart mincer attachment. The resulting spaghetti-like strings

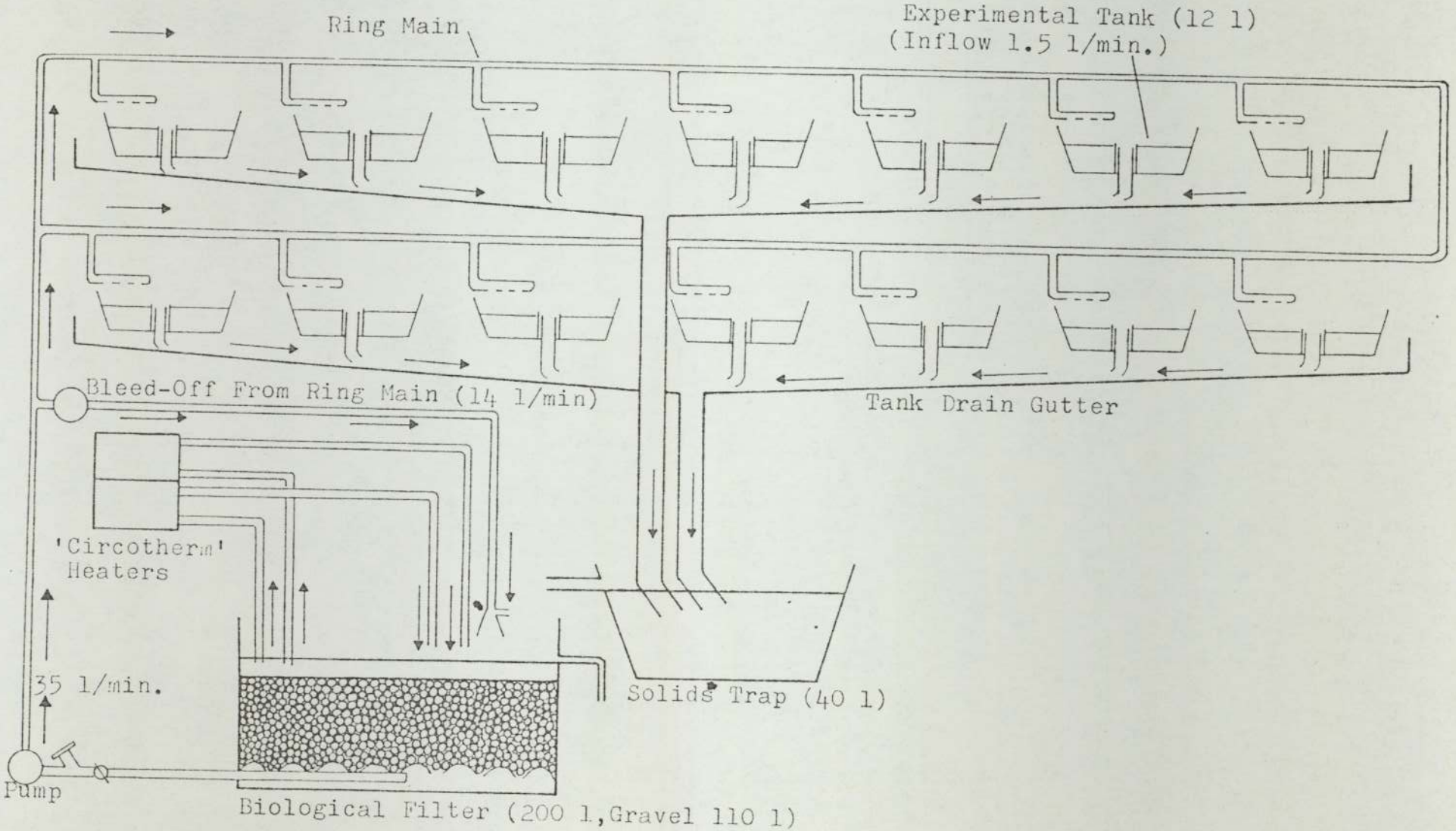

Fig 2.5 . A diagrammatic representation of ' System 2 '.

Table 2.4. Mineral Supplement Composition.

From test diet H44Ø Western Fish Nutrition Laboratory (NAC, 1973).

A combination of;-

Premix No. 5 (Mineral) in grams	
Aluminium chloride	Ø.Ø15
Potassium iodide	Ø.Ø15
Cuprous chloride	Ø.Ø1Ø
Manganese sulphate	Ø.Ø8Ø
Cobalt chloride	Ø.1ØØ
Zinc sulphate	Ø.3ØØ
and Salt mixture No. 2 in grams	
Calcium biphosphate	13.58
Calcium lactate	32.7Ø
Ferric citrate	2.97
Magnesium sulphate	13.2Ø
Potassium phosphate	23.98
Sodium biphosphate	8.7Ø
Sodium chloride	4.35
Total	1ØØ.ØØ

Table 2.5. Composition of Vitamin Premix.

This premix was supplied as a commercial fish additive (formulated by Coopers Nutrition Products) obtained from B.P.Nutrition.

Vitamin A	Ø.Ø12 m.i.u
Vitamin D_3	Ø.ØØ15 m.i.u
Vitamin E	6Ø
Vitamin K	15
Thiamine	1Ø
Riboflavine	25
Pyridoxine	15
Biotin	6Ø
Vitamin B_{12}	2
Nicotinic acid	15Ø
Pantothenic acid	5Ø
Folic acid	4
Choline chloride	113Ø
Vitamin C	6Ø
BHT (antioxidant)	1ØØØ
Iron	2Ø
Cobalt	2ØØ
Manganese	3Ø
Copper	2ØØ
Zinc	5Ø
Iodine	45ØØ

were laid on trays and air dried in a heated cabinet at 4Ø C; when almost dry to the touch, the strings were broken into pieces 3-4 mm in length then were further dried. The dry diets were passed through a sieve to remove fines, a sample was taken for proximate analysis (Section 2.5.) and the reminder stored in sealed polythene bags at - 2Ø C until required for feeding.

2.4. Quarantine Procedures.

After arrival each batch of fish was placed in prepared clean and disinfected tanks. A green house was used as a quarantine area which was separated from the main fish culture laboratory, where all experiments were carried out.

For the first ten days after arrival the fish were fed a specially prepared antibiotic diet, to eliminate bacterial pathogens, and for the remainder of the time in quarantine were fed a commercial trout diet.

On the 5th day after arrival the fish were bathed in 15Ø ppm formalin for one hour. On the 1Øth day the fish were treated twice on successive days by immersing in 2 ppm of malachite green for one hour in a strongly aerated tank. These treatments with malachite green and formalin were used to eliminate external parasites.

Throughout the quarantine period fish were

carefully observed for any abnormalities. Fish were held in quarantine for total of 21 days befor being transferred to the experimental tanks.

2.5. Tagging and Weighing Procedurs.

Before tagging, fish were anaesthetised with benzocain. When benzocain was used, it was first dissolved in a small amount of acetone before mixing with water in a bucket. Benzocain was used at a concentration of 1 g / 2Ø L. Fish were placed in the anaesthetic solution until they lost equilibrium (usually 2 min) after which they were marked, and then returned to well-aerated water. In all experiments, the fish were individualy tagged, a 15 mm x 5 mm numbered tag (Charles Neal Ltd., Finchley, London) was attached to a plastic thread which was inserted through the dorsal muscle at point midway along the dorsal fin. The thread was driven through the fish with a tagging gun as used in the clothing industry (Kimbal System Ltd., Leics, UK). The needle of this gun was dipped in absolute alcohol before tagging to prevent infection. The tags did not appear to affect the behaviour of the fish in any way and were still in place after two years.

Individual weighing of fish permitted the distribution of growth rates in different populations to be

statistically compared. In addition tagging enabled instant identification of any fish which succeeded in escaping from the experimental tank, thus allowing, in the case of mortalities, adjustment of daily feeding rate and calculation of growth parameters (Experiment 1).

Individual weighing of fish was undertaken in all experimental work. This was achieved by removing the fish from their tank and anaesthetising them, a few at a time, in benzocain. They were then removed from the anaesthetic, lighly blotted on a paper towel, and placed on a tared small rectangular pad of absorbent material placed on the pan of a Sartorus 3719MP dual range balance. The fish and pad were weighed to an accuracy of ± Ø.Ø1 g and the fish returned to their tank, where complete recovery occurred within three minutes. The absorbent pad was then weighed and the difference between this weight and the weight of the pan and fish was taken as the live weight of the fish.

In Experiments 1, 8, 9, 1Ø a Sartorius 4 kg balance accurate to Ø.1Ø g was used and in experiments 2, 3, 4, 5, 6, 7 the balance was a Sauter 1Ø side pen balance weighing to 1 g.

In all experiments the fish were starved for twelve hours before weighing.

2.6. Methods of Proximate Analysis.

Proximate analysis of diets, dietary ingredients, fish and faeces were carried out by the following procedures.

Moisture

Moisture content was determined by air drying samples in an oven at 105 C for 48 hours.

Crude lipid

Crude lipid content was determined by extracting dried samples for four hours, using a soxhlet apparatus (A. Gallenkamp & Co Ltd.), with 40 to 60 C boiling range petroleum ether and the soluble material extracted was weighed.

Crude Protein

Crude protein content was determined by the microkjeldahl method for determining nitrogen and applying the empirical factor of 6.25 to the results to convert total nitrogen to total crude protein.

Ash

Ash content was determined by heating samples in a muffle furnace (FR 610, A. Gallenkamp & Co. Ltd.) for twelve hours at a temperature of 500-600 C.

Nitrogen Free Extractives (NFE)

NFE was determined by calculation.

NFE = 1ØØ - (% moisture + % crude lipid + % crude protein + % ash)

2.7. Analysis of Experimental Data.

2.7.1. Specific Growth Rate.

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

$$W_2 = W_1 . e^{g(T-t)}$$

Where W_2 is the final weight (at time T)and W_1 the initial size (at time t); T and t are expressed in units of time (usually days); W_2 and W_1 in units of weight (usually grams) ; e is the base of natural logarithms; g is a constant for a particular curve known as the specific, instantaneous, geometrical or multiplicative growth rate.

Rearrangement of the above equation, to obtain g, and multiplying by 1ØØ gives the rate of change in weight of the fish, expressed as percent per day; this is commonly termed the Specific Growth Rate (SGR).

$$SGR\ (\%\ /\ day) = \frac{Log_e W_2 - Log_e W_1}{T - t} \times 1ØØ$$ (Brown, 1957)

2.7.2. Food Conversion Ratio.

The food conversion ratio (FCR) is defined as the amount of dry food fed per unit live weigh gain of fish.

$$FCR = \frac{\text{Food Fed, g dry food}}{\text{Live Weight Gain, g wet fish}}$$

In calculation of FCRs for Experiment 1 the true weight of dry food fed was used with correction being made for the analysed moisture content of each diet.

2.7.3. Protein Utilization.

The efficiency with which fish were able to utilize dietary protein was determined by calculation of values for Protein Efficiency Ratio (PER), defined as the gain in wet weight of fish per gram of crude protein consumed (Osborn et al., 1919).

$$PER = \frac{\text{Weight Gain , g wet fish}}{\text{g Crude Protein Fed}}$$

Although PER values give a somewhat better indication of the nutritional status of the fish than FCRs, they do not take into account the proportion of ingested protein used for maintenance and are based on the assumption that the growth of the fish consists of tissues with identical composition in all groups. An improved assessment of the nutritional status of the fish, with respect to dietary

protein utilization, is the the apparent efficiency of deposition of dietary protein as body tissue, the NPU .

In experiment 1 NPU was determined by the carcass analysis method of Bender & Miller (1953) and Miller & Bender (1955) where a low protein diet was fed in order to enable estimation of endogenous nitrogen losses and thus calculation of true NPU. The feeding of a low protein diet in fish in order to overcome the problems of feeding a non-protein diet, was first proposed by Cowey et al., (1974). Hence ;

$$\text{True NPU (\%)} = \frac{B - (BK - IK)}{I}$$

Where B is the total body nitrogen of the fish fed the test diet and BK the total body nitrogen of the fish fed the low protein diet, with nitrogen intake I and IK respectively.

Obtaining a value of True NPU in this way, together with the determination of true protein digestibility (Section 2.6.4), permits calculation of the BV of the dietary protein.

$$BV = \frac{\text{True NPU}}{\text{True Digestibility}}$$ (Bender & Miller, 1953)

2.7.4. Digestibility Determination.

The use of inert indicators, which pass unaffected by digestion through the alimentary tract, has provided a convenient method of measuring digestibility in a number of animals without the need to collect the faeces quantitatively. This method has been successfully applied to fish digestion studies using chromium (III) oxide as the indicator (Furukawa & Tsukahara, 1966; Nose, 1967).

For the purpose of determining digestibility the diet used in experiments 1 & 5 contained 1% chromium oxide. After the terminal weighing of each of these experiments feeding was continued at the same rate for six days, with faeces being stripped from the fish once per day, and the six day samples for each group pooled.

Faecal stripping was accomplished by lightly anaesthetising the fish and gently applying pressure on the abdomen from the pelvic fins to the anus. Faeces obtained in this way were collected on filter paper, dried at 1Ø5 C for 12 hours and transferred to sealed dry containers .

Chromium oxide determinations by the spectrophotometric method of Furukawa & Tsukaha (1966) were carried out on the diet and faeces.

Apparent digestibility was derived from the following equation (Maynard & Loosli, 1969);

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

In experiment 1 a low protein diet was fed which enabled approximation of endogenous losses and thus calculation of true digestibility assuming the low protein diet to be 100% digestible .

2.7.5. Statistical Analysis.

Statistical comparison between means was made by one way analysis of variance (Snedecor and Cochran, 1980).

CHAPTER 3.

Chapter 3.

3. Experiment 1. Bioassay Method for Measuring Protein Quality in Carp Diets.

3.1. Introduction.

In recent years there has been much interest in the possibility of using various unconventional protein sources as a replacement for fish meal in compounded fish feeds.

Most of this interest has centered on feeds for salmonids, as this family includes the most commonly reared species for which artificial diets are required. Many of the results obtained so far have proved encouraging. Partial replacement of fish meal with yeast, bacteria and soybean proteins seems possible (Hoshiai, 1972; Andruetto et al., 1973; Bergstrom, 1978; Spinelli et al., 1978). However, attempts to replace all of the fish meal in piscean rations have not so far proved successful. Thus, when soybean meal was substituted on an isonitrogenous basis for menhaden meal in catfish (Ictalurus punctatus) diets, growth and food conversion efficiency fell significantly (Andrews & Page, 1974). Addition of methionine, cystine and lysine (the most limiting AA) to these soybean substituted diets did not enhance growth or food conversion. Comparative studies of different types of SCP have already been made (Atack and Matty, 1978, Spinelli et al., 1978).

Comparatively little information exists on the relative nutritional quality of food proteins for fish, and few

of the available methods have yet been rigorously applied to fish. There is a considerable need for such information as well as for knowledge of the most limiting AAs in food proteins given to fish. Such knowledge appears mandatory for the rational formulation of the protein component of practical fish diets.

Studies by Hauser (1975) and Bayne et al., (1976) support the general belief that vegetable proteins can be used in large quantities successfully in tilapia diets. Further research in this area is important not only in the culture of tilapia, but to fish culture in general.

Since there is now a trend towards the intensive culture of warm-water species in thermal effluents, specialised compounded feeds will soon become necessary. In view of their digestive system, it seems possible that species such as carp may be able to use protein sources of lower quality than those normally included in trout and salmon rations. Hence the following experiment was undertaken to determine the value of whole egg protein, casein and Pruteen against a fish meal control in semipurified diets for carp. Carp are stomachless fish, lacking pepsin digestion, and serve as an interesting comparison with fish having a functional stomach .

3.2. Material and Methods.

3.2.1. The Experimental System and Animals.

The experimental facility used in the present study was "System 2" as described in detail in section 2.1.2.

300 fingerling common carp (5-8 cm) were obtained from

Newhay Fish Farm, England. The fish were subjected to quarantine and prophylaxis, as described in section 2.4, and then transferred to two 230 L tanks of system 1 at the prevailing ambient temerature of 13 °C.

The temperature was then raised at approximately 3 °C per day to 25 °C; during this period the fish fed actively on a commercial trout ration and showed no signs of stress. 30 fish at a time were then allocated to each of ten experimental tanks. 5 fish were taken from each tank for proximate carcass analysis (section 2.6.).

The fish in each tank were then individually tagged (section 2.5.) and the temperature of the recycling system adjusted to 25 ± 1 °C.

No losses were experienced during tagging or temperature acclimation and the fish fed actively over this period.

3.2.2. Experimental Diets

The protein sources to be tested were casein (Sigma, Dorset, England), whole egg protein, a methanophilic bacterium ("Pruteen", ICI Ltd., Billingham, Great Britain). The control diets contained fish meal (Ewos Baker Ltd, U.K.).

A low (2.6%) protein diet was also included in the experiment so that BV & NPU values (Section 2.7.3) could be obtained by the method of Bender and Miller (1953) and also that true digestibilities (section 2.7.4.) could be evaluated .

The diets (Table 3. 1) were formulated to be identical (ca 38% protein, recommended for carp), Nose (1978).

Table 3.1. Composition of Experimental Diets for Carp fed Different Protein Sources With circa 38% protein.

Component	Weight (g)				
	1	2	3	4	5
Casein	-	44	-	-	-
Whole Egg Protein	-	-	39	-	-
Fish meal	-	-	-	56	-
Pruteen	-	-	-	-	53
Dextrin	45	2Ø	22	1Ø	12
Starch	35	17	22	11	13
Cod liver oil	9	9	9	9	9
Mineral mixture	2	2	2	2	2
Vitamin mixture	1	1	1	1	1
Carboxy methyl Cellulose	1	1	1	1	1
Chromic Oxide	1	1	1	1	1
Cellulose	6	5	3	9	8
	1ØØ	1ØØ	1ØØ	1ØØ	1ØØ
Proximate analysis (%)					
Moisture	4.4Ø	2.8Ø	12.1Ø	2.6Ø	2.ØØ
Protein	2.6Ø	39.3Ø	39.3Ø	39.37	38.3Ø
Lipid	8.9Ø	9.Ø2	1Ø.5Ø	12.9Ø	13.5Ø
Ash	7.ØØ	7.ØØ	5.ØØ	8.ØØ	9.ØØ
NFE	77.1Ø	41.88	33.1Ø	37.13	37.2Ø

A low protein diet (diet 1), casein , whole egg protein, fish meal and Pruteen diets (2, 3, 4 and 5 respectively) were prepared as described in section 2.2.
The diets were formulated to be isonitrogenous, and as far as possible isoenergetic for total energy.

3.2.3. Feeding Rate.

During this experiment it was only practicable to feed the fish four times per day between Ø8.3Ø h and 18.3Ø h. In addition to this, feeding a stomachless fish, such as carp, to satiation presents difficulties as they are, in nature, continuous feeders. They were fed on a fixed feeding regime of 4% of their body weight (dry food / whole fish) per day, divided into four equal feeds, though with certain diets the fish aften showed signs of being unwilling to consume the whole day's ration. If this occurred they were fed only the amount they would eat within a 3Ø-min feeding period.

3.2.4. Weighing and Sampling.

Details of the weighing procedure employed are presented in section 2.5. Fish were individually weighed (±Ø.Ø5 g), under anaesthesia after 12 h starvation, every ten days for forty days. At the end of this period 8 fish were killed at 4 and 6 h after the final feeding. The alimentary canal was separated, measured and dissected into 2 equal parts. The contents were removed from each part after ligaturing at selected places. Samples from the

separate parts of the intestine were pooled together, according to the diet and time after feeding, and were analysed for dry matter content and total nitrogen (section 2.6.) and chromic oxide (section 2.7.4). Three fish were removed from each group for proximate carcass analysis (section 2.6.). Faeces were obtained from the remaining fish, as described in section 2.7.4. and analysed for protein (section 2.6.) and chromic oxide (section 2.7.4.).

3.2.5. Statistical Methods and Analysis of Growth Data.

These were performed as detailed in section 2.7.

3. Results

3.1. Growth Performance.

The increase in average weight of fish through the experiment is shown in Fig 3.1, whilst the average specific growth rate for the whole period is tabulated in Table 3.2. Statistical analysis showed a significant difference ($p<Ø.Ø5$) between fish meal, casein and low protein diets, while, no significant differences ($p>Ø.Ø5$) between fish meal, pruteen and whole egg protein diets were noted.

Table 3.2. shows that the fish meal diet produced the highest weight gain (1Ø9.75%) which was considerably greater than that of fish fed diets 3, 5, 2 and 1, ranging from 1Ø5.12, 99.83 , 43.9Ø and - Ø.2% of the initial weight.

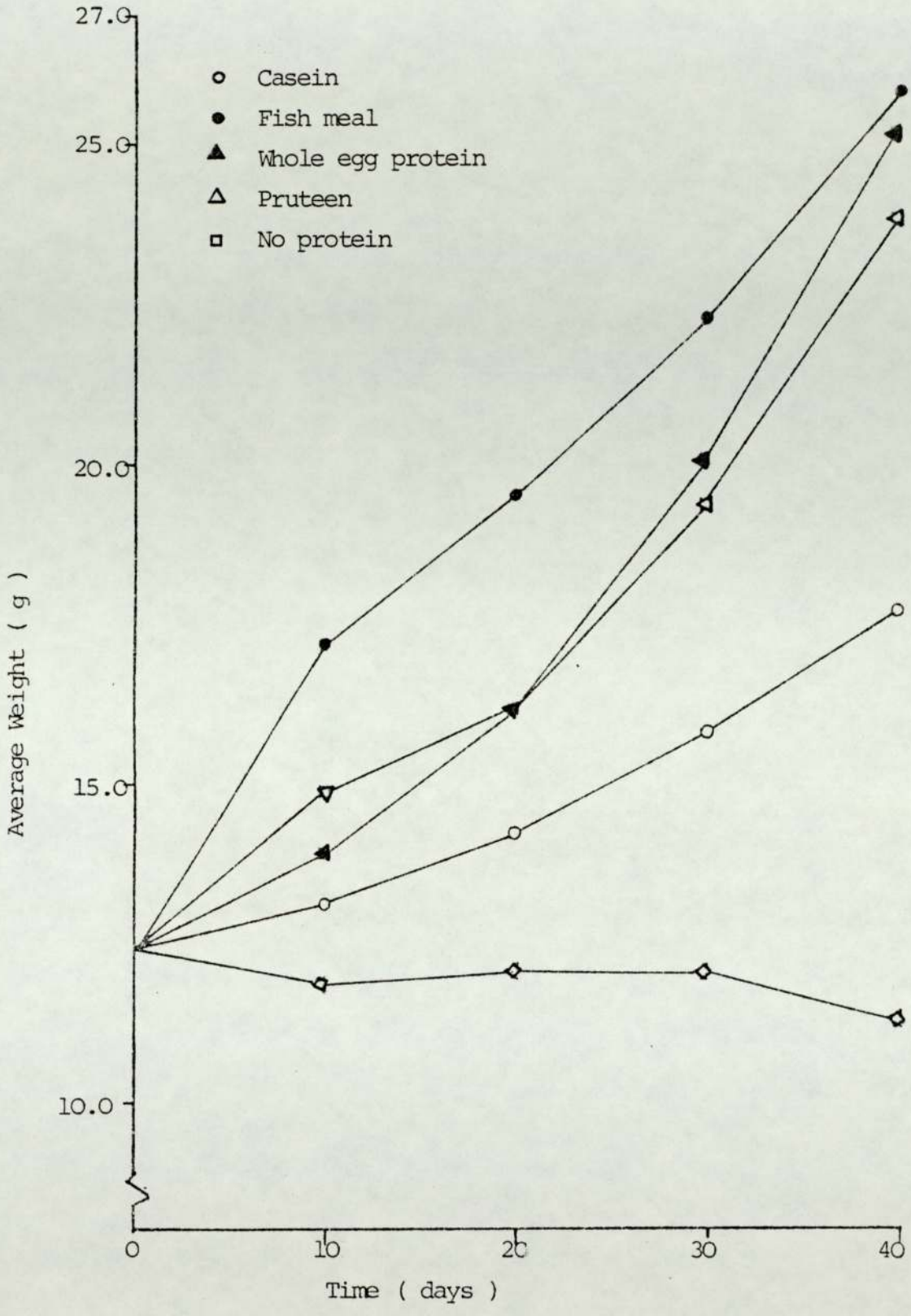

Fig.3.1. Change in Average Fish Weight During the Experimental Period.

Table 3.2. Growth Data For Carp on Different Diets For Experimental Period of 40 Days.

Diet	Initial Weight g	Final Weight g	Weight Gain g	% Weight Gain	SGR(1) % day
Casein	12.3±7.6*	17.7±7.69	5.4±6.50	43.9±5.6	0.41±0.23
Fish meal	12.3±8.6	25.8±8.44	13.5±6.07	109.75±5.4	0.82±0.21
Egg Protein	12.3±8.6	25.23±8.44	12.93±7.80	105.12±12.6	0.8±0.18
Pruteen	12.3±8.6	24.58±8.36	12.28±7.02	99.83±6.69	0.77±0.2
Low Protein	12.3±5.6	11.36±5.20	-0.95±5.8	-7.64±4.8	-0.09±0.01

(1) LSD_{05} = 0.0257

* Mean ± SD

3.2. Food Conversion Ratios (Table 3.3).

Mean Food Conversion Ratios (FCRs, section 2.7.2.) were obtained for each group and are presented in Table 3.3. Statistical analysis showed a significant difference($p < Ø.Ø5$) between casein and low protein; whole egg protein and low protein ; Pruteen and fish meal & low protein . The Pruteen gave significantly ($p < Ø.Ø5$) the lowest FCR followed by casein, whole egg protein, fish meal and low protein diet .

3.3.3. Specific Growth Rates.

SGRs (Table 3.2) very much reflect the same pattern as final average weights and percentage weight gains (Table 3.2.). The fish fed the fish meal diet produced significantly ($p<Ø.Ø5$) the highest SGR, followed by whole egg protein, Pruteen, casein and no protein diet. Statistical analysis of the SGRs (section 2.7.1.) are presented in Table 3.2.

3.3.4. Protein Efficiency Ratios.

PER (section 2.7.3.) were calculated for each group and presented in Table 3.3. The low protein feed gave significantly ($p<Ø.Ø5$) the lowest PER, which was to be expected as Ogino & Saito (197Ø) have shown, in carp , that PER increases as dietary protein decreases. However, consideration of PER at very low dietary protein shows that PER must fall as the protein decreases. PER would be zero when the dietary protein was equal to the maintenance requirement. With still lower amounts, the fish would lose weight. Fish fed a low-protein diet would

Table 3.3. Protein Utilization Data For Carp on Various Diets.

Diet	FCR(1)	PER(2)	NPU(3) %	Digestibility (4) %	BV %
Casein	1.92	1.33	56.92	91.ØØ	62.54
Fish meal	2.12	1.21	72.47	94.ØØ	77.ØØ
Whole egg Protein	2.Ø1	1.26	65.39	93.ØØ	7Ø.31
Pruteen	1.91	1.36	63.58	93.ØØ	68.36
Low Protein	3.2Ø	-2.4	____	____	_____

(1) $LSD_{Ø5}$ = Ø.286

(2) $LSD_{Ø5}$ = Ø.181

(3) $LSD_{Ø5}$ = 6.Ø5

(4) $LSD_{Ø5}$ = 4.38

be expected to exhbit a negative PER approximating to the endogenous nitrogen excretion.

3.3.5. Net Protein Utilization.

True NPU (section 2.7.3.) values were calculated for each group and means of results for duplicate tanks are presented in Table 3.3. Since the body composition of the various groups is fairly stable the results in general reflect the PER values. Statistically, the NPU value was significantly different ($p<0.05$) between all groups except between whole egg protein and Pruteen.

3.3.6. Protein Digestibility.

True protein digestibilities were determined for each group, as described in section 2.7.4., and the results are presented in Table 3.3. Protein digestibility was fairly high for all the experimental diets. No significant difference ($p>0.05$) was observed between all groups.

3.3.7. Body Composition.

The results of proximate analysis of final carcass composition are presented in Table 3.4. Gross body composition was little affected by the dietary regime except in the group fed the low protein diet. These fish had a significantly higher ($p<0.05$) body lipid content and lower moisture content than fish from other groups due to the higher dietary carbohydrate. Fish fed the lowest dietary protein (2.60%) tended to have lower protein contents (in some cases significantly).

Table 3.4. Effect of Protein Source on Body Composition (%)[a].

Protein Source	Composition of Dry Matter (%)			
	Moisture[b]	Protein[c] NX6.25	Ether[d] Extract	Ash
Initial	77.76	65.51	25.90	9.10
Casein diet	77.09	61.25	27.80	9.10
Fish meal diet	77.10	67.03	23.90	9.20
Whole egg protein	77.25	65.60	25.40	9.10
Pruteen	76.98	64.62	27.20	9.00
Low protein	74.65	58.98	32.10	9.47

a Values are the means of three samples per diet.

b LSD_{05} = 2.47

c LSD_{05} = 8.17

d LSD_{05} = 5.99

3.3.8. Biological Value.

BVs (section 2.7.3.) were determined for each group and the means of results for duplicate tanks are presented in Table 3.3. Most of the values thus obtained proved similar although statistically significant ($p<Ø.Ø5$) diff-ererence could be detected between certain of the protein sources.

3.3.9. Digestion of Protein.

If the length of the intestine of the fish is plotted against body weight (Fig 3.2) and body length (Fig 3.3.) variation is seen, but it appears that some correlation does occur. Gut length does affect evacuation time.

However, it remains to be seen if functional segments of stomachless fish (Stroband et al., 1979) change in the same proportion as the whole gut.

The values obtained for the percentage content of chromium oxide, dry matter and protein from digesta of each intestine region 4 and 6 h after feeding are presented in Table 3.5 and 3.6. There was a general trend of decrease in protein content and increase in Cr_2O_3 content from the first to the second half of the intestine at both times. The decrease in protein content indicates that protein is absorbed (presumably as AAs following its digestion) in the second half of the intestine, although it is said that the intestinal bulb (or enlargement forming a substantial part of the first 2Ø% of the gut) has a considerable role in AA

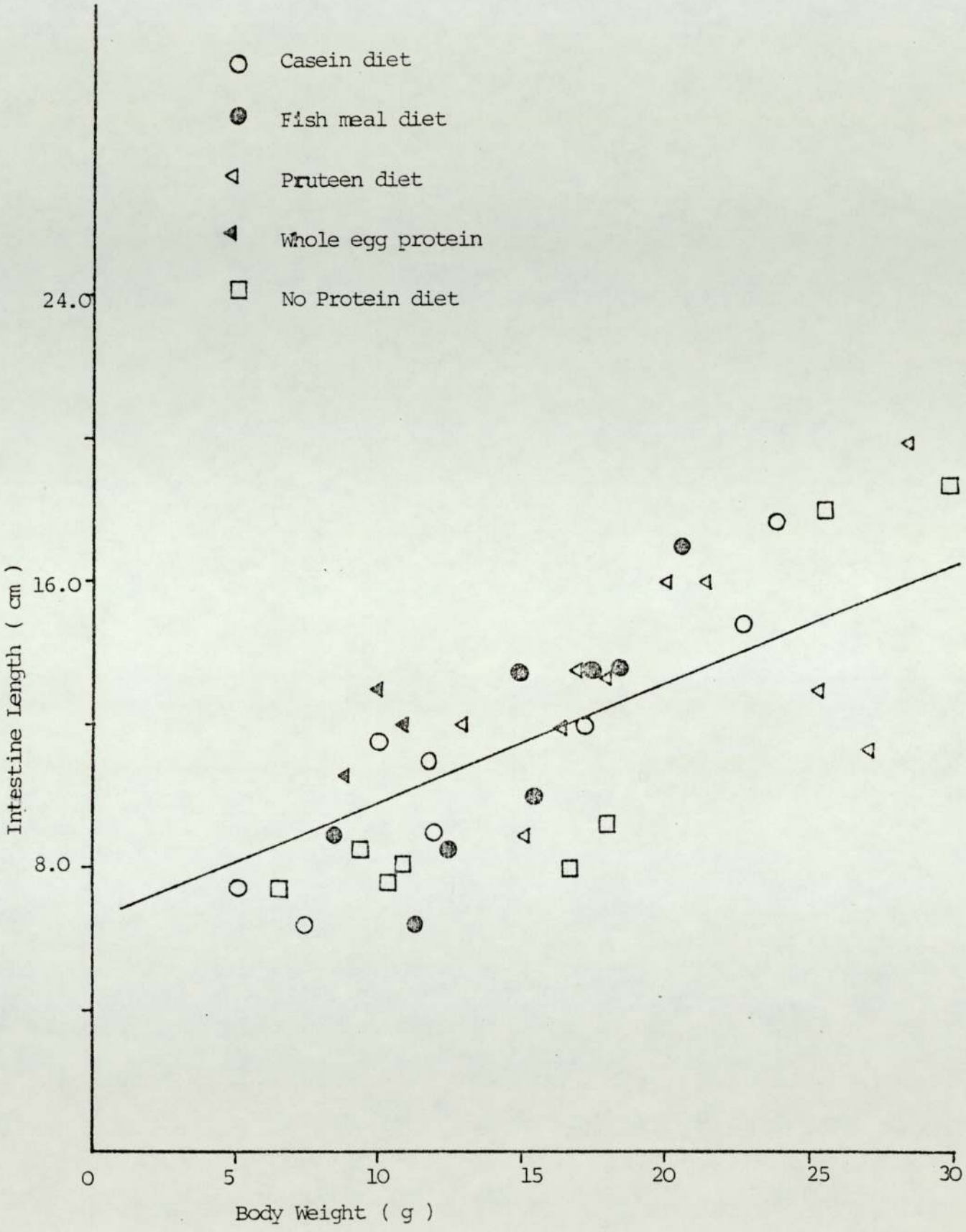

Fig 3.2. Relationship Between Carp Body Weight and Intestine Length.

Correlation Coefficient. ($r = + 0.599$)

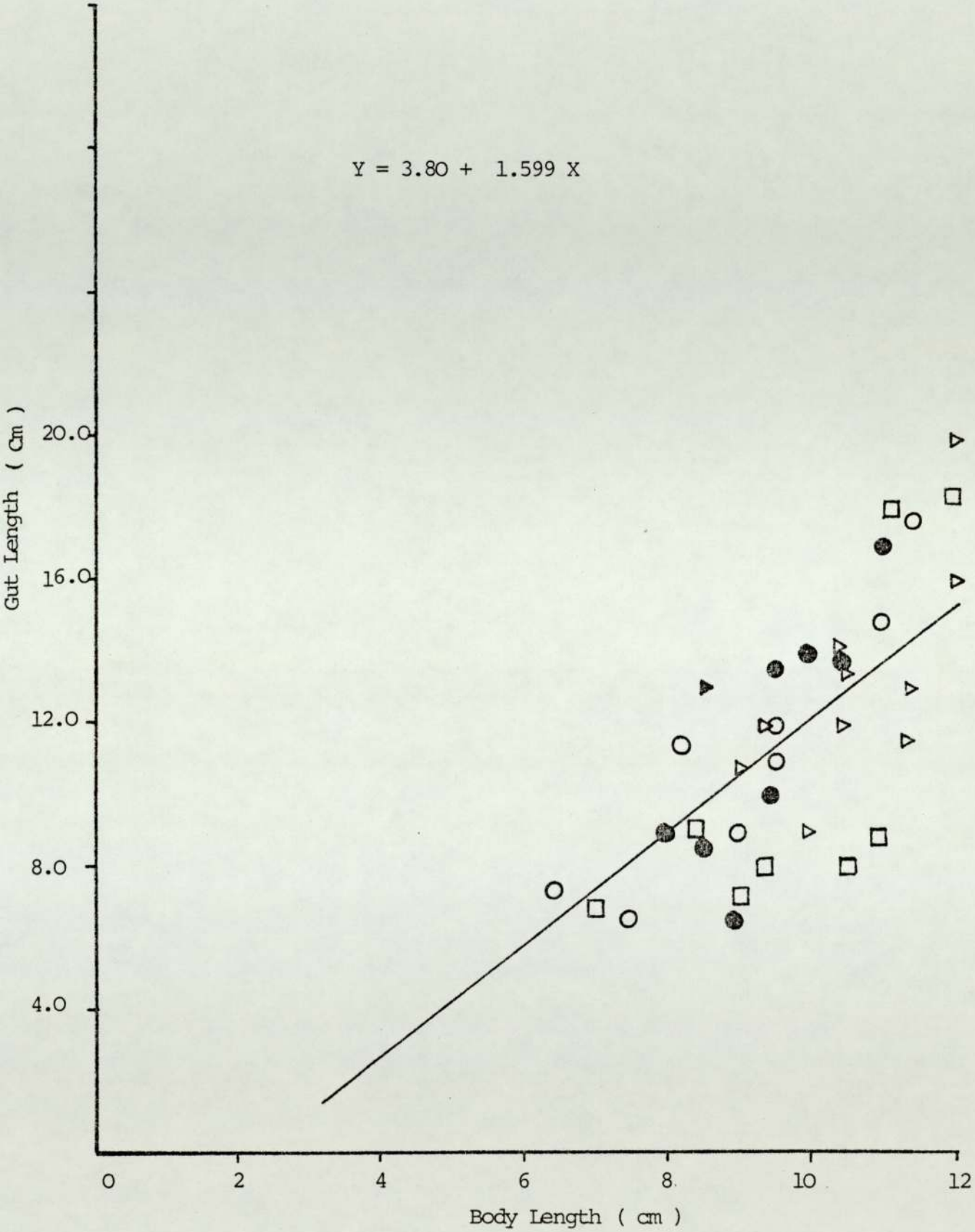

Fig 3.3. Relationship between Carp Length and Intestine Length.
Correlation Coefficient (r = + 0.654).

Table 3.5. Chemical Composition of The Digesta from Anterior (1) and Posterior (2) Parts of Carp Intestine 4 h After Feeding. (n = 8)

Diet	Gut segment	Dry matter	Protein*	Cr_2O_3 *
Casein	1	25.07	30.88	00.80
	2	58.95	16.83	01.10
Fish meal	1	25.00	28.43	00.62
	2	40.50	17.89	00.90
Whole egg	1	28.26	14.58	00.75
	2	51.72	9.25	00.95
Pruteen	1	28.07	38.28	00.64
	2	26.98	10.15	01.10

* As % of dry matter.

Table 3.6. Chemical Composition of The Digesta From Anterior (1) and Posterior (2) Parts of Carp Intestine 6 h after Feeding. (n = 8)

Diet	Gut segment	Dry matter	Protein *	Cr_2O_3 *
Casein	1	83.75	13.12	Ø.89
	2	87.23	7.95	1.2Ø
Fish meal	1	75.96	3Ø.62	ØØ.5Ø
	2	83.56	16.9Ø	1.26
Whole egg	1	57.14	12.3Ø	ØØ.54
	2	62.12	1Ø.93	1.1Ø
Pruteen	1	71.29	12.15	ØØ.85
	2	81.81	8.1Ø	1.12

* As % of dry matter.

absorption. Absorption of protein was greater 6 h after feeding.

3.4. Discussion and Conclusion.

3.4.1. Evaluation of Experimental Diets.

NPU value for casein is higher than that found by Ogino and Saito (197Ø) of about 46%, and all diets exhibited higher values than those reported by Sin (1973) for a compounded ration.

BV for casein and egg protein are very similar to those reported by Ogino and Chen (1973a), 62 and 7Ø for (casein, and egg protein).

Body water and lipid appeared to be inversely related as has been noted elsewhere (Dabrowska and Wojno, 1978, Grayton and Beamish , 1977; Murray et al., 1977; Atack et al., 1979, Jauncey, 1982 , Margit et al., 1984). The lower protein content of fish fed low or zero protein diets has also been reported by Atack et al., (1979). Body ash was unaffected by dietary regime, as has been noted with other fish species (Cowey et al., 1974; Elliot, 1976; Dabrowska and Wojno, 1978; Yu et al., 1977; Atack et al., 1979, Schwarz et al., 1985).

An attempt was made to correlate the BVs obtained in the experiment with the AA profiles of the test proteins. The results (Table 3.7) show that in absolute terms, none of the chemical methods accurately predicted the BVs found. The explanation of this probably lies in the fact that the BV of protein varies with its concentration in the diets and also varies in relation to other dietary and

Table 3.7. Comparison of BVs With Chemical Predictions of Protein Utilization.

	BV Experimental	EAAI (1)	Chemical Score(2)	Chemical Score(3)	Chemical Score(4)
Casein	62	88	77	53	75
Egg Protein	7Ø	1ØØ	58	96	52
Fish meal	77	121	153	73	1Ø6
Pruteen	68	62	71	33	66

(1) EAAI- Reference Whole Egg Protein. "The geometric mean of the ratios between the concentration present in the diet and whole egg protein". (Mitchell, 1954).

(2) Chemical score- reference carp body protein.

(3) Chemical score- reference AA requirements of Chinook salmon (Mertz, 1969).

(4) Chemical score- reference AA requirements of carp (Nose, 1978).

environmental criteria. Different sources of protein at the same concentration in diets will give differing BVs and EAAI. However, as the BVs of various proteins appear to increase at an equivalent rate (Ogino and Chen, 1973b) they should always be ranked in the same order provided they are fed at equivalent rates, and this ranking should essentially be dependent on their AA composition. In fact, Table 3.8 shows that all the chemical analysis methods used correctly predicted the fish meal as the best protein source, but overall the chemical scores were more consistent with the experimental results than the EAAI.

The observed differences in NPU (Table 3.3) may be looked at in relation to the quality of the dietary proteins, as expressed by their EAA composition (Table 3.8). This gives some indication as to those AAs which are most limiting. The casein diet is low in arginine, the whole egg protein in methionine, and pruteen does not cover the requirement of carp for 4 EAA. Using such information it will be possible to make least-cost feed formulations using a combination of several protein sources.

The experiments of Nose (1974) with yeast SCP have shown that, on addition of Ø.5% cystine + arginine, growth rate of trout increased by 25% compared to unsupplemented diets. Methionine and lysine had benefical effects, phenylalanine an adverse influence, whereas the combination of methionine + lysine + arginine did not have any noticeable effect. In a subsequent trial (Nose, 1974),

Table 3.8. Calculated Amounts (%) of EAA in 38.5% Diets, and The Dietary Requirement for Carp. (38.5% protein Diets)

Dietary protein	Amino acid								
	Arg	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val
Casein	1.28	1.80	3.30	1.71	1.51	1.63	1.54	0.60	2.06
Egg protein	2.40	1.85	3.33	3.07	0.42	2.02	1.60	1.09	2.27
Fish meal	2.22	1.79	2.92	2.92	1.17	1.63	1.59	4.29	2.02
Pruteen	1.41	1.26	1.99	1.57	0.53	0.99	1.26	0.38	1.53
Requirement for carp	1.70	0.90	1.30	2.20	0.80	1.30	1.50	0.30	1.40

AA below the carp requirement are underlined.

favourable effects of cystine and a depressive effect of methionine supplementation were demonstrated. Other investigations have shown contradictory results. Methionine alone (Spinelli et al., 1978) or in association with lysine (Bergstrom, 1978) when added to SCP diets improved growth rate. Similarly, results of successive trials by Beck et al., (1978) of the supplementation of SCP diets with methionine, cystine or arginine were contradictory. Only the simultaneous addition of DL-methionine and DL-arginine to SCP diets was found to improve growth rate and feed efficiency .

Successful replacements by other protein feedstuffs have been reported, mostly of animal origin: milk or whey powder (Meske et al., 1977), feather meal and meatmeal (Tiews et al., 1976), Krillmeal (Pfeffer and Meske, 1978) SCP (Atack et al., 1979), and algae meal (Sandbank and Hepher, 1978).

It is interesting to compare the protein utilization data for carp and trout (Table 3.9) though of course the effects of different feeding regimes and environmental conditions have to be considered. Similarly, diet ingredients other than the proteinaceous sources especially carbohydrates, are used to a different extent by the two species and so the diets would not have been equivalent on a metabolic energy basis. For instance, carp can digest crude cellulose to a certain extent (Shcherbina and Kazlauskene, 1971) and also digest starch more efficiently than trout (Chiou and Ogino, 1975). However,

Table 3.9. Comparison of Results Obtained for Carp With Those Obtained For Trout.

Diet	BV		PER		NPU		Digestibility	
	Carp	Trout	Carp	Trout	Carp	Trout	Carp	Trout
Casein	52(6)	41(7)	2.48(6)	1.97(7)	49(6)	40(7)	93(6)	98(7)
		46.3(1)		2.15(1)		45.4(1)		98(1)
	65(2)			3.5(5)		56(5)		
	62(3)		1.33(3)		57(3)		91(3)	
				2.89(4)		47(4)		
Fish meal(1)		50.4(1)		2.17(1)		46(1)		91(1)
		64(2)						
	77(3)		1.21(3)		72(3)	94(3)		
Hering meal(6)	79(6)	41(6)	2.82(6)	1.91(6)	64 38(6)	80.3(6)		91.2(6)
Egg protein	70(3)		1.26(3)	3.80(5)	65(3)	61(5)	93(3)	95(5)
Pruteen	52(6)	40(7)	2.54(6)	1.62(7)	49(6)	37(7)	95(6)	93(6)
	68(3)		1.36(3)		63(3)		93(3)	

(1) Nose (1971).

(2) Ogino & Chen (1973a).

(3) Present work .

(4) Watanabe et al., (1979).

(5) Ogino & Nanri (1980).

(6) Atack et al., (1979).

(7) Atack & Matty (1978).

the comparison does produce some interesting results. The absolute carp PER values for fish meal, Pruteen and casein are all higher than their corresponding values for trout, whereas PER and digestibility of whole egg are higher in trout than in carp.

Again, as for PER values, the NPU's rank the proteins in roughly the same order for both species, though the absolute values are appreciably higher for carp, probably due to the dietary energy effect .

Digestibilities were also similar, whilst the herring meal was slightly less well assimilated. BVs for trout were remarkably constant, whilst for carp significant differences could be determined between many of the values, which again may be due to the greater available energy in the carp diets.

One further point must be considered: the effect of non-protein nitrogen in the diets on fish growth. Certain of the novel proteins, notably the Pruteen, contain high amounts of NPN in the form of nucleic acids. As the total nitrogen digestibility was high for Pruteen, it appears that at least a portion of these nucleic acids are absorbed. If it is then assumed that these are not utilized to any great extent, then the retention of the true dietary protein may have been appreciably greater than the values shown here. However, as there is a lack of information on the metabolism of large quantities of dietary nucleic acids, and especially any protein-sparing effect that they may have on protein utilization,

values based on crude protein analysis may be assumed to be a sufficiently reliable estimate of their true values. In conclusion, the results of this experiment indicate that carp, like trout, require a diet containing high quality protein for optimal growth in tanks, and that a major part of this could possibly be Pruteen or a partial replacement of fish meal by other protein sources .

3.4.2. Chromium Oxide.(Cr_2O_3).

The decrease in the Cr_2O_3 content of the digesta from the first part of the intestine (Table 3.1Ø) as compared with the content in the food (1%), may be due either to secretion of material (eg. via the pancreatic duct) into the intestine, or a selective removal of Cr_2O_3, or a combination of both. Removal of Cr_2O_3 could be via the mouth of the fish during consumption of the food, or by selectively faster passage of the marker particles along the intestine.

With fish meal and whole egg, dilution of Cr_2O_3 in the first part of the gut is greater at 6 h than at 4 h, suggesting (1) that these two diets may be more difficult to digest and require continued secretion of digestive juices, or (2) that Cr_2O_3 is removed selectively more readily from these diets. The reverse is seen with casein and pruteen.

In the second part of the gut, absorption of material has taken place (compared with the first part, hardly surprising). However, with fish meal and whole egg at 4 h, the gut contents are still more diluted than the food,

Table 3.1Ø. Cr_2O_3 Content (as % of dry matter) of digesta.

Diet	Gut segment	4 h after feeding	6 h after feeding
Casein	1	Ø.8Ø	Ø.89
	2	1.1Ø	1.2Ø
Fish meal	1	Ø.62	Ø.5Ø
	2	Ø.9Ø	1.26
Whole egg	1	Ø.75	Ø.54
	2	Ø.95	1.1Ø
Pruteen	1	Ø.64	Ø.85
	2	1.1Ø	1.12

so the fish seems to be secreting (losing) more than it gains. At 6 h, things look rather better.

Dry matter increases very markedly 4-6 h in all cases (Table 3.5 and 3.6).

3.4.3. Caution Concerned With Cr_2O_3 Usage .

The simultaneous movement of a marker and other food components through the digestive tract is a necessary condition needed to satisfy the use of the indirect method to establish food digestibility. According to Possomes *et al.*, (1975) if only one meal per day is given, the appearance of the marker in the faeces is at a maximum for only a short time, whereas feeding a number of meals prolongs this time considerably (ultimately a steady-state condition would be achieved). However, it has been indicated that food components in the digestive tract of rainbow trout are variable (Kionka & Windell, 1972) and the significant increase of protein in the anterior part over that in the posterior part of the gut of carp in the present experiment confirmed this phenomenon. Bowen (1978) noticed that in the stomach of *Tilapia mossambica*, Cr_2O_3 was greatly diminished, reaching 1.66% in comparison to 6.35% in the diet. This was suggested to be due to the selective rejection of the high specific gravity marker during disintegration of food in the fish mouth. Dabrowski & Dabrowska (1981) also observed a significantly lowered chromium oxide in fish stomachs when compared to the amount in the diets, and

this supports the present findings with carp. Since the anterior part content was composed of bigger particles than that recovered in other parts of the digestive tract it is clear that the chromium oxide powder was removed selectively faster from the anterior part of the gut than other components. Consequently, a misleading increase of digestibility coefficient in subsequent parts of the digestive tract due to this phenomenon should be a consideration. In stomachless fish, the digestibility coefficient can also be regarded as having a rhythm based on the kind of food, number of meals and species specificity. A similar phenomenon of digestibility variation in the fish with a stomach, Seriola quinqueradiata was observed Furukawa (1976) although an explanation was not given.

3.4.4. Protein and AA Absorption in Carp Digestive Tract.

Keeping in mind difficulties of chromium oxide usage, the interpretation of the results must be made with extreme caution. Certain differences appeared in the digestbility of protein in subseqent parts of the digestive tract, depending on the kind of diet and time after feeding.

The results suggest that under these experimental conditions, the time after a meal when the digest should be analysed is essential for a proper interpretation of the site and extent of AA absorption. Scherbina & Sorvacev (1969) analysed carp digesta 2-3 h after feeding as recommended by Scherbina (1967). The fish were 255-365

g live weight and kept at 22-23 °C; in the first 13% of gut length, lysine was absorbed to a small extent, 1.8% and 4.2% from sunflower and cottonseed protein respectively, whereas 57.Ø and 36.5% of arginine was absorbed. Carp used by Plakas & Katayama (1981) were 34.8-38.8 g wet weight; it was claimed that the first section of the gut, (erroneously called "functional stomach") was essentially of the same length as described by Scherbina & Sorvacev (1969). At 4.5 h after feeding a casein-based diet, most of the EAA were more than 7Ø% absorbed in the first segment of the intestine. Absorption of a number of AA in the anterior 1Ø% of grass carp gut was higher than 5Ø%, methionine, lysine, histidine and arginine being dominant (Stroband & Van der Veen, 198Ø). A similar phenomenon was observed in the present work. It appears that most of the food was digested in the first half of the gut length after 4 h. Similar results were obtained by Dabrowski (1983).

CHAPTER 4.

Chapter 4.

Experiment 2. A short Term Urea Assay to Evaluate The Protein Quality of Food.

4.1. Introduction.

As mentioned previously, the blood urea concentration of rats is highly correlated with PER (Schoeneberger & Gross, 1982) and thus related to the protein content of the diet, rather than to the dietary nitrogen consumption as in the case of the BV (Eggum, 197Ø). Blood urea may therefore be used as an alternative and more economical indicator of the protein quality of food.

The present study was undertaken to apply this to the evaluation of protein quality in carp diets. It was designed to use low amount and low quality of protein and compare these sources with good quality given in larger quantity.

4.2. Materials and Methods.

4.2.1. The Experimental System and Animals.

The experimental facility used was "System 1" as described in detail in section 2.1.1. The fish used were common carp (Cyprinus carpio), weight range 1.66-1.9Ø kg. The fish were divided into four groups in duplicate tanks, four fish in each tank.

The temperature was maintained at 2Ø-22 °C. The fish in each tank were then individually tagged (Section 2.5). No losses were experienced during tagging, and they were fed commercial trout ration (4Ø% protein) for a further week.

The fish were starved 48 h before starting the experiment.

4.2.2. The Experimental Diet.

Formulation of the diets was carried out by the general procedure described in section 2.2, and they were prepared by wet extrusion as detailed in section 2.3. Proximate analysis (section 2.6.) was carried out on samples of the diets, and the results are presented in Table 4.1 along with the ingredients used. In order to determine if in fish a low quality protein increased plasma urea production, a diet containing only wheat middlings (see AA profile Table 4.2) was compared with diets containing fish meal and casein as their protein sources.

The fish meal and casein diets were made up at high (45%) and low (15%) protein concentration in order to determine if protein concentration influenced plasma urea.

4.2.3. Feeding Rates.

The fish in each group were fed a diet at a rate of 2.5% of their body weight per day for six days.

4.2.4. Blood Sampling, Urea and Protein Determination.

The fish in each tank were removed and anaesthetized with benzocaine, and blood was obtained by cardiac puncture using heparin as an anticoagulant. The blood was centrifuged at 3000 rpm for 10 min. The plasma thus obtained was stored at -20 °C until analysis. The blood samples were taken every two days. Plasma urea was

Table 4.1. Ingredient Composition of Diets Used in Experiment 3.

Ingredient %	Diet			
	1	2	3	4
Fish meal	2Ø	-	6Ø	-
Casein	-	-	-	4Ø
Wheat middlings	-	87	-	-
Mineral Mix	2	2	2	2
Vitamin Mix	1	1	1	1
Cod Liver Oil	5	5	5	5
Corn Oil	4	4	4	4
Cellulose	15	1	1Ø	1Ø
Dextrin	53	-	18	38
Proximate Analysis (%).				
Moisture	2.6Ø	7.9Ø	4.2Ø	8.7Ø
Protein	14.2Ø	16.8Ø	42.ØØ	38.8Ø
Lipid	11.24	1Ø.64	14.2Ø	9.77
Ash	9.1Ø	1.9Ø	1Ø.2Ø	9.3Ø
NFE	62.86	62.76	29.4Ø	33.43

Table 4.2. Amino Acid Profile of Experimental Diets (Calculated).

AA %	1	2	3	4
Arginine	1.2Ø	6.ØØ	3.6Ø	1.68
Cystine	2.ØØ	1.2Ø	6.ØØ	Ø.16
Glycine	1.34	4.43	4.Ø2	Ø.8Ø
Histidine	Ø.48	1.82	1.44	1.16
Isoleucine	Ø.8Ø	2.87	2.4Ø	2.28
Leucine	1.4Ø	5.Ø4	4.2Ø	4.2Ø
Lysine	1.48	3.56	4.44	3.48
Methionine	Ø.56	1.39	1.68	1.ØØ
Phenylalanine	Ø.78	3.13	2.34	2.16
Threonine	Ø.84	2.69	2.52	1.84
Tryptophan	Ø.26	1.13	Ø.78	Ø.56
Tyrosine	Ø.66	2.43	1.98	2.52
Valine	Ø.96	4.17	2.88	2.88

measured by the method of Chaney and Marbach (1962). To 2Ø micro L of serum, plasma or standard in the range Ø - 2Ø mg %, add Ø.2Ø ml urease, leave 3Ø min at room temperature or 1Ø min at 37 °C. Add 2.5 ml dye, mix, add 2.5 ml alkali, mix, leave 1 h, measure absorbance at 625 nm.

Protein content was analysed by the method of Lowry et al., (1951). Bovine serum albumin was used as standard.

4.2.5. Statistical Methods.

These were performed as detailed in section 2.7.5.

4.3. Results.

Fish in all groups fed vigorously throughout the experiment, appearing to consume all of their daily ration. Therewere no mortalities during the experiment.

4.3.1. Effect of Protein Quality on Blood Urea Concentration.

Table 4.3 and Fig 4.1 show the daily mean of blood urea concentration in the fish. The differences between dietary groups were apparent by the second day, and increased up to the sixth day. Statistical analysis (Table 4.4) shows a significant difference ($p<Ø.Ø5$) between dietary groups. The higher-protein diet (42%) caused a significant increase in blood urea. It was not possible to prepare a high-protein diet based on wheat middlings.

4.3.2. Effect of Protein Source on Blood Protein Concentration.

Table 4.5 shows the daily means of blood protein concentration. The differences between dietary groups

were apparent by the fourth day (significant $p<0.05$) but were not significant (Table 4.6) at 6 days ($p>0.05$).

Table 4.3. Blood Urea Concentration (mg/100 ml) of Carp (Cyprinus carpio) Fed Four Diets.

Mean ± SD (Numbers of fish 4)

Diet	Day			
	0	2	4	6
Fish meal,1,	7.48±0.89	8.97±1.46	9.58±1.30	10.10±0.75
Fish meal,3,	7.49±1.03	10.90±1.29	11.83±0.66	12.93±1.10
Casein,4,	7.44±0.63	9.93±1.32	10.14±1.36	12.82±1.22
Wheat middlings,2,	7.42±1.04	9.18±1.24	10.07±1.00	10.40±0.98

2 LSD $_{0.05}$ = 1.22

4 LSD $_{0.05}$ = 1.01

6 LSD $_{0.05}$ = 0.86

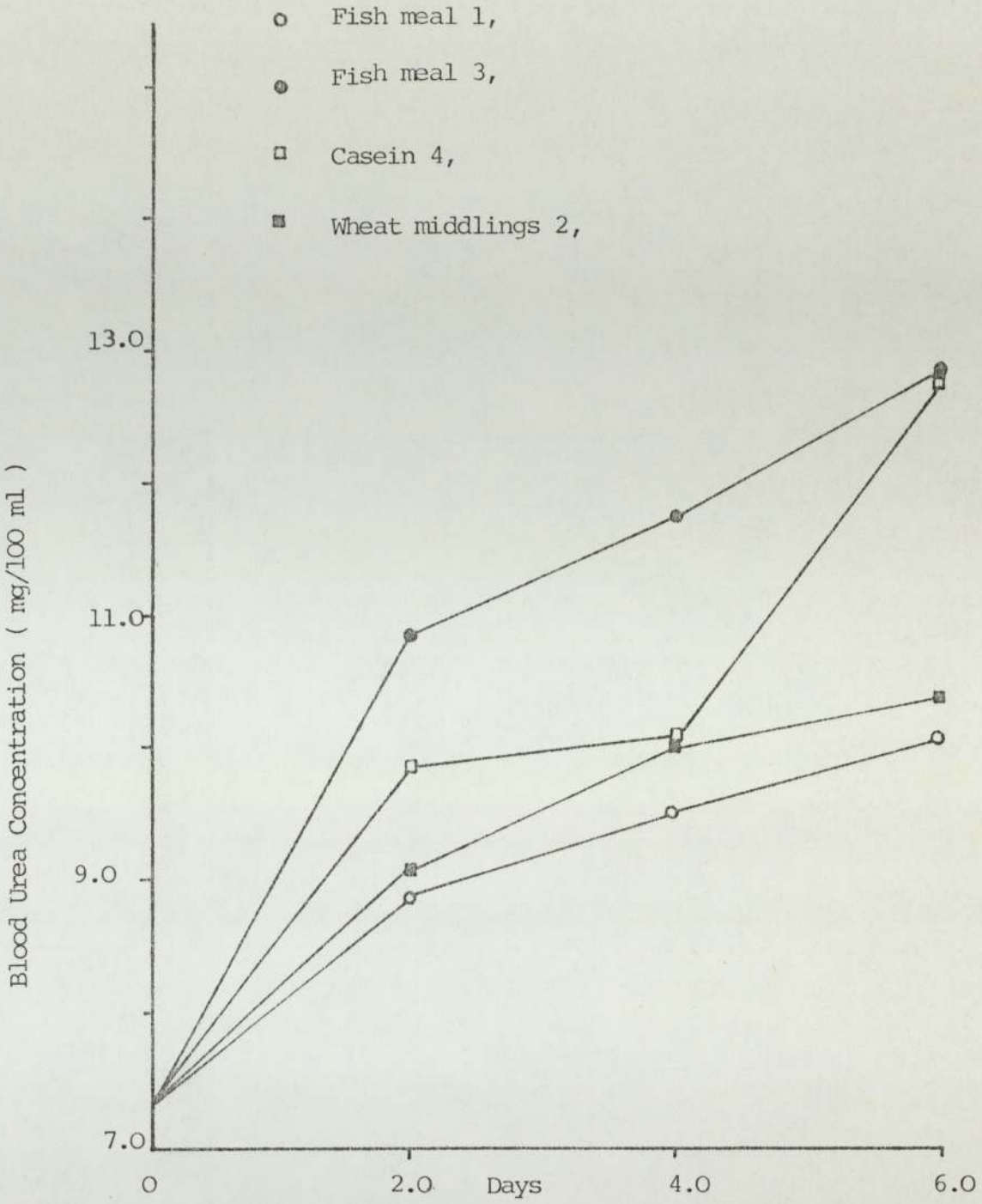

Fig 4.1 Changes of Blood Urea Concentration of Carp Fed with Four diets for up to six days.

Table 4.4. Analysis of Variance for Urea Concentration.

Day	Source of Variance	SS(1)	D.F(2)	M.S(3)	F(4)
	Between treatment	16.732	3	5.57	3.96Ø*
2	Within treatment	39.428	28	1.41	
	Total	56.16Ø	31		
	Between treatment	23.182	3	7.72	7.868*
4	Within treatment	27.497	28	Ø.98	
	Total	5Ø.676	31		
	Between treatment	37.423	3	12.47	17.693*
6	Within treatment	19.741	28	Ø.7Ø	
	Total	57.164	31		

* Significant at < Ø.Ø5.

(1) Sum of square.

(2) Degrees of freedom.

(3) Mean square.

(4) F value.

Table 4.5. Plasma Protein concentration (g/100ml) in Carp Fed with Four Diets. (mean ± SD)

Diet	Days			
	Ø	2 #	4	6
Fish meal,1	3.50±Ø.34	3.14±Ø.32	2.89±Ø.18	2.90±Ø.27
Fish meal,3	3.56±Ø.20	3.51±Ø.17*	3.56±Ø.23	3.60±Ø.30
Casein,4	3.53±Ø.18	3.61±Ø.18*	3.60±Ø.20	3.37±Ø.20
Wheat middlings,3	3.57±Ø.24	3.44±Ø.25*	3.38±Ø.25	3.21±Ø.33

* Significant <Ø.Ø5

LSD = Ø.31

Table 4.6. Analysis of Variance for Protein Concentration.

Day	Source of Variance	SS	DF	MS	F
	Between treatment	Ø.1142	3	Ø.ØØ38	Ø.5847
2	Within treatment	1.8255	28	Ø.Ø651	
	Total	1.9397	31		
	Between treatment	Ø.61Ø4	3	Ø.2Ø34	4.3368*
4	Within treatment	1.3157	28	Ø.Ø469	
	Total	1.9261	31		
	Between treatment	Ø.3811	3	Ø.127Ø	Ø.6775
6	Within treatment	5.2519	28	Ø.1875	
	Total	5.633Ø	31		

* Significant at < Ø.Ø5 .

4.4. Discussion & Conclusion.

This study indicates a significant difference ($p < 0.05$) between the fish meal diets 1 and 3, but not between diet 1 and the wheat middlings and casein diets 2 and 4. Similar results were obtained by Preston *et al.*, (1965) when they observed that blood urea increased linearly with the protein intake of wether lambs, based on body weight. Pastuszewska (1967) obtained similar results with suckling pigs. This relationship is consistent with the demonstration that an increase in dietary protein content caused a decline in BV (Barnes *et al.*, 1946, Forbes *et al.*, 1958). Miller & Payne (1961) found in experiments with rats that a negative correlation exists between NPU and calories from the protein fraction. This was shown by increasing the amounts of protein from beef, casein and wheat gluten from 10 to 40% in the diet. The regression coefficient for beef was - 1.68, for casein - 1.39 and for wheat gluten - 0.79.

Measurements of nitrogen excretion of feeding fish provide a near approximation of the exogenous fraction, while records of nitrogen excretion rate of starved animals give the endogenous fraction (Cowey & Sargent, 1972). From the results of this experiment it was decided to investigate the excretory products of feeding metabolism of carp in relation to dietary protein quality (Chapter 6, Experiment 4).

Also, it was found that plasma protein concentration is not affected by the dietary protein. Similar results were

obtained by Zeitoun et al., (1974) when they reduced the protein concentration of trout food from 60 to 30% and found no influence on serum protein. In contrast, Bulatovich et al., (1976) recorded a decreased blood protein content after supplying a diet short of the main nutritional components.

CHAPTER 5.

Chapter 5.

Experiment 3. Evaluation of Soybean, Maize and Rice bran for Carp (Cyprinus carpio) Diets and The Effect of Protein Quality on Blood Constituents.

5.1. Introduction.

Using various analytical procedures Perrier et al., (1974) identified several serum proteins of rainbow trout. They emphasised the impossibility of correlating any of those proteins with mammalian serum albumin. This is, at least partly, because fish serum proteins are distinct from those of mammals in physiological function and chemical composition. To minimize any confusion resulting from the serum protein differences of fish species, the definition of the terms "albumin" and "globulin" should be understood. However, in the absnce of any specific methodology for fish albumin and globulin determinations, mammalian techniques will be used. In clinical chemistry, A/G (albumin/globulin) ratio is widely used as an activity index of physiological state. This experiment was designed to evaluate soybean, maize and rice bran for carp and study their effect on blood protein components and A/G ratio.

5.2. Materials and Methods.

5.2.1. The Experimental System and Animals.

The experimental facility used in the present study was "system 1" as described in detail in section 2.1.1.
The fish used in the present study were common carp (Cyprinus carpio), of average weight 2.5 kg. Eight

individuals were transferned to three 23Ø L tanks of system 1 at the prevailing ambient temperature of 15 °C. The temperature was then raised approximately 3 °C / day to 22 °C. The fish in each tank were then individually tagged (section 2.5) and fed on a commercial trout ration for a further week. During the period of temperature acclimation and tagging there were no mortalities.

5.2.2. The Experimental Diet.

The diet were prepared exactly as before (section 4.2.2). Results of analysis and ingredients used are presented in Table 5.1.

5.2.3. Feeding Rates.

The fish were fed once a day at Ø9ØØ h, with feed being distributed over a period of 15-2Ø min. All groups were fed 2.5% of their body weight per day. The quantity of food delivered per day was adjusted after each weekly weighing and fed for the subsequent six days. For 18 days the feeding experiment was conducted on all three groups of fish. Fish in all groups fed throughout the experiment appearing to consume all of their daily ration. No mortalities occurred during the experiment.

5.2.4. Blood Sample, Urea and Protein Determination.

Details of blood sampling, urea and protein determination are presented in section 4.2.4.

5.2.5. Albumin and Globulin Determination.

Albumin was measured by the method of Doumas et al., (1971). Globulins were measured by the method of Goldenberg and Drewes (1971).

Table 5.1. Ingredient Composition of The Diets Used in Experiment 5.

Ingredient %	1	2	3
Soybean meal	18	-	-
Maize	-	91	-
Rice bran	-	-	75
Cod liver oil	5	3	2
Corn oil	4	3	-
Vitamin Mix	2	2	2
Mineral Mix	1	1	1
Starch	7Ø	-	2Ø
Proximate analysis (%)			
Moisture	3.2Ø	3.4Ø	3.8Ø
Protein	8.75	9.62	9.18
Lipid	9.18	7.82	3.5Ø
Ash	2.Ø8	9.19	12.8Ø
NFE	76.79	69.97	7Ø.72

5.2.6. Statistical Methods.

These were performed as detailed in section 2.7.5.

5.3. Results.

5.3.1. Effect of Protein Quality on Urea Concentration.

Table 5.2 and Fig 5.1. show the daily mean of blood urea concentration. No significant difference between groups was observed (Table 5.3).

The blood urea concentration in the three groups of common carp decreased from 7.8Ø to 5.52 and 5.72 mg /1ØØ ml for Soybean meal, Maize and Rice bran diets respectively, and this is most likely to relate to the reduction in the protein content of the diet from 4Ø% protein at day Ø rather than from any difference in protein quality.

Table 5.2. Blood Urea Concentration (mg/1ØØ ml) in Common Carp Fed Three Sources of Protein.(mean ± SD)

Diet	Day			
	Ø	6#	12	18
Soybean	7.7Ø±Ø.77	4.76±Ø.77	5.32±Ø.88	5.32±Ø.83
Maize	7.8Ø±Ø.8Ø	5.22±Ø.8Ø	5.51±Ø.78	5.52±Ø.76
Rice bran	7.8Ø±Ø.79	*5.53±Ø.79	5.44±Ø.78	5.72±Ø.8Ø

* Significant at p <Ø.Ø5 .

LSD $_{Ø5}$ = Ø.58

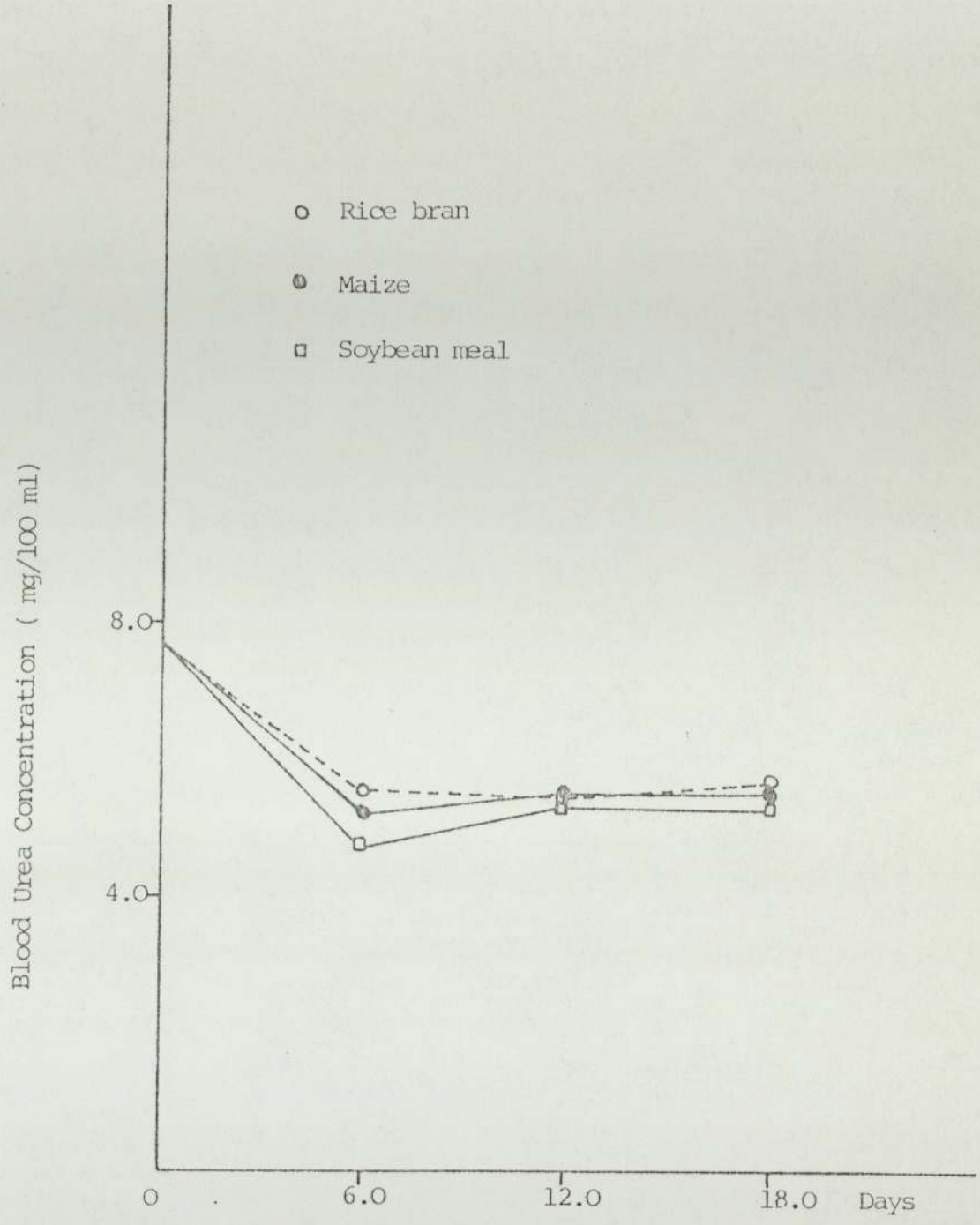

Fig 5.1. Change of Blood Urea Concentration in Carp Fed Three Different Proteins for up to 18 Days.

Table 5.3. Analysis of Variance for Urea at 6, 12 and 18 Days.

Day	Source of Variation	SS	DF	MS	F
6	Between treatment	2.4324	2	1.2162	3.8281 *
	Within treatment	6.6726	21	Ø.3177	
	Total	9.1Ø5Ø	23		
12	Between treatment	Ø.1424	2	Ø.Ø712	Ø.1597
	Within treatment	9.3626	21	Ø.4458	
	Total	9.5Ø5Ø	23		
18	Between treatment	Ø.64ØØ	2	Ø.32ØØ	1.Ø9Ø2
	Within treatment	6.165Ø	21	Ø.2935	
	Total	6.8Ø5Ø	23		

* Significant at p<Ø.Ø5 .

5.3.2. **Effect of Protein Quality on Blood Protein Concentration.**

Table 5.4. shows the blood protein concentration, the albumin, the globulin, and A/G ratios. No significant differences ($P > Ø.Ø5$) between any groups were found (Tables 5.5, 5.6 and 5.7).

5.4. **Discussion & Conclusion.**

This study indicates no significant differences between blood urea concentration on Soybean meal, Maize and Rice bran diets. The results of these experiments confirm the thesis that blood urea increases as the protein content of the diet increases (Munchow and Bergner, 1968). Preston et al., (1965) investigated whether blood urea nitrogen could be determined by dietary protein intake, in an attempt to contribute to an understanding of protein nutrition through an evaluation of the relative amount of protein catabolized from changes in blood urea nitrogen. They observed that the blood urea increased linearly with the protein intake of wether lambs, based on the body weight. The starting point of the linear protein coincided with the critical point of the daily gain curve plotted against dietary protein. This finding could be very important as the data suggest the possibility of a relationship between protein requirements of animals and protein quality of the diet. It should be examined further under various conditions.

Table 5.8. shows the relationship between protein intake and blood urea concentration of groups of common carp

Table 5.4. The Relationship Between Albumin & Globulin and Total Protein.

Diet		Days			
		Ø	6	12	18
Soybean	T.Protein*	3.Ø5±Ø.48	2.78±Ø.35	3.Ø5±Ø.16	3.1Ø±Ø.1Ø
	Albumin*	2.11±Ø.56	1.92±Ø.28	2.13±Ø.33	2.19±Ø.19
	Globulin*	Ø.85±Ø.16	Ø.83±Ø.27	Ø.83±Ø.18	Ø.7Ø±Ø.11
	A / G ratio	2.63±Ø.79	2.53±Ø.84	2.72±Ø.91	2.79±Ø.61
Maize	T.Protein*	3.Ø5±Ø.36	2.86±Ø.33	3.21±Ø.18	3.Ø5±Ø.15
	Albumin*	2.11±Ø.43	1.78±Ø.32	2.33±Ø.14	2.18±Ø.23
	Globulin*	Ø.85±Ø.22	Ø.85±Ø.61	Ø.78±Ø.81	Ø.77±Ø.12
	A / G ratio	2.64±Ø.97	2.18±Ø.64	3.Ø9±Ø.55	2.89±Ø.65
Rice bran	T.Protein*	3.Ø7±Ø.24	3.Ø3±Ø.27	3.15±Ø.27	3.1Ø±Ø.16
	Albumin*	2.11±Ø.36	1.91±Ø.3Ø	2.22±Ø.29	2.22±Ø.24
	Globulin*	Ø.85±Ø.19	Ø.88±Ø.15	Ø.85±Ø.12	Ø.73±Ø.79
	A / G	2.78±1.11	2.23±Ø.62	2.67±1.Ø3	3.Ø9±Ø.72

* mg / 100 ml

Table 5.5. Analysis of Variance for Total Protein at 6, 12 and 18 Days .

Day	Source of Variation	SS	DF	MS	F
6	Between treatment	Ø.25	2	Ø.125	1.225
	Within tretment	2.15	21	Ø.1Ø2	
	Total	2.4Ø	23		
12	Between treatment	Ø.11	2	Ø.Ø5	1.37
	Within treatment	Ø.93	21	Ø.Ø4	
	Total	1.Ø4	23		
18	Between treatment	Ø.Ø15	2	Ø.ØØ7	Ø.352
	Within treatment	Ø.441	21	Ø.Ø21	
	Total	Ø.456	23		

Table 5.6. Analysis of Variance for Albumin at 6, 12 and 18 Days.

Day	Source of Variation	SS	DF	MS	F
6	Between treatment	Ø.1Ø	2	Ø.Ø5	Ø.55
	Within treatment	1.97	21	Ø.Ø9	
	Total	2.Ø7	23		
12	Between treatment	Ø.16	2	Ø.Ø8	1.14
	Within treatment	1.6Ø	21	Ø.Ø7	
	Total	1.76	23		
18	Between treatment	Ø.Ø12	2	Ø.ØØ6	Ø.122
	Within treatment	1.Ø78	21	Ø.Ø51	
	Total	1.Ø9Ø	23		

Table 5.7. Analysis of Variance for Globulin at 6, 12 and 18 Days.

Day	Source of variance	SS	DF	MS	F
6	Between treatment	Ø.Ø11	2	Ø.ØØ5	Ø.135
	Within treatment	Ø.887	21	Ø.Ø42	
	Total	Ø.898	23		
12	Between treatment	Ø.Ø17	2	Ø.ØØ8	Ø.311
	Within treatment	Ø.587	21	Ø.Ø27	
	Total	Ø.6Ø4	23		
18	Between treatment	Ø.Ø16	2	Ø.ØØ8	Ø.589
	Within treatment	Ø.293	21	Ø.Ø13	
	Total	Ø.3Ø9	23		

Table 5.8. The Relationship Between Protein Content in The Diet and Blood Urea concentration.*

Source of protein	Protein content in the diet %	Urea concentration mg/100ml
Casein	38.80	12.82 ± 1.22
Fish meal	14.20	10.10 ± 0.75
Fish meal	42.00	12.93 ± 1.10
Wheat middlings	16.80	10.40 ± 0.98
Soybean meal	8.75	5.32 ± 0.83
Maize	9.62	5.52 ± 0.76
Rice bran	9.18	5.72 ± 0.80

* From the previous work.

(Cyprinus carpio) fed with different sources of protein at different concentrations. The relationship between protein content of the diet and blood urea is shown in Fig 5.2. The correlation equation is :

Blood Urea (mg/100ml)= 4.83+0.20(Protein concentration%)

A positive correlation (r= 0.89) has been found between protein concentration and blood urea. This is in agreement with other findings and must be taken into account if blood urea is to be used as a measure of protein quality. Accordingly, all the experiments on the effect of protein quality were carried out using diets containing the same amount of protein.

It was found in mammals by using blood urea technique for evaluating protein quality that at least three factors influence blood urea : concentration,the quality and quantity of protein in the diet and the time of sampling after feeding. Eggum (1973) found that proteins with low digestibilities would produce lower blood urea nitrogen values due to less nitrogen absorption. The rate of passage of food through the gastrointestinal tract of fish could influence the time after feeding at which proteins would be absorbed and blood urea nitrogen would be maximum. Blood urea nitrogen would be elevated when caloric intakes were low and AAs used to supply energy. Therefore, more research is needed to establish the appropriate uses of blood urea nitrogen in fish nutritional studies.

Also, these results indicate that the protein source has

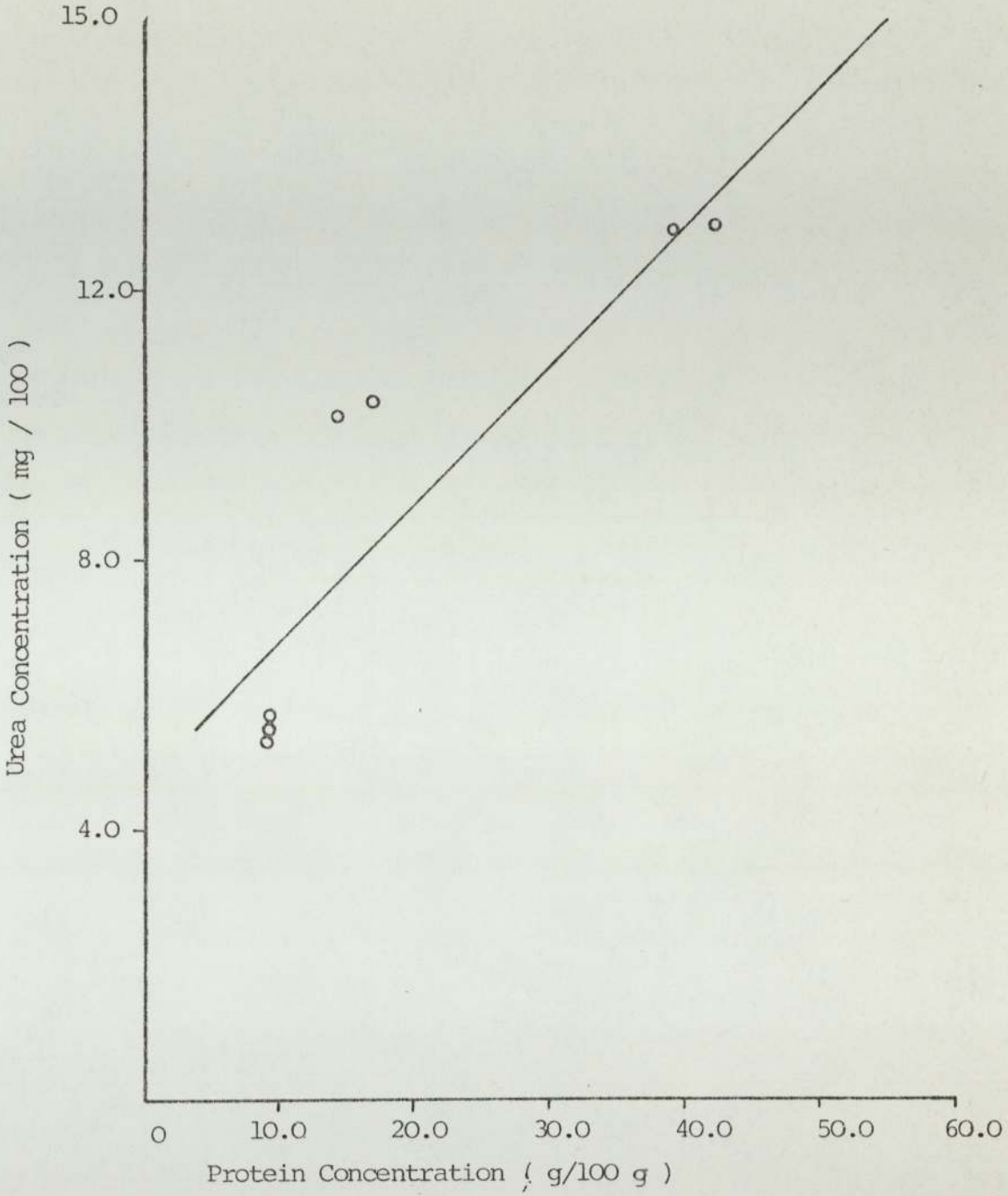

Fig 5.2. Correlation between Protein Concentration and Blood Urea Concentration.

no effect on blood albumin and globulin. Some studies in man found that the total protein concentration in the plasma is often greately decreased to as little as 4 g /1ØØ (Waterlow et al., 196Ø). The decrease is mainly due to a decrease in the albumin fraction. Brock (1961) stated that the most sensitive biochemical index of mild or impending protein deficiency is a drop in serum albumin into the marginal range. The total globulins show little change. According to Waterlow (196Ø), a fall in serum albumin concentration would be a relatively late event in protein depletion, since there seem to be mechanisms which tend to protect the total circulating albumin mass when protein supplies are short. Schendel et al., (1962) found that, even with a poor quality food, such as maize, serum albumin concentration was maintained for several weeks before it began to decrease, and this supports the finding in the present study. The results suggest that plasma albumin concentration is probably not a very sensitive index of protein adequacy.

CHAPTER 6.

Chapter 6.

Experiment 4. Ammonia Excretion Rate As Index For Protein Quality Evaluation For Carp Diets.

6.1. Introduction.

Efficient utilization of dietary protein for growth is not among the economically important traits which have been considered for genetic selection in fish. In this regard, the development of a simple and effective methodology for measurement of nutrient efficiency in such studies is urgently needed (Gjedrem, 1983).

Principal end-products of nitrogen metabolism have been used to measure efficiency of dietary protein utilization in both terrestrial and aquatic species. This has been discussed in the previous chapter. Eggum (197Ø) found that, following consumption of different feed stuffs, the plateau concentration of blood urea in the pig was negatively correlated with protein BV for the feed stuffs. Miles and Featherston (1974) working with chicks used the inflection point in the dose-response graph of uric acid excretion relative to dietary lysine concentration to determine its optimum supplementation and results obtained by this indirect method agreed with those based on weight gain. Garcia et al., (1981) compared efficiency of protein utilization for different diets fed to groups of rainbow trout of the same strain and found agreement between PER and PPV ranking based on total ammonia excreted in 24 h. Ming (1985) obtained similar results, as did Rychly and Marina (1977) and

Kaushik (198Ø).

Brett and Zala (1975) reported that Sockeye salmon exhibit a sharp peak in ammonia excretion rate 4-4.5 h after a single daily meal. Some information is available on the quantitative relationship between the composition of natural diets and nitrogen excretion (Gerking, 1955; Elliott, 1976).

On the basis of these observations, ammonia excretion would appear to have potential as an index of dietary protein utilization for carp. The present study investigates the ammonia excretion of carp fed various different diets and attempts to relate it to the digestibility of the diets.

6.2. Materials & Methods.

6.2.1. The Experimental System and Animals.

The experimental facility used in the present study was "System 2" as described in detail in section 2.1.2.

6Ø yearling common carp of mean weight 21Ø.8 g were divided into six groups in six tanks (1Ø fish each). All groups were acclimated to experimental tanks with flowing aerated water, at a temperature of 25±1 °C. All fish were fed twice daily with a commercial trout diet. Starting 1 week before the experiment, the fish were fed once daily (at Ø9.3Ø h).

6.2.2. Experimental Protocol.

Six tanks were used, one for each group. Water volumes were approximately the same. Fish were weighed individ-

ually on the day preceding the experiment and returned to the same tank, which had been throughly cleaned. All weighings were done at least 12 h after the previous meal, and after fish were anaesthetized (2-phenoxyethanol, Ø.5 ml/L water) and rolled on cloth to remove excess water.

Before being fed, fish were starved for 3 days. Nitrogen excretion rates of starved fish are the lowest (Savitz, 1971) and throughout this manuscript they will be referred to as the maintenance nitrogen excretion.

On the four trial days, the fish were fed the diets (Table 6.1) once daily, at Ø9.3Ø, at a fixed percentage of initial body weight (Table 6.2). Feeding was done with the water flow to the tanks turned off. Feeding activity could therefore be monitored carefully to ensure total consumption of all the fed offered, and its even distribution among all experimental animals in each tank. Feeding of the fish in all tanks was completed in about 2Ø min.

Similar to the method of Brett and Zala (1975), an alternating water flow cycle (1 h on and 1 h off) was used to prevent excessive elevation of ammonia concentration during the daily 6-h experimental period (Ø9.3Ø h-15.ØØ h). Water flow was on at all other times, and off during feeding (Ø9.3Ø-Ø9.5Ø h). Ammonia excretion was only measured during the 1-h intervals when water flow was turned off, giving three daily measurements of ammonia production for each tank. On alternate

Table 6.1. Experimental diets.

Ingredient %	Diet					
	1	2	3	4	5	6
Fish meal	43	-	-	-	-	-
Cottonseed meal	-	86	-	-	-	-
Pruteen	-	-	42	-	-	-
Groundnut	-	-	-	75	-	-
Rapeseed meal	-	-	-	-	75	-
Sunflower seed meal	-	-	-	-	-	85
Dextrin	18	2	19	6	6	3
Potato starch	17	2	17	6	6	2
Cod liver oil	14	2	14	5	5	2
Mineral mix	4	4	4	4	4	4
Vitamin mix	2	2	2	2	2	2
Carboxyl methyl Cellulose	1	1	1	1	1	1
Chromic oxide	1	1	1	1	1	1
Proximate analysis (%)						
Moisture	5.85	3.16	1.92	6.49	5.91	10.20
Protein	29.28	30.10	30.24	31.50	28.20	30.60
Lipid	13.08	5.50	17.50	7.90	6.50	3.10
Ash	7.19	7.00	7.00	8.00	8.00	12.49
NFE	44.60	54.24	43.34	46.11	51.39	43.61

Table 6.2. Body Weights and Feed Consumption for Carp Over a 4-day Period.

	Diets					
	1	2	3	4	5	6
Mean live body wt						
Initial (g)	210.8	210.8	210.8	210.7	210.8	210.8
Final (g)	214.9	212.2	213.2	212.0	211.9	211.3
Feed consumption						
(g as fed)						
Day 1	49.00	32.00	32.58	45.00	38.00	50.00
Day 2	48.00	32.20	26.50	37.10	38.00	42.00
Day 3	37.00	30.00	25.00	37.00	32.00	40.00
Day 4	34.00	23.00	25.00	37.00	32.00	40.00

days the cycle was reversed. Over a 2-day period, therefore, ammonia excretion was determined for each of six hourly intervals. The fish were reweighed at the termination of the 4-day experiment.

6.2.3. Water Sampling, Ammonia and Oxygen Analysis.

For determining ammonia production and oxygen consumption, four water samples of 125 ml each were taken hourly from each tank, between Ø9.3Ø h and 15.3Ø h, just before turning water flow on, or just after turning it off. The first two samples were taken immediately after feeding (at about Ø9.5Ø h) so as not to disrupt feeding behaviour. Samples were taken from either side of the central standpipe by moving the glass sampling bottle backwards in a slow sweeping arc from the back of the tank to the front, about halfway to the bottom. The duplicate samples from each tank were mixed together for ammonia and oxygen determination.

Ammonia analysis was carried out by direct Nesslerization. Oxygen content was determined by modified Winkler method using the average of the two closest titrations.

6.2.4. Statistical Analysis.

Statistical comparisons between means were made by two way analysis of variance (Snedecor and Cochran, 198Ø).

6.3. Results.

Table 6.2. shows the mean body weight and feed consumption for each tank of fish. There was a slight increase in body weight in all groups of fish, during the 4 days

of the experiment. Composition of the gain was not determined.

The appetites of all groups were different; groups fed fish meal, groundnut and sunflower consumed more diet (circa 2.3% of body weight). Actual feed consumed per unit body weight per day was different for the different groups in the first 2 days. After the second day, feeding rates for all groups had been standardized at 1.5% of the initial weight per day.

6.3.1. Nitrogen Excretion Rates.

Hourly rates of NH_3-N excretion were expressed as mg ammonia-N excreted per Kg/h, (Table 6.3, Fig 6.1 and 6.2).

Hourly points of the graph for each group represent measurements made on different days (day 1 & 3, or day 2 & 4). The ammonia excretion of fish fed on Pruteen and fish meal was almost constant; that of the cottonseed group was similar, except for a peak at 4 h, when ammonia production was doubled. By contrast, the fish fed on groundnut, sunflower and rapeseed showed a rapid and sustained increase in ammonia production, to around ten times that of the fish meal group, the difference being (very) significantly different ($P > Ø.Ø1$).

The picture correlates well with the protein quality of the feed. Pruteen, cottonseed meal and fish meal are high-quality protein sources, whereas the other three are lower quality, the term"quality" being defined in terms of the amount of ammonia production. It was found

that ammonia production increased with a decline in protein quality.

Table 6.3. Development of Ammonia Production (mg/Kg/h) for Carp Fed Six Protein Sources (circa 30%).

	Hours after feeding						
Diets	Ø	1	2	3	4	5	6
Fish meal	15.18	15.18	18.21	15.18	15.18	15.18	15.18
Cottonseed meal	15.18	18.21	18.97	18.97	39.46	16.69	18.97
Pruteen	15.18	15.18	18.21	15.18	18.21	15.18	21.25
Groundnut	15.18	18.22	91.12	144.28	136.68	136.68	151.87
Rapeseed meal	15.18	42.50	88.04	98.67	113.85	121.44	129.03
Sunflower	15.18	42.50	91.08	129.03	129.03	136.62	136.62

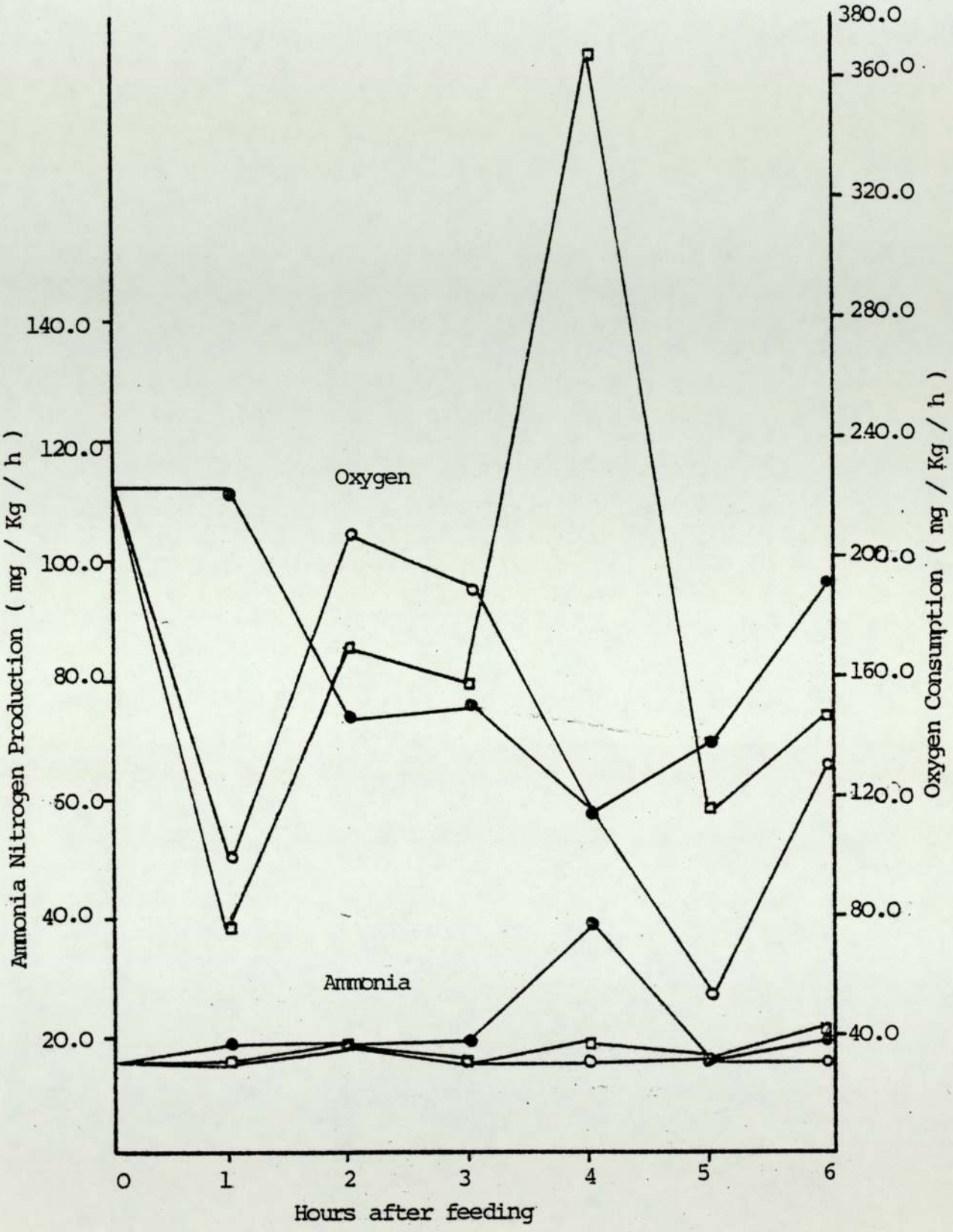

Fig 6.1. Change in Rate of Ammonia-N Production and Oxygen Consumption with Time after Feeding Fish meal —o—, Cotton seed meal —●—, and Pruteen —□— .

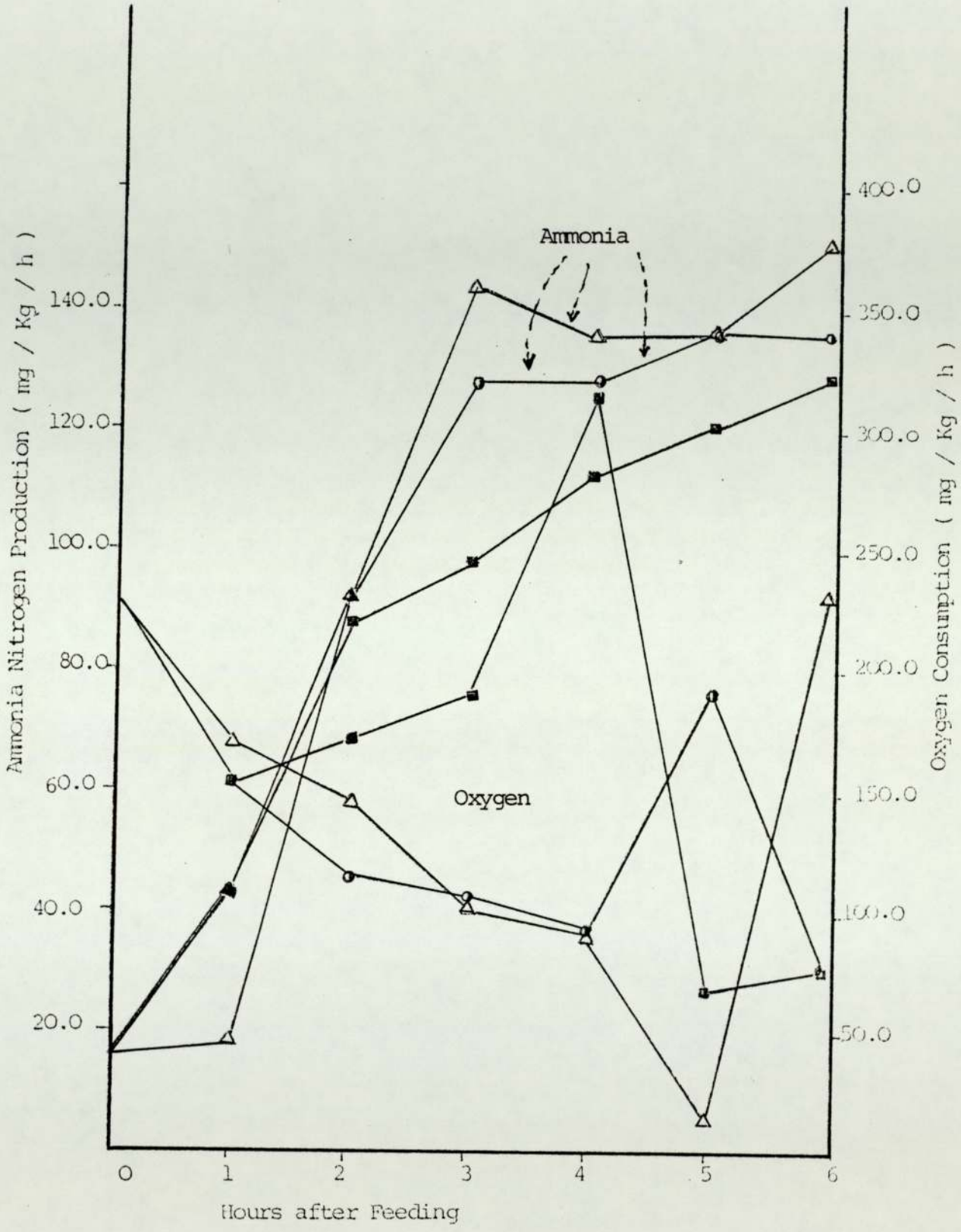

Fig 6.2. Change in Rate of Ammonia-N Production and Oxygen Consumption with Time after Feeding Rapeseed meal (—■—), Groundnut (—△—), and Sunflower (—○—) diets.

6.3.2. Oxygen Consumption Rates.

Metabolic rates as expressed by oxygen consumption showed a large fluctuation, both in magnitude and in time of appearance of the maximum value (Table 6.4, Fig 6.1 & 6.2). There was a significant difference between all diets at all times. Since protein is an important energy source for fish, it might be expected that ammonia production would increase in direct proportion to the metabolic rate. A negative correlation was found between ammonia production and oxygen consumption (r= -Ø.3Ø5) Fig 6.3.

Since there is such a clear distinction between the ammonia excretion on the diets, it might be worthwhile to treat them separateley for oxygen consumption, which is done for (A) Fish meal, Pruteen, cottonseed meal; (B) Groundnut, rapeseed, sunflower giving Fig 6.1 & 6.2.

6.4. Discussion & Conclusion.

The present study shows that carp fed on various different diets show peaks in ammonia excretion that vary both in size and time of occurrence after feeding. This supports the work of other authors, who found that the timing of the peak of ammonia excretion varied between species. Salmonid nitrogen excretion rates were measured by Burrows (1964), Brett and Zala (1975), Rychly and Mariana (1977) and Ming (1985) who all found a post-prandial increase in ammonia excretion, the peak values being observed at different intervals, 4 h in

Table 6.4. Oxygen Consumption (mg/kg/h) for Carp Fed Six Protein Sources (ca 30% protein).

Diet	Hours after feeding						
	Ø	1	2	3	4	5	6
Fish meal	225.8Ø	1ØØ.94	2Ø9.48	19Ø.6Ø	116.12	55.Ø8	131.3Ø
Cottonseed meal	225.8Ø	222.25	147.24	151.87	116.12	139.65	193.54
Pruteen	225.8Ø	77.41	17Ø.ØØ	159.46	369.32	116.12	147.25
Groundnut meal	225.8Ø	17Ø.77	147.24	1ØØ.99	93.35	15.93	232.25
Rapeseed meal	225.8Ø	154.83	17Ø.77	186.Ø4	316.15	69.82	77.41
Sunflower meal	225.8Ø	154.83	116.12	1Ø8.59	92.59	193.54	77.41

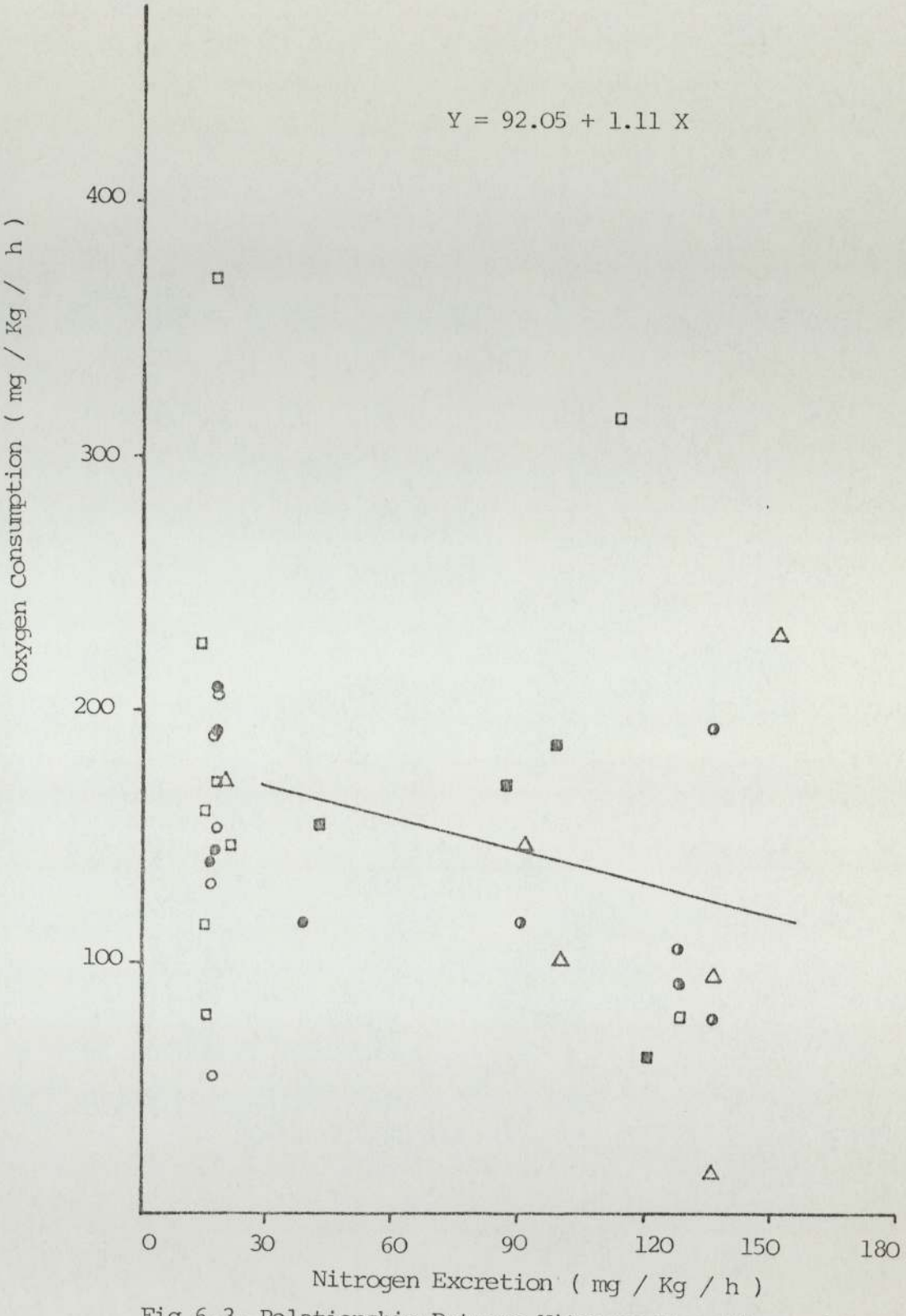

Fig 6.3. Relationship Between Nitrogen Excretion and Oxygen Consumption for Carp Fed Six Protein Sources (30 % Protein). r = -0.305

Sockeye salmon fed fish meal diet (33% protein), 6 h in the trout, 11 h in Coho salmon, and 6 h in Carp fed a commercial salmonid diet.

In the present study, significant differences in ammonia-N excretion among carp fed six protein diets were the results of poor utilization of groundnut, rapeseed meal and sunflower diets. The large pulse in ammonia excretion appears to be entirely the product of food intake (exogenous fraction), and not of metabolic rate. Such an effect of food has been observed by only a few authors as mentioned previously (Burrows, 1964, McLean & Fraser, 1974; Brett & Zala, 1975; Durbin, 1976).

The post-prandial times of peak ammonia excretion rate (Fig 6.2 & 6.3) are compatible with the 6 h appetite cycle reported for rainbow trout (Fange and Groves, 1979), and the value 6 h for carp (Ming, 1985) as well as the post-prandial time of peak ammonia excretion for Sockeye salmon (Brett & Zala, 1975). Ranking based on maximum rate of excretion (Fig 6.2 & 6.3) is, therefore, a good indicator of the relative efficiency with which dietary protein is used for growth by carp.

Rychly and Marina (1977), in a single 24 h trial observed multiple peaks for ammonia excretion rate in rainbow trout. However, their supernumerary peaks may have been an artifact of the experimental design, by which individual fish were tested in standing water continuously for 24 h. Under similar conditions (with the exception that the trout used were of a larger body weight, and fasted),

the increase in ammonia concentration in the water appeared to suppress the normal rate of ammonia excretion within 2 h. Rates returned to normal after flushing the closed system with fresh water (Wright and Wood, 1985). An intermittent flushing/ standing water regimen (see Materials & Methods), similar to that described by Brett and Zala (1975), was used to minimize negative feedback of ammonia in the current experiment.

This study indicates that ammonia excretion rate by carp may be used as the basis for comparing the relative efficiency of dietary protein utilizaton.

CHAPTER 7.

Chapter 7.

7. Experiment 5. An In Vitro Method For Measuring Protein Digestibility.

7.1. Introduction.

It is well known that a long term feeding trial is the most dependable method of measuring the nutritive value of fish feed, but is often too slow and expensive. *In vitro* methods of evaluating protein digestibility are important, not only because they are rapid and less expensive, but also because they allow close observation of the dynamics of the breakdown of protein by using only small amounts of raw materials.

There exist a number of test systems (Satterlee *et al.*, 1977, Hsu *et al.*, 1977) using individual proteolytic enzymes or a combination of them (Hsu *et al.*, 1977). All are conducted under artificial conditions, for example, the *in vitro* digestion with papain (Buchanan & Byers, 1969), an approximation of the condition of the alimentary system of mammals using pepsin (Sheffner *et al.*, 1956), pancreatin (Menden and Caremer, 1966), pepsin-pancreatin (Akeson and Stahman, 1964), or successively pepsin-pancreatin-trypsin and erepsin digestion (Ford and Salter, 1966).

The enzyme systems described by Akeson and by Buchanan were reinvestigated by Saunders *et al.* (1973). They found that the values obtained from the enzyme system used by Akeson showed an excellent correlation with *in vivo* data ($r = Ø.88$) whereas the results from the papain

digestion showed very poor correlation. Saunders developed a papain-trypsin-system, the *in vitro* results of which correlated well with *in vivo* digestibility (r = Ø.91). Maga *et al.* (1973) pointed out that initial rates of hydrolysis by trypsin on some commonly used protein sources were good indicators of their digestibilities. Rhinehart (1975) modified Mago's procedure and investigated the correlation between *in vitro* and *in vivo* protein digestibility. Rhinehart examined several enzyme systems which included trypsin, pepsin-trypsin, trypsin-chymotrypsin, and trypsin-chemotrypsin-peptidase combinations. The results were encouraging, with correlation coefficients of Ø.79, Ø.72 , Ø.8Ø and Ø.74 for these four systems.

Horn *et al.* (1976) suggested that *in vitro* protein decomposition should be, in fact, a copy of natural conditions in order to obtain the truest possible picture of the first phase of protein metabolism. The *in vitro* digestion method reported here is oriented to this suggestion, working from a fish-specific physiological background. The purpose of the research was to develop a reliable and rapid *in vitro* method which can determine protein digestibility.

7.2. MATERIALS & METHODS.

7.2.1. Samples.

Carp (Cyprinus carpio), weighing about 900 g were used.

7.2.2. Diet.

Either whole compounded diet (Table 7.1) or pure protein sources (casein, fish meal, sunflower, albumin, maize and whole egg protein) were used. The actual amount assayed was the total of the contained protein in the natural product sample (section 2.6).

7.2.3. Preparation of enzyme solution.

Ten carp were killed 2 h after feeding. The intestine from each carp was rinsed with distilled water after discarding the gut contents. The tissue was homogenized in an adequate quantity of distilled water using a Potter-Elvehjem homogenizer. The homogenate was centrifuged at 10,000 X g for 20 minutes. The supernatant was diluted ten fold with Tris-HCl buffer pH 9.5 containing a trace of merthiolate.

7.2.4. Assay of Protease Activity.

Total proteolytic activity was determined at 37 °C by means of a modified method of Charney & Tomarelli (1947), using 25 mg of azocasein and 5 mg of sodium bicarbonate per ml (pH 9.5) as substrate. 1 ml of the diluted gut fluid was mixed with 1 ml of azocasein. A substrate blank was prepared by substituting 1 ml of bicarbonate buffer for the test sample. At the end of 30 minutes the digestion was stopped and undigested azocasein precipitated from the solution by adding 5 ml of 5% trichloro-

Table 7.1. Experimental Diets Used for In Vitro Measurements of Protein Digestibility.

Ingredient %	Diet Number								
	1	2	3	4	5	6	7	8	9
Fish meal	2Ø	4Ø	-	-	-	-	15	-	-
Casein	-	-	2Ø	4Ø	-	-	-	-	-
Soybean meal	-	-	-	-	2Ø	4Ø	15	-	-
Sunflower	-	-	-	-	-	-	-	2Ø	4Ø
Potato starch	2Ø	2Ø	2Ø	2Ø	2Ø	2Ø	2Ø	2Ø	2Ø
Dextrin	39	19	39	19	39	19	29	39	19
Corn Oil	9	9	9	9	9	9	9	9	9
Mineral Mix	4	4	4	4	4	4	4	4	4
Vitamin Mix	2	2	2	2	2	2	2	2	2
Cellulose	5	5	5	5	5	5	5	5	5
Chromic Oxide	1	1	1	1	1	1	1	1	1
Proximate Analysis.									
Moisture%	2.6Ø	2.8Ø	8.1Ø	8.7Ø	7.9Ø	8.2Ø	5.2Ø	8.4Ø	8.6Ø
Protein %	14.25	27.2	18.7	36.8	1Ø.1Ø	2Ø.1Ø	18.2	8.1Ø	16.1Ø
Lipid %	11.24	13.4	9.7	9.7Ø	9.2Ø	9.3Ø	9.2Ø	9.4Ø	9.8Ø
Ash %	9.1Ø	9.3Ø	9.2Ø	9.3Ø	9.5Ø	9.4Ø	9.3Ø	9.4Ø	9.3Ø
NFE %	62.81	47.3	54.3	35.5	63.3Ø	53.Ø	58.1Ø	64.7Ø	56.2Ø

acetic acid to each tube, including the substrate blank tubes. The absorbance of the centrifuged supernatant, diluted with the same volume of 1 N NaOH was measured at 44Ø nm. Proteolytic activity is expressed as units per ml gut fluid or units per mg protein in the gut fluid, one unit corresponding to a defined increase in absorbance over a period of one minute. One azocasein-unit corresponds to 7,464 BAEE-trypsin units.

7.2.5. In Vitro Incubation.

In order to model the physiological action of gut protease enzymes, an assay was prepared by incubating a 1ØØ mg protein sample contained in compounded diets (Table 7.1), or 1ØØ mg pure protein sources, with 4 ml of whole gut extract (activity 2985 BAEE unit) and 25 ml of Tris-HCl buffer pH 9.5 at 37 °C for 24 h. Enzyme blanks were prepared by incubation under the same conditions with protein omitted. Fifty parts per million merthiolate were added to prevent growth of microorganisms and did not interfere with the digestion and subsequent analysis. A rapid decline in pH occurred immediately caused by the freeing of AA carboxyl groups from the protein chain by the proteolytic enzymes. The pH drop was recorded over a 1Ø minute period (Table 7.2).
At the end of the incubation period, the undigested protein and peptides were precipitated with 15 ml of 14% sulphosalicylic acid and the flasks were then shaken for another 15-3Ø minutes. The solids were separated by

Table 7.2. pH Values Obtained by Incubation of Protein Stated with Whole Gut Enzyme Extract.

Diet No	Time(min)											
	Ø	25 Sec	1	2	3	4	5	6	7	8	9	1Ø
Casein,3	9.5	8.7	8.6	8.3	8.2	8.Ø	7.8	7.5	7.5	7.5	7.5	7.5
Casein,4	9.5	8.7	8.5	8.2	8.Ø	7.8	7.7	7.5	7.5	7.5	7.5	7.5
Fish meal,1	9.5	8.8	8.7	8.6	8.5	8.4	8.4	8.3	8.3	8.2	8.2	8.Ø
Fish meal,2	9.5	8.8	8.6	8.5	8.4	8.4	8.3	8.2	8.2	8.1	8.1	8.Ø
Sunflower,8	9.5	9.Ø	8.8	8.6	8.6	8.6	8.5	8.5	8.5	8.5	8.5	8.5
Sunflower,9	9.5	8.7	8.7	8.7	8.6	8.5	8.5	8.5	8.5	8.5	8.5	8.5
Soybean,5	9.5	8.7	8.6	8.5	8.4	8.4	8.4	8.4	8.3	8.3	8.3	8.2
Soybean,6	9.5	8.8	8.7	8.6	8.5	8.4	8.4	8.4	8.3	8.2	8.2	8.1
Soybean+ Fish meal,7	9.5	8.7	8.6	8.5	8.5	8.4	8.4	8.3	8.3	8.3	8.3	8.2

centrifuging 5 min at 20000 g and washing with water (5 X 30 ml), centrifuging and removing supernatant after each washing. The solids were finally filtered through a 1.2 µm filter (Millipore), air dried, weighed, and analysed for nitrogen (section 2.6).

The calculation is as follows :

In vivo apparent digestibility =

$$\frac{\text{N in diet(g)} - \text{undigested N(g)}}{\text{N in diet(g)}} \times 100$$

7.2.6. In Vivo Apparent Digestibility Determination.

Cyprinus carpio varying in weight from 120-130 g were used. Fish were randomly distributed at a rate of 10 fish per tank, two tanks per dietary treatment (System 2 section 2.1.2.1). Over the course of the 2-week experimental feeding period water temperature was 25 ± 1 °C.

All fish were fed twice daily at a fixed rate, 1.5% body weight per day, 7 days a week. Faecal samples were collected from individual fish by hand stripping during the 2-week feeding period, and pooled for each experimental tank sampled. The pooled faecal samples were oven dried at 105 °C for 24 h, and stored in air-tight containers for subsequent chemical analysis (Section 2.7.4), results are presented in Table 7.3.

7.2.7. Diets.

Nine experimental diets were formulated (Table 7.1), containing varying protein concentrations.

Table 7.3. In Vivo and In Vitro Measurements of Protein Digestibility in Compounded Diets.

Diet No *	In vivo	In vitro
Fish meal, 1	91.3Ø ± 2.2Ø	9Ø.2Ø ± 2.3Ø
Fish meal, 2	94.2Ø ± 1.8Ø	93.ØØ ± 1.95
Casein , 3	93.2Ø ± 2.2Ø	91.5Ø ± 2.2Ø
Casein , 4	94.8Ø ± 1.8Ø	93.2Ø ± 1.8Ø
Soybean , 5	85.2Ø ± 1.95	84.2Ø ± 1.4Ø
Soybean , 6	86.1Ø ± 2.3Ø	85.3Ø ± 2.2Ø
Soybean+Fish meal,7	87.3Ø ± 1.6Ø	86.5Ø ± 1.95
Sunflower,8	65.3Ø ± 2.3Ø	64.2Ø ± 1.65
Sunflower,9	68.2Ø ± 1.75	66.1Ø ± 2.2Ø

* (See Table 7.1).

7.2.8. Protein Digestibility.

Apparent protein digestibilities were determined as described in section 2.7.4.and are presented in Table 7.3.

7.2.9. Correlation between In Vitro Parameters and In Vivo Digestibility.

All enzymatic digestions were characterized by (1) the rate of pH drop 15 seconds after enzyme addition, (2) the extent of the pH drop 1Ø min after enzyme addition (Fig 7.1); and (3) an equation describing the pH drop (Hsu et al., 1977). The pH drop at 1Ø min and the derived equation were analyzed for correlation with in vivo digestibility. A multiple regression program was run to determine the correlation coefficients and standard error of estimates to determine the best correlation of the in vitro method with in vivo apparent digestibilities.

7.3. Results.

7.3.1. In Vitro Protein Digestibility for Diets.

Table 7.2 illustrates the pH changes at various time intervals after incubating the diets with gut extract.

Table 7.3 illustrates the in vitro protein digestibilities of 9 diets and their respective in vivo digestibilities. These data were used to construct the equations shown in Table 7.4.

It was found that there is a positive correlation (r = Ø.99) between in vitro and in vivo protein

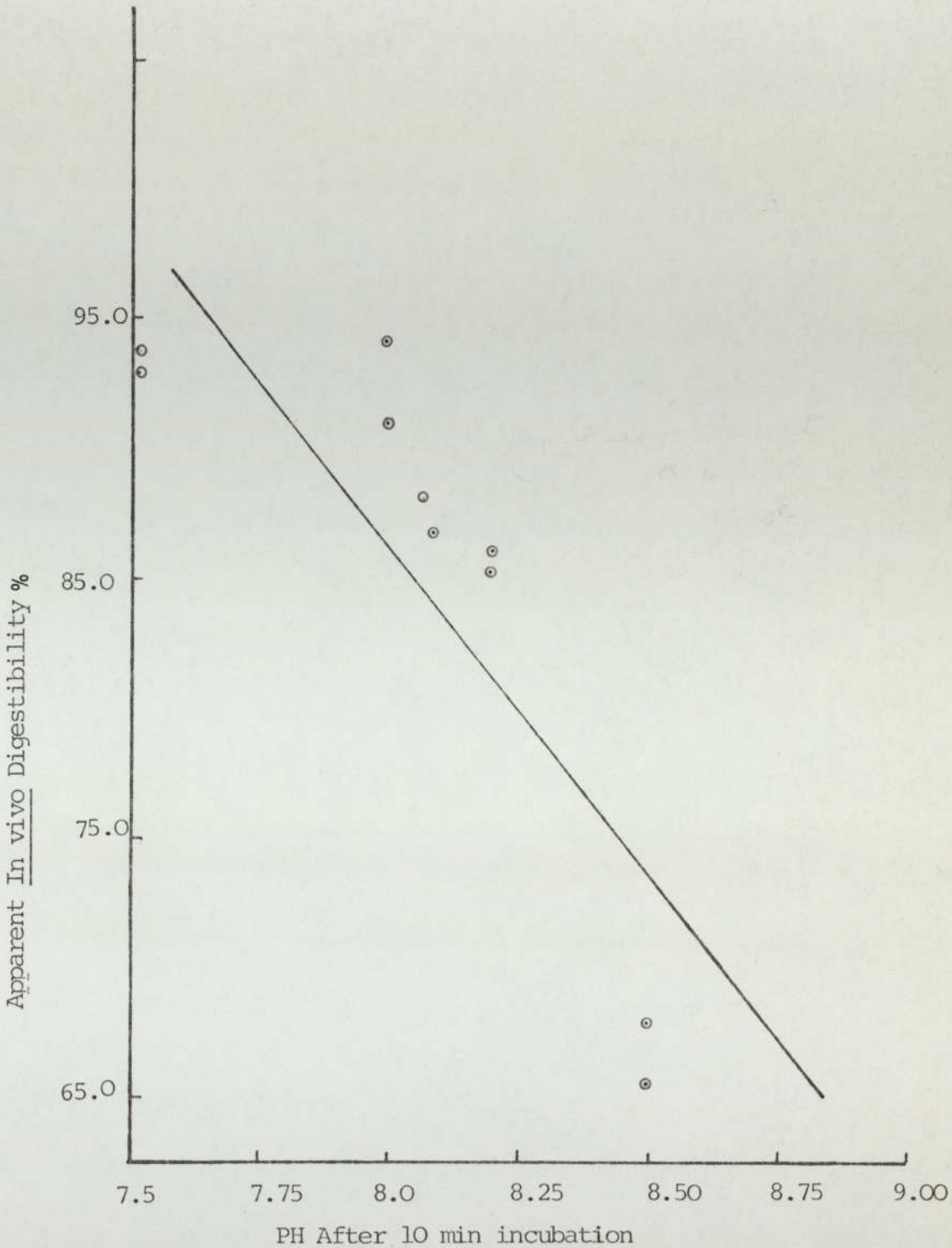

Fig 7.1. Relationship of PH at 10 min and Apparent Protein Digestibility.

Table 7.4. Regression Analysis of pH After 25 Seconds and 1Ø minutes In Vitro and In Vivo Protein Digestibility.

Correlation Coefficient Between pH after 25 Sec and In Vivo Protein Digestibility	Ø.63
The Regression Equation is	
In Vivo = 765 - 77.5 pH at 25 Sec	
F Value for Analysis of Variance (D.F.1, 7)	6.23
Correlation Coefficient Between pH after 1Ø min and In Vivo Protein Digestibility	Ø.81
The Regression Equation is	
In Vivo = 288 - 25.2 pH at 1Ø min	
F For Analysis of Variance (D.F. 1, 7)	16.39
Standard Deviation Within Carp In Vivo Digestibility	±Ø.17
Standard Deviation Within Carp In Vitro Digestibility	± Ø.17
Correlation Coefficient Between In Vivo and In vitro Protein Digestibility	Ø.99
The Regression Equation is	
pH at 1Ø min = 1Ø.7 - Ø.515 In Vivo + Ø.49 In Vitro	
F For Analysis of Variance (D.F 2, 6)	9.1Ø

digestibilities. Also, it was found that *in vivo* apparent digestibility had a higher correlation with the pH drop at 1Ø min after enzyme addition (r = - Ø.81) than it did with pH drop at 15 seconds after enzyme addition (r= - Ø.63). The correlation coefficients, standard errors, and other statistical data of the regression of *in vitro* digestion parameters (pH at 1Ø min) and various apparent digestibilities are shown in Table 7.4. The data indicate that pH at 1Ø min, when used alone, gave a correlation coefficient of Ø.81 with standard deviation of Ø.17. The linear regression curve describing the equation using the pH at 1Ø minute values is shown in figure 7.1. When two variables (pH at Ø.25 and 1Ø min) were selected, the correlation coefficient was Ø.82.

7.3.2. *In Vitro* Protein Digestibility For Pure Protein Sources.

Table 7.5 illustrates the *in vitro* protein digestibilities of pure protein sources and their respective carp *in vivo* apparent digestibilities. Like the compounded diet the digestibility of pure protein sources also correlated well with *in vivo* values (r = Ø.99).

7.4. Discussion.

Generally the utilization of protein by carp and trout is as good as that by farm animals (Steffens, 1981). For many of the conventional feed proteins, high values of digestibility, often above 9Ø%, have been reported (Nose, 1967; Ogino and Chen, 1973a, Table 7.6).

Table 7.5. In Vivo and In vitro Measurements of Protein Digestibility for Pure Protein sources.

Source of Protein	In Vivo %	In Vitro%
Casein	92.2Ø ± 1.9Ø	91.2Ø ± 1.3Ø
Fish meal	91.3Ø ± 1.1Ø	9Ø.3Ø ± 1.2Ø
Soybean meal	83.2Ø ± 1.2Ø	81.9Ø ± 1.4Ø
Sunflower	63.2Ø ± 1.8Ø	61.4Ø ± Ø.9Ø
Albumin	93.2Ø ± 2.3Ø	9Ø.3Ø ± Ø.95
Maize	4Ø.9Ø ± 2.4Ø	36.3Ø ± 1.2Ø

Table 7.6. In Vitro Measurements of Protein Digestibility in Carp Compared With In Vivo Digestibility Determinations Made by other Authors.

Protein Source	In Vitro Protein in diet%	In Vitro Digest-ibility	In Vivo Protein in diet%	In Vivo Digest-ibility	Reference
Casein	35.8	97.21	4Ø.ØØ	96.ØØ	Syazuki, 1956.
			3Ø.ØØ	95.ØØ	Syazuki, 1956.
Casein	18.8	95.4Ø	2Ø.ØØ	95.ØØ	Syazuki, 1956.
			3Ø.24	93.ØØ	Atack et al., 1979
Fishmeal	26.25	93.24		95.2Ø	Ogino & Chen, (1973 a)
Fishmeal	12.25	9Ø.1Ø	12.25	91.3Ø	
Sunflower	13.12	67.69	13.12	68.2Ø	Present
Sunflower	6.56	6Ø.47	6.56	65.3Ø	Study
Soybean meal	12.5	86.15	38.ØØ	95.5Ø	Ogino & Chen, (1973 a)
			29.93	83.7Ø	Atack et al., 1979.
Soybean+ Fishmeal	17.8Ø	87.7Ø			

The protein digestibility figures in Table 7.3 show that *in vitro* determinations of digestibility have a high degree of correlation with *in vivo* findings, for example Syazuki (1956) reported that the digestibility of casein was 95-96%, Ogino and Chen (1973a) gave it to be 98.6% and Atack *et al.* (1979) reported a figure of 98% (Table 7.6). Ogino and Chen (1973a) reported that protein digestibility of fish meal ranged between 95.2 and 95.5%. Atack *et al.* (1979) reported a protein digestibility of soybean meal of 83.7% in carp and 43.6% in trout in contradiction to the high digestibility of 80% reported by Sandholm *et al.* (1976). Non-defined residues and trypsin inhibitors may contribute to such discrepancies. In the present study the digestibility of soybean meal was 86.15% for carp.

Digestibility of protein in beans is highly dependent on their treatment. Raw beans contain powerful protease inhibitors which reduce their digestibility (Block and Mitchell, 1946), and the method by which the inhibitors are removed can influence the availability of AAs. Dry heating causes reduced digestibility (Rackis, 1974) as well as the formation of a lysine carbohydrate complex (Eldred and Rodney, 1946; Carpenter, 1958) indigestible by fish (Viola *et al.*, 1982). Consequently, undefined processing conditions have led to difficulties in the interpretation of soya digestion experiments (Dabroweski and Kozak, 1979).

The present *in vitro* experiment agreed with other

findings. Hsu *et al.* (1977) used a multienzyme *in vitro* method for the estimation of protein digestibility, consisting of trypsin, chymotrypsin and peptidase. It was found that the pH of a protein suspension immediately after 10 min digestion with the multienzyme solution was highly correlated with *in vivo* apparent digestibility in rats. Regression analysis of 23 samples tested showed that the correlation coefficient between pH at 10 min and *in vivo* apparent digestibility was 0.90.

Any assay of protein quality in which animal growth is measured only yields a comparison of the growth with the product under assay relative to a standard protein. Many different techniques have been used to determine and express this comparison; however, no single animal assay will give data to indicate whether a low quality of the protein under assay is due to its poor digestibility or lack of enough AAs, or to damage to one or more of the EAAs. In contrast, the enzymatic methods for protein quality evaluation which use the digestive enzymes, but not plant or microbial enzymes, have the advantage that a single assay gives information on the digestibility and quality of the product. If such a enzyme assay was combined with AA analysis then it could give an indicator of which EAA may be low. Thus enzymes not only give a measure of the protein quality of a product which correlates well with *in vivo* animal assays, but also, in those assays in which AA release is determined, can be used to indicate how the product might be improved by

the addition of AA. These merits of the enzyme assays and their speed and low cost would suggest that they should be used to monitor the effect of processing operations on protein quality, in order to tell how to blend ingredients in food products for the highest protein values, as well as to evaluate the quality of the finished processed food.

CHAPTER 8.

Chapter 8.

Experiments 6 & 7. The Use of Plasma AA Concentrations as Indices of Dietary Protein Quality.

8.1. Introduction.

The nutritive value of a dietary protein depends on its AA content and on the extent of absorption of the constituent AA into the bloodstream. In terrestrial animals, a significant amount of research has therefore been directeds toward the correlation of plasma AA with the adequacy of the test diet (Leathem, 1968). However, only a few publications have dealt with this subject in fish (See Section 1.1Ø.3.8). Fish require a high percentage of protein in their diet for optimum growth, and it is of particular importance to employ some biochemical method such as plasma EAAI, in addition to growth studies, in order to evaluate the quality of a test protein. While AA composition of a test protein has been mainly used for evaluating its quality, it is evident that other important factors, such as the rate of absorption of the constituent AA from a protein during digestion, will influence the quality of dietary protein. Hence, to use only AA composition of a test protein appears inadequate for evaluating its quality.

As described in the Introduction (Section 1.1Ø.3.8), the interrelationship between the plasma AA change and AA composition of the protein ingested has been demonstrated by an in vivo plasma AA technique which would take into account the availability of the AA and the

digestibility of the protein. AA deficiencies of a protein and proper supplementation to overcome these deficiencies can be evaluated by this technique. Several researchers have noted that although certain protein test diets are able to sustain the normal growth of carp (Cyprinus carpio), crystalline AA mixtures which duplicated the AA composition of the protein sources failed to sustain their growth (Nose et al., 1974). Plakas et al., (198Ø) examined plasma AA patterns after feeding casein and corresponding FAA diet to support normal growth of carp.

For plasma AA studies with terrestrial animals, force-feeding methods have been commonly used (Herbert and Coulson, 1975). A few researchers have developed force-feeding techniques with fish (Jones and Geen, 1976, Yamada et al., 1981), but such a technique has not yet been used for a plasma AA study in fish where it would lessen the variability of feed intake between animals. The present studies used force-feeding techniques to determine the nutritive value of different proteins at different concentrations.

8.2. Materials & Methods.

8.2.1. The Experimental System and Animals.

The experimental facility used in the present study was "System 1" as described in detail in Section 2.1.1.

The fish used in the present study were common carp (Cyprinus carpio). Twenty four fish with a mean weight

of 2.14 Kg were divided into 6 groups with 4 fish per tank (Experiment 6) and another sixteen fish divided into four groups (Experiment 7) with 4 fish per tank. The fish in each tank were individually tagged (Section 2.5). The fish were fed daily with a commercial trout pellet diet for 3 weeks in order to acclimatize them to the experimental conditions. Water temperature was maintained at 17±1 °C. After 48 h of fasting the fish were force-fed.

8.2.2. The Experimental Diets.

Formulation of the diets was carried out by the general procedure described in section 2.2 and the ingredients used are listed in Table 8.1 (Experiment 6) and Table 8.2 (Experiment 7). Protein concentration in the diets was ca 15% (Table 8.1) & 30% (Table 8.2). Proximate analysis (section 2.6) was carried out on samples of the diets and results are presented in Tables 8.1 & 8.2. AA composition of experimental diets is shown in Tables 8.3 & 8.4. The EAAI for each diet was calculated using the whole egg protein and requirements of carp as reference protein according to the method of Mitchell (1954).

8.2.3. Force-Feeding Method.

All fish in each group were fed 2% of body weight in a slurry by a force-feeding method based on Dodd et al. (1959). 50 ml disposable plastic syringes with a teflon tube, 5 mm in diameter and 180 mm long, were used to deliver the slurry into the gut of the fish. The slurry was mixed throughly just before drawing it into the

syringes.

Table 8.1. Experimental Diets (15% protein) used for Force-Fed Carp (Cyprinus carpio) g/100 g diet.

Ingredient	Weight (g)					
	1	2	3	4	5	6
Casein	17	-	-	-	-	-
Fish meal	-	22	-	-	-	-
Whole Egg protein	-	-	15	-	-	-
Soybean meal	-	-	-	30	-	-
Rapeseed meal	-	-	-	-	41	-
Sunflower meal	-	-	-	-	-	50
Dextrin	20	20	25	20	20	19
Potato starch	43	38	40	30	13	-
Cod liver oil	9	9	9	9	9	9
Mineral Mix	4	4	4	4	4	4
Vitamin Mix	2	2	2	2	2	2
Cellulose	5	5	5	5	11	16
	100	100	100	100	100	100
Proximate Analysis(%)						
Moisture	10.94	10.65	10.19	11.71	10.79	8.93
Protein	15.30	14.75	14.55	13.10	15.75	16.50
Lipid	9.00	11.09	9.99	9.33	9.36	10.15
Ash	4.27	5.93	4.13	3.16	5.29	4.44
NFE	60.49	57.58	61.14	62.70	58.81	59.98

Table 8.2. Experimental Diets (ca. 30% protein) used for Force-Fed Carp (Cyprinus carpio) g/100 g diet.

Ingredient	Weight (g)			
	7	8	9	10
Casein	34	-	-	-
Fish meal	-	43	-	-
Whole Egg Protein	-	-	30	-
Rapeseed meal	-	-	-	85
Dextrin	20	20	20	2
Potato starch	20	10	25	2
Cod liver oil	9	9	9	3
Mineral Mix	4	4	4	4
Vitamin Mix	2	2	2	2
Cellulose	11	12	10	2
	100	100	100	100
Proximate Anaylsis(%)				
Moisture	10.53	5.85	9.19	10.20
Protein	30.10	29.25	27.60	30.60
Lipid	9.00	13.08	9.66	3.100
Ash	9.73	7.19	4.13	12.49
NFE	40.64	44.63	49.42	43.61

Table 8.3. AA Composition of the 15% Protein Test Diets for Carp.

	Diet No					
AA %	1	2	3	4	5	6
Asp	1.799	1.649	1.113	Ø.ØØ2	1.277	1.24Ø
Thr	Ø.488	Ø.758	Ø.561	Ø.787	Ø.645	Ø.446
Ser	1.27Ø	Ø.752	Ø.7Ø4	Ø.899	Ø.796	Ø.5Ø9
Glu	5.95Ø	2.438	1.446	3.642	3.358	2.929
Pro	2.233	Ø.556	Ø.473	Ø.99Ø	Ø.957	Ø.574
Gly	Ø.538	1.Ø67	Ø.48Ø	Ø.9Ø1	Ø.996	Ø.883
Ala	Ø.81Ø	1.132	Ø.593	1.189	Ø.9Ø8	Ø.774
Val	1.634	Ø.824	Ø.632	Ø.863	Ø.873	Ø.677
Met	1.137	Ø.576	Ø.433	Ø.4Ø2	Ø.514	Ø.495
Ile	1.359	Ø.772	Ø.575	Ø.81Ø	Ø.774	Ø.643
Leu	2.443	1.246	1.182	2.Ø12	1.748	1.229
Tyr	1.3Ø8	Ø.517	Ø.447	Ø.696	Ø.456	Ø.312
Phe	1.347	Ø.7Ø2	Ø.52Ø	Ø.999	Ø.747	Ø.657
His	Ø.692	Ø.38Ø	Ø.2Ø2	Ø.279	Ø.41Ø	Ø.27Ø
Lys	1.283	Ø.74Ø	Ø.31Ø	Ø.434	Ø.267	Ø.147
Arg	3.Ø8Ø	1.Ø29	3.7Ø3	3.491	Ø.772	1.658
TFAA	27.371	15.138	13.374	18.396	15.498	13.443
TEAA	13.463	7.Ø27	8.118	1Ø.Ø77	6.75Ø	6.222
TNEAA	13.9Ø8	8.111	5.256	8.319	8.748	7.221
EAA/NEAA	Ø.968	Ø.866	1.544	1.211	Ø.771	Ø.861
EAAI Using						
Whole egg	96.4Ø	86.66	1ØØ	99.15	82.2Ø	49.18
Carp Edible						
Protein	85.7Ø	64.88	48.46	62.66	57.84	46.71
Requirements	4Ø.25	21.75	17.45	24.18	19.2Ø	12.Ø2

Tabl 8.4. AA Composition of 30% Protein Test Diets for Carp.

	Diet No			
AA %	7	8	9	10
Asp	2.355	2.842	1.341	2.376
Thr	1.537	1.434	0.569	1.656
Ser	1.953	1.427	0.796	1.611
Glu	7.390	4.831	1.706	6.224
Pro	2.320	1.242	0.798	2.618
Gly	0.689	2.326	0.453	1.905
Ala	1.050	2.349	0.711	2.239
Val	2.147	1.560	0.798	1.753
Met	0.983	1.148	0.619	0.824
Ile	1.667	1.343	0.777	1.509
Leu	2.901	3.164	1.417	2.533
Tyr	1.158	0.960	0.492	0.927
Phe	1.587	1.241	0.656	1.415
His	0.816	0.445	0.207	0.683
Lys	1.592	1.444	0.239	0.526
Arg	5.710	3.928	6.135	1.883
TFAA	35.855	31.684	17.714	24.458
TEAA	18.940	15.707	11.417	12.294
TNEAA	16.915	15.977	6.297	12.164
EAA/NEAA	1.119	0.983	1.813	1.010
EAAI Using				
Whole egg protein	99.20	95.15	100	67.90
Carp edible protein	96.35	90.36	50.92	84.67
Carp requirement	50.35	43.33	48.96	33.07

8.2.4. Blood Sampling.

Immediately before and at intervals (6, 24 & 48 h) after force-feeding, the fish were removed from tanks, immediately anesthetized with benzocaine (1 g/2Ø L) and separate blood samples were withdrawn with heparinized syringes by cardiac puncture. The fish were then put back into tanks. The blood samples were centrifuged at 3,ØØØ r.p.m for 2Ø minutes to collect the blood plasma. The samples were then deproteinized by adding Ø.5 ml 3% (W/V) 5-sulphosalicylic acid solution to Ø.1Ø ml of plasma and centrifuging to remove percipitated protein. Ø.1Ø ml of supernatant was taken for analysis on a Locarte amino acid analyzer using a 23 cm column with a sodium citrate buffer system.

8.2.5. Plasma Protein.

Plasma protein was determined by the method of Lowry et al. (1951) using bovine serum albumin as standard.

8.2.6. Statistical Methods.

Statistical analysis on the plasma protein results was performed as detailed in section 2.7.

8.3. Results of Carp Force-Fed Six Protein Diets (ca 15% Protein).

Table 8.5 to 8.1Ø show the plasma EAA after force-feeding the six different protein sources, together with the total, EAA & NEAA, and the EAA/NEAA ratio.

8.3.1. Total FAA (Fig 8.1).

It is seen that the FAA decreased from Ø to 6 h, more on some diets than others, and then remained nearly

Table 8.5. Plasma FAA Concentrations of Carp at Various Intervals after Force-Feeding Casein Diet (ca. 15% protein).

(μ mol/ml of blood plasma)

	Hours after force-feeding			
AA	Ø	6	24	48
Asp	Ø.316	Ø.59Ø	1.243	Ø.Ø77
Thr	Ø.341	1.138	2.415	Ø.188
Ser	Ø.4Ø4	Ø.612	2.612	Ø.Ø78
Glu	Ø.633	1.344	2.484	Ø.7Ø1
Pro	Ø.5Ø8	1.394	2.363	Ø.961
Gly	Ø.72Ø	1.697	2.Ø97	1.Ø71
Ala	Ø.73Ø	1.239	2.2Ø6	Ø.264
Val	Ø.375	Ø.892	1.626	Ø.211
Met	Ø.Ø95	Ø.487	Ø.875	Ø.332
Ile	Ø.24Ø	Ø.687	1.628	Ø.212
Leu	Ø.421	1.7Ø7	2.947	Ø.858
Tyr	Ø.Ø86	Ø.483	1.164	Ø.568
Phe	Ø.133	Ø.645	1.32Ø	Ø.682
His	Ø.221	Ø.693	1.388	Ø.72Ø
Lys	Ø.423	Ø.335	Ø.854	2.829
NH_3	1.147	Ø.523	1.853	Ø.898
Arg	Ø.376	Ø.Ø75	Ø.286	Ø.279
TFAA	6.Ø22	18.148	26.863	1Ø.Ø31
EAA	2.625	6.659	13.339	6.311
NEAA	3.397	11.489	13.524	3.72Ø
EAA/NEAA	Ø.772	Ø.579	Ø.986	1.696

Table 8.6. Plasma FAA Concentrations of Carp at Various Intervals after Force-Feeding Fish meal Diet (ca 15% protein).

	(u mol/ml of blood plasma)			
AA	Hours after force-feeding			
	Ø	6	24	48
Asp	Ø.315	Ø.316	Ø.515	Ø.432
Thr	Ø.458	Ø.371	Ø.79Ø	Ø.Ø4Ø
Ser	Ø.473	Ø.384	Ø.766	1.Ø98
Glu	Ø.564	Ø.458	Ø.589	Ø.556
Pro	Ø.574	Ø.6Ø9	Ø.312	Ø.366
Gly	Ø.441	Ø.412	Ø.63Ø	Ø.739
Ala	Ø.7Ø3	Ø.685	Ø.844	1.Ø36
Val	Ø.377	Ø.317	Ø.586	Ø.727
Met	Ø.126	Ø.Ø91	Ø.185	Ø.345
Ile	Ø.241	Ø.276	Ø.359	Ø.462
Leu	Ø.389	Ø.343	Ø.597	1.ØØ1
Tyr	Ø.Ø65	Ø.Ø52	Ø.241	Ø.662
Phe	Ø.134	Ø.1Ø1	Ø.314	Ø.239
His	Ø.133	Ø.Ø85	Ø.368	Ø.385
Lys	Ø.961	Ø.818	1.699	Ø.979
NH_3	1.141	1.381	1.777	1.1Ø9
Arg	Ø.913	Ø.938	1.Ø38	Ø.281
TFAA	6.867	6.256	9.833	9.348
EAA	3.732	3.34Ø	5.936	4.459
NEAA	3.135	2.916	3.897	4.889
EAA/NEAA	1.19Ø	1.145	1.523	Ø.912

Table 8.7. Plasma FAA Concentrations of Carp at Various Intervals after Force-Feeding Whole Egg Protein Diet (ca 15% protein).

	(μ mol/ml of blood plasma)			
AA	Hours after force-feeding			
	Ø	6	24	48
Asp	Ø.745	Ø.487	Ø.7ØØ	Ø.83Ø
Thr	Ø.717	Ø.5Ø2	Ø.47Ø	Ø.5ØØ
Ser	Ø.736	Ø.521	Ø.561	Ø.5Ø6
Glu	Ø.989	Ø.566	Ø.44Ø	Ø.615
Pro	Ø.513	Ø.671	Ø.642	Ø.461
Gly	Ø.737	Ø.453	Ø.451	Ø.563
Ala	Ø.993	Ø.677	Ø.613	Ø.661
Val	Ø.594	Ø.346	Ø.3Ø6	Ø.381
Met	Ø.32Ø	Ø.121	Ø.1Ø6	Ø.1Ø8
Ile	Ø.484	Ø.256	Ø.182	Ø.214
Leu	Ø.663	Ø.332	Ø.299	Ø.391
Tyr	Ø.154	Ø.1Ø1	Ø.1Ø6	Ø.148
Phe	Ø.323	Ø.15Ø	Ø.187	Ø.172
His	Ø.351	Ø.Ø56	Ø.169	Ø.2Ø2
Lys	Ø.122	1.248	1.162	1.142
NH_3	1.396	1.142	Ø.851	1.289
Arg	2.644	Ø.481	Ø.4Ø2	Ø.371
TFAA	11.Ø85	6.968	6.796	7.265
EAA	6.218	3.492	3.283	3.481
NEAA	4.867	3.476	3.513	3.784
EAA/NEAA	1.277	1.Ø04	Ø.934	Ø.919

Table 8.8. Plasma FAA Concentrations of Carp at Various Intervals after Force-Feeding Soybean meal Diet (ca 15% protein).

(u mol/ml of blood plasma)

AA	Hours after force-feeding			
	Ø	6	24	48
Asp	Ø.491	Ø.36Ø	Ø.41Ø	Ø.379
Thr	Ø.173	Ø.477	Ø.596	Ø.558
Ser	Ø.715	Ø.468	Ø.6Ø9	Ø.58Ø
Glu	Ø.416	Ø.499	Ø.382	Ø.472
Pro	1.Ø7Ø	Ø.478	Ø.926	Ø.624
Gly	Ø.799	Ø.6Ø1	Ø.7Ø4	Ø.516
Ala	Ø.899	Ø.668	Ø.9Ø7	Ø.52Ø
Val	Ø.384	Ø.427	Ø.265	Ø.433
Met	Ø.177	Ø.237	Ø.178	Ø.24Ø
Ile	Ø.269	Ø.275	Ø.226	Ø.349
Leu	Ø.411	Ø.484	Ø.386	Ø.575
Tyr	Ø.149	Ø.153	Ø.123	Ø.15Ø
Phe	Ø.227	Ø.216	Ø.226	Ø.376
His	Ø.271	Ø.259	Ø.25Ø	Ø.259
Lys	1.319	Ø.428	Ø.361	Ø.749
NH_3	Ø.883	Ø.8Ø4	Ø.728	Ø.8Ø6
Arg	Ø.562	Ø.5Ø4	Ø.531	Ø.587
TFAA	8.332	6.534	7.Ø8Ø	7.367
EAA	3.793	3.3Ø7	3.Ø19	4.126
NEAA	4.539	3.227	4.Ø61	3.241
EAA/NEAA	Ø.835	1.Ø24	Ø.743	1.273

Table 8.9. Plasma FAA Concentrations of Carp at Various Intervals after Force-Feeding Rapeseed meal Diet (ca 15 % protein).

(μ mol/ml of blood plasma)

AA	Hours after force-feeding			
	Ø	6	24	48
Asp	1.191	Ø.38Ø	Ø.527	Ø.469
Thr	1.Ø81	Ø.552	Ø.31Ø	Ø.435
Ser	Ø.739	Ø.438	Ø.365	Ø.432
Glu	Ø.94Ø	Ø.4Ø4	Ø.675	Ø.626
Pro	1.189	Ø.3Ø6	Ø.365	Ø.542
Gly	1.486	Ø.559	Ø.443	Ø.455
Ala	1.225	Ø.816	Ø.554	Ø.561
Val	1.138	Ø.3Ø3	Ø.Ø83	Ø.317
Met	Ø.122	Ø.166	Ø.154	Ø.168
Ile	Ø.273	Ø.1Ø4	Ø.199	Ø.181
Leu	Ø.421	Ø.352	Ø.3Ø3	Ø.432
Tyr	Ø.977	Ø.116	Ø.Ø95	Ø.127
Phe	Ø.187	Ø.239	Ø.171	Ø.2Ø8
His	Ø.276	Ø.357	Ø.326	Ø.2Ø8
Lys	Ø.421	Ø.118	Ø.1Ø3	Ø.1Ø4
NH_3	1.4Ø3	Ø.688	Ø.618	Ø.689
Arg	1.58Ø	1.62Ø	1.693	1.835
TFAA	13.246	6.83Ø	6.366	7.1ØØ
EAA	5.499	3.811	3.342	3.888
NEAA	7.747	3.Ø19	3.Ø24	3.212
EAA/NEAA	Ø.7Ø9	1.262	1.1Ø5	1.21Ø

Table 8.1Ø. Plasma FAA Concentration of Carp at Various Intervals after Force-Feeding Sunflower Diet (ca 15% protein).

(µ mol/ml of blood plasma)

AA	Hours after force-feeding			
	Ø	6	24	48
Asp	Ø.542	Ø.453	Ø.4Ø6	Ø.32Ø
Thr	Ø.448	Ø.524	Ø.54Ø	Ø.975
Ser	Ø.526	Ø.529	Ø.531	Ø.743
Glu	Ø.852	Ø.7Ø3	Ø.556	Ø.546
Pro	Ø.196	Ø.759	Ø.368	1.136
Gly	1.Ø35	Ø.639	Ø.598	Ø.696
Ala	1.Ø73	Ø.851	Ø.798	Ø.978
Val	Ø.351	Ø.351	Ø.367	Ø.556
Met	Ø.153	Ø.144	Ø.157	Ø.153
Ile	Ø.23Ø	Ø.187	Ø.452	Ø.277
Leu	Ø.4Ø6	Ø.467	Ø.383	Ø.485
Tyr	1.934	Ø.Ø85	Ø.1Ø3	Ø.148
Phe	Ø.184	Ø.166	Ø.215	Ø.293
His	Ø.25Ø	Ø.231	Ø.223	Ø.238
Lys	Ø.936	1.Ø21	Ø.9Ø4	Ø.343
NH_3	1.144	1.11Ø	1.156	1.484
Arg	Ø.771	Ø.945	Ø.912	Ø.975
TFAA	9.927	8.Ø55	7.513	8.862
EAA	3.769	4.Ø36	4.153	4.295
NEAA	6.158	4.Ø19	3.36Ø	4.567
EAA/NEAA	Ø.612	1.ØØ4	1.236	Ø.94Ø

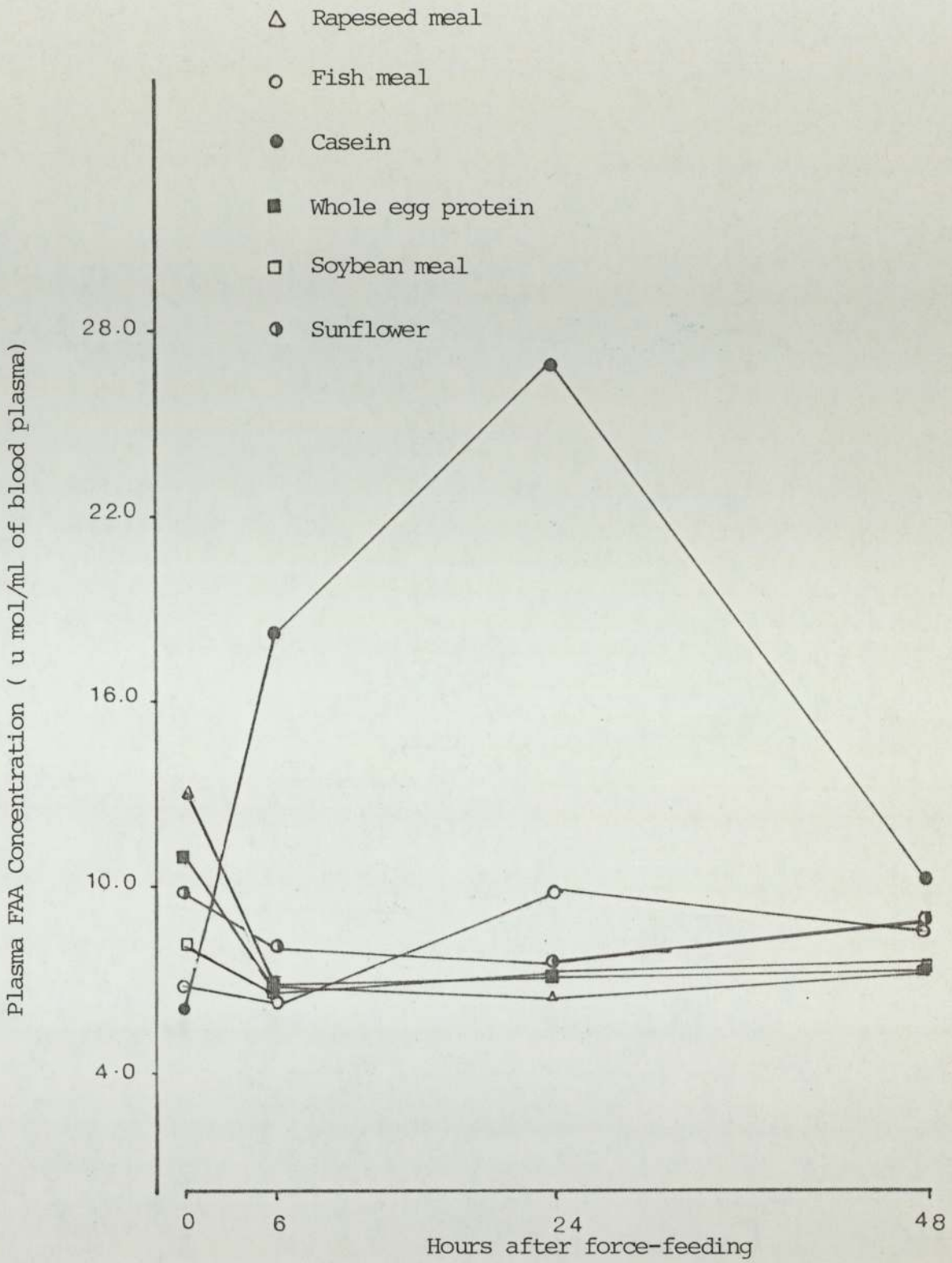

Fig. 8.I. Change in plasma total FAA for Carp after Force-Feedin Six Protein diets ca. I5% Protein.

constant throughout, except with casein and to a lesser extent with the fish meal diets, where there was an increase at 24 h; in addition the values with the casein diet were much higher than all others up to 24 h. At 48 h all diets were closely similar.

8.3.2. Total EAA (Fig 8.2).

All values for TEAA concentration were very much alike for five of the diets at all times, except for whole egg protein at time Ø and fish meal at 24 h, which were elevated. The results with casein were markedly higher than the other diets with two low (6, 48 h) and two high (Ø, 24 h) values.

8.3.3. NEAA and EAA/NEAA Ratio (Fig 8.3).

The NEAA concentrations showed little variation, except for the sunflower and rapeseed diets, which were rather higher initially, and the casein, which remained high for 24 h and then decreased sharply to join the rest.
No pattern at all could be discerned in the ratio of EAA/NEAA.

8.3.4. Individual FEAA.

In the casein diet (Fig.8.4) all EAA except lysine show a decrease in concentration at 6 h, then an increase to 24 h, followed by a fall again at 48 h, similar to the pattern seen with the total AA (Fig 8.1). With lysine, however, there was a marked increase in concentration at 48 h.

The fish meal diet (Fig 8.5) is similar to casein up to 24 h (decrease, then rises in concentrations). Most AA

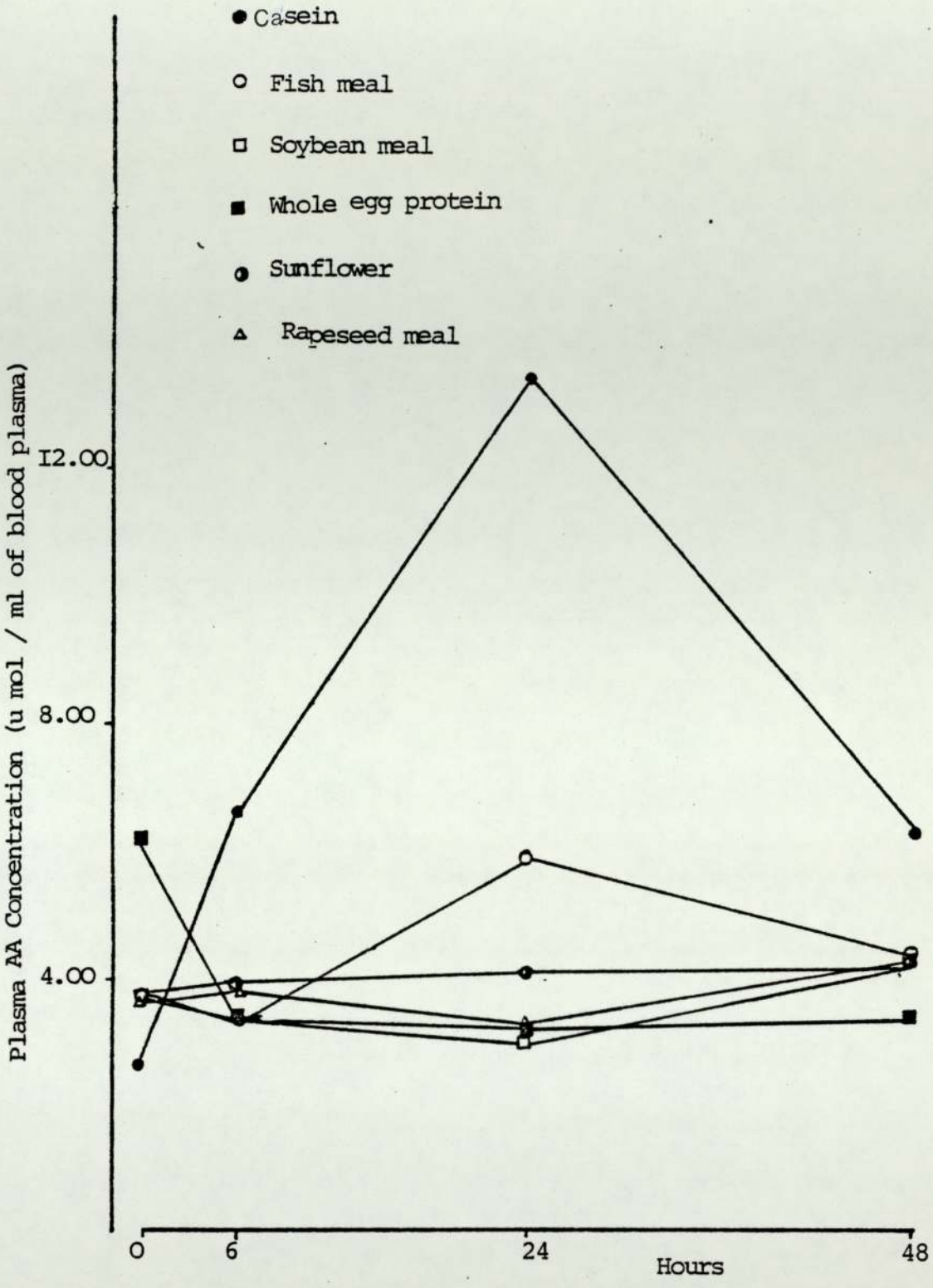

Fig 8.2. Change in plasma EAA of Carp at Various Intervals after Force-Feeding Six Protein Diets ca 15 % Protein.

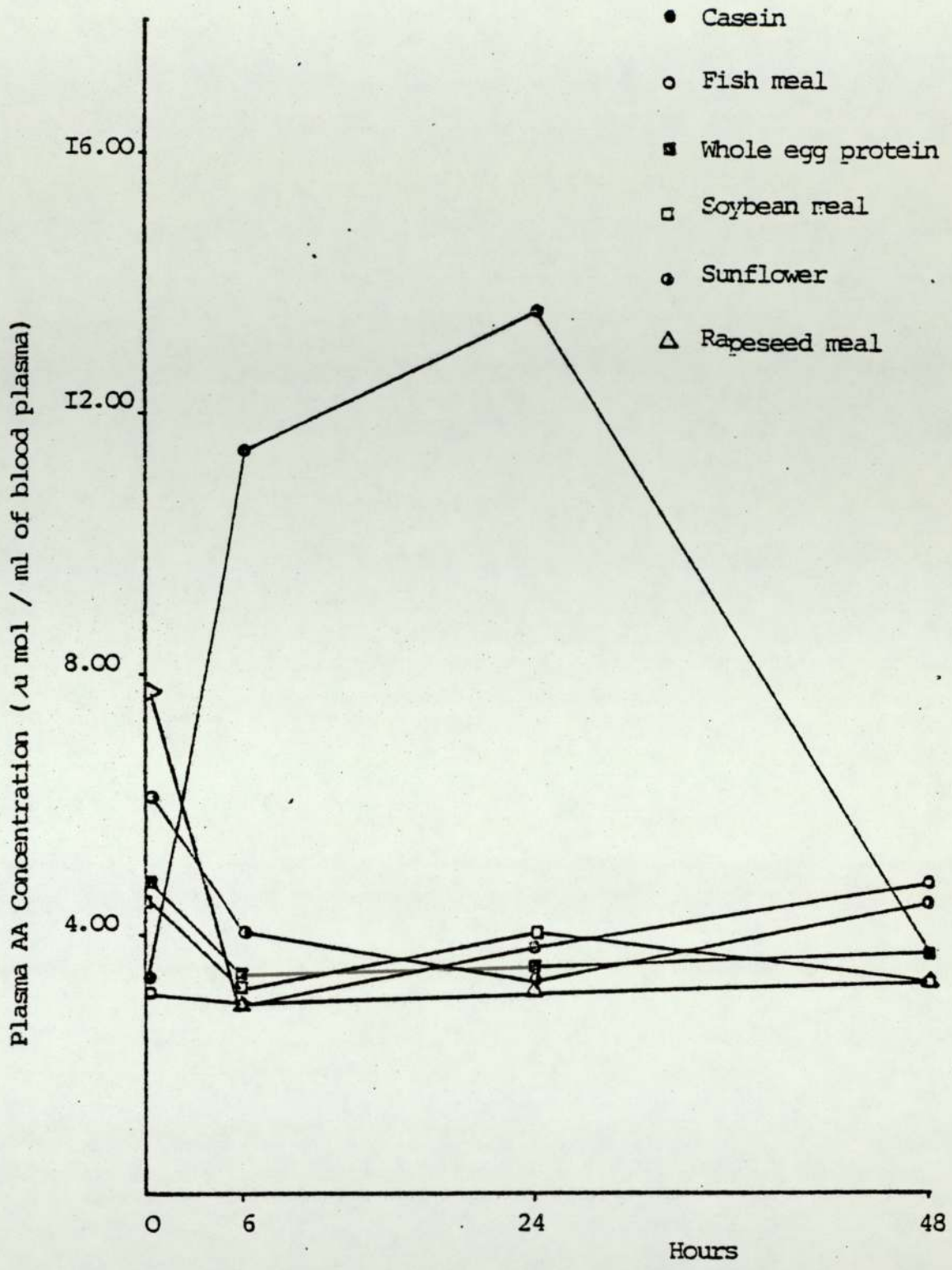

Fig 8.3. Change in Plasma FNEAA of carp at various Intervals after Force-Feeding Six Diets ca I5 % Protein.

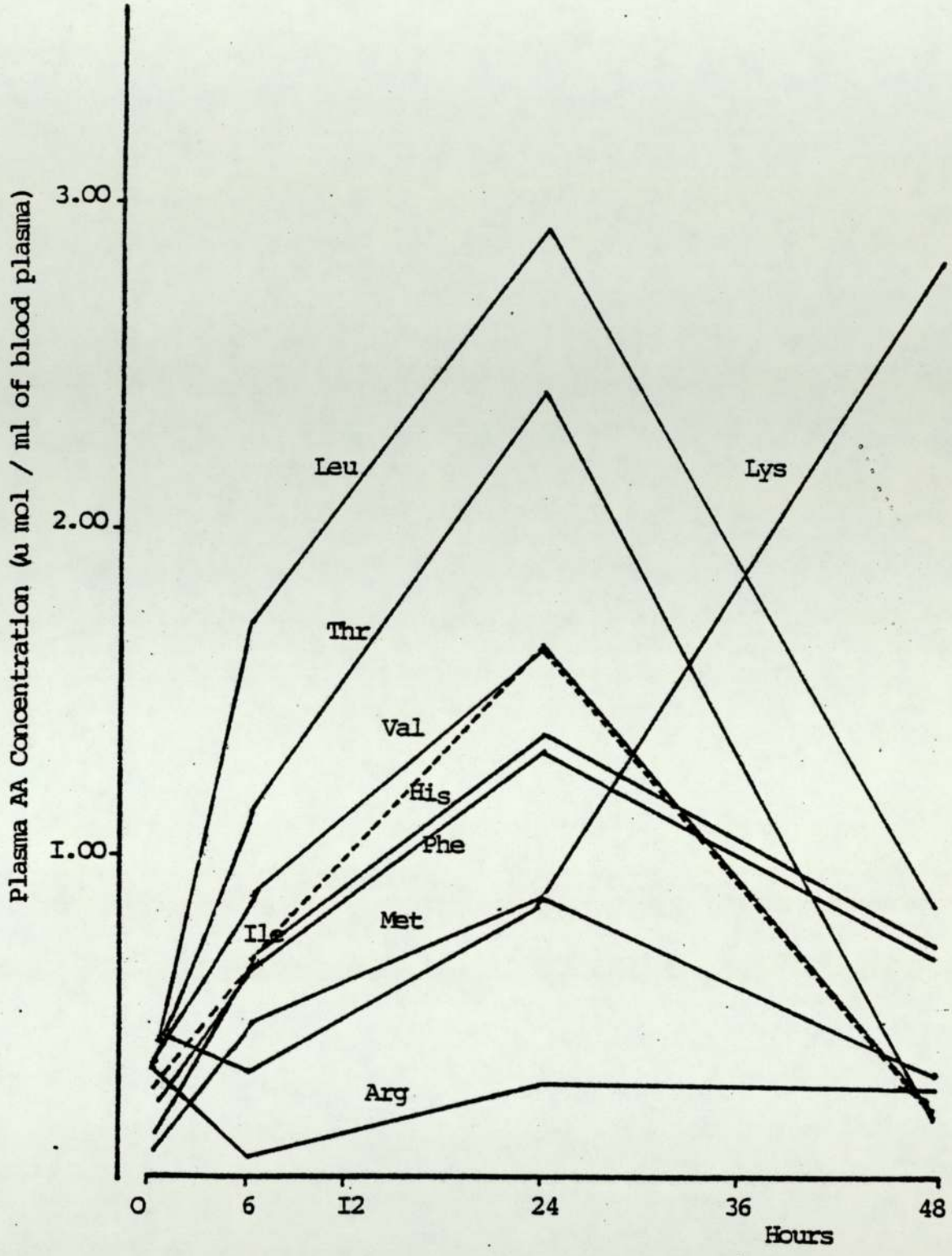

Fig 8.4. Change in Plasma EAA of Carp at Various Intervals after Force-Feeding Casein Diet ca 15 % Protein .

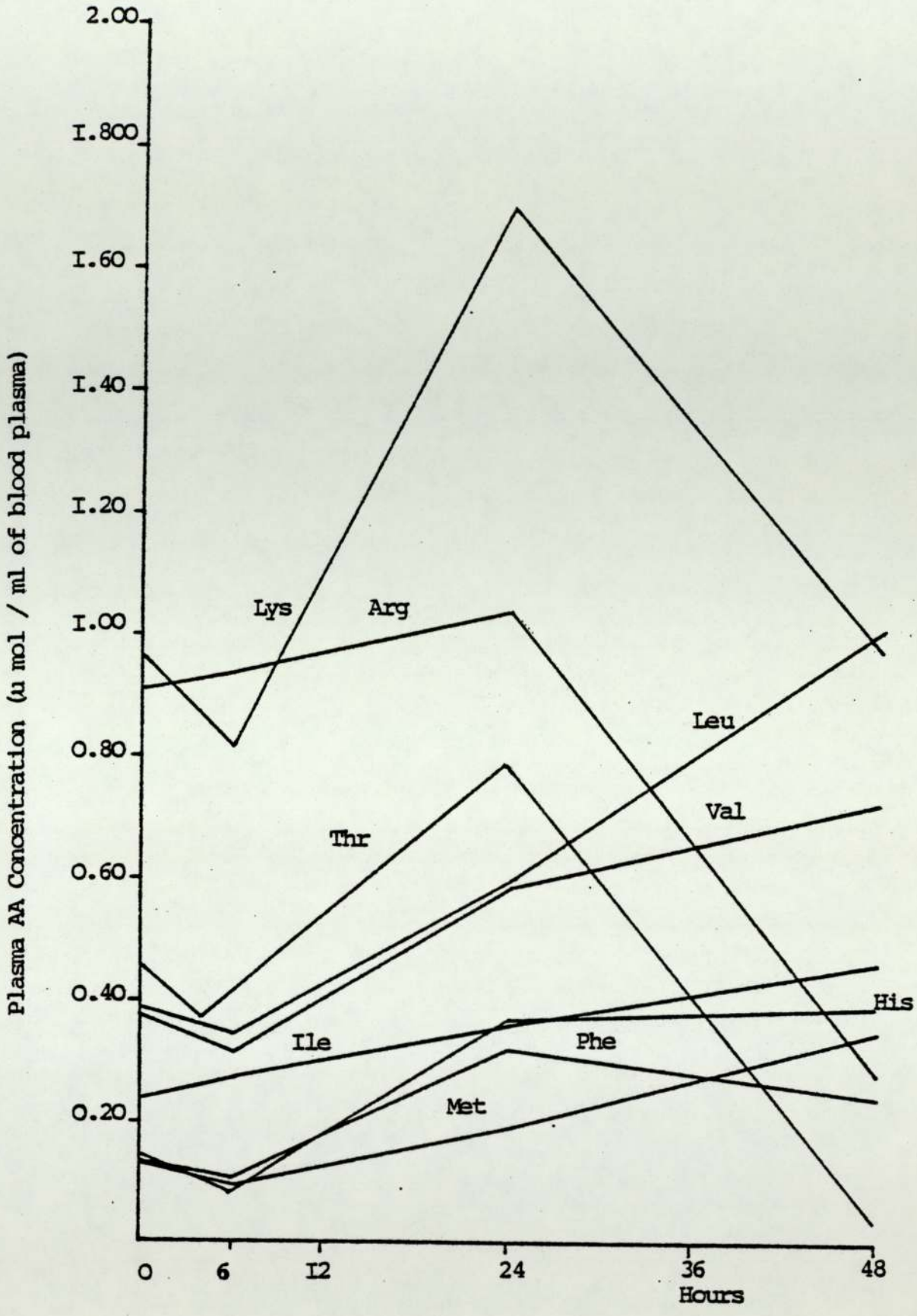

Fig 8.5. Change in Plasma EAA of Carp at Various Intervals after Force-Feeding Fish meal diet ca I5 % Protein.

then increase or remain approximately the same, though three AA (Lys, Arg, Thr) concentrations fall considerally to around the 6 h value.

On the whole with egg protein, rapeseed, and soybean diets, most plasma concentrations of EAA were bunched fairly closely together. With whole egg (Fig 8.6), arginine was higher initially, and fell sharply at 6 h, while lysine was the reverse, beginning low and then increasing above all the others. From 6 to 48 h, the concentration of each AA remained almost constant.

On the soybean meal diet (Fig 8.7), the lysine concentration was much greater than the rest at zero time, dropping sharply, then rising again at 48 h. Threonine was low at first and increased at 6 h.

With the rapeseed meal diet (Fig 8.8), threonine, valine, and to a lesser extent lysine began higher and decreased at 6 h; arginine remained uniformly at a much higher concentration than the others throughout.

On the sunflower meal diet (Fig 8.9), the EAA concentrations were distributed fairly widely, with only a few individual variations, notably lysine and threonine at 48 h, and isoleucine at 24 h.

The overall picture seen in Fig 8.2 is reflected by the data for each individual EAA. On four out of the six diets, most of the AA show little or no change in concentration either with time or with diet; lysine, and to a lesser extent valine, are the most variable (Fig 8.1Ø). With the casein diet, most plasma EAA concentra-

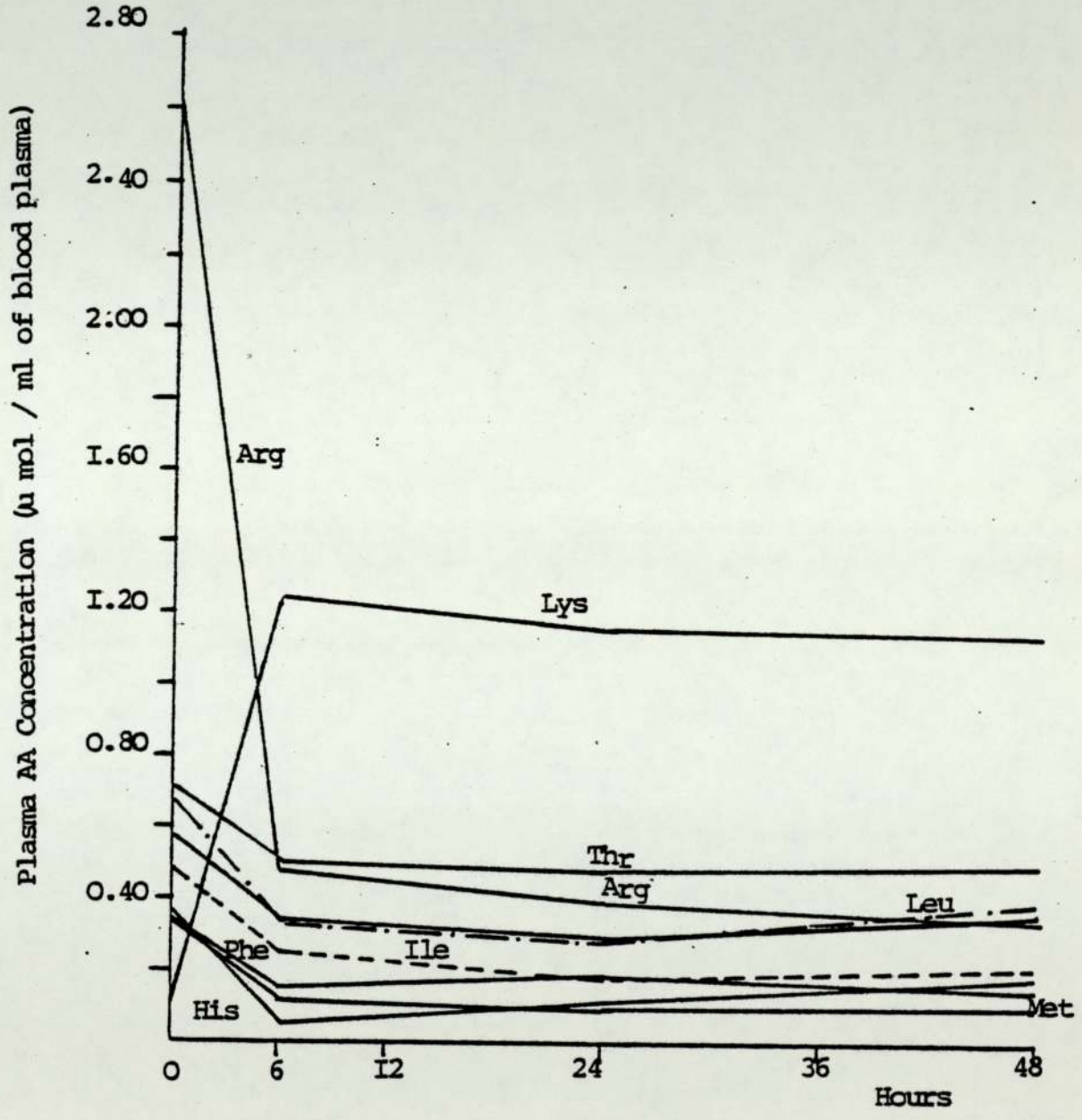

Fig 8.6. Change in Plasma FEAA of Carp at Various Intervals after Force-Feeding Whole egg protein ca I5 % Protein.

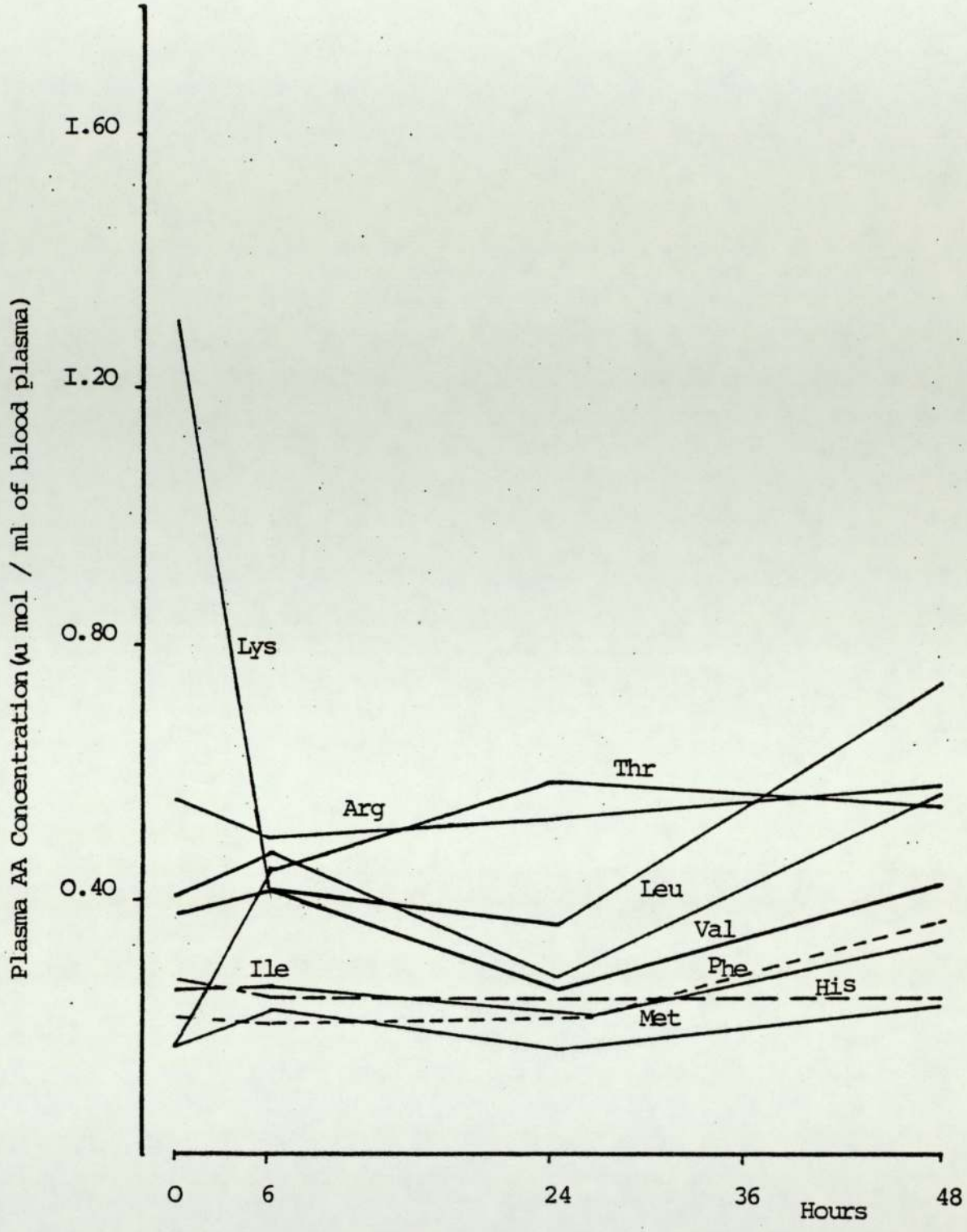

Fig 8.7. Change in Plasma EAA of Carp at Various Intervals after Force-Feeding Soybean meal ca I5 % Protein.

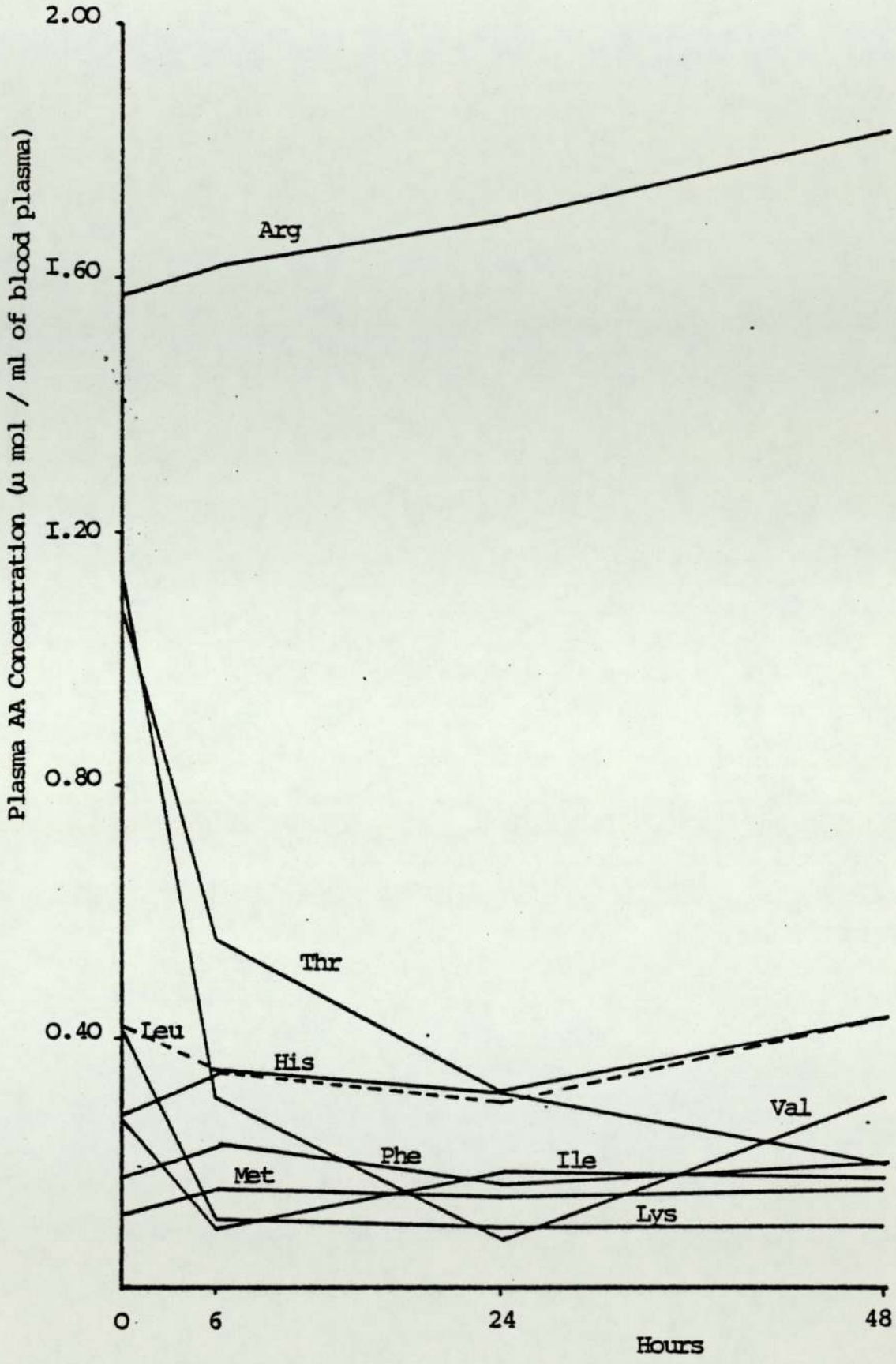

Fig 8.8. Change in Plasma FEAA of Carp at Various Intervals after Force-Feeding Rapeseed meal ca 15 % Protein.

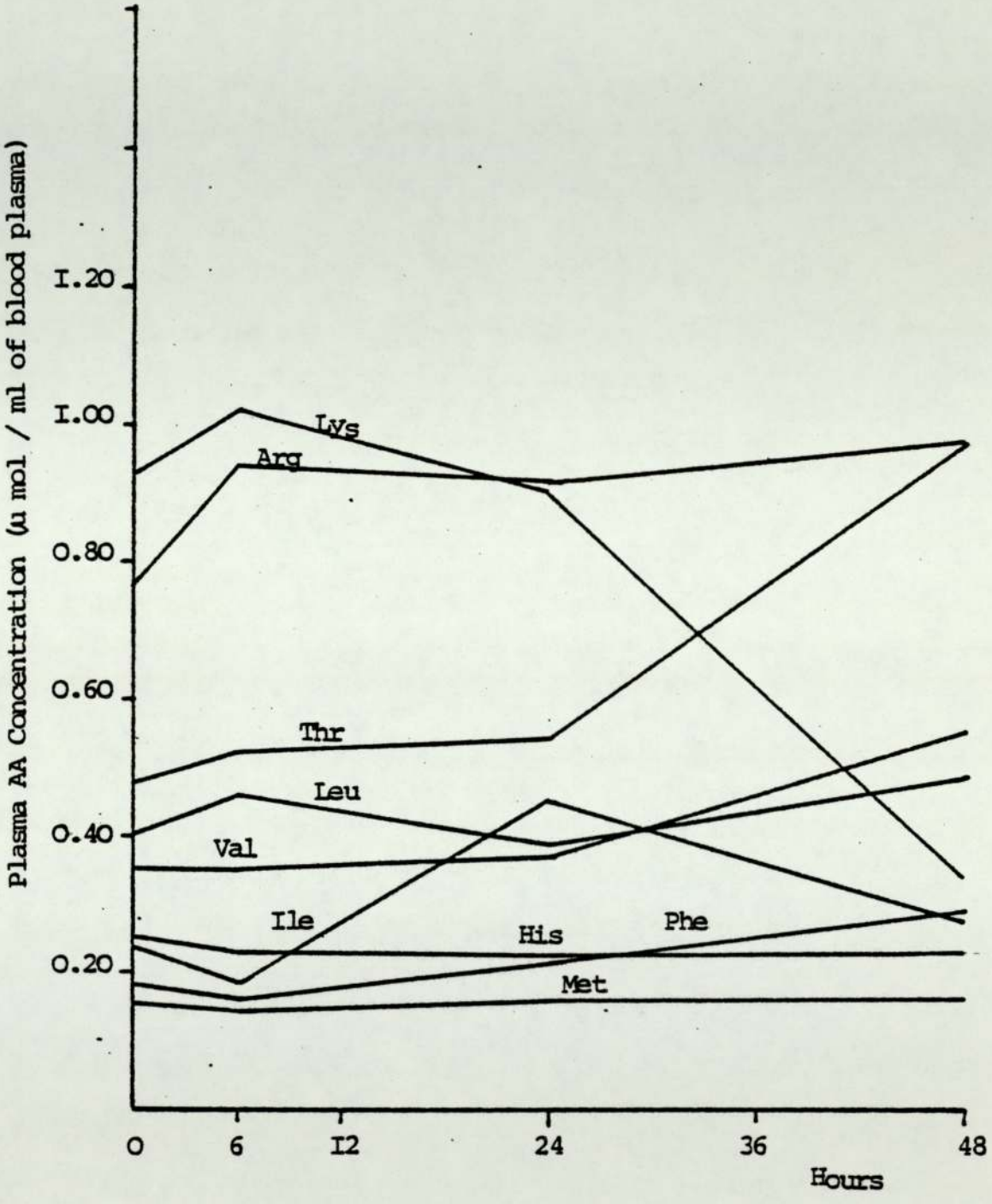

Fig 8.9. Change in Plasma EAA of Carp at Various Intervals after Force-Feeding Sunflower diet ca I5 % Protein.

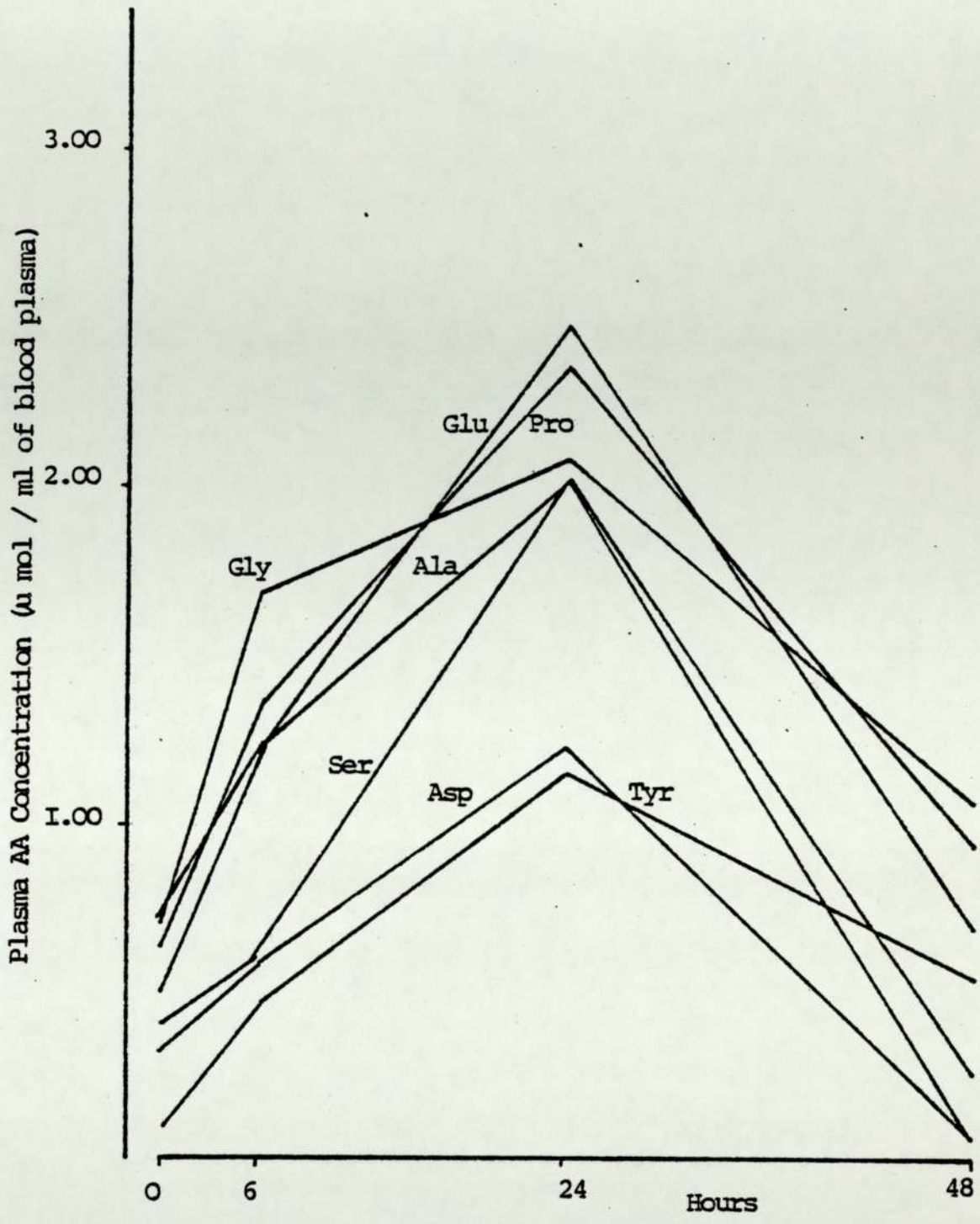

Fig 8.10. Change in Plasma NEAA of Carp at Various Intervals after Force-Feeding Casein diet ca 15 % Protein.

tions are much higher than those of the other diets, they drop at 6 h, rise again at 24 h, and at 48 h fall towards the same values as those found with the other four diets. However, the leucine concentration is low to begin with, and is unchanged at 6 h; methionine does not show the rise at 24 h. By contrast with the other AA, lysine values are similar on all diets except at 48 h, when the figure for casein is higher than the rest; arginine on the casein diet is lowest of all throughout.

8.3.5. Individual FNEAA.

On the casein diet (Fig.8.1Ø), the individual FNEAA follow the pattern seen with the total AA (Fig.8.1), and they do so rather more closely then the EAAs; there is a fall in concentration at 6 h, rising to around the fasting value at 24 h, and falling again to below the 6 h figure at 48 h.

With fish meal (Fig.8.11), the AA concentrations decrease slightly, if anything, at 6 h, increase at 24 h, and then either increase, or remain much the same at 48 h. There is one exception, proline, whose 24 h and 48 h concentrations are about half the Ø and 6 h values. A part from this one, the NEAA pattern is similar to 6 out of the 9 EAAs (Fig.8.5).

The results from the egg diet for individual NEAA (Fig. 8.12) reflect the pattern of the total AA (Fig.8.1); there is a fall in concentration from Ø to 6 h, and then little change except for proline, which rises at 6 h and then falls at 48 h, and aspartic acid, whose concentra-

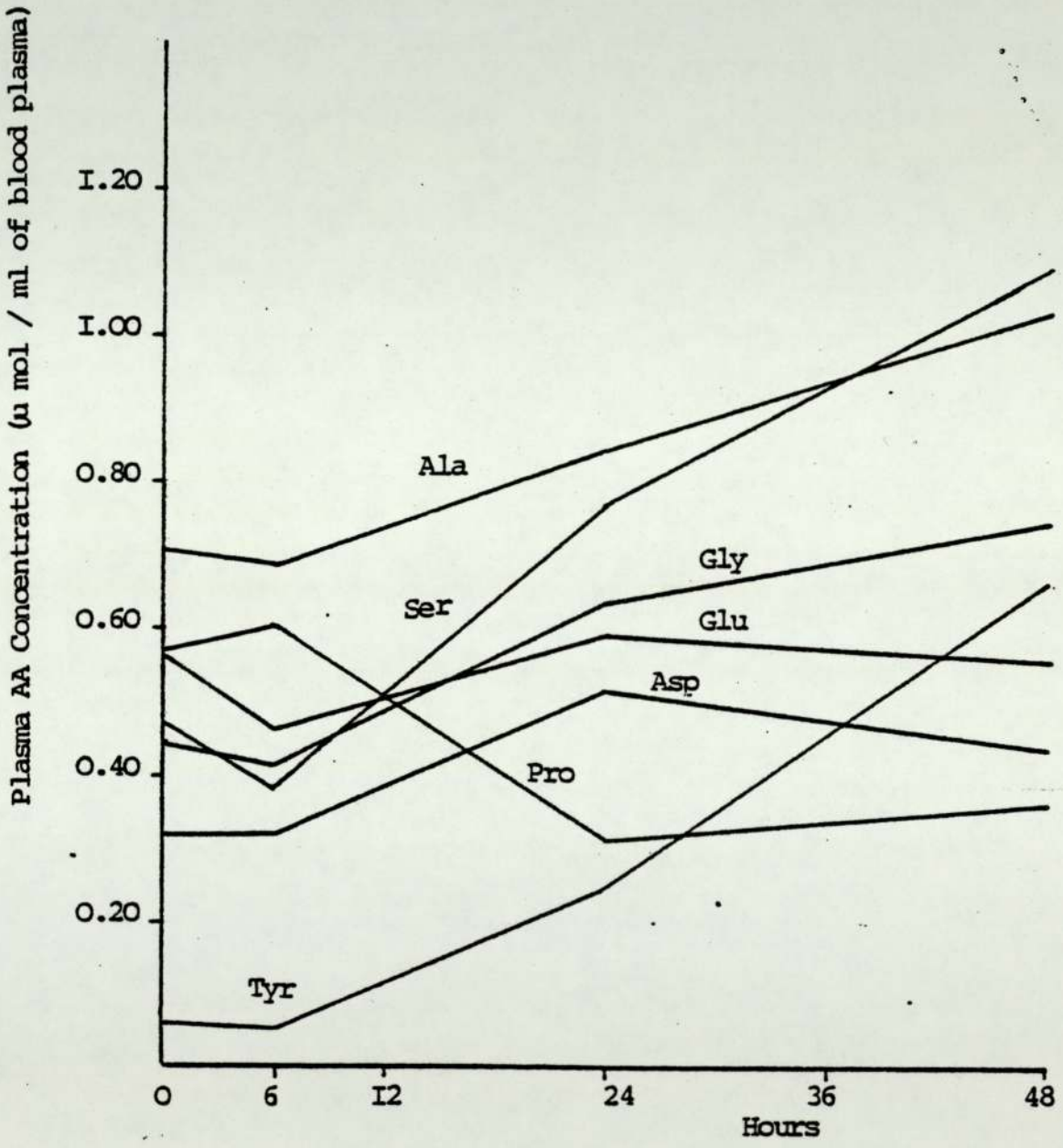

Fig 8.II. Change in Plasma FNEAA of Carp at Various Intervals after Force-Feeding Fish meal diet ca I5 % Protein.

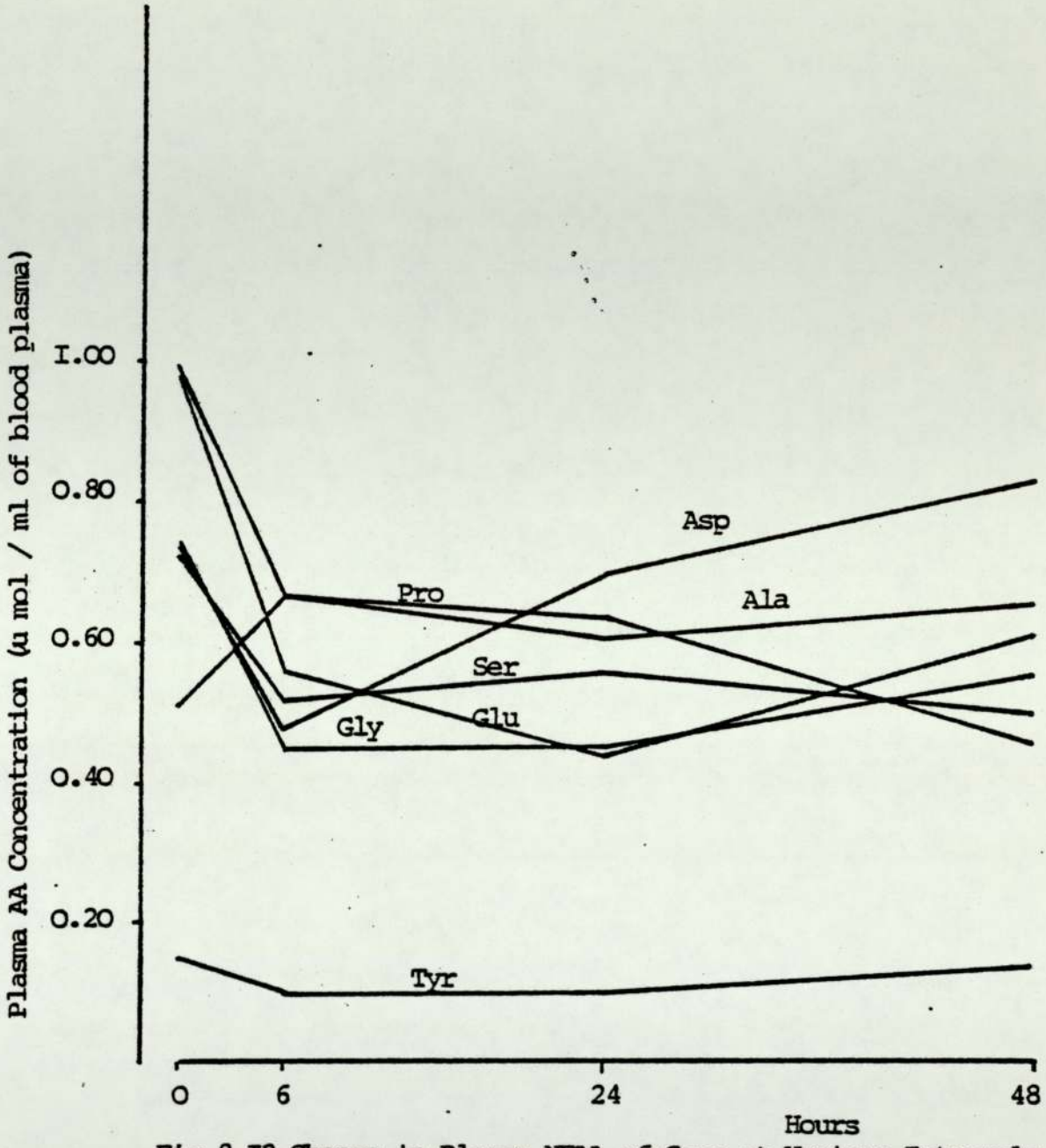

Fig 8.I2. Change in Plasma NEAA of Carp at Various Intervals after Force-Feeding Whole egg protein diet ca I5 % Protein.

tion rises at 48 h. The NEAA concentrations are generally higher than those of the EAA (Fig.8.6), except for tyrosine which is markedly lower than the other NEAA.

On the soybean diet (Fig.8.13), NEAA concentrations generally decrease at 6 h, except for glutamic acid, and there is a tendency for an increase at 24 h, followed by a fall, seen more with some than others. Tyrosine, again, has a much lower concentration than the others, virtully constant throughout.

With rapeseed, there is a marked drop in NEAA concentration at 6 h (Fig.8.14), which then remains more or less constant at 24 h and 48 h, not dissimilar to the EAA picture. Again, there is noticeably less tyrosine than on the remaining diets.

The results from feeding the sunflower diet (Fig.8.15) do not reveal any particular trend, and as with the EAA, the individual NEAA concentrations are spread fairly widely.

8.3.6. Total Free Ammonia.

Fig 8.16 shows the change of plasma free ammonia after force-feeding carp with 6 protein diets. Maximal concentration occurred at 24 h for carp force-fed casein and fish meal diets, while for soybean, egg protein, sunflower and rapeseed, it attained maximal concentration at 48 h after force feeding. Sequential changes in plasma ammonia observed in all treatments (Fig.8.16) were generally similar to those of total amino acids.

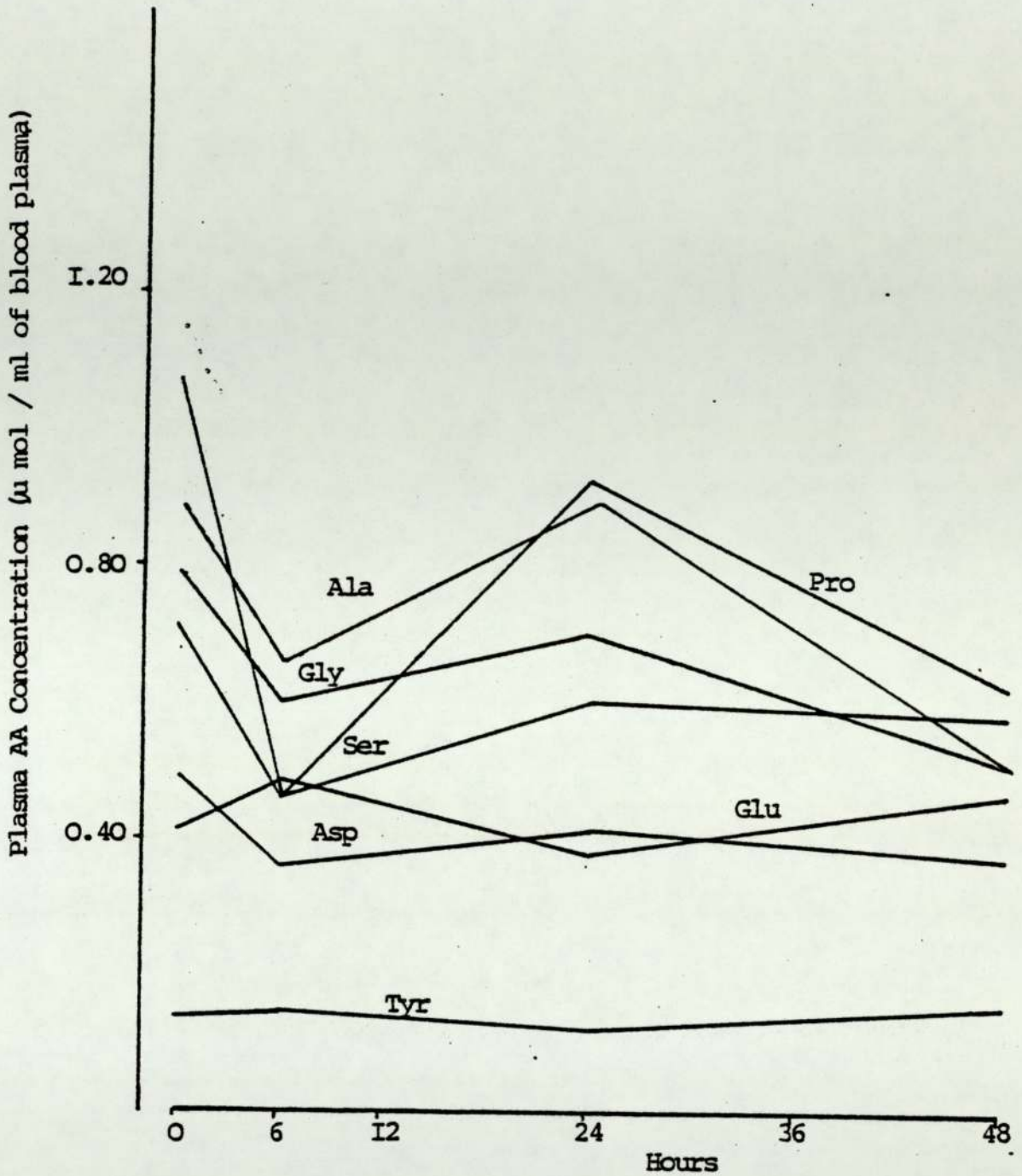

Fig 8.I3. Change in Plasma FNEAA of Carp at Various Intervals after Force-Feeding Soybean meal diet ca I5 % Protein.

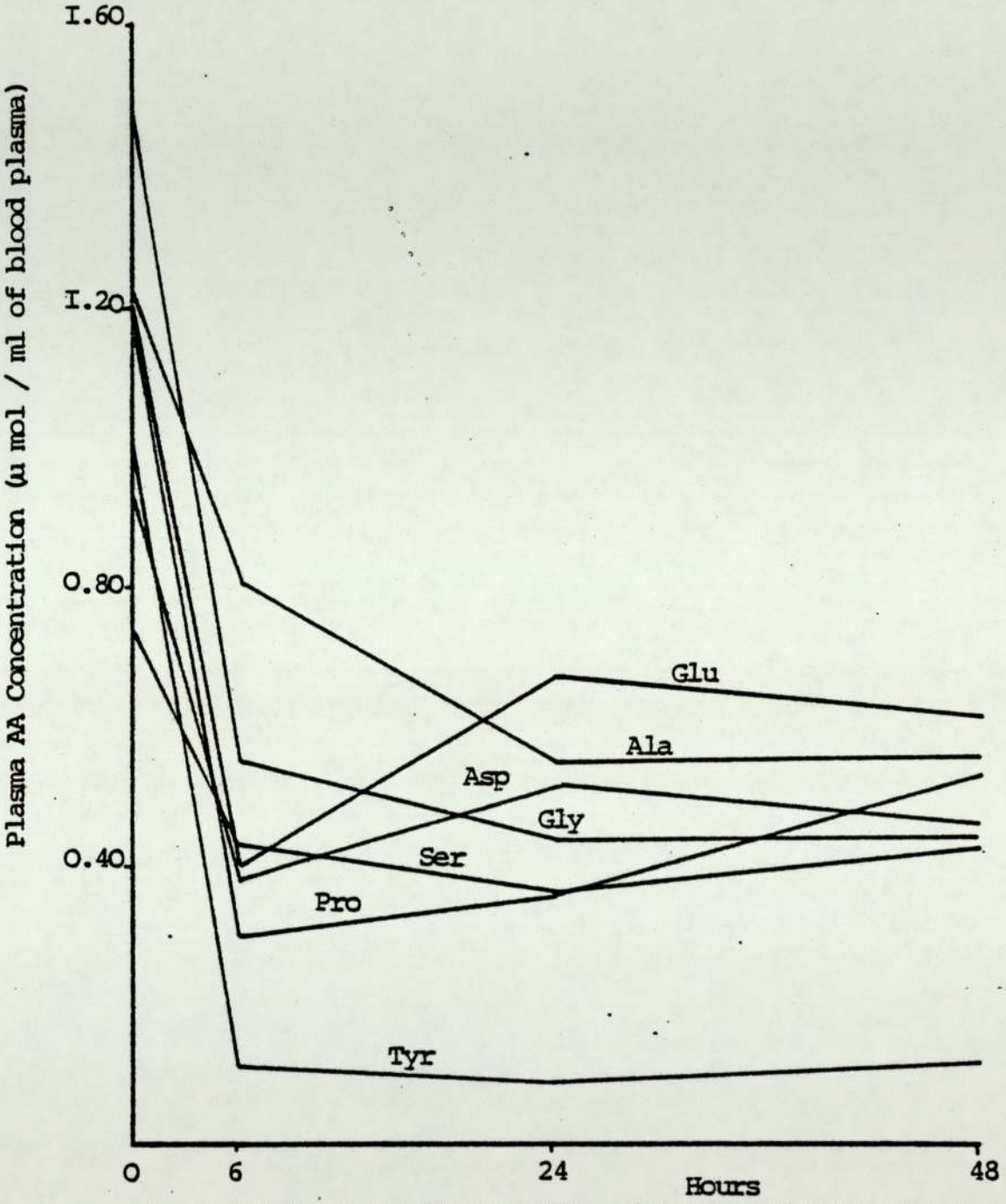

Fig 8.I4. Change in Plasma FNEAA of Carp at Various Intervals after Force-Feeding Rapeseed meal diet ca I5 % Protein.

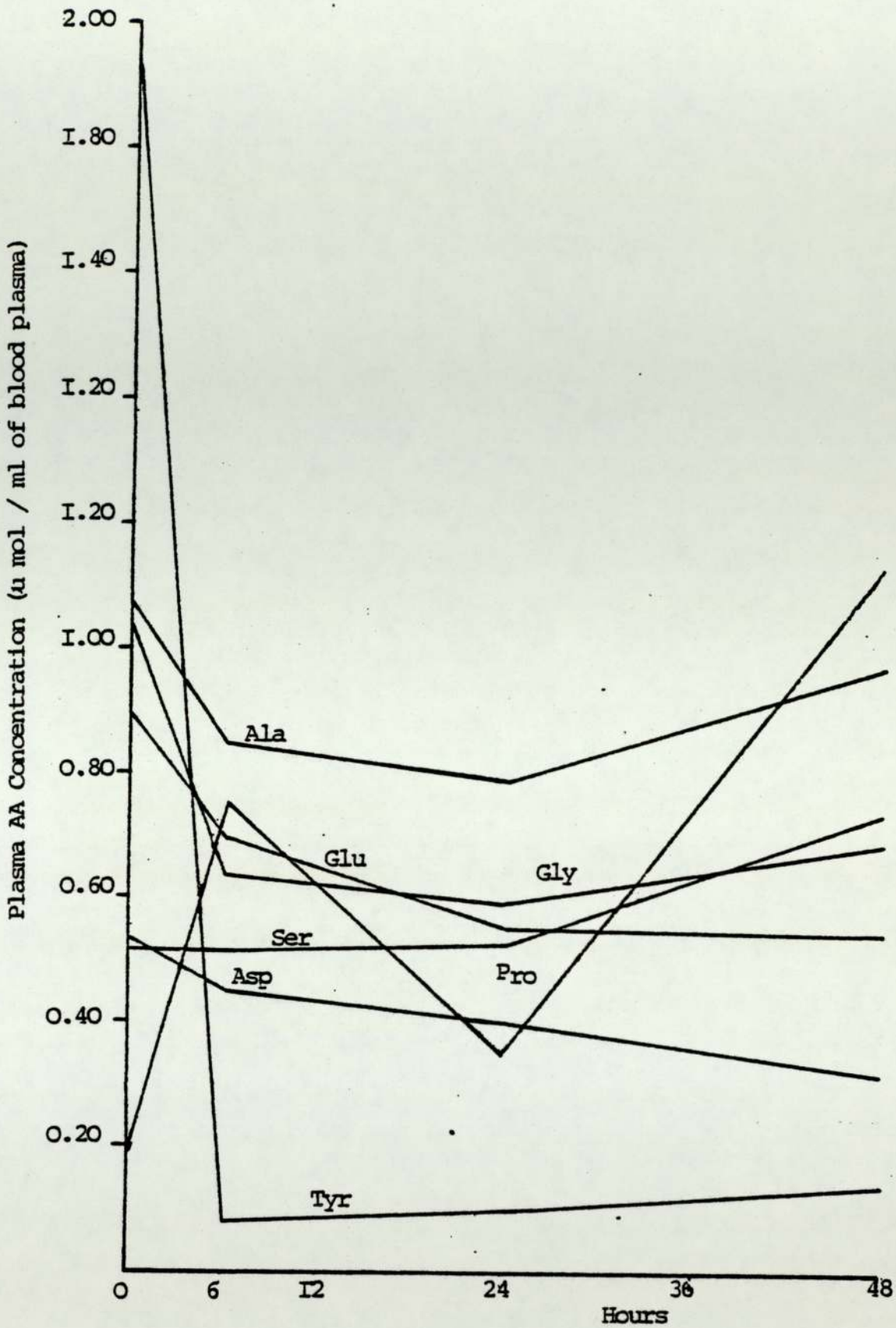

Fig 8.I5. Change in Plasma FNEAA of Carp at Various Intervals after Force-Feeding Sunflower diet ca I5 % Protein.

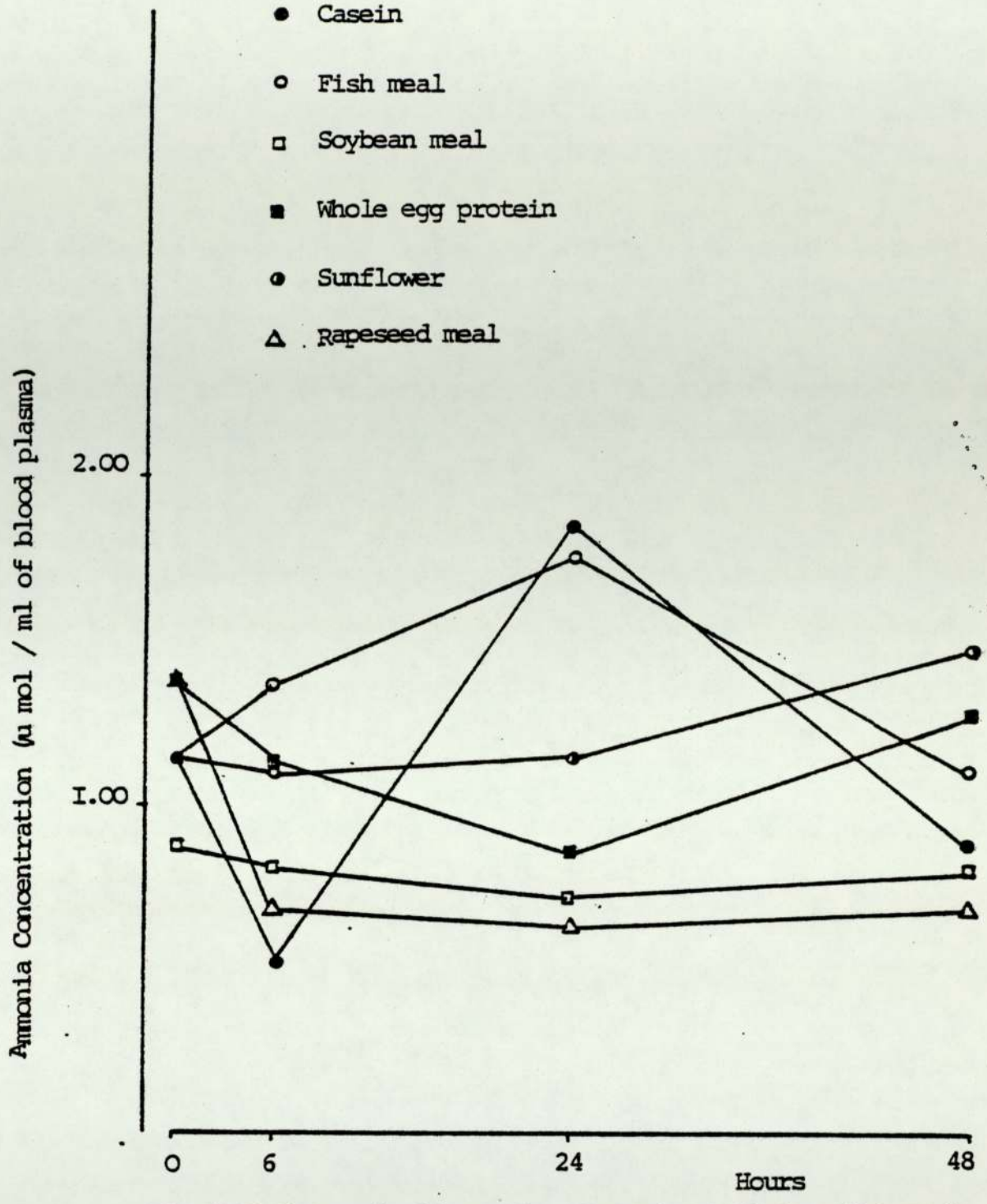

Fig 8.16. Change in Plasma Free Ammonia at Various Intervals after Force-Feeding Carp with Six Protein Diets ca 15 % Protein.

Table 8.11. The Change in Plasma Protein (mg/1ØØ ml)in Carp after Force-Feeding Six Protein Diets at (15% protein).

Mean ± SD; Number of fish = 3

Diets	Hours after force-feeding			
	Ø	6	24	48
Casein, 1	3.1Ø±Ø.75	3.3Ø±Ø.44	3.24±Ø.51	3.2Ø±Ø.79
Fish meal, 2	3.ØØ±Ø.45	3.27±Ø.24	3.2Ø±Ø.26	3.ØØ±Ø.15
Whole egg, 3	3.1Ø±Ø.1Ø	3.34±Ø.21	3.24±Ø.19	3.1Ø±Ø.21
Sunflower, 6	3.ØØ±Ø.Ø1	3.17±Ø.15	3.29±Ø.24	3.1Ø±Ø.1Ø
Soybean, 4	3.ØØ±Ø.21	3.16±Ø.24	3.26±Ø.24	3.12±Ø.23
Rapeseed, 5	3.1Ø±Ø.1Ø	3.1Ø±Ø.26	3.1Ø±Ø.26	3.17±Ø.25

8.3.7. Total Plasma Protein.

Plasma protein (Table 8.11) is maximal at 6 h with the casein, fish meal and egg diets; with the sunflower and soybean it shows a maximum at 24 h, but with the rape-seed, it is almost constant throughout. None of the differences, however, were shown to be significant.

8.4. Results of Carp Force-Fed Four Protein Diets at ca 30% Protein.

8.4.1. Total FAA.

Fig 8.17 illustrates the changes in TFAA at various intervals after force-feeding carp with four protein diets. Sequential changes in TFAA from fish fed diets 7 and 8 had a similar pattern, maximal values being attained at 24 h, while for diets 9 and 10 TFAA gradually decreased until 24 h and rose again at 48 h. Diet 9 always had higher concentrations than those found in the other three diets (Tables 8.12 to 8.15).

8.4.2. Total EAA.

Fig 8.17 illustrates the change in plasma TEAA after force-feeding four protein diets. Maximal values occurred at 24 h after force-feeding with diets 7 and 8, were lower at 48 h. While for diets 9 and 10 maximal concentrations were attained at 48 h after force-feeding. The concentrations of TEAA for diet 9 were always higher than those from the other diets. The picture is very similar to what was seen with the TFAA, but the trends are rather less marked. Again, the figures found for the egg diet (No 3) were always higher than the others.

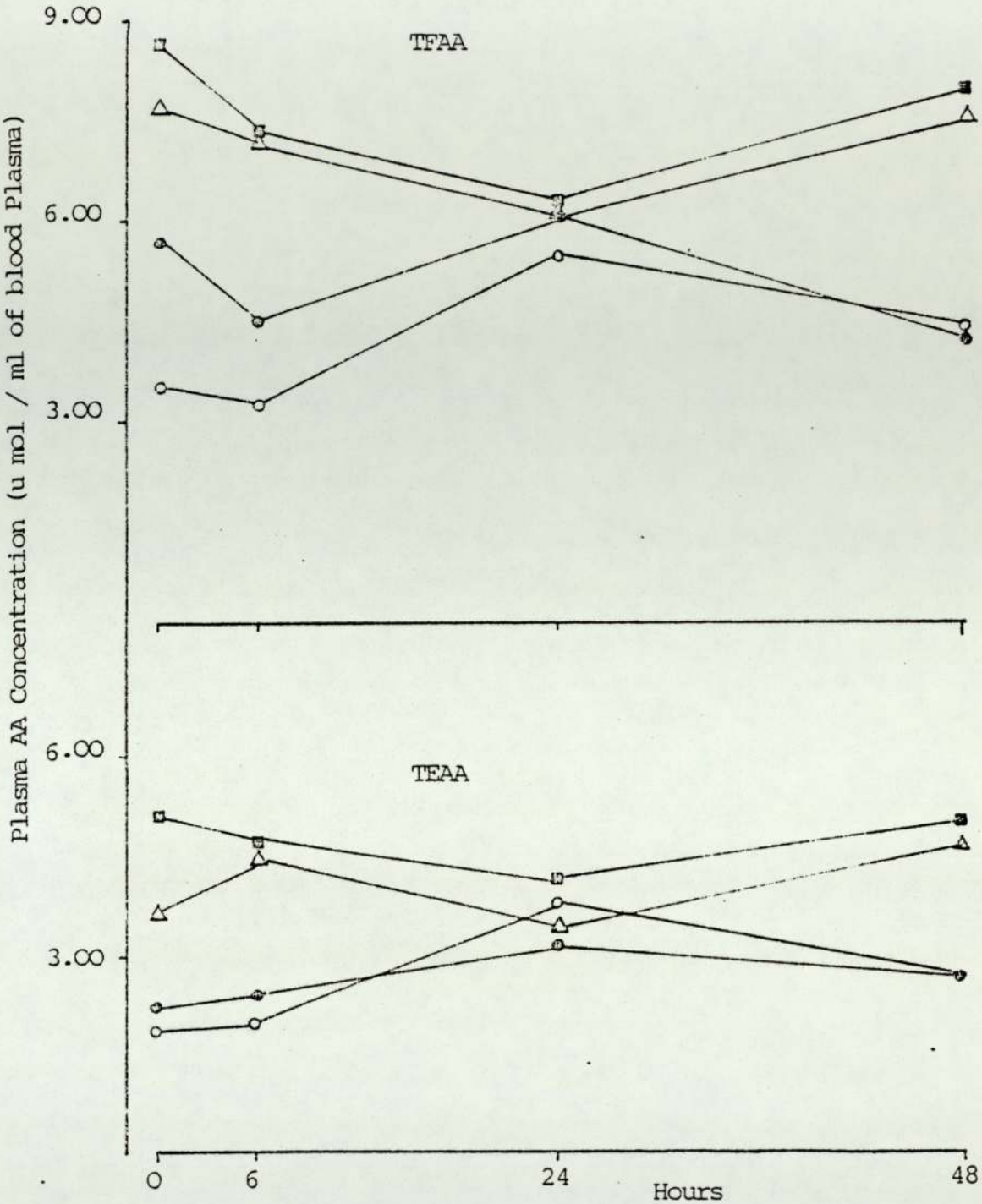

Fig 8.17. Change in Plasma TFAA and TEAA after Force-Feeding Carp with Casein (—●—), Fish meal (—○—), Egg protein (—■—) and Rapeseed meal (—△—) ca 30 % Protein.

Table 8.12. Plasma FAA Concentrations of Carp at Various Intervals after Force-Feeding Casein Diet at 30% Protein.

(μ mol/ml of blood plasma)

AA	Hours after force-feeding			
	Ø	6	24	48
Asp	1.414	Ø.1ØØ	Ø.269	Ø.187
Thr	Ø.563	Ø.438	Ø.6Ø1	Ø.3Ø3
Ser	Ø.322	Ø.4Ø5	Ø.597	Ø.387
Glu	Ø.218	Ø.154	Ø.377	Ø.21Ø
Pro	Ø.962	Ø.9Ø9	1.Ø21	Ø.391
Gly	Ø.289	Ø.269	Ø.275	Ø.263
Ala	Ø.2Ø6	Ø.2ØØ	Ø.246	Ø.2Ø4
Val	Ø.216	Ø.226	Ø.3Ø8	Ø.241
Met	Ø.1Ø1	Ø.97Ø	Ø.176	Ø.115
Ile	Ø.1Ø8	Ø.211	Ø.288	Ø.276
Leu	Ø.2Ø4	Ø.317	Ø.371	Ø.239
Tyr	Ø.Ø47	Ø.Ø52	Ø.Ø94	Ø.Ø85
Phe	Ø.Ø98	Ø.118	Ø.146	Ø.122
His	Ø.194	Ø.19Ø	Ø.252	Ø.178
Lys	Ø.53Ø	Ø.548	Ø.745	Ø.939
NH_3	Ø.3Ø2	Ø.264	Ø.574	Ø.374
Arg	Ø.276	Ø.3Ø7	Ø.351	Ø.313
TFAA	5.748	4.541	6.117	4.453
EAA	2.29Ø	2.452	3.238	2.726
NEAA	3.458	2.Ø89	2.879	1.727
EAA/NEAA	Ø.662	1.173	1.124	1.578

Table 8.13. Plasma FAA Concentrations of Carp at Various Intervals after Force-Feeding Fish meal Diet at 30% protein.

(μ mol/ml of blood plasma)

AA	Hours after force-feeding			
	Ø	6	24	48
Asp	Ø.Ø98	Ø.Ø94	Ø.164	Ø.1Ø2
Thr	Ø.341	Ø.386	Ø.425	Ø.385
Ser	Ø.27Ø	Ø.23Ø	Ø.34Ø	Ø.426
Glu	Ø.183	Ø.158	Ø.287	Ø.143
Pro	Ø.657	Ø.536	Ø.4Ø6	Ø.237
Gly	Ø.195	Ø.166	Ø.253	Ø.3Ø3
Ala	Ø.131	Ø.145	Ø.139	Ø.161
Val	Ø.191	Ø.2ØØ	Ø.412	Ø.428
Met	Ø.Ø94	Ø.Ø86	Ø.1Ø6	Ø.115
Ile	Ø.172	Ø.2Ø4	Ø.186	Ø.215
Leu	Ø.177	Ø.185	Ø.25Ø	Ø.2Ø7
Tyr	Ø.Ø37	Ø.Ø37	Ø.1Ø3	Ø.511
Phe	Ø.Ø88	Ø.1ØØ	Ø.3Ø6	Ø.Ø73
His	Ø.194	Ø.2Ø2	Ø.2Ø4	Ø.4Ø8
Lys	Ø.4ØØ	Ø.364	1.1ØØ	Ø.549
NH_3	Ø.346	Ø.353	Ø.459	Ø.29Ø
Arg	Ø.282	Ø.287	Ø.9Ø4	Ø.327
TFAA	3.51Ø	3.38Ø	5.585	4.59Ø
EAA	1.939	2.Ø14	3.893	2.7Ø7
NEAA	1.571	1.366	1.692	1.883
EAA/NEAA	1.234	1.474	2.3Ø3	1.437

Table 8.14. Plasma FAA Concentrations of Carp at Various Intervals after Force-Feeding Whole Egg Protein 30% protein).

(u mol/ml of blood plasma)

AA	Hours after force-feeding			
	Ø	6	24	48
Asp	Ø.426	Ø.347	Ø.419	Ø.429
Ser	Ø.735	Ø.431	Ø.4Ø5	Ø.254
Ser	Ø.6Ø4	Ø.475	Ø.515	Ø.469
Glu	Ø.519	Ø.411	Ø.416	Ø.542
Pro	Ø.515	Ø.387	Ø.115	Ø.334
Gly	Ø.7Ø8	Ø.517	Ø.129	Ø.6Ø6
Ala	Ø.721	Ø.551	Ø.47Ø	Ø.477
Val	Ø.412	Ø.388	Ø.41Ø	Ø.683
Met	Ø.2Ø3	Ø.214	Ø.173	Ø.2ØØ
Ile	Ø.51Ø	Ø.577	Ø.231	Ø.25Ø
Leu	Ø.395	Ø.354	Ø.411	Ø.436
Tyr	Ø.Ø49	Ø.Ø8Ø	Ø.1Ø9	Ø.133
Phe	Ø.188	Ø.16Ø	Ø.232	Ø.424
His	Ø.262	Ø.328	Ø.264	Ø.758
Lys	1.38Ø	1.167	1.Ø79	1.1Ø2
NH_3	1.618	Ø.647	Ø.8Ø1	1.Ø71
Arg	1.Ø85	1.1Ø2	Ø.971	Ø.911
TFAA	8.757	7.489	6.346	8.ØØ8
EAA	5.17Ø	4.721	4.176	5.Ø18
NEAA	3.587	2.768	2.17Ø	2.99Ø
EAA/NEAA	1.441	1.7Ø5	1.924	1.678

Table 8.15. Plasma FAA Concentrations of Carp at Various Intervals after Force-Feeding Rapeseed meal Diet (30% protein).

(μ mol/ml of blood plasma)

AA	Hours after force-feeding			
	Ø	6	24	48
Asp	Ø.398	Ø.3Ø4	Ø.327	Ø.323
Thr	Ø.544	Ø.448	Ø.417	Ø.398
Ser	Ø.574	Ø.412	Ø.352	Ø.1Ø3
Glu	Ø.685	Ø.37Ø	Ø.53Ø	Ø.415
Pro	Ø.718	Ø.334	Ø.414	Ø.461
Gly	Ø.712	Ø.496	Ø.4Ø4	Ø.463
Ala	Ø.889	Ø.653	Ø.515	Ø.526
Val	Ø.449	Ø.364	Ø.413	Ø.717
Met	Ø.1Ø8	Ø.172	Ø.154	Ø.478
Ile	Ø.314	Ø.236	Ø.258	Ø.223
leu	Ø.612	Ø.349	Ø.443	Ø.859
Tyr	Ø.Ø87	Ø.Ø83	Ø.Ø9Ø	Ø.186
Phe	Ø.167	Ø.191	Ø.114	Ø.167
his	Ø.276	Ø.23Ø	Ø.2Ø8	Ø.215
Lys	Ø.771	1.561	Ø.899	1.28Ø
Nh_3	1.659	Ø.938	Ø.525	1.834
Arg	Ø.411	Ø.96Ø	Ø.571	Ø.824
TEAA	7.715	7.163	6.1Ø9	7.638
EAA	3.652	4.511	3.472	4.761
NEAA	4.Ø63	2.652	2.632	2.877
EAA/NEAA	Ø.898	1.7ØØ	1.321	1.654

8.4.3. Total NEAA & EAA/NEAA (Fig 8.18).

TNEAA, however, were different. The casein diet produced a decrease from Ø-6 h, then an increase, followed by further decrease in concentration at 48 h. On the egg diet, there was a decrease to 24 h, then an increase. With rapeseed the concentration decreased at 6 h, then showed little change. There was only slight variation throughout on the fish meal diet .

The EAA/NEAA ratio was highest at 24 h with fish meal and egg, and at 48 h with casein (Fig.8.18).

8.4.4. Individual FEAA.

On the casein diet (Fig 8.19), the lysine concentration increases steadily throughout; threonine peaks at 24 h after an initial decrease, then decreases even more at 48 h. The other FAA show maximal values at 6-24 h, but the changes are not very marked.

With the fish meal diet (Fig 8.2Ø), lysine and arginine show a very pronounced peak concentration at 24 h, as does phenylalanine, through somewhat less so. Valine increases at 24 h, and remains decreased at 48 h, and histidine increases at 48 h. The other EAA are virtually unchanged.

The results obtained with the other two diets are rather different. With the egg diet (Fig 8.21), lysine, arginine, threonine and isoleucine concentrations decreased to a minimum at 48 h, whereas valine, histidine and phenylalanine increased to a maximum at this time. Leucine and methionine showed little change.

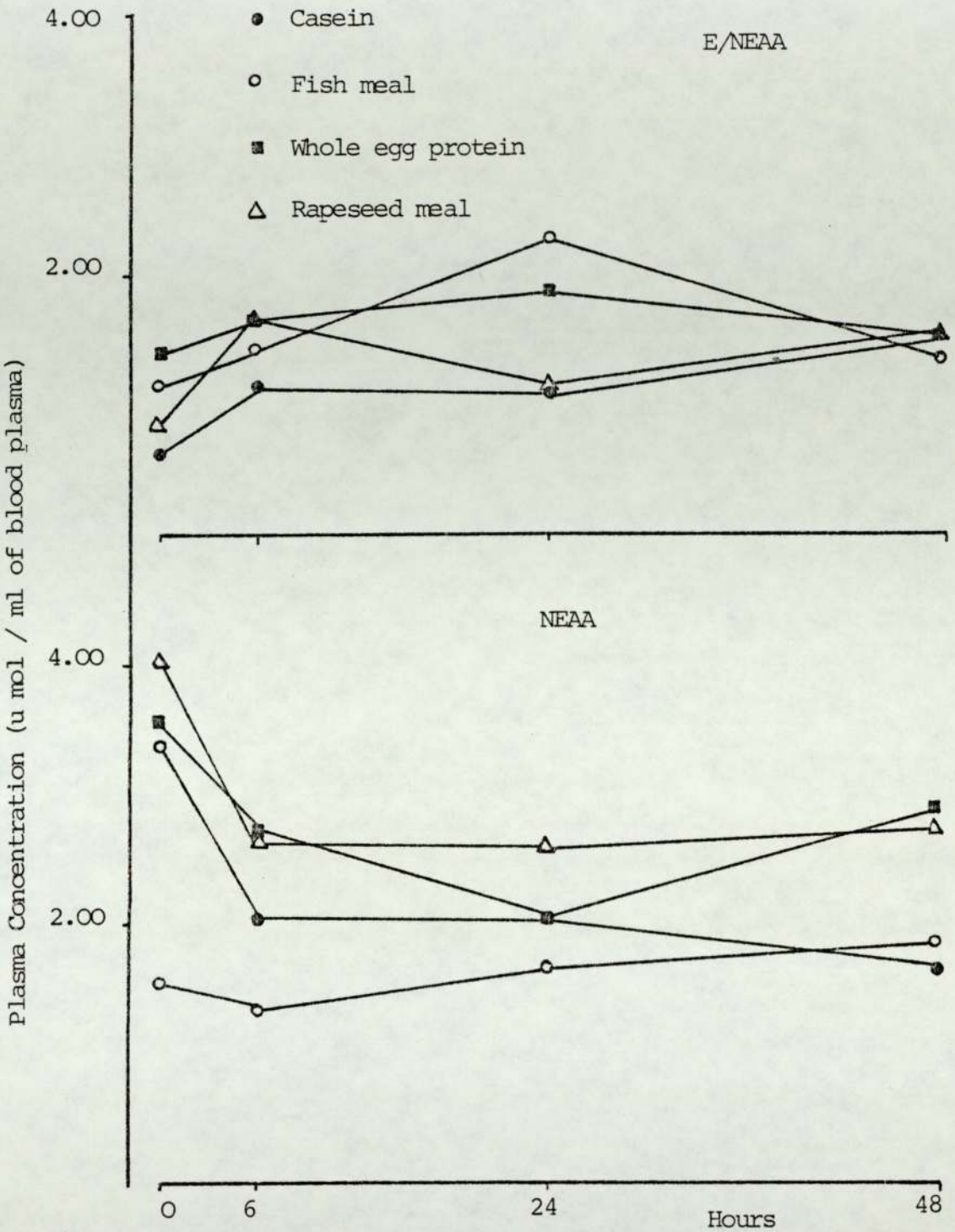

Fig 8.I8. Change in Plasma TFNEAA and E/NEAA of Carp at Various Intervals after Force-Feeding Four Diets ca 30 % Protein.

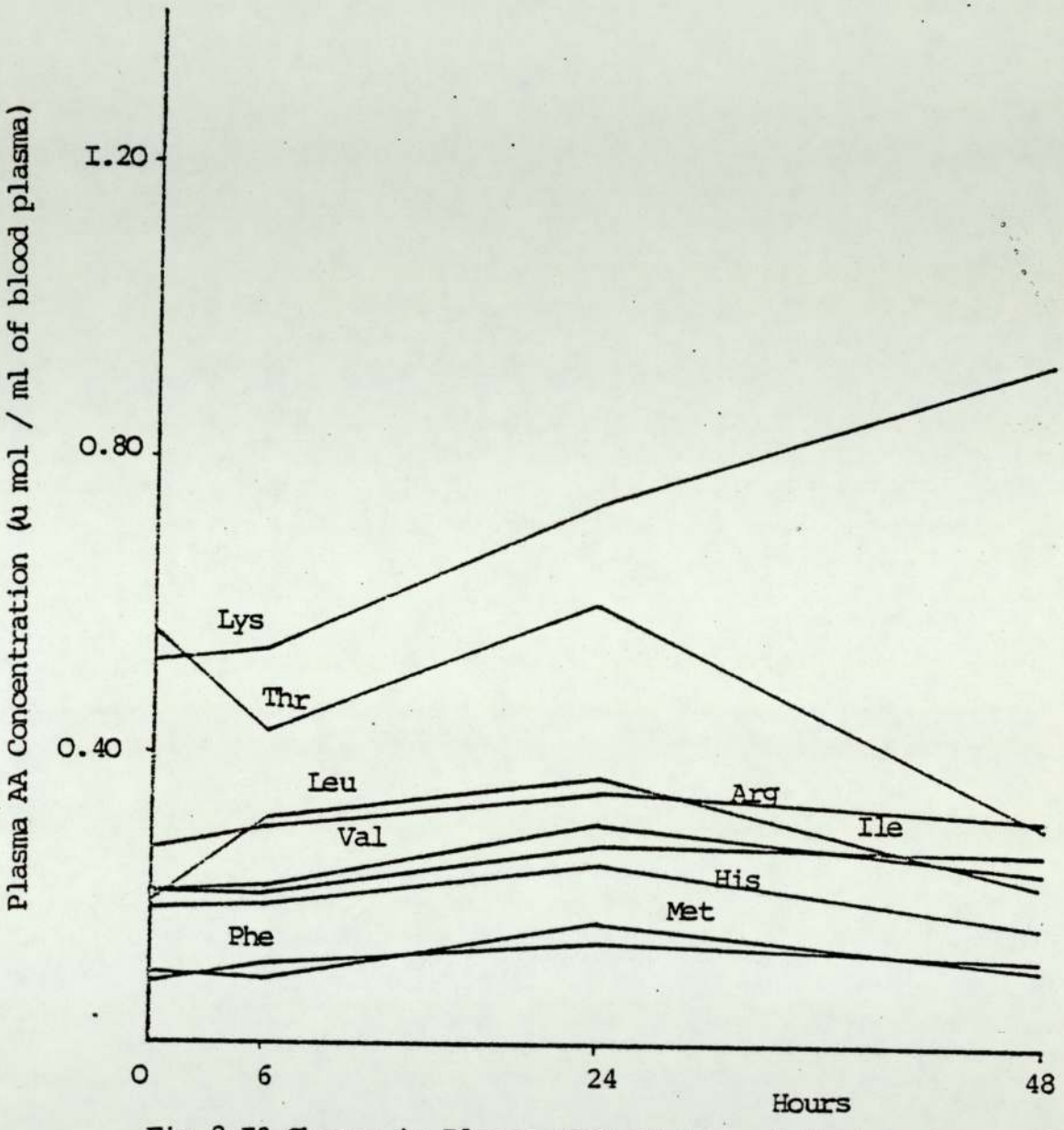

Fig 8.I9. Change in Plasma FEAA of Carp at Various intervals after Force-Feeding Casein Diet ca 30 % Protein.

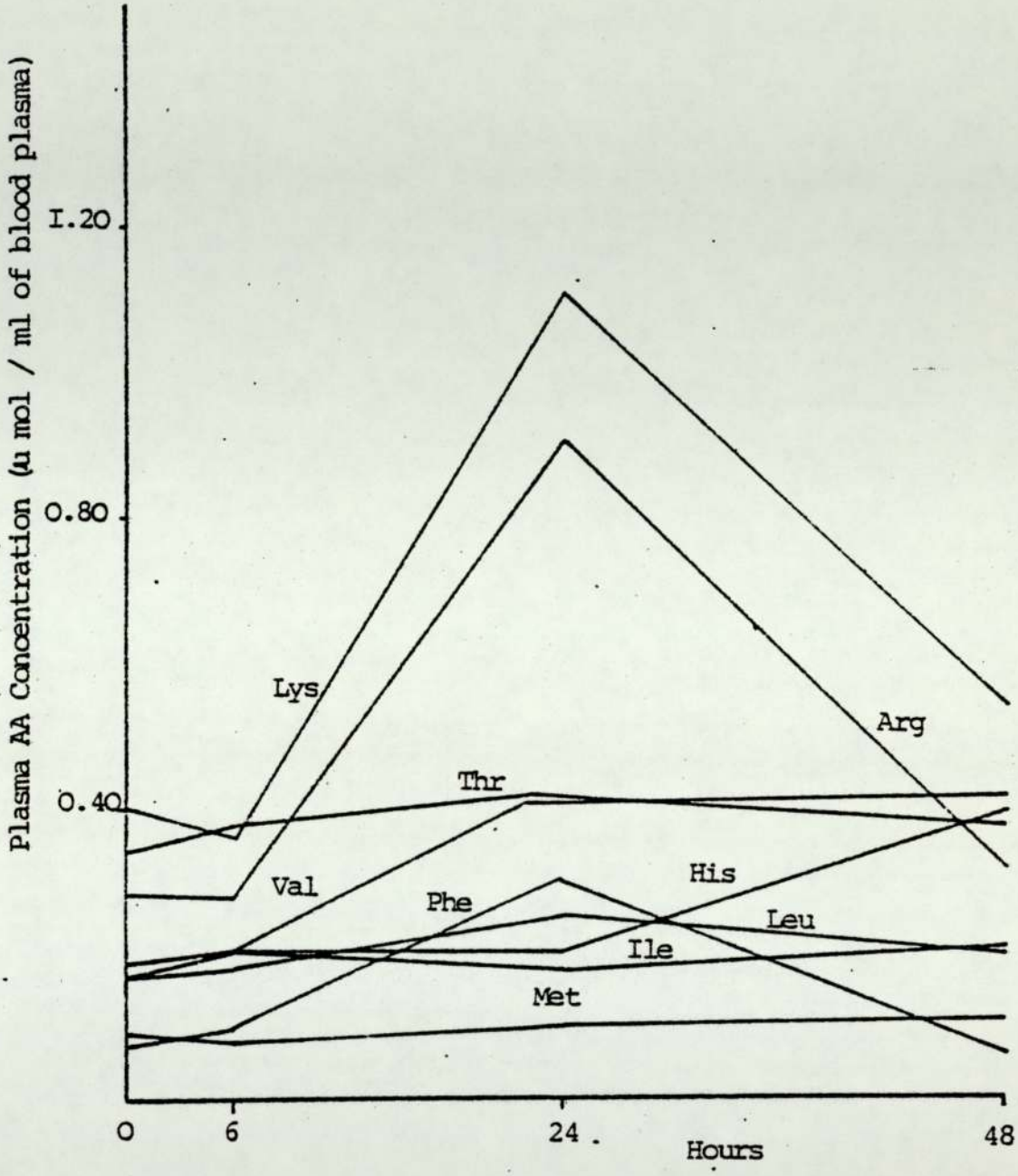

Fig 8.20. Change in Plasma FEAA of Carp at Various Intervals after Force-Feeding Fish meal diet ca 30 % Protein .

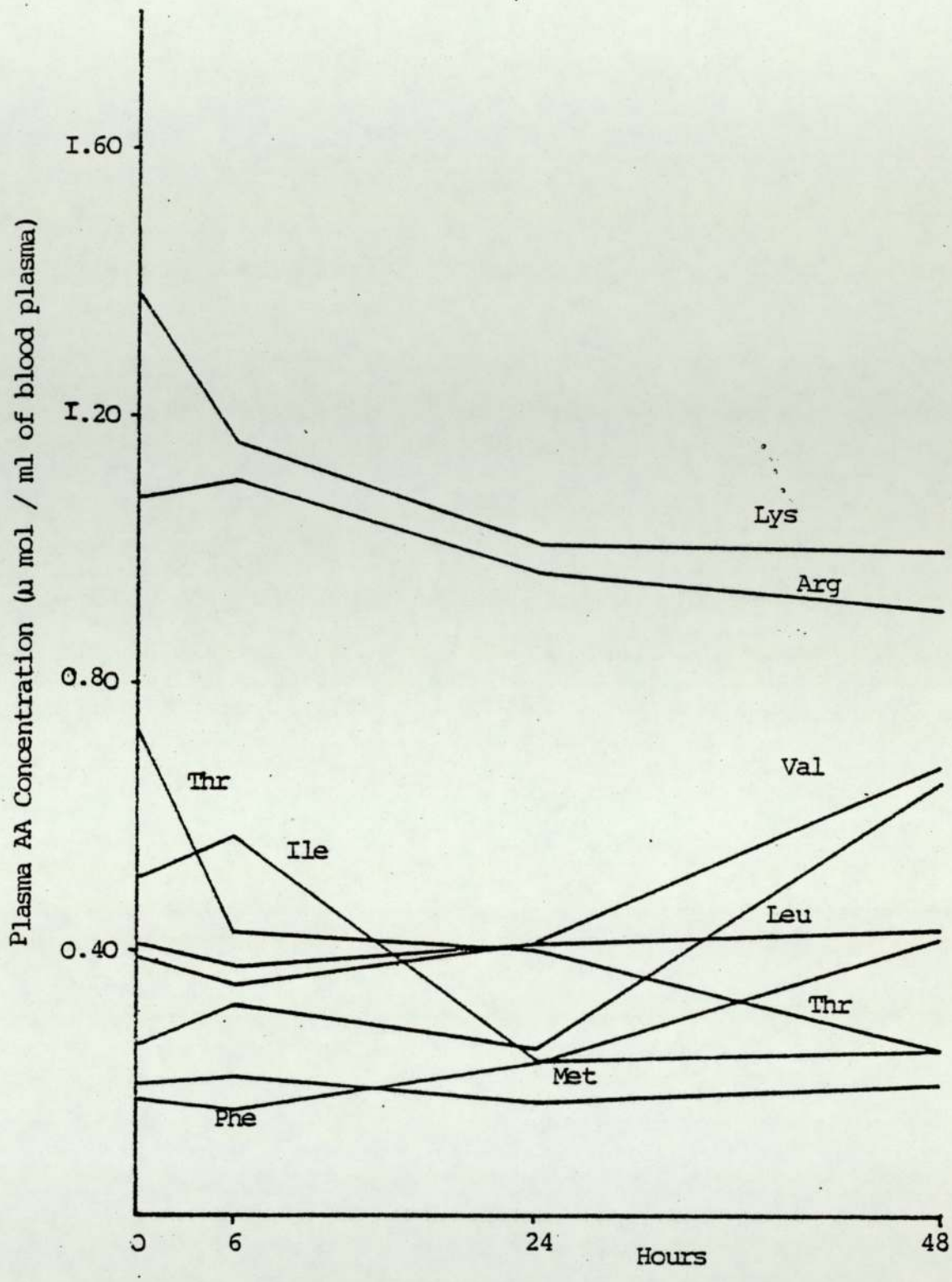

Fig 8.2I. Change in Plasma FEAA of Carp at Various Intervals after Force-Feeding Whole egg protein ca 30 % Protein.

On the rapeseed diet, arginine and lysine peaked very markedly at 6 h. Leucine, valine and methionine increased to maximal concentrations at 48 h, the first two after an initial decline. Threonine, histidine and isoleucine decreased slighly throughout, and phenylalanine showed little change (Fig 8.22).

It may be noted that on three of the four diets, lysine and arginine concentrations are much higher than any of the other AA, except for arginine with the casein diet. Figs 8.23-8.26 show that the plasma concentrations of most of the EAA were fairly consistent with reflecting both the time after feeding and the type of diet. Arginine, phenylalanine and lysine concentrations, however, were more variable.

Plasma concentration of valine from fish force-fed casein diet attained a maximal value at 24 h, while for fish meal, egg protein and rapeseed it was maximal at 48 h after force-feeding (Fig 8.23).

Fig 8.23 shows that the highest concentration for leucine was at 24 h for casein and fish meal diets, and for egg protein and rapeseed diets at 48 h after force-feeding. Maximal concentration for isoleucine occurred at 6 h for fish force-feed egg diet; 24 h for fish fed casein and rapeseed diet and 48h for fish on the fish meal diet (Fig 8.23).

The peak of phenylalanine occurred at 24 h for fish force-fed casein and fish meal diets, at 6 h for rapeseed and 48 h for egg protein diet (Fig 8.24).

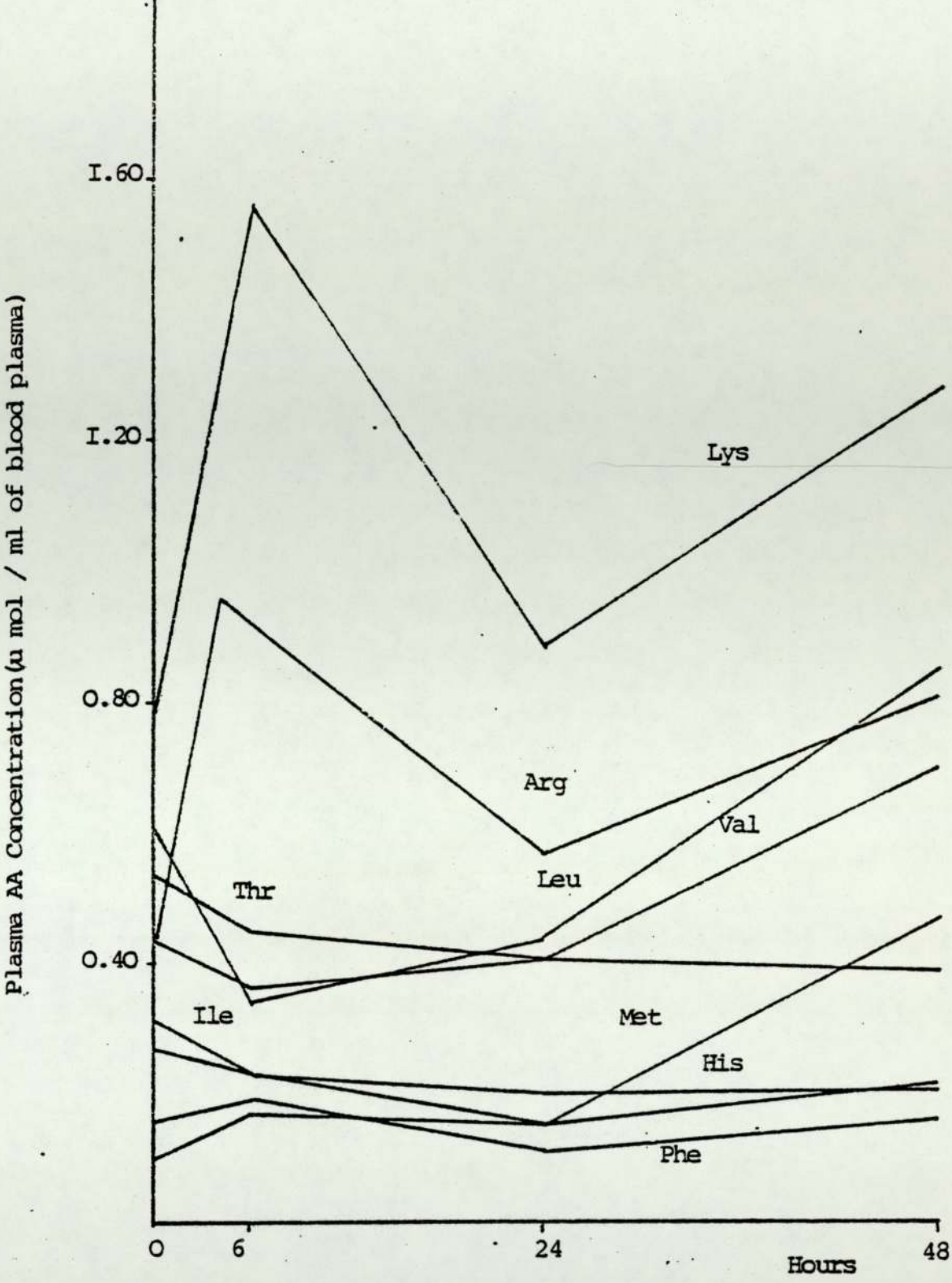

Fig 8.22. Change in Plasma FEAA of Carp at Various Intervals after Force-Feeding Rapeseed meal diet ca 30 % Protein.

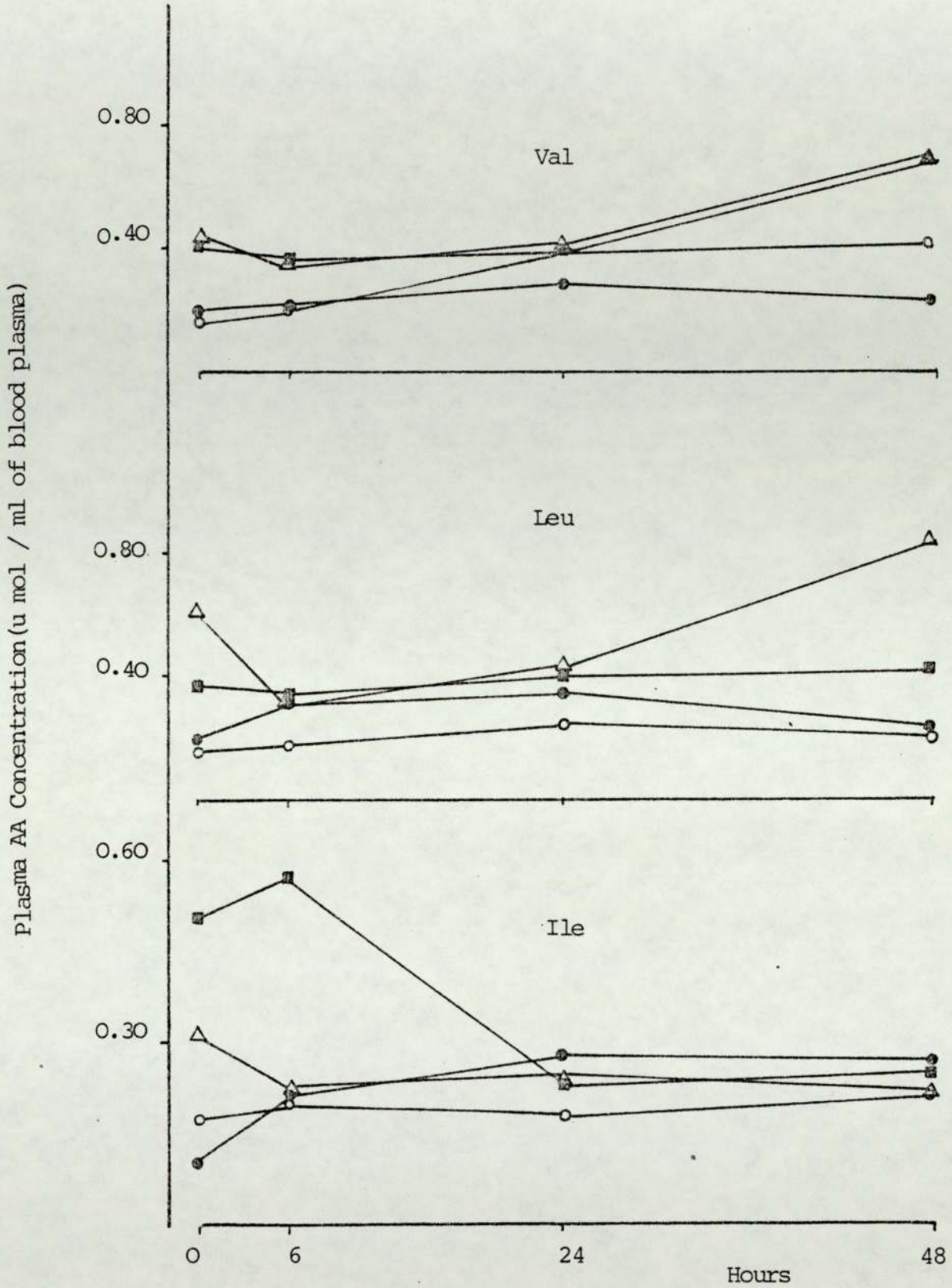

Fig 8.23. Change in Plasma Free Valine, Leucine and Isoleucine of Carp after Force-Feeding Casein (—●—), Fish meal (—○—), Egg protein (—■—) and Rapeseed meal (—△—) diets ca 30 % protein.

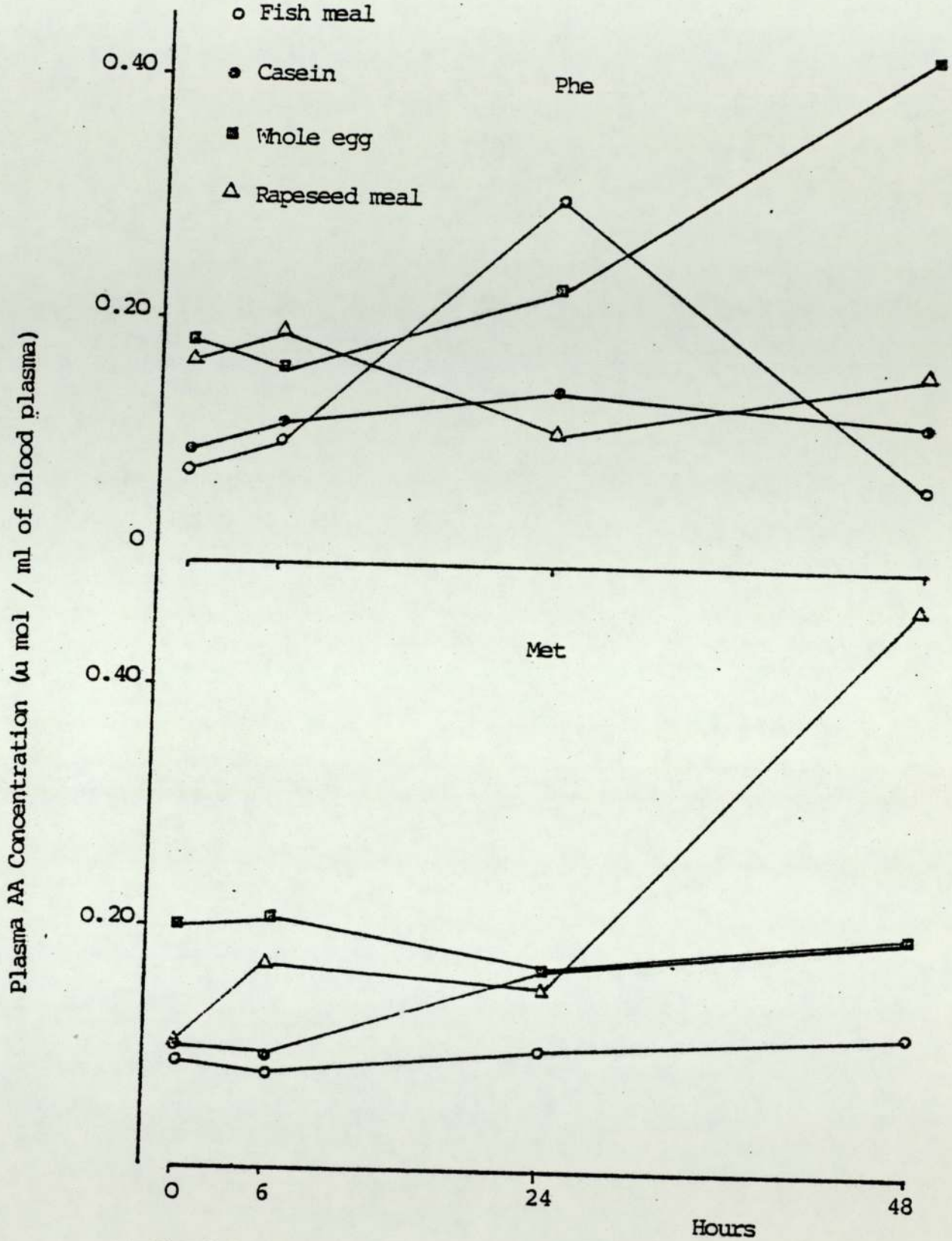

Fig 8.24. Change in plasma Phenylalanine and Methionine of Carp at Various intervals after Force-Feeding Four Protein diets ca 30 % Protein.

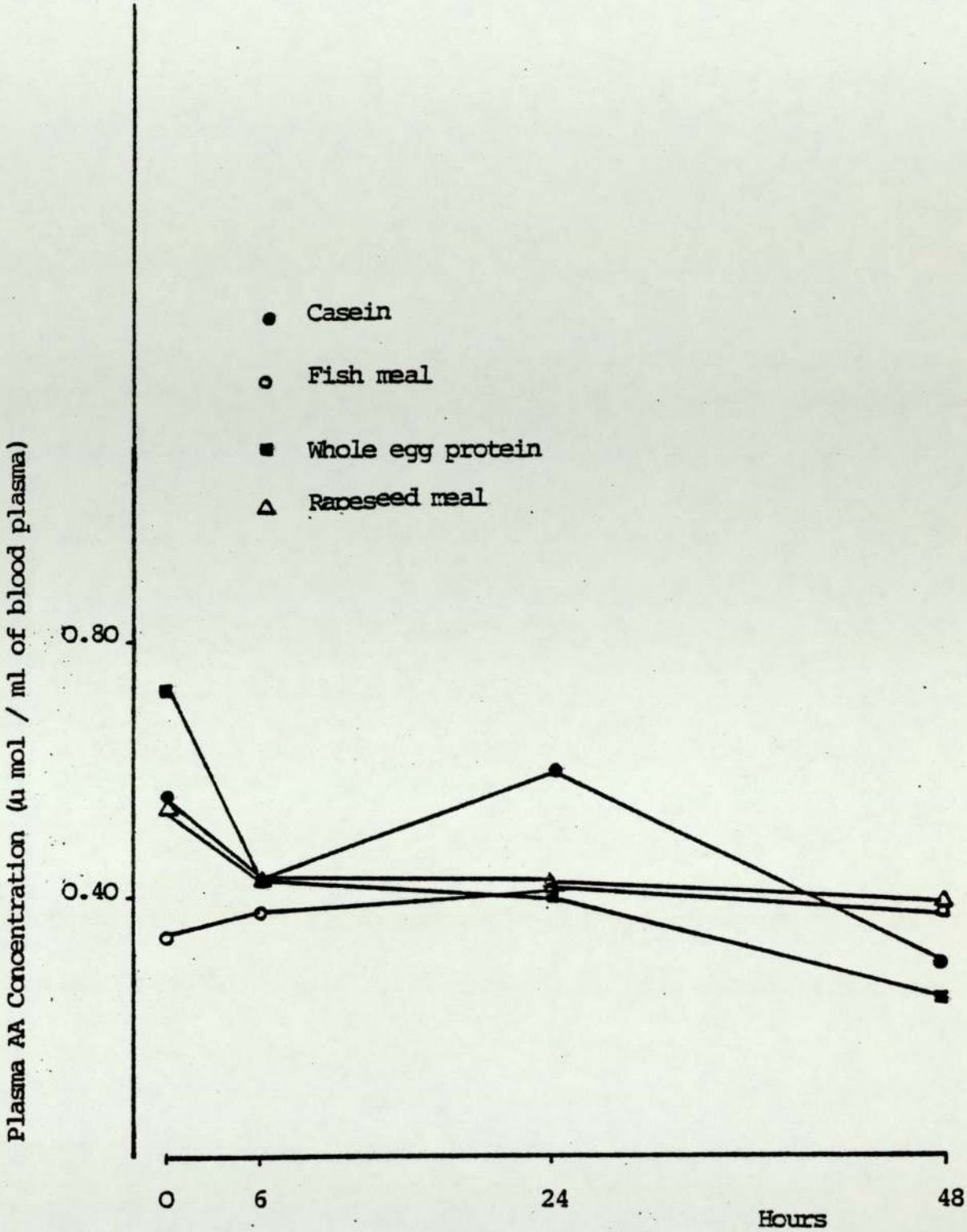

Fig 8.25. Change in Plasma free Threonine in Carp at Various Intervals after Force-Feeding Four Diets ca 30 % Protein.

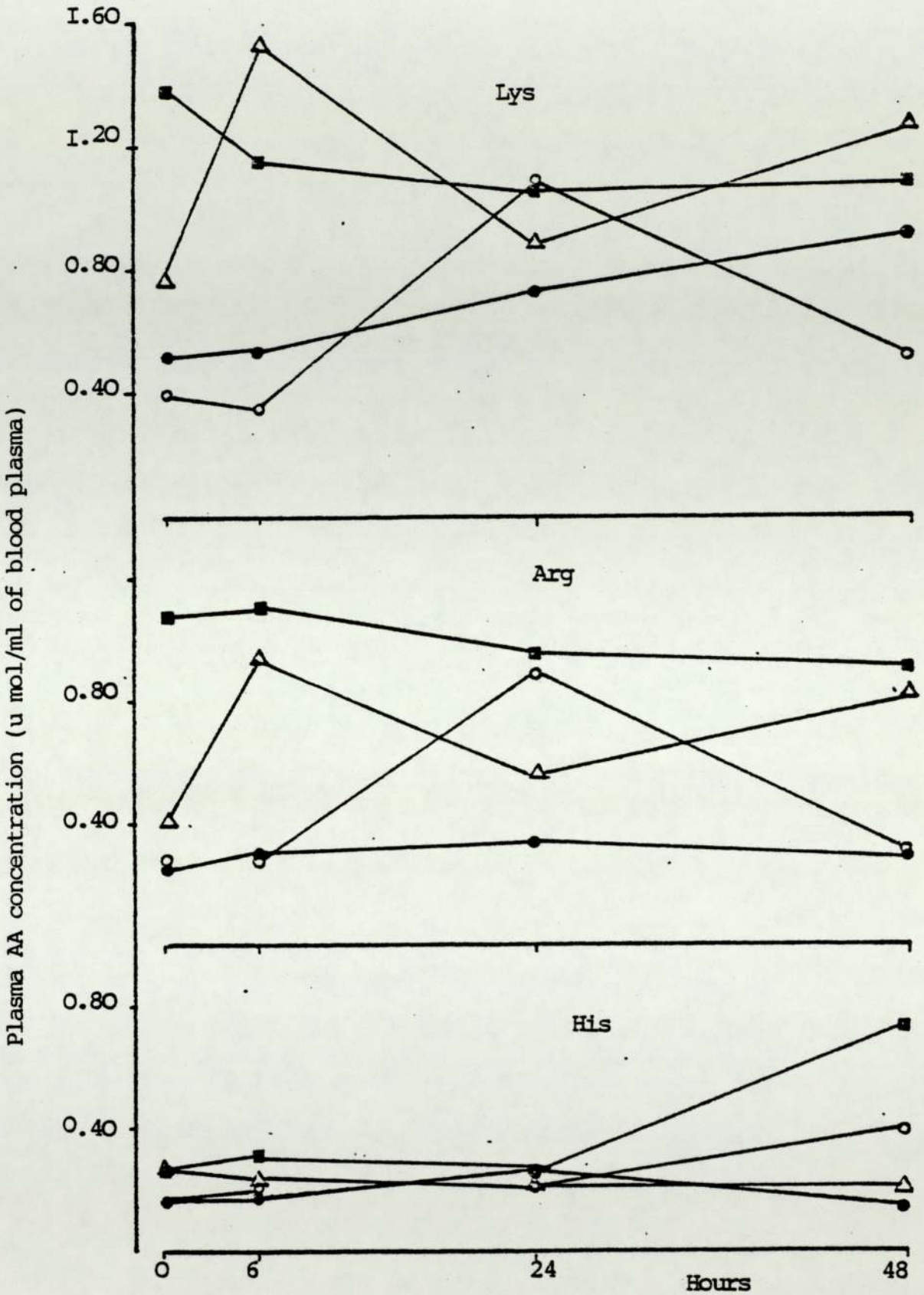

Fig 8.26. Change in Plasma Free Lysine, Arginine and Histidine of Carp at Various intervals after Force-Feeding with Casein (—●—), Fish meal (—○—), Egg protein (—■—), and Rapeseed meal (—△—) diets ca 30 % Protein.

Maximal concentration for methionine was at 6 h for egg diet; 24 h for casein diet and 48 h for fish meal and rapeseed diet (Fig 8.24).

Fig 8.25 illustrates the changes in threonine. Maximal value occurred at 6 h for fish force-fed egg protein and rapeseed meal diets, and dropped to below fasting at 48 h. For casein and fish meal diets, maximal concentrations were attained at 24 h after feeding.

In Fig 8.26 lysine was maximal at 6 h after force-feeding carp with rapeseed and egg protein diets ; 24 h for fish meal diet and 48 h for the casein diet. For arginine a maximum for egg protein and rapeseed meal diet occurred at 6 h, and for fish meal and casein at 6 h (Fig 8.26). Histidine reached a maximal concentration at 6 h for rapeseed meal diet; 24 h for the casein and 48 h for egg protein and fish meal diets.

8.4.5. Free NEAA.

On the casein diet (Fig 8.27), the NEAA concentrations are maximal at 24 h or are virtually unchanged (glycine, alanine). The result with aspartate is curious, showing a precipitous fall at 6 h to less than one tenth of its high starting concentration.

With fish meal (Fig 8.28) the concentrations of serine, glycine and tyrosine rise to a maximum at 48 h; glutamate increases, then falls and proline decreases throughout. Alanine and aspartate show little marked change.

The results for the egg diet (Fig 8.29) show a fairly steady picture between 6-48 h for alanine, serine,

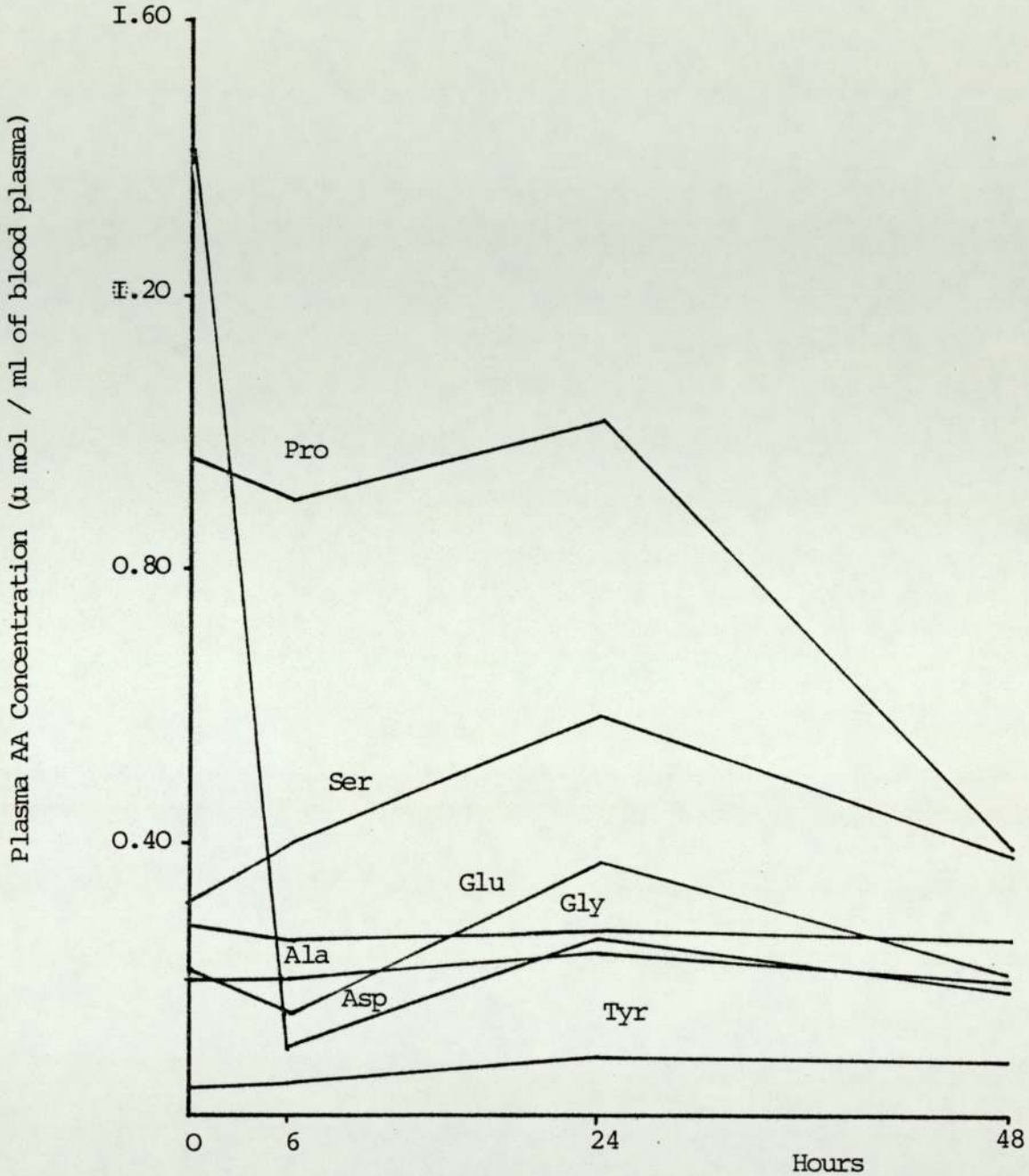

Fig 8.27. Change in Plasma FNEAA of Carp at Various intervals after Force-Feeding Casein diet ca 30 % Protein.

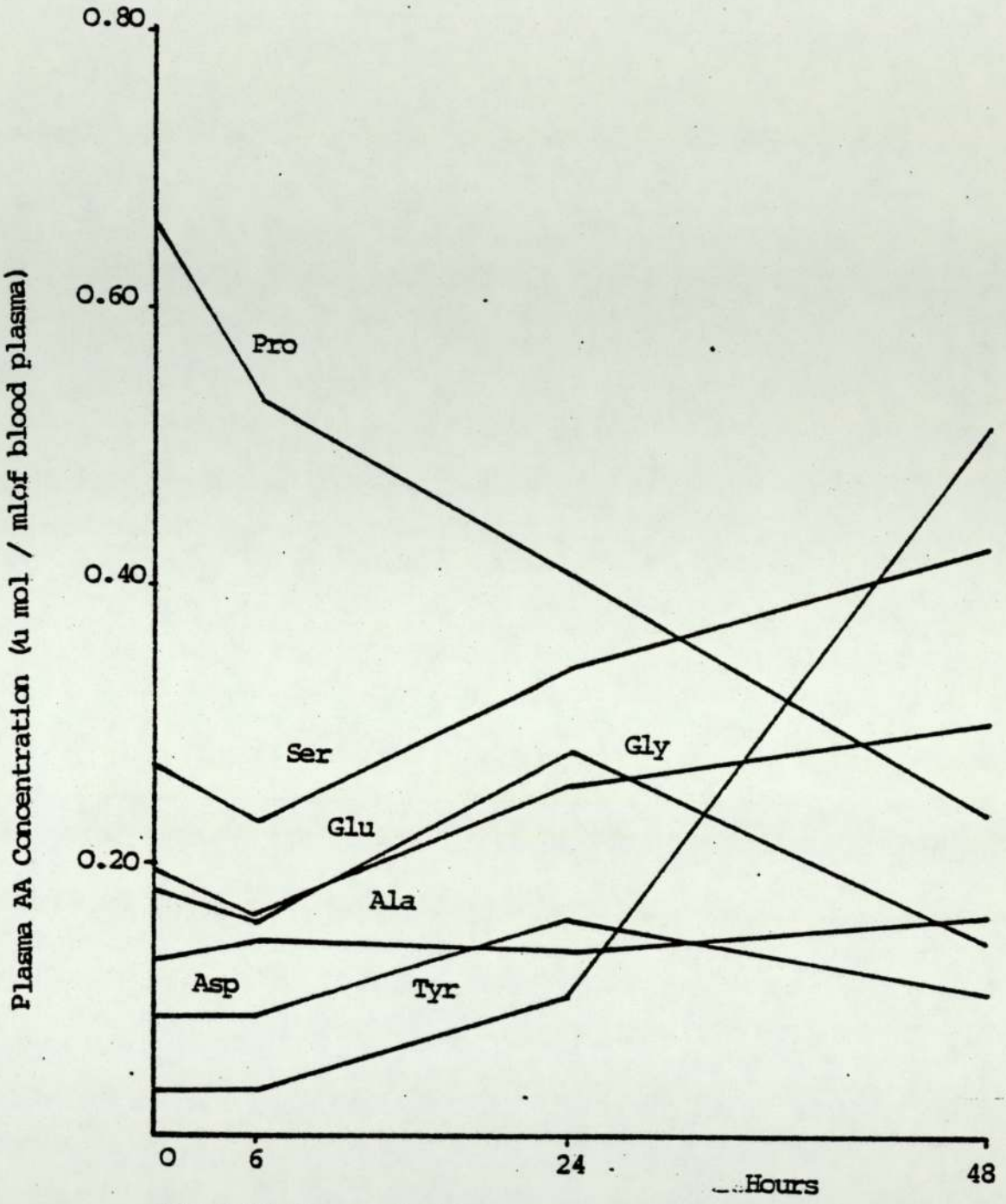

Fig 8.28. Change in Plasma FNEAA of Carp at Various intervals after Force-Feeding Fish meal diet ca 30 % Protein.

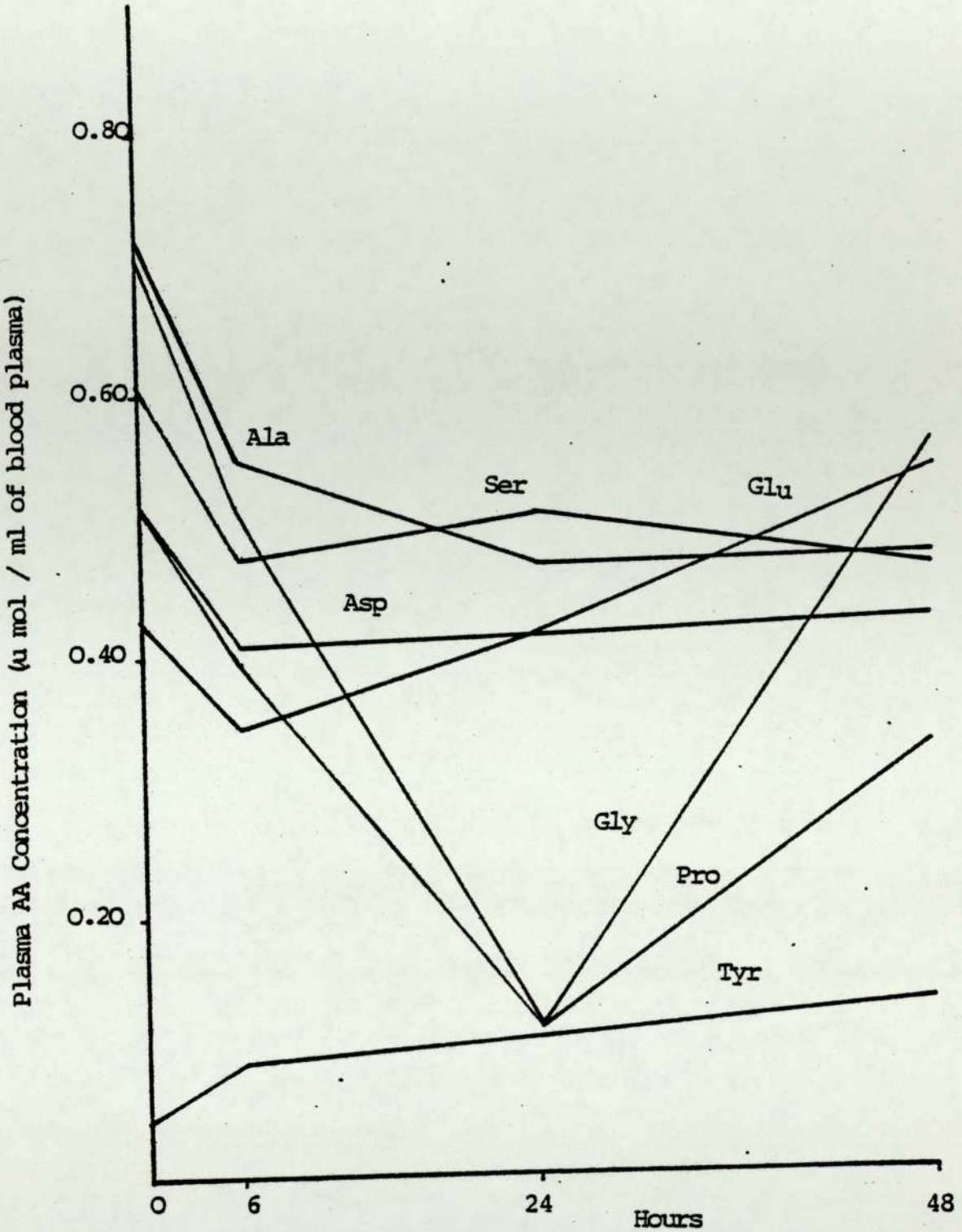

Fig 8.29. Change in Plasma FNEAA of Carp at Various intervals after Force-Feeding Whole egg protein ca 30 % Protein.

aspartate and tyrosine. Glutamate increases over this period. Glycine and proline concentrations fall very sharply at 24 h, then increase again at 48 h to around their 6 h values.

On the rapeseed diet (Fig 8.3Ø) there is a generally an initial decrease in NEAA concentrations at 6 h. Proline then rises slightly to 48 h, glutamine rises at 24 h and falls again, and serine continues to decrease to 48 h.

8.4.6. Total Free Ammonia.

Fig 8.31 shows the change of plasma free ammonia after force-feeding carp with four protein diets. Maximal concentration occurred at 24 h for carp fed casein and fish meal diets, and at 48 h for fish fed egg protein and rapeseed meal diets.

8.4.7. Total Protein Concentration.

The plasma protein concentration was found to increase slightly at 6 h after force-feeding (Table 8.16). It then gradually decreased and stabilized at 48 h postfeeding. There was no significant difference between the diets at all times of sampling.

8.5. Discusion & Conclusions.

Fujita et al., (1981) suggested that FAA do not directly reflect the AA composition or amount of ingested protein, but rather indirectly point to the total metabolism pattern in the tissues and their response to the quality and quantity of protein consumed. Nose (1972) found most AAs to attain maximum values between 12 and 24 h after feeding a commercial diet to trout, and then

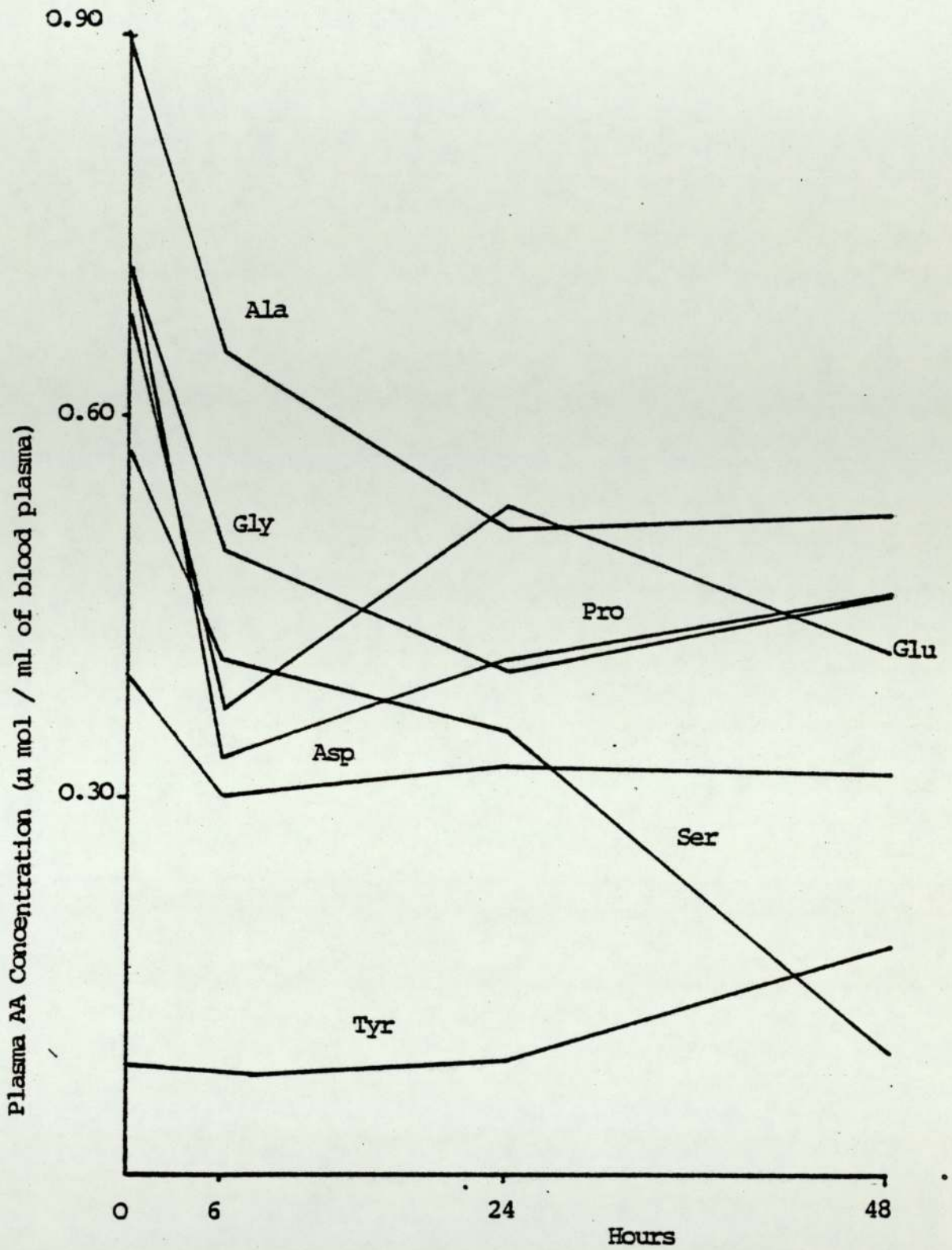

Fig 8.30. Change in Plasma FNEAA of Carp at Various Intervals after Force-Feeding with Rapeseed meal diet ca 30 % Protein.

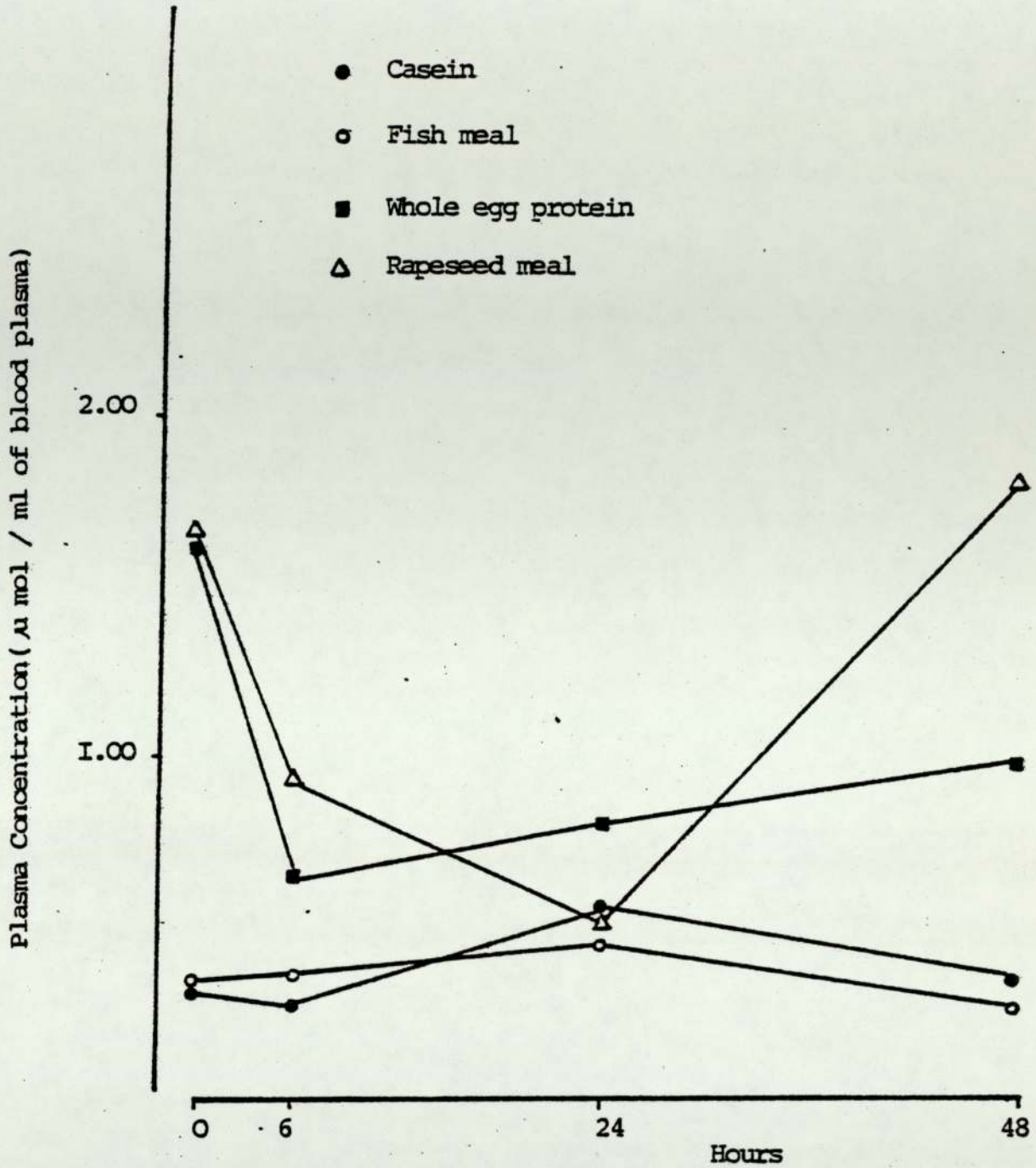

Fig 8.3I. Change in Plasma Ammonia of Carp at Various Intervals after Force-Feeding four Diets ca 30 % Protein.

Table 8.16. Change in Plasma Protein (mg/100 ml) in Carp after Force-Feeding Four Protein Diets at 30% protein.

Mean ± SD, Number of fish = 3

Diets	Hours after force-feeding			
	0	6	24	48
Casein, 7	3.10±0.10	3.40±0.24	3.30±0.25	3.10±0.10
Fish meal, 8	3.00±0.21	3.30±0.43	3.20±0.21	3.22±0.25
Whole egg, 9	3.06±0.26	3.32±0.21	3.10±0.32	3.10±0.33
Rapeseed, 10	3.10±0.20	3.20±0.10	3.20±0.20	3.10±0.10

to decline to their lowest values 72 h after feeding. Schlisio and Nicolai (1978) also found the same with trout fed on a diet containing free amino acids. These results are similar to those found in the present study with carp fed the casein, and to a lesser extent the fish meal diets. TFAA were virtually unchanged by the other four diets. This indicates that the first two diets are digested well and absorbed faster than the others.

The EAA concentrations in the casein diet (Table 8.3) are not all of the same order as in the plasma (Fig 8.4 and 8.5). Sometimes plasma amino acids are at a higher or lower concentration than those found in the casein diet. If NEAA undergo interconversion reactions it might be expected that their concentrations will become more similar; this appears to be borne out by Fig 8.1Ø & 8.11.

Frame (1958) reported that plasma AAs of normal adult man attained a maximum 1 to 4 h after a meal and reduced to the fasting value to 6 to 8 h afterwards. Using dogs, Longenecker and Hause (1959) tested the average of 5 h plasma AA concentration after 18 h fasting immediately before the meal to predict the limiting AAs in food. In rats, Yamamoto et al., (1974) reported that nitrogen derived from excess dietary AA was released from the tissues into the blood circulation in the form of glutamine, alanine, glycine and serine, and that these NEAA were considered to be the end products of AA metabolism

within the tissues and to serve as the nitrogen carriers from organ to organ.

The 6 h lag period prior to the increase in the FEAA in the plasma after force-feeding may be related to the digestive process. As already seen with the total FAA, there was a marked increase in plasma concentration 24 h after feeding casein and fish meal diets, because these were digested better and more quickly than diets 3-6.

Plakas et al., (198Ø) compared the plasma AA pattern of carp (weight 3Ø g) after feeding casein and corresponding AA mixture diets in investigating the reason for inability of FAAs to serve as the dietary nitrogen source for normal growth of carp. EAAs in the plasma of carp were at maximum concentration 4 h after ingestion of a casein diet, whereas with the AA diet, arginine and lysine peaked at 2 h post-feeding, then declined to pre-feeding values by 4 h, while leucine, isoleucine and valine reached maximum concentrations at 4 h post-feeding. The rates of absorption of individual pure AA may have differed markedly. Casein is digested slowly, and the absorption of AA takes place gradually, according to the rate of enzymatic liberation. In the present study with large carp (2.14 kg weight) the individual plasma EAA after feeding diets 1 and 2 peaked at 24 h, and the same AA with Diets 3, 4, 5 and 6 peaked at 48 h due to low absorption rate in large carp. Dabrowski (1983) reported that the absorption rate in carp larvae appears to be 21 times higher than in

adult carp of an individual weight 5ØØ g, and absorption rates of AAs in 5ØØ g carp at 22 °C appear slightly lower than in crocodilians (1ØØ g Wt. 28 °C). Assimilation of dietary protein into fish body protein is a highly efficient process if the carp body increases 1Ø^{6} in size in one year. Dabrowski stated this figure is the higest in the animal kingdom. The results presented in the present study agrees with Dabrowski (1983) as unlike the work of Plakas et al., (198Ø).

In the present study with carp it was found that the peak of ammonia production is similar to those of TFAA for all diets. This indication of a higher generation rate of ammonia at 24 h for casein and fish meal diets (at ca 15 & 3Ø% protein), at 48 h for diets 3-6 at ca 15% and diet 9 and 1Ø at ca 3Ø% protein, would support the hypothesis of a high rate of AA catabolism occurring after force-feeding.

The overall plasma AA patterns of big carp in the present study appear to differ from those of other fish species to varying degrees. In channel catfish (Wilson et al., 1985), plasma AA peaked within 4 h after feeding purified diets with intact protein, and returned to baseline values within 24 h after feeding. With each diet tested in channel catfish, most of the plasma FAA increased within 2 h after feeding, remained elevated for up to 12 h, and returned to fasting values within 24 h after feeding. Rainbow trout (Salmo gairdneri) plasma FAA have been seen to peak much later (24 to 36 h)

after feeding and not return to baseline until 48-96 h after feeding (Schlisio and Nicolai, 1978; Yamada et al. 1981). The differing responses indicated by these studies may, in part, be attributed to different experimental procedures. In both rainbow trout, carp and channel catfish, the conditioning diets and experimental diets differed in composition. In the comparison of the different sources of protein and different protein concentrations (Experiment 7 & 8), the concentration of FAA in the blood seems to be related to the nature of the protein ingested. In view of several factors which interact in the plasma AA picture (AA withdrawal from the blood by the tissues, AA catabolism in the liver, degree of absorption at the intestinal wall, etc.), it is difficult to assign a definite significance to these different blood plasma AA concentrations as they relate to the AA make-up of the dietary proteins. Moreover, increasing the protein in carp diets did not have a significant effect on in the blood AA pattern. It was initially expected to see a greater increase in circulating AA to correspond to the increasing protein fed, and to see a more rapid removal of circulating EAA by the tissues at the lower dietary protein; however, these changes did not occur. Perhaps if one could measure the AA in portal blood after feeding, one could observe a graded response of the absorbed AA to increasing dietary protein. It appears that the liver must exert a major control function in regulating circulating FAA

after a meal, because the circulating TFAA and TFEAA were not similar, regardless of the amount of protein fed (in experiment 7 & 8). This apparent regulatory effect of the liver would appear to function in ensuring that the extrahepatic tissues could make maximum utilization of dietary AA for protein synthesis. There was no apparent effect of the amount in the test diets on circulating AA patterns. Similar results were found by Wilson et al., (1985). They found that varying dietary protein to energy ratios in the diets did not profoundly affect postprandial systemic serum FAA patterns for channel catfish.

The sequential phase of fluctuation in FAA composition and content in plasma AA due to food intake might differ among species of experimental animals used and also with rearing temperature. Quality of experimental diets and time after feeding can also affect the response of fish. Especially, the last factor can drastically affect tissue FAA profiles (Ogata, 1986). Therefore, when a study on FAA dynamics under various nutritional and physiological conditions is conducted, the nutritional history of the experimental fish should be thoroughly studied in advance.

The results of experiment 8 were similar to those obtained by Nose (1972) and Schlisio and Nicolai (1978) for casein and fish meal diets.

8.6. Plasma Amino Acid Index (PAAI).

The peaksat 24 h represent absorption of AA released by

digestion of dietary protein, and PAA values at 48 h after force-feeding represent a new steady state and no longer reflect the AA composition of the dietary protein.

The change of plasma EAAs after 24 h for the fish fed the diets given in experiments 7 and 8 were calculated. An index of protein quality using whole egg protein values as standard was computed for casein (Table 8.17), fish meal (Table 8.18), soybean meal (Table 8.19), rapeseed meal (Table 8.2Ø) and sunflower (Table 8.21) for experiment 7 (ca 15 % protein) and for experiment 8, casein (Table 8.22), fish meal (Table 8.23) and rapeseed meal (Table 8.24). This index is an adaptation of the method of Oser (1951) for EAAI to PAA. PAAI is the geometric mean of the egg ratios of 24 h PAA changes.

Also, PAAI was calculated from PAA data for one carp at 48 h for in both experiments 7 and 8 (Table 8.25 and 8.26). Moreover, EAAI for all experimental diets were calculated using AA composition of whole egg protein and carp edible protein or EAA requirements for carp (Tables 8.3 & 8.4). It was found that EAAI using whole egg protein as standard for blood (PAA) shows the same trend of EAAI for all diets except for sunflower. From these result of experiment 7, a positive correlation was found between EAAI for diets 3, 4, 5 and 6 and the corresponding values at 48 h (r= Ø.9Ø6), and a strong negative correlation between EAAI for diets 1 and 2 and corresponding values at 24 h (r= - 1ØØ). A positive

Table 8.17. Calculation of PEAAI Using Egg ratios of 24h Changes in Plasma EAA after Force-Feeding Carp with Casein Diet at ca 15% Protein.

AA	Egg protein μ mol/ml	Casein μ mol/ml	Egg ratio	Corrected ratio	Log ratio
Thr	Ø.47Ø	2.415	513.82	1ØØ.ØØ	2.ØØØ
Val	Ø.3Ø6	1.626	531.37	1ØØ.ØØ	2.ØØØ
Met	Ø.1Ø6	Ø.875	825.47	1ØØ.ØØ	2.ØØØ
Ile	Ø.182	1.628	894.5Ø	1ØØ.ØØ	2.ØØØ
Leu	Ø.299	2.947	985.61	1ØØ.ØØ	2.ØØØ
Phe+					
Tyr	Ø.293	2.484	847.78	1ØØ.ØØ	2.ØØØ
His	Ø.169	1.388	821.3Ø	1ØØ.ØØ	2.ØØØ
Lys	1.162	Ø.854	73.49	73.49	1.866
Arg	Ø.4Ø2	Ø.286	71.14	71.14	1.852
				Total	17.718
				Ave.Log	1.968

PAAI is the Geometric Mean: Antilog 1.968 = 92.89

Table 8.18. Calculation of PEAAI Using Egg ratios of 24h Change in PEAA after Force Feeding Carp With Fish meal Diet at ca 15% protein.

AA	egg protein	Fish meal	egg	Corrected	Log
	u mol/ml	u mol/ml	ratio	ratios	ratio
Thr	Ø.47Ø	Ø.79Ø	168.Ø8	1ØØ.ØØ	2.ØØØ
Val	Ø.3Ø6	Ø.586	191.5Ø	1ØØ.ØØ	2.ØØØ
Met	Ø.1Ø6	Ø.185	174.52	1ØØ.ØØ	2.ØØØ
Ile	Ø.182	Ø.359	197.25	1ØØ.ØØ	2.ØØØ
Leu	Ø.299	Ø.597	199.66	1ØØ.ØØ	2.ØØØ
Phe+					
Tyr	Ø.293	Ø.555	189.41	1ØØ.ØØ	2.ØØØ
His	Ø.169	Ø.368	217.75	1ØØ.ØØ	2.ØØØ
Lys	1.162	1.699	146.21	1ØØ.ØØ	2.ØØØ
Arg	Ø.4Ø2	1.Ø38	258.2Ø	1ØØ.ØØ	2.ØØØ
				Total	18
				Av.Log	2

PAAI is the Geometric Mean: Antilog 2 = 1ØØ

Table 8.19. Calculation of Plasma EAAI Using Egg Ratios of 24h change in Plasma EAA after Force-Feeding Carp with Soybean meal Diet at ca.15% Protein.

AA	Egg protein μ mol/ml	Soybean meal μ mol/ml	Egg ratio	corrected ratios	Log ratio
Thr	Ø.47Ø	Ø.596	126.8Ø	1ØØ.ØØ	2.ØØØ
Val	Ø.3Ø6	Ø.265	86.6Ø	86.6Ø	1.937
Met	Ø.1Ø6	Ø.178	167.92	1ØØ.ØØ	2.ØØØ
Ile	Ø.182	Ø.226	124.17	1ØØ.ØØ	2.ØØØ
Leu	Ø.299	Ø.386	129.Ø9	1ØØ.ØØ	2.ØØØ
Phe+					
Tyr	Ø.293	Ø.349	119.11	1ØØ.ØØ	2.ØØØ
His	Ø.169	Ø.25Ø	147.92	1ØØ.ØØ	2.ØØØ
Lys	1.162	Ø.361	31.Ø6	31.Ø6	1.492
Arg	Ø.4Ø2	Ø.531	132.Ø8	1ØØ.ØØ	2.ØØØ
				Total	17.42
				Av.Log	1.936

Plasma AAI is the Geometric Mean: Antilog 1.936 = 86.29

Table 8.2Ø. Calculation of PEAAI Using Egg Ratios of 24 h after Force-Feeding Carp With Rapeseed meal Diet at ca. 15% protein.

AA	Egg protein µ mol/ml	Rapeseed µ mol/ml	Egg ratio	Corrected ratio	Log ratio
Thr	Ø.47Ø	Ø.31Ø	65.95	65.95	1.819
Val	Ø.3Ø6	Ø.ØØ83	27.12	27.12	1.433
Met	Ø.1Ø6	Ø.154	145.28	1ØØ.ØØ	2.ØØØ
Ile	Ø.182	Ø.199	1Ø9.34	1ØØ.ØØ	2.ØØØ
Leu	Ø.299	Ø.3Ø3	1Ø1.33	1ØØ.ØØ	2.ØØØ
Phe+					
Tyr	Ø.293	Ø.266	9Ø.78	9Ø.78	1.958
His	Ø.169	Ø.326	192.89	1ØØ.ØØ	2.ØØØ
Lys	1.162	Ø.1Ø3	8.86	8.86	Ø.947
Arg	Ø.4Ø2	1.693	421.14	1ØØ.ØØ	2.ØØØ
				Total	16.157
				Av.Log	1.795

PAAI is the Geometric Mean: Antilog 1.795 = 62.4Ø

Table 8.21. Calculation of PEAAI Using Egg Ratios of 24h Change in Plasma EAA after Force-Feeding Carp With Sunflower Diet at ca. 15% Protein.

AA	Egg protein µ mol/ml	Sunflower µ mol/ml	Egg ratio	Corrected ratio	Log ratio
Thr	Ø.47Ø	Ø.54Ø	114.89	1ØØ.ØØ	2.ØØØ
Val	Ø.3Ø6	Ø.367	119.93	1ØØ.ØØ	2.ØØØ
Met	Ø.1Ø6	Ø.157	145.2Ø	1ØØ.ØØ	2.ØØØ
Ile	Ø.182	Ø.452	248.35	1ØØ.ØØ	2.ØØØ
Leu	Ø.299	Ø.383	128.Ø9	1ØØ.ØØ	2.ØØØ
Phe+					
Tyr	Ø.293	Ø.318	1Ø8.53	1ØØ.ØØ	2.ØØØ
His	Ø.169	Ø.223	131.95	1ØØ.ØØ	2.ØØØ
Lys	1.162	Ø.9Ø4	77.79	77.79	1.89Ø
Arg	Ø.4Ø2	Ø.912	226.86	1ØØ.ØØ	2.ØØØ
				Total	17.89
				Av.Log	1.987

PAAI is the Geometric Mean: Antilog 1.987 = 97.22

Table 8.22. Calculation of PEAAI Using Egg Ratios of 24h Change in Plasma EAA after Force-Feeding Carp With Casein Diet at ca. 30% Protein.

AA	Egg protein µ mol/ml	Casein µ mol/ml	Egg ratio	Corrected ratio	Log ratio
Thr	Ø.4Ø5	Ø.6Ø1	148.39	1ØØ.ØØ	2.ØØØ
Val	Ø.41Ø	Ø.3Ø8	75.12	75.12	1.875
Met	Ø.173	Ø.176	1Ø1.73	1ØØ.ØØ	2.ØØØ
Ile	Ø.231	Ø.288	124.67	1ØØ.ØØ	2.ØØØ
Leu	Ø.411	Ø.371	9Ø.26	9Ø.26	1.955
Phe+					
Tyr	Ø.341	Ø.24Ø	7Ø.38	7Ø.38	1.847
His	Ø.264	Ø.252	95.45	95.45	1.979
Lys	1.Ø79	Ø.745	69.Ø4	69.Ø4	1.839
Arg	Ø.971	Ø.351	36.14	36.14	1.558
				Total	17.Ø5
				Av.Log	1.894

PAAI is the Geometric Mean: Antilog 1.894 = 78.48

Table 8.23. Calculation of PEAAI Using Egg Ratios of 24 h Change in Plasma EAA after Force-Feeding Carp With Fish meal Diet at ca. 30% Protein.

AA	Egg protein µ mol/ml	Fish meal µ mol/ml	Egg ratio	Corrected ratio	Log ratio
Thr	0.405	0.425	104.93	100.00	2.000
Val	0.410	0.412	100.48	100.00	2.000
Met	0.173	0.106	61.27	61.27	1.787
Ile	0.231	0.186	80.51	80.51	1.905
Leu	0.411	0.250	60.82	60.82	1.784
Phe+					
Tyr	0.341	0.409	119.94	100.00	2.000
His	0.264	0.204	77.72	77.27	1.888
Lys	1.079	1.100	101.94	100.00	2.000
Arg	0.971	0.904	93.09	93.09	1.968
				Total	17.23
				Av.Log	1.914

PAAI is the Geometric Mean: Antilog 1.914 = 82.24

Table 8.24. Calculation of PEAAI Using Egg Ratios of 24 h Change in Plasma EAA after Force-Feeding Carp With Rapeseed meal diet at ca. 30% Protein.

AA	Egg protein µ mol/ml	Rapeseed µ mol/ml	Egg ratio	Corrected ratio	Log ratio
Thr	Ø.4Ø5	Ø.417	1Ø2.96	1ØØ.ØØ	2.ØØØ
Val	Ø.41Ø	Ø.413	1ØØ.73	1ØØ.ØØ	2.ØØØ
Met	Ø.173	Ø.154	89.Ø1	89.Ø1	1.949
Ile	Ø.231	Ø.258	111.86	1ØØ.ØØ	2.ØØØ
Leu	Ø.411	Ø.443	1Ø7.78	1ØØ.ØØ	2.ØØØ
Phe+					
Tyr	Ø.341	Ø.2Ø4	59.82	59.82	1.776
His	Ø.264	Ø.2Ø8	78.78	78.78	1.896
Lys	1.Ø79	Ø.899	83.31	83.31	1.92Ø
Arg	Ø.971	Ø.571	58.8Ø	58.8Ø	1.769
				Total	17.3Ø5
				Av.Log	1.922

PAAI is the Geometric Mean: Antilog 1.922 = 83.71

Table 8.25. Comparison of PAAI Values at 24 & 48 h after Force-Feeding Carp with 6 diets (ca. 15% protein)

Diets	24 h	48 h
Casein, 1	92.89	81.26
Fish meal, 2	100.00	73.20
Egg protein, 3	100.00	100.00
Soybean meal, 4	86.29	95.41
Rapeseed meal, 5	62.40	91.43
Sunflower, 6	97.22	87.49

Table 8.26. Comparison of PAAI Values at 24 & 48 h after Force-Feeding diet at ca. 30% Protein.

Diets	24 h	48 h
Casein, 7	78.48	48.01
Fish meal, 8	82.24	100.00
Egg protein, 9	100.00	100.00
Rapeseed meal, 10	83.71	75.80

correlation was found between PEAAI and BV (r = Ø.773). Table 8.27 illustrates a comparison of EAAI from the previous study and data from Atack et al., (1979) and Ogino & Chen (1973a). A positive correlation was found (r = Ø.853) between PEAAI and corresponding values of EAAI obtained by Atack et al., (1979). Also, a positive correlation between PEAAI and BV (r = Ø.56) was obtained by Ogino & Chen (1973a).

Table 8.27. Comparison of Biological Values with Chemical Predictions of Protein Utilization.

Protein Source	PEAAI (1)	EAAI (3)	BV (2)
Casein	78.48	61	65
Fish meal	83.24	74	72
Whole egg protein	1ØØ.ØØ	-	7Ø
Soybean meal	86.4Ø	71	74
Fish meal	1ØØ.ØØ	-	76

(1) EAAI-Reference Whole egg protein.

(2) Data from Ogino & Chen (1973a).

(3) Data from Atack et al., (1979).

CHAPTER 9.

Chapter 9.

9. Experiment 8. The Adaptation of Digestive Proteases of Carp to Diets. (a) Long Term Adaptation.

9.1. Introduction.

Questions of the adaptation of the digestive system of fish to the qualitative composition of food have been insufficiently explained in the literature. Generally, the reports available refer to changes in the activity of the digestive enzymes in connection with type of feeding. It is noted that in carnivorous fish, the activity of the proteolytic enzymes is higher than in herbivorous. Thus, for instance, it has been established (Vonk, 1927) that in the pike, Esox lucius, the activity of the enzymes which break down the proteins is higher than in the carp, but that the amylolytic activity of the intestinal mucosa is 1000 times higher in the carp than in the pike.

The low amount of protein available to herbivorous fish and the resistant nature of plant material require adaptive mechanisms of digestion. Such mechanisms are represented by an elongated intestine (Silver carp, grass carp, Tilapia) or by the mechanical breakdown of plants with the help of pharyngeal teeth (Cyprinids). Another possibility of making the best use of the scarce proteins in the plants would appear to be the secretion of large amounts of proteases into the gut. However, the literature abounds with reports that specific proteolytic activity in the digestive tract of fish is

proportional to the protein content of the diet, and is thus higher in carnivorous than in herbivorous species (Kawai and Ikeda, 1972; Hofer, 1979). Hofer & Schiemer (1981) reported that taking into account specific activity, volume of the gut fluid and the number of gut fillings per day, food turns out to be exposed to higher proteolytic activity in the digestive tracts of herbivorous than in carnivorous fish. Buzinova (1971), studying the activity of the digestive enzymes in the grass carp and silver carp, established seasonal changes in the correlation of the activity of the amylases and trypsin connected with adaptation of these fish to vegetable food.

Thus, the study of the adaptation of the digestive enzymes to quality of food has hitherto been carried out mainly on various fish for the purpose of explaining their species differences or determining the changes in enzyme activity connected with seasonal changes in the nature of the food. Published data on the adaptation of proteolytic enzymes in carp reared on commercial and artificial foods are lacking.

The main purpose of this experiment was to study the adaptation of proteolytic enzymes in carp gut to protein quality.

9.2. Materials & Methods.

9.2.1. The experimental System, Animal and Enzyme Preparation.

The experimental facility used in the present study was

"System 2" as described in detail in section 2.1.2. The temperature was maintained at 25 ± 1 °C.

Eighty six young carp weighing 4Ø-6Ø g were divided into four groups (groups of 2Ø fish); 6 fish were killed at the beginning of the experiment. At 2, 4, 6, 8 and 16 days, four fish from each group were killed and dissected. The intestine and pancreas were removed, pooled, weighed, homogenized, centrifuged for 5 min at 15,ØØØ g and the supernatant diluted 1Ø- fold with Tris-HCl buffer pH 9.5. The supernatant was frozen for further analysis. Soluble proteins of gut fluid were determined by means of the Folin phenol method (Lowry et al., 1951).

9.2.2. The Experimental Diets.

Formulation of the diets was carried out by the general procedure described in section 2.2, and the ingredients used are listed in Table 9.1. The diets were formulated to be isonitrogenous (3Ø% protein) and as far as possible isoenergetic for total energy. The diets were prepared by wet extrusion as detailed in section 2.3. Proximate analysis (section 2.6) was carried out on samples of the diets and the results are presented in Table 9.1.

9.2.3. Feeding Rate.

The fish were fed twice a day between Ø8.3Ø h and 18.3Ø h with each feed being distributed over a period of 15-3Ø minutes. All groups were fed 4% of their body weight per day with allowance made for the moisture

Table 9.1. Composition of Experimental Diets.

	Diet No			
Ingredient %	1	2	3	4
Casein	35	-	-	-
Whole egg protein	-	31	-	-
Fish meal	-	-	44	-
Pruteen	-	-	-	43
Cod liver oil	9	9	9	9
Dextrin	2Ø	25	15	15
Starch	22	24	15	17
Cellulose	5	2	8	7
Chromic oxide	1	1	1	1
Binder (1)	2	2	2	2
Vitamin Mix (2)	4	4	4	4
Mineral Mix (3)	2	2	2	2
	1ØØ	1ØØ	1ØØ	1ØØ
Proximate Analysis(%).				
Moisture	1Ø.6Ø	9.2Ø	6.2Ø	1.9Ø
Protein	3Ø.2Ø	28.5Ø	3Ø.1Ø	31.ØØ
Lipid	9.ØØ	1Ø.1Ø	13.42	11.ØØ
Ash	9.8Ø	4.6Ø	7.4Ø	7.3Ø
NFE	4Ø.4Ø	47.6Ø	42.88	48.8Ø

(1) Carboxymethylcellulose, Sodium Salt, High Viscosity.

(2) Composition given in Table 2.3 Section 2.2.

(3) Composition given in Table 2.4 Section 2.2.

content of the diets. All carp had a good appetite throughout the experimental period. The experiment was conducted for 16 days.

9.2.4. Assay of Protease Activity.

All enzyme activities are expressed as total activities (amount of product produced/ml of gut fluid). Total proteolytic activity was determined by the method described in section 7.2.4.

9.2.5. Statistical Methods.

These were performed as detailed in section 2.7.5.

9.3. Results.

9.3.1. Effect of Protein Source on Total Proteolytic Activity in carp Intestine.

All groups of carp fed casein, whole egg protein and Pruteen showed a decrease in total proteolytic activity on the second day (Table 9.2, Fig 9.1). There is a significant difference ($P<Ø.Ø5$) between dietary groups except between casein and fish meal; Whole egg protein and fish meal ; whole egg protein and Pruteen.

At the 4th day total proteolytic activity increased for casein, whole egg protein and fish meal. There is a significant difference ($P<Ø.Ø5$) between whole egg protein and Pruteen; fish meal and Pruteen; and casein and Pruteen.

The highest proteolytic activity for casein and whole egg protein occurred at 6 days, while activity decreased for Pruteen and fish meal. There is a significant

Table 9.2. Effect of Protein Source on Total Proteolytic activity in Carp Intestine.(U ml^{-1} Gut Fluid).

Diet	Casein	Whole egg	Fish meal	Pruteen
Days				
Ø	4.57±Ø.21 (for all diets)			
2 a	4.5Ø ± Ø.26	3.Ø9 ± Ø.94	4.8Ø ± 1.16	3.Ø8 ± Ø.55
4 b	4.54 ± Ø.37	4.89 ± Ø.3Ø	4.81 ± Ø.58	2.52 ± Ø.28
6 c	5.34 ± 5.52	5.52 ± Ø.6Ø	4.73 ± Ø.13	1.63 ± Ø.21
8 d	3.8Ø ± Ø.42	2.41 ± Ø.6Ø	4.73 ± Ø.81	1.44 ± Ø.91
16e	4.95 ± Ø.58	4.25 ± Ø.16	4.98 ± Ø.26	3.16 ± Ø.13

a LSD $_{Ø.05}$ = Ø.83

b LSD $_{Ø.05}$ = Ø.66

c LSD $_{Ø.05}$ = Ø.84

d LSD $_{Ø.05}$ = Ø.35

e LSD $_{Ø.05}$ = Ø.32

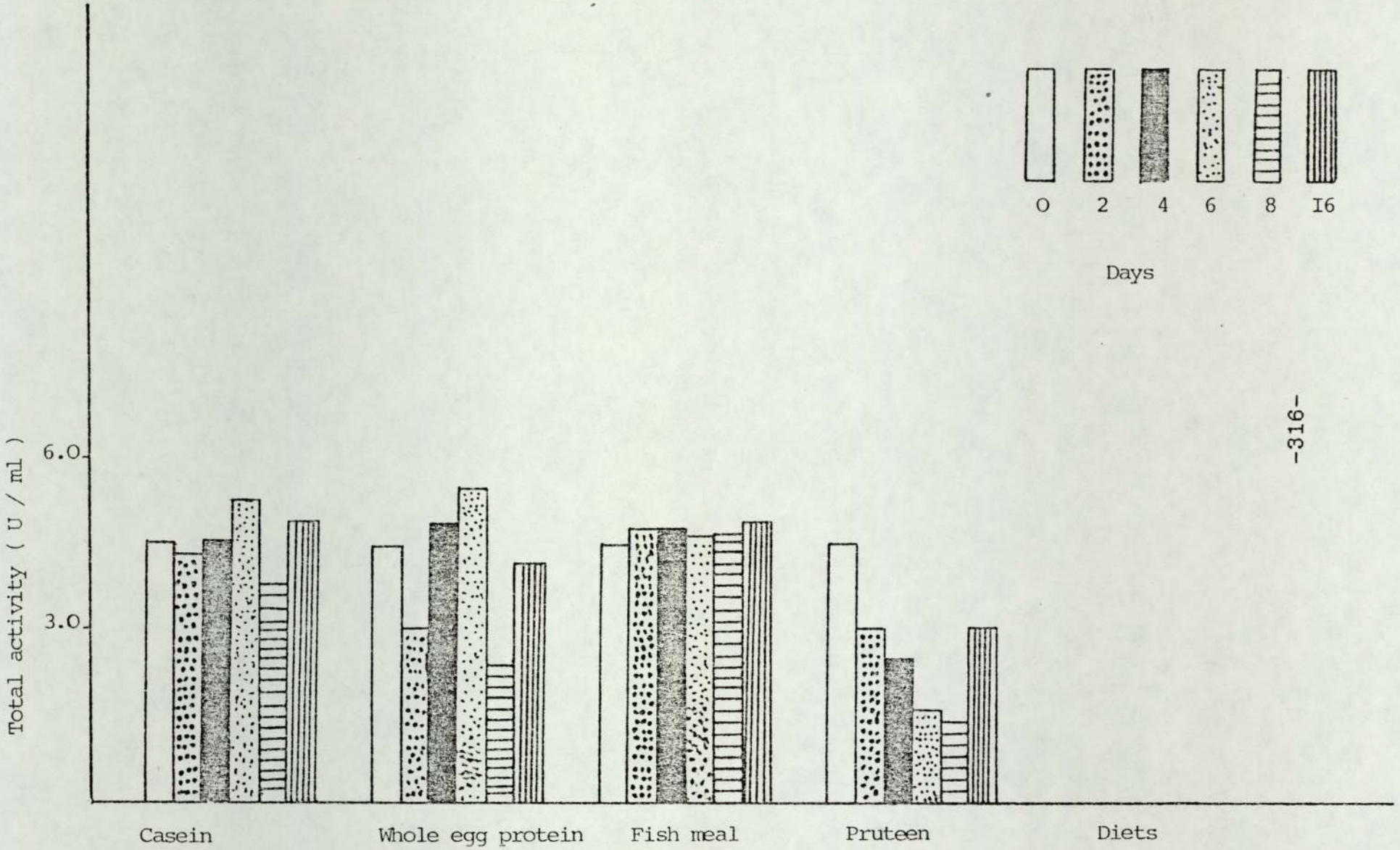

Fig 9.I.Effect of protein source on total proteolytic activity in carp intestine.

difference (P<Ø.Ø5) between all groups except between casein and whole egg protein; casein and fish meal; and whole egg and fish meal.

At the 8th day total proteolytic activity decreased for all groups except fish meal; there is a significant difference (P<Ø.Ø5) between all groups of carp fed casein, whole egg protein, fish meal and Pruteen. Total proteolytic activity attained its maximum value at 16 days for carp fed fish meal and Pruteen. There is a significant difference (P<Ø.Ø5) between dietary groups except for casein and fish meal.

9.3.2. **Effect of Protein Source on Total Proteolytic Activity for Carp Pancreas.**

Table 9.3 and Fig 9.2 show that total proteolytic activity decreased for all groups except casein. There was a significant difference (P<Ø.Ø5) between casein and whole egg protein; casein and fish meal; casein and Pruteen and whole egg protein, fish meal at 2 days. At the 4th day activity increased for all groups except the Pruteen; there is a significant difference (P<Ø.Ø5) between all groups fed casein, whole egg pruteen, fish meal and Pruteen at 4, 6, 8 and 16 days after feeding. The peak values for casein were seen at 8 days; for whole egg at 16 days and for fish meal at 6 days.

Total proteolytic activity for carp intestine was higher than in the hepatopancreas, which contains the inactive state of enzymes until they enter the alimentary canal.

Table 9.3. Effect of Protein Source on Total Proteolytic Activity in Carp Pancreas (U ml^{-1} gut fluid).

Diets		Casein	Whole egg	Fish meal	Pruteen
Days					
Ø			2.84 ± Ø.62		
2	a	2.97 ± Ø.26	1.79 ± Ø.38	2.33 ± 1.Ø9	1.73 ± Ø.87
4	b	3.21 ± Ø.36	1.97 ± Ø.36	2.49 ± Ø.92	1.32 ± Ø.64
6	c	3.59 ± Ø.63	1.45 ± Ø.14	2.87 ± Ø.59	1.53 ± Ø.3Ø
8	d	3.75 ± Ø.38	1.67 ± Ø.16	2.49 ± Ø.63	1.Ø5 ± Ø.Ø2
16	e	3.6Ø ± Ø.14	2.95 ± Ø.21	2.32 ± Ø.26	1.82 ± Ø.38

a LSD $_{05}$ = Ø.1Ø

b LSD $_{05}$ = Ø.25

c LSD $_{05}$ = Ø.24

d LSD $_{05}$ = Ø.25

e LSD $_{05}$ = Ø.Ø9

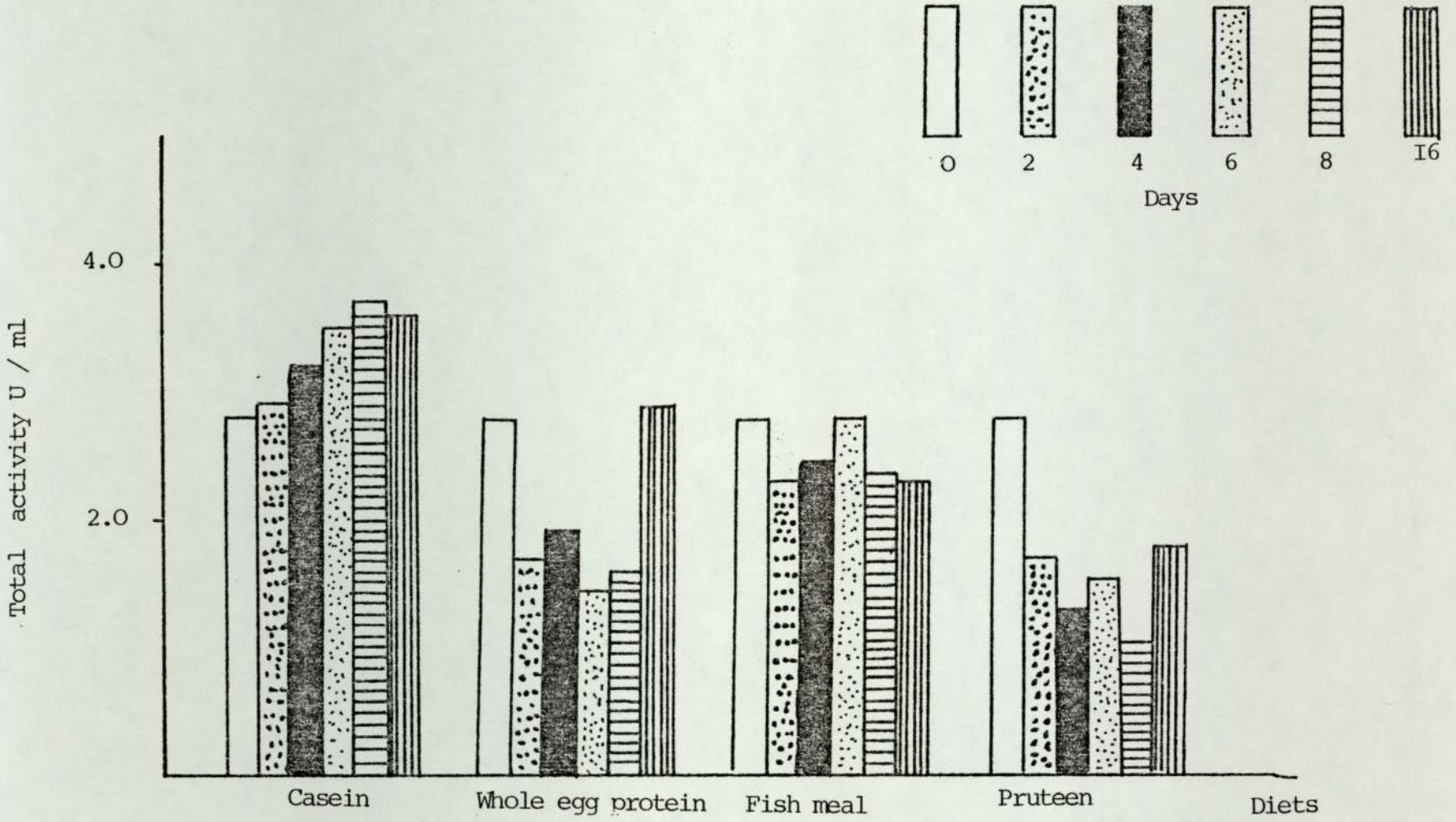

Fig 9.2.Effect of protein source on total proteolytic activity in carp pancreas.

9.4. Discussion.

The problems connected with rearing certain species of fish on artificial diets formed the background of the present investigation. Total proteolytic activity in the gut of carp depends on the quality of protein (Table 9.2 and 9.3). Similar results were found by Hofer (1979). Shambaugh (1954) found a positive correlation between the blood ingested and the subsequent protease activity in the midgut of the mosquito. However, Guth et el., (1956) found no such correlation between specific pancreatic enzymes in dogs and the kind of food they were fed within a few hours after feeding. This negative result may be due to the fact that adaptation to the diet was not possible because of the short interval of time (Prosser and Van Weel, 1958).

Lee et al., (1984) studied the relationships between source and amount of protein, size, and digestive protease enzyme activities of marine shrimp (Penaeus vannamei Boone). Three sizes of shrimp (4.Ø, 9.8, 2Ø.8 g) were fed with isocaloric diets. The protein sources were varied by changing the animal to plant protein ratio (a/p ratio), 2:1 and 1:1, while each of these two series was composed of three diets having protein at 22, 3Ø and 38% . The amount of protein in the diet had a greater effect on the total enzyme activities (activity per g wet tissue) in the large shrimp (17-3Ø g) than in small shrimp (<1Ø g). When the specific activities (activity per mg of protein in the extract)

of the fed shrimp were evaluated, small shrimp (<1Ø g) fed the 1:1 a/p ratio diets displayed lower activities than those fed the 2:1 ratio diet. The protein concentration influenced the enzyme activities in shrimp of all sizes while the protein source had a greater effect on the enzyme activities in small shrimp (<1Ø g). This differing proteolytic response to protein as a function of size supports the formulation of specific diets for shrimp of different sizes, taking into consideration the changes in digestive physiology as the shrimp grow. This might be applied to fish.

Kawai and Ikeda (1973 b) found that proteolytic activity of young carp showed adaptation to fish meal in the diet within a week, when the starch content in the diet was kept constant. This supports the finding in the present experiment.

According to Shlyin (1977), if there was no adaptation the digestive organ would produce higher amounts of all the enzymes; this would lead inevitably to undesirable alterations in the relationships of the digestive rates of different dietary components, and thus to changes in the absorption kinetics of nutrients and their further metabolism. Snook (1974) believes that adaptation would be useful in decreasing enzyme activity and thus in reducing endogenous loss. She also proposed that the physiological utility of adaptation could not be evidenced simply because the digestion-absorption processes are not a limiting step in the use of dietary components.

Total proteolytic activity for carp fed fish meal diet (Table 9.3) showed a better adaptation than the other diets; fish meal is a very good protein source and well utilized by fish. Similar results were obtained by Hofer (1979). Thus the digestive system of carp adapts to changes in the qualitative composition of the diet. This adaptation is expressed in changes of total proteolytic activity of the gut and hepatopancreas. Stroganov and Buzinova (1969) found in grass carp that tryptic activity in the intestine was higher than that in the pancreas again supporting the finding in the present study.

9.5. Experiment 9. The Adaptation of Digestive Enzymes to Protein Quality. (b) Short Term Adaptation.

9.5.1. Introduction.

Protein hydrolysis in vertebrates starts with "acid digestion" in the stomach. Hydrochloric acid denatures the proteins by destroying their tertiary structure. The increased molecular surface thus formed allows a faster and more complete hydrolysis, not only by pepsin in the stomach itself, but also by pancreatic endopeptidases (trypsin and chymotrypsin), which act in the neutral or slightly alkaline environment of the intestine. But in some families of fish, like Cyprinids, a stomach is not present at all. The proteins ingested by these fish are subjected to hydrolysis by the pancreatic endopeptidases without any preliminary treatment.

There are many physiological and biochemical consequences of the function of digestive processes in these fishes. Basically it is possible to distinguish two processes: digestion and absorption, but in stomachless fish, especially during early life, these processes are connected (Iwai, 1969). Documented data now exist demonstrating that the absorption of protein macromolecules in the intestine plays a considerable role in the feeding process of crucian carp (Yamamoto, 1966, Gauthier and Landis, 1972) common carp (Noaillac-Depeyre and Gas, 1973), and tench (Noadillac-Depeyre and Gas ,1973). Analysis of this phenomenon in fish nutrition requires the use of two types of techniques, microscopic-

cytological and analytical-biochemical, since it relates two problems: intracellular and extracellular digestion. The understanding of digestive enzyme activity in relation to diet is important in nutritional studies. This understanding must take into account the ability of enzymes to specifically hydrolyse individual materials in the diet, and variation in enzyme activity with age and season. The importance of hydrolytic activity in the digestive system of an animal is subordinate only to the composition and form of food and degree of mechanical trituration. The chemical breakdown of the food ultimately determines the types of nutrients which are available for absorption.

The principal objective of this research was to obtain information concerning the proteolytic enzymes present and changes in activities that occur in the digestive tract of carp (Cyprinus carpio), in response to type of protein in the diet.

9.5.2. Materials and Methods.

9.5.2.1. The Experimental System and Animals.

The experimental facility used in the present study was "System 2" as described in detail in section 2.1.2.

5Ø common carp (6-8 cm) were selected from those remaining from Experiment 1 (Chapter 3) that had been maintained on five diets.

Water temperature was maintained at 25 ± 1 °C. At 4 and 6 h after feeding, 5 fish from each group were killed and

dissected. The intestine was removed, pooled, weighed, divided into two parts, homogenized and centrifuged (Section 9.2.1).

9.5.2.2. The Experimental Diets.

Formulation of the diets was carried out by the general procedure described in section 2.2 and the ingredients used are listed in Table 3.1 (Chapter 3). The diets were formulated to be isonitrogenous (circa 39%) and as far as possible isoenergetic for total energy.

9.5.2.3. Feeding Rate.

The fish were fed once a day at 0900 am with feed being distributed over a period of 15-30 minutes. All groups were fed 4% of their body weight per day with allowance made for the moisture content of the diets. All carp had a good appetite throughout the experimental period. The experiment was conducted for 7 days.

9.5.2.4. Assay of Protease Activity.

All enzyme activities are expressed as total and specific (units per mg protein in the gut fluid). Total proteolytic activity was determined by the method described in section 7.2.4.

9.5.2.5. Statistical Methods.

These were performed by a T test (Snedecor & Cochran, 1980).

9.6. Results.

Highest total and specific proteolytic activities (Table 9.4 & 9.5) were found in the first half of the intestine, whereas towards the anus, activity decreased 4

and 6 h after feeding.

Total and specific proteolytic activity was higher for carp fed fish meal diet, followed by those fed casein, whole egg protein, Pruteen and low protein diet. A significant difference was found ($P<Ø.Ø5$) for specific activity in the first half of the intestine at 4 and 6 h after feeding.

In the second half of the intestine, total and specific activity was higher after 4 h in carp fed casein followed by those fed whole egg protein, Pruteen, fish meal and low protein diet. There was a significant difference ($P<Ø.Ø5$) for total proteolytic activity in the second half of the intestine between 4 and 6 h.

Protein content in the gut fluid was higher in the first half of the intestine than the second half (Table 9.4 & 9.5) 4 and 6 h after feeding.

Protein content in the crude enzyme was higher in fish fed casein followed by whole egg protein, Pruteen, fish meal and low protein diet (Table 9.4 & 9.5) at both times.

Analysis of the rate of absorption of protein breakdown products (Table 9.6) shows that the most active absorption is found in the second half of the gut.

A positive correlation ($r= Ø.87$) was found between total proteolytic activity and protein content in gut extract.

Table 9.4. Total and Specific Proteolytic Activity of Gut Homogenate Supernatant of Carp 4 h after Feeding (Mean of 5 Samples ± SD).

Diets	1st half of intestine		
	Protein in crude enzyme mg/ml	Total activity U/ml	Specific activity U/mg protein
Casein	22.8Ø ± 4.44	7.12 ± 2.1Ø	Ø.31 ± Ø.11
Whole egg	22.5Ø ± 3.74	5.11 ± 1.34	Ø.23 ± Ø.Ø7
Fish meal	19.2Ø ± 3.54	7.79 ± 2.98	Ø.41 ± Ø.Ø9
Pruteen	2Ø.2Ø ± 2.34	5.1Ø ± 1.6Ø	Ø.25 ± Ø.Ø8
No protein	13.25 ± 3.53	1.Ø1 ± 1.21	Ø.Ø8 ± Ø.Ø2

Diets	2nd half of intestine		
	Protein in crude enzyme mg/ml	Total activity U/ml	Specific activity U/mg protein
Casein	17.2Ø ± 4.34	4.81 ± 2.2Ø	Ø.28 ± Ø.22
Whole egg	17.1Ø ± 4.25	4.74 ± 1.98	Ø.27 ± Ø.11
Fish meal	14.2Ø ± 3.76	3.4Ø ± 1.12	Ø.24 ± Ø.22
Pruteen	16.28 ± 1.31	3.59 ± 1.32	Ø.22 ± Ø.16
No protein	1Ø.2Ø ± 3.25	Ø.85 ± 1.1Ø	Ø.Ø8 ± Ø.Ø6

Table 9.5. Total and Specific Proteolytic Activity of Gut Homogenate Supernatant of Carp 6 h after Feeding.

Diets	1st Half of Intestine		
	Protein in crude enzyme mg/ml	Total Proteolytic activity U/ml	Specific activity U/mg protein
Casein	23.25 ± 4.97	7.92 ± Ø.Ø6	Ø.34 ± Ø.16
Whole egg	22.79 ± 4.28	5.77 ± 2.24	Ø.25 ± Ø.22
Fish meal	19.41 ± 3.95	8.1Ø ± 1.75	Ø.41 ± Ø.Ø7
Pruteen	2Ø.26 ± 3.72	5.2Ø ± Ø.25	Ø.25 ± Ø.11
No protein	13.25 ± 5.13	Ø.38 ± Ø.16	Ø.Ø2 ± Ø.Ø8

	2nd half of intensin		
Diets	protein in crude enzyme mg/ml	Total activity U/ml	Specific activity U/mg protein
Casein	17.38 ± 4.97	4.9Ø ± 2.17	Ø.28 ± Ø.16
Whole egg	17.27 ± 4.26	4.Ø2 ± 1.75	Ø.23 ± Ø.Ø6
Fish meal	14.74 ± 3.6Ø	4.2Ø ± 1.Ø9	Ø.28 ± Ø.Ø7
Pruteen	16.91 ± 3.6Ø	3.6Ø ± 1.22	Ø.21 ± Ø.Ø7
No protein	1Ø.66 ± 3.29	Ø.29 ± Ø.16	Ø.Ø2 ± Ø.Ø8

Table 9.6. Protein Content (mg/ml) in the Gut Extract for Carp Fed 4 Protein Sources (Mean ± SD.)

Diet	Time after Feeding	
	4 h	6 h
Casein		
Anterior	22.2Ø ± Ø.95	21.5Ø ± Ø.71
Posterior	19.5Ø ± Ø.7Ø	2Ø.ØØ ± Ø.61
Whole egg protein		
Anterior	22.5Ø ± Ø.5Ø	21.ØØ ± 1.ØØ
Posterior	21.ØØ ± 1.53	19.ØØ ± 1.ØØ
Fish meal		
Anterior	21.5Ø ± 1.52	22.ØØ ± 1.ØØ
Posterior	2Ø.ØØ ± 1.ØØ	2Ø.5Ø ± Ø.5Ø
Pruteen		
Anterior	21.ØØ ± Ø.5Ø	22.ØØ ± Ø.5Ø
Posterior	21.ØØ ± Ø.7Ø	21.5Ø ± Ø.5Ø
No Protein		
Anterior	12.ØØ ± 1.ØØ	12.5Ø ± Ø.5Ø
Posterior	12.5Ø ± Ø.5Ø	12.5Ø ± Ø.5Ø

9.7. Discussion.

A decrease of proteolytic activity in the hindgut occurs both in stomachless fish and in those with stomachs, and also in mammals (Hofer & Schiemer, 1981; Diamond, 1978). Bitterlich (1985) found that tryptic activity was higher in the first one-third of the gut in silver carp. The same phenomenon has been observed in tilapia (Hofer & Schimer, 1981) but its cause is not clear.

Hofer and Uddin (1985) found tryptic activity was positively correlated with the diet under natural conditions. A similar relationship between proteolytic activity and diet was found with adult roach (Hofer, 1979). Enzyme activities in the intestine are a function of diet, filling of the gut, temperature, water chemistry, pH, age of fish and oxygenous enzymes (Reichenbach-Klinke, 1969; Hofer, 1979). The activities of the "major" alkaline proteases in the digestive tract of teleosts adapt to the quantitative and qualitative protein content in the diet (Brett and Higgs, 197Ø) and this supports the finding in the present experiment. Thus the digestive system of the carp adapts to changes in the qualitative composition of the diet. This adaptation is expressed in changes of total proteolytic activity of the intestinal mucous coat and also in rate of absorption of products of the breakdown of crude protein. A comparison of the data on protein reabsorption with the distribution of protease activity along the alimentary canal, allows the assumption that high indicators of

protein resorption in the anterior part of the alimentary canal are conditioned not so much by high activity of proteolytic enzymes as by other reasons (Pegel et al., 1971). Towards the second half of the alimentary canal, the rate of reabsorption of crude protein decreases, irrespective of the increasing number of enzymes absorbed by the mucous coat with all diets. This also agrees with the data of the above-mentioned authors, who demonstrated absorption of enzymes from the hind portion of the intestine in fish.

9.8. Experiment 9. Studies on Digestive Proteases of Carp Gut. (c) Enzyme Activity throughout the intestine.

9.8.1. Introduction.

Digestion processes in fish are little known and this may be one of the reasons for several difficulties in fish feeding. Proteolytic enzymes in the intestine enable the animal to utilize the proteins in the diet and consequently, they directly affect the growth of an organism. Properties of these enzymes are best known in carp (Onishi et al., 1973 a, b), rainbow trout (Kawai and Ikeda, 1973 a; Rungrungsak and Utne, 1981), mackerel (Ooshiro, 1971), tilapia (Negase, 1964), roach & rudd (Hofer, 1979). Activity of the enzymes was observed in the youngest developmental stages (Kawai & Ikeda, 1973 a, b). The effect of the diet on proteolytic activity of digestive organs was also studied in relation to the moment of application of food (Kawai & Ikeda, 1972; Onishi et al., 1973 b). The principal objective of this section of the research was to obtain information concerning the activity of the proteolytic enzyme in different sections of the gut and changes in the activities that occur in the digestive tract of carp.

9.9. Material & Methods.

9.9.1. The Experimental System and Animals.

The experimental facility used in the present study was "System 2" as described in detail in section 2.1.2.

Six adult carp from laboratory stock 2.40-3.50 kg body

weight, were divided into two tanks (3 fish per tank). Water temperature was maintained at 25 ± 1 °C.

9.9.2. The Experimental Diet.

Formulation of the diet was carried out by the general procedure described in section 2.2 and the ingredients used are listed in Table 9.7. The carp were fed a diet contaning 30% protein for three weeks in order to acclimatize them to a diet known to meet the protein requirement for their size.

9.9.3. Feeding Rates.

The fish were fed once a day at 1000 h, feed being distributed over a period of 15-20 minutes. Both groups were fed 2% of their body weight per day with allowance made for the moisture content of the diet.

9.9.4. Preparation of Enzyme Solution.

The first group of 3 fish was killed five hours after feeding, and the second group 24 hours after feeding. The intestine, pancreas and liver were removed and weighed. The intestine was divided into 12 equal sections. The enzyme extract was prepared as described in section 9.2.1.

9.9.5. Proteolytic Activity.

Total and specific proteolytic activity were determined by the method discribed in section 5.

9.9.6. Statistical Methods.

These were performed as detailed in section 9.5.2.5.

Table 9.7. Composition of Experimental Diet.

Ingredient	%
Fish meal	44
Cod liver oil	9
Dextrin	15
Starch	15
Cellulose	8
Binder (1)	2
Vitamin Mix (2)	4
Mineral Mix (3)	2
Chromic Oxide	1
Proximate Analysis (%).	
Moisture	6.2Ø
Protein	3Ø.1Ø
Lipid	13.42
Ash	7.4Ø
NFE	42.88

(1) Carboxymethylcellulose, Sodium Salt, High Viscosity.

(2) Composition given in Table 2.5.

(3) Composition given in Table 2.4.

9.1Ø. Results.

The gut of common carp is 2.2 times longer than its body (Table 9.8). Total (U/ml of gut fluids) and specific proteolytic activity (U/mg protein of gut) increased from the first to the third gut segment and decreased rapidly thereafter (Table 9.9, 9.1Ø; Fig 9.3 & 9.4). Total and specific proteolytic activity was higher in the first and third segments of the gut at 5 h than at 24 h after feeding.

Fig 9.3 shows that specific activity was high in segment 1-8 after 5 h and low in 1Ø-12; but after 24 h, activity was high in segments in 1-6 only and low in segments 7-12. Thus between 5 and 24 h, segments 7,8 and 9 change their activity significantly ($P<Ø.Ø5$). A similar picture is shown by Fig 9.4. Here, the 5 h activity in segments 1-8 is slightly higher than the 24 h in segments 1-6.

The average proteolytic activity (Total & Specific) is higher 5 h after feeding than 24 h (Table 9.9). However, for an understanding of the digestive process, not only the enzymatic activity but also the retention time of food in the gut is relevant. Unfortunately, little information is availabe on the gut passage time in fish (Table 9.11).

The proteolytic activity generally decreased from foregut to hindgut. This is illustrated in Fig 9.3 & 9.4. The difference between proteolytic activity in the first and last segments of the intestinal tract is here considered to reflect the "reabsorption" of proteolytic

Table 9.8. Data on Carp used for Enzyme Assay (Mean ± SD).

Group	Time after feeding	
	5h	24h
Body wt (kg)	3.32±Ø.32	2.6Ø±Ø.48
Body length (cm)	56.5Ø±2.12	57.ØØ±1.5Ø
Gut wt (g)	162.94±44.6Ø	13Ø.Ø5±42.7Ø
Gut length (cm)	126.5Ø±36.1Ø	126.ØØ±8.48

Table 9.9. Protein concentration and Proteolytic Activity of Intestinal Homogenate Supernatant Fluid in Different Gut Sections of Carp, 5 h After Feeding.

Gut Section No	Protein in Gut Fluid U/ml	Total Activity U/ml	Specific Activity U/ mg
1	2Ø.2Ø ± Ø.12	4.58 ± Ø.59	Ø.23 ± Ø.12
2	18.3Ø ± Ø.32	4.8Ø ± Ø.83	Ø.26 ± Ø.Ø8
3	18.2Ø ± Ø.37	4.5Ø ± Ø.4Ø	Ø.25 ± Ø.Ø8
4	18.3Ø ± Ø.4Ø	4.53 ± Ø.34	Ø.24 ± Ø.Ø9
5	18.5Ø ± Ø.22	4.56 ± Ø.22	Ø.24 ± Ø.11
6	18.1Ø ± Ø.34	4.5Ø ± Ø.11	Ø.24 ± Ø.15
7	16.5Ø ± Ø.3Ø	4.Ø1 ± Ø.46	Ø.24 ± Ø.18
8	17.5Ø ± Ø.24	4.25 ± Ø.1Ø	Ø.24 ± Ø.18
9	16.5Ø ± Ø.36	3.Ø5 ± Ø.43	Ø.18 ± Ø.17
1Ø	15.5Ø ± Ø.35	Ø.44 ± Ø.Ø2	Ø.Ø3 ± Ø.27
11	16.2Ø ± Ø.44	Ø.32 ± Ø.Ø7	Ø.Ø2 ± Ø.32
12	16.5Ø ± Ø.54	Ø.3Ø ± Ø.13	Ø.Ø2 ± Ø.34

Table 9.1Ø. Protein Concentration and Proteolytic Activity of intestinal Homogenate Supernatant Fluid in Different Gut Sections of the Carp, 24 h After Feeding (Mean ± SD).

Gut Section No	Protein in Gut fluid mg/ml	Total Activity U/ml	Specific Activity U/mg Protein
1	2Ø.1Ø ± Ø.22	4.ØØ ± Ø.Ø5	Ø.2Ø ± Ø.36
2	18.2Ø ± Ø.23	4.24 ± Ø.Ø6	Ø.23 ± Ø.36
3	17.8Ø ± Ø.88	3.89 ± Ø.Ø5	Ø.22 ± Ø.36
4	17.7Ø ± Ø.24	4.24 ± Ø.Ø6	Ø.24 ± Ø.36
5	16.5Ø ± Ø.33	4.1Ø ± Ø.Ø5	Ø.25 ± Ø.15
6	16.5Ø ± Ø.72	3.72 ± Ø.Ø5	Ø.23 ± Ø.16
7	16.ØØ ± Ø.5Ø	Ø.9Ø ± Ø.Ø5	Ø.Ø6 ± Ø.22
8	17.ØØ ± Ø.54	Ø.64 ± Ø.22	Ø.Ø4 ± Ø.21
9	16.1Ø ± Ø.77	Ø.68 ± Ø.13	Ø.Ø4 ± Ø.Ø4
1Ø	15.1Ø ± Ø.65	Ø.64 ± Ø.Ø6	Ø.Ø4 ± Ø.23
11	15.5Ø ± Ø.62	Ø.32 ± Ø.Ø5	Ø.Ø2 ± Ø.25
12	14.1Ø ± Ø.58	Ø.32 ± Ø.Ø4	Ø.Ø2 ± Ø.22

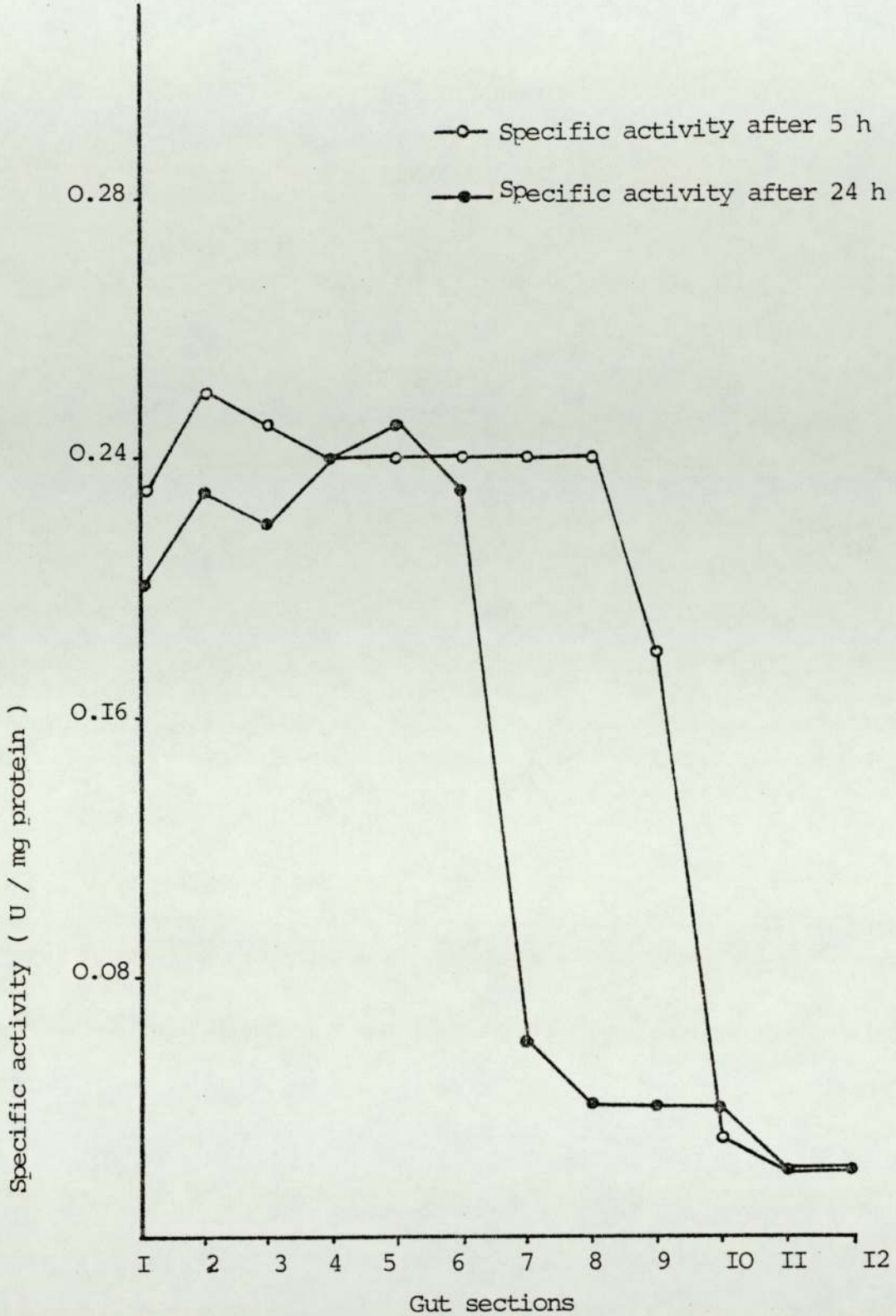

Fig 9.3. Specific Proteolytic Activity (per mg protein in the gut fluid) from different gut section of Carp.

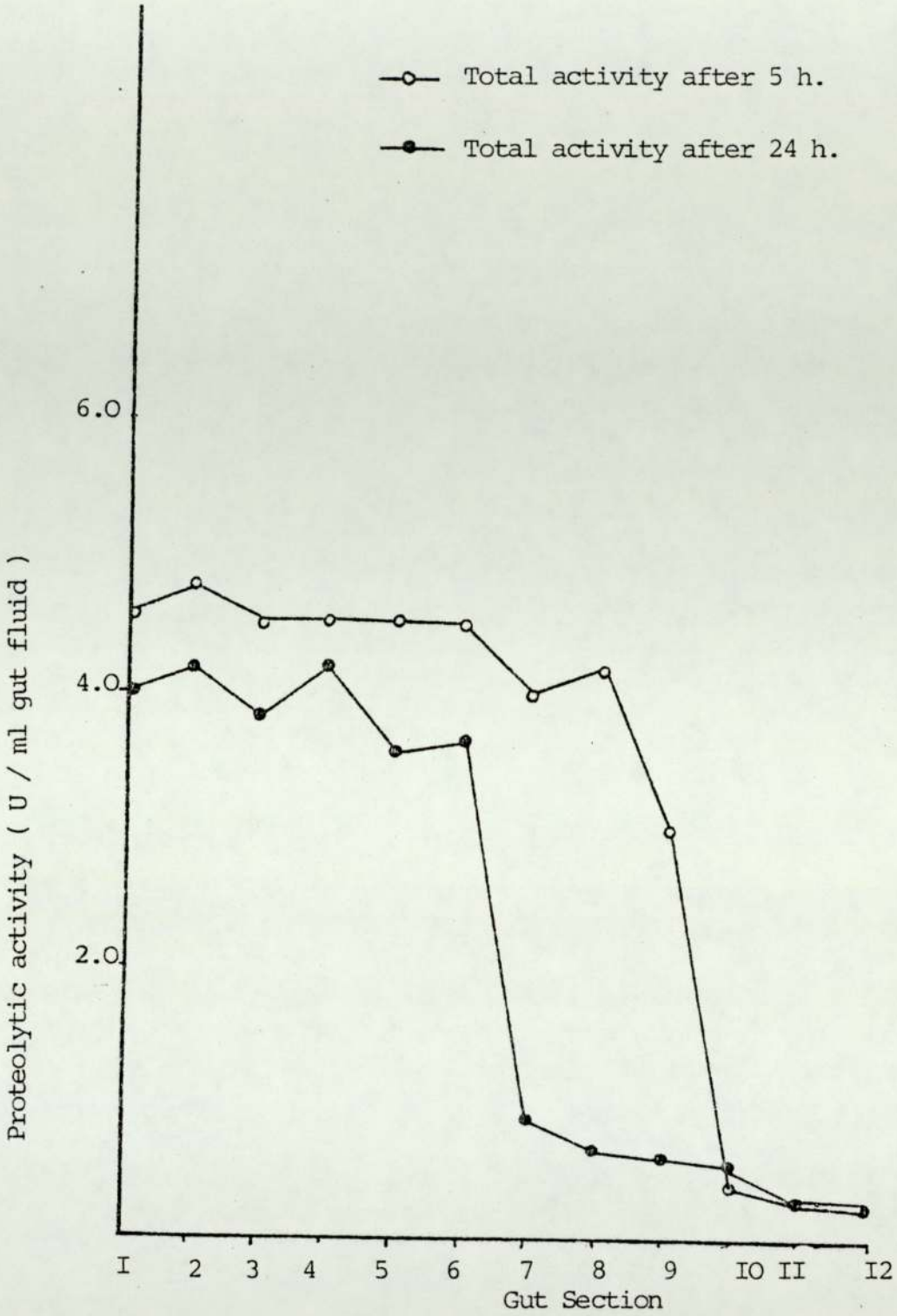

Fig 9.4. Total Proteolytic Activity from different Gut Sections of Carp.

Table 9.11. Duration of Gut Passage in Some Species of Fish.

Species	Temperature (°C)	Time (h) to 100% empty	Reference
Salmonidae			
Salmo gairdneri	8	49-51	
	11	46	
	13.5	35	Grove et al.
	15	40	(1978).
	18	30.5	
Cyprinidae			
Ctenopharyngodon Sp.	9	7	Hickling (1966)
Barbus liberiensis.	22-25	6-8	Payne (1975)
Rutilus rutilus.	3	30	
	6	31	
	10	21-22	Karzinkin (1932)
	14	22	Bokova (1938)
	17	12	
	20	9-10	
	25	6-8	
Cyprinus carpio.	12	60	Lane & Jackson
	23	48	(1969)
	12.5	22-50	Kevern (1966)
	25	16-25	
	10	18	Maltzan (Cited
	26	4-5	in Hickling, (1970)

enzymes (see discussion). An interesting relationship was found between relative gut length as a percentage of body length and the efficiency of "reabsorption" (difference of activity between the first one third and last one third of the gut as a percent of the first part). As shown in Fig 9.5, optimum efficiency of reabsorption of proteolytic enzymes is attained at a gut length of 1.83-2.23 fold body length. A further increase of gut length does not influence reabsorption. Protein content in the gut fluids tended to be higher in the anterior part and decrease posteriorly. It was found that a negative correlation existed between protein content in gut fluids and gut section 5 and 24 h after feeding ($r = -0.88$ and -0.92) respectively (Fig 9.6).

Total proteolytic activity for pancreas and liver exhibited higher values ($P<0.05$) 5 and 24 h after feeding-(Table 9.12).

9.8. Discussion.

Total and specific proteolytic activity increased from the first to the third segment of the fore-gut (Fig 9.3 & 9.4). Similar results were obtained by Bitterlich (1985) for silver carp and bighead carp. The same phenomenon has been observed in tilapia (Hofer & Schiemer, 1981), but its cause is not clear. Activation of zymogens may require a running period, but in fish with a diffuse pancreatic system, the actual entrance

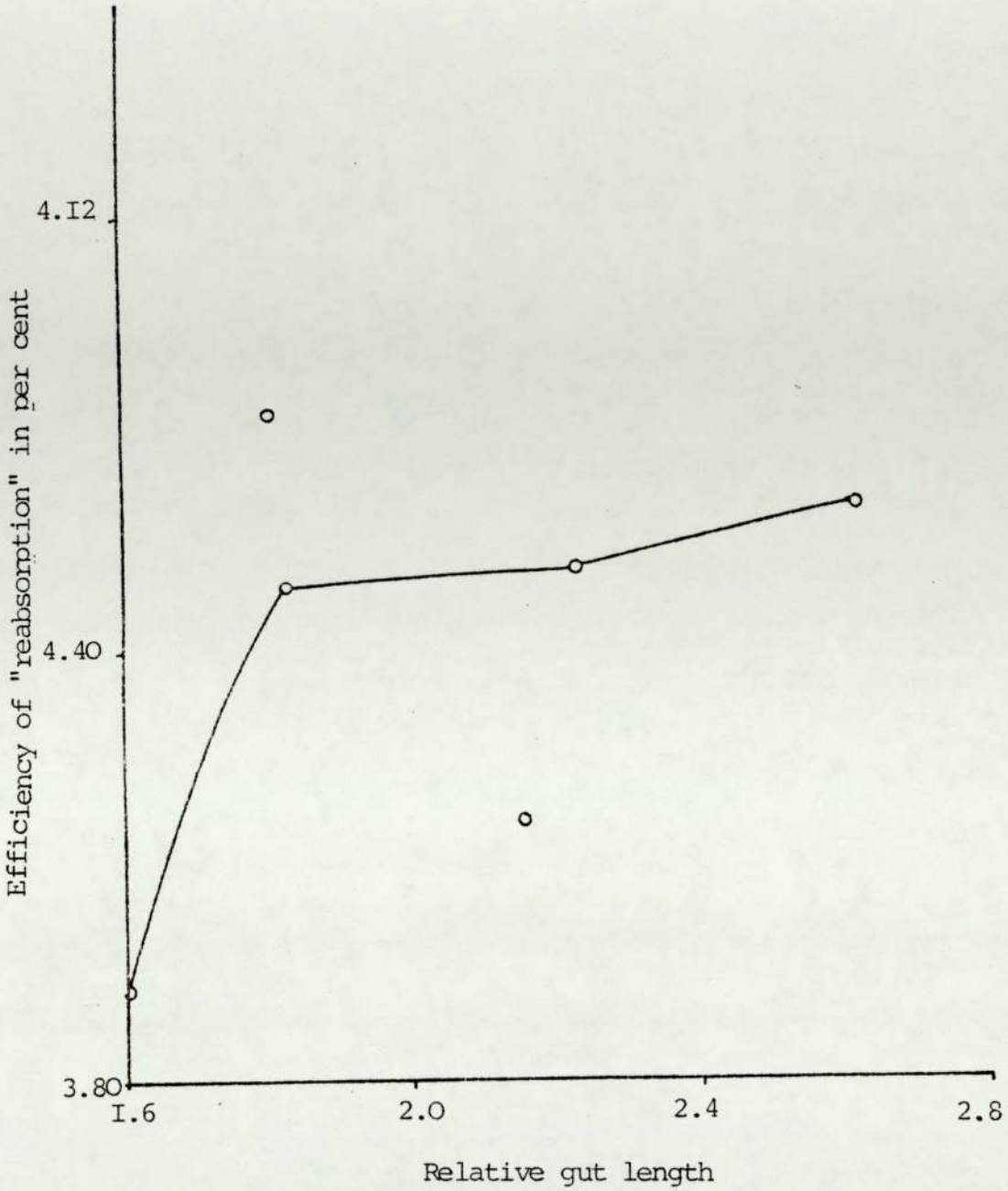

Fig 9.5. Efficiency of "reabsorption" of proteolytic Enzymes (difference of activity between the first and last part of the gut in per cent of the first part) in relation to relative length of the gut.

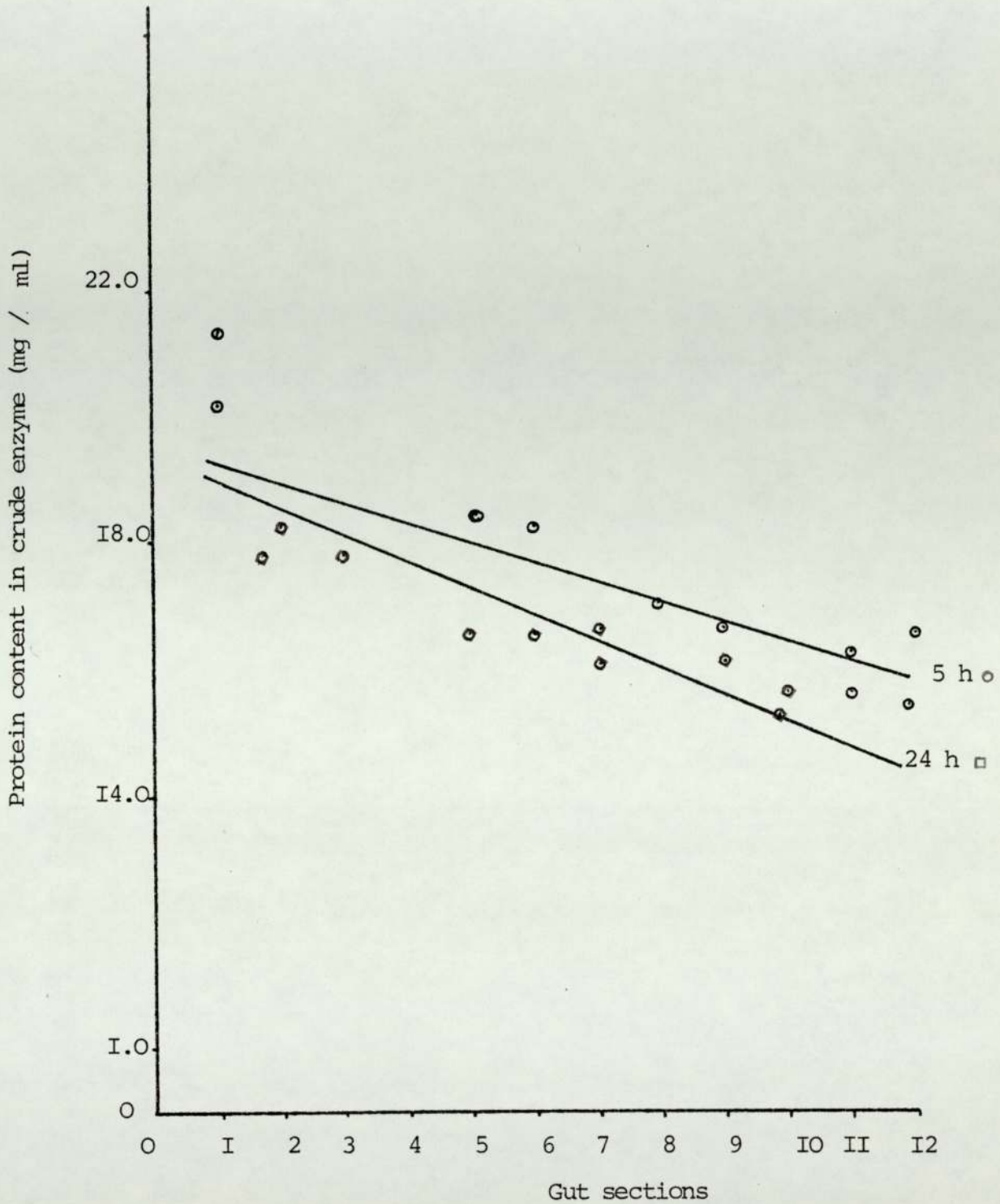

Fig 9.6. Correlation between gut sections and protein content in gut fluids (mg / ml) 5 and 24 h after feeding.

Table 9.12. Protein Concentration and Proteolytic Activity of Liver and Pancreas in Carp; 5 and 24 h after Feeding (Mean ± SD).

	5 h after Feeding		24 h after Feeding	
	protein mg/ml	Proteolytic activity U/ml	protein mg/ml	proteolytic activity U/ml
Liver	2.96 ± Ø.59	1.4Ø ± 1.Ø3	2.93 ± Ø.79	Ø.76 ± Ø.96
Pancreas	4.24 ± Ø.83	4.93 ± 1.21	4.92 ± Ø.77	1.Ø3 ± 1.Ø2

site of the ductus pancreaticus into the alimentary canal has not yet been explored. The decrease of specific activity has been investigated in more detail (Beynon & Kay, 1976; Hofer & Schiemer, 1981) and is generally interpreted as a reabsorption mechanism by which enzymes are salvaged. This may indicate an active reabsorption process by the mucosa, the mechanism of which remains unclear. The carnivorous fish Puntius dorsalis reabsorbs proteolytic enzymes with an efficiency of 42% in comparison to the herbivorous tilapia Sarotherodon mossambicus where RE is about 93% . Again, omnivores have an intermediate range.

Onishi et al. (1973 a & b) reported that total proteolytic activity in the intestine of carp gradually increased after feeding and reached a maximum after 5-7.5 h, returning to normal 1Ø.5 h after feeding; this supports the finding in the present experiment.

Total proteolytic activity in the pancreas was significantly ($P<Ø.Ø5$) higher at 5 h than 24 h. Similar results were obtained by Onishi et al. (1973 a & b).

CHAPTER 1Ø.

Chapter 1Ø. General Discussion & Conclusion.

Before discussing the value and use of protein quality figures derived from fish in order to standardize assays, it is necessary to review briefly three aspects which apply to all species as well as to all available methods of protein quality evaluation.

First, it is accepted that the primary function of dietary proteins is to furnish a mixture of amino acids of the proper pattern and some additional nitrogen for synthesis and maintenance of tissue proteins. The relative efficiency of a protein in making the required amino acid pattern available to the animal will determine the magnitude of measurable biological responses. According to this concept, practically all methods of protein quality evaluation are directly measuring the relative efficiency of protein sources in satisfying EAA requirements.

Most of the methods used today in evaluating protein quality are concerned with comparison of biological responses obtained under carefully controlled conditions. The usefulness of any particular method chosen for the assay of protein quality will depend on successfully ensuring that, under the experimental conditions employed, protein is the only factor limiting the response chosen to be measured. This implies the subjects must not constitute a variable; a condition easily attained with experimental animals.

The second generally accepted aspect concerns the

purpose of protein quality assays. This is to classify biologically the EAA balance of proteins as a whole in relation to well-identified standards. In this sense the result of an assay represents biological confirmation of the EAA content of the protein and allows one to predict the usefulness of the protein in meeting nutrient needs alone or as a component of diets. It identifies the protein as good, intermediate or poor, just as protein content indicates whether the material corresponds to a protein isolate, animal protein or cereal protein.

Thus, biological evaluation of protein quality has various applications:

(a) To monitor processing techniques that are used in food preparation or preservation.

(b) To measure the effectiveness of a protein source in supplying the nutrients needed for specific physiological functions, such as protein repletion, reproduction, production and growth.

(c) Protein quality evaluation techniques have been found valuable for monitoring changes in protein quality brought about by breeding improved varieties of cereal grains, particularly since chemical methods do not give entirely satisfactory assessment.

(d) The methods have also been extremely useful in establishing optimum amounts of protein and of AA supplementation of deficient proteins, and in studies of AA interrelationships.

(e) Protein quality assays are also valuable in

assessing the nutritive value of the protein in diets as consumed by the animal. Although such determinations might be thought to be the only objective of the various assays, interpretation of the results obtained with diets may be better understood and explained if knowledge is available about the quality of individual components.

(f) Finally, protein quality assay techniques can also be used to estimate the minimum needs for AA and protein.

The third aspect that should be recognized is that all protein quality assay techniques, whether or not applied to experimental animals, are interrelated and are affected by the same group of factors. For example, protein intake affects in the same manner PER and NPU, NB and BV; an energy, mineral or vitamin deficiency in the diet decreases the determined values in animals. The age, sex and physiological state of the subject also affect the results obtained by the various techniques.

In the present study with carp, the results obtained from each experiment have already been discussed fully in their relevant sections. Hence the main purpose of this section is to take an overall view of the results and the methods used for measuring protein quality and arrive at some general conclusions on the subject under discussion.

In chapter 3, there are two important points related to the methodology which are worth noting here. The first

is the fate of the non-protein nitrogen commonly present in high amounts in SCP. No account was taken of this in evaluating NPU. Strictly NPU should be based on the utilization of true protein not Kjeldahl N. However, as already mentioned, the total N digestibility of the test materials tended to be rather high, even for those containing large quantities of non-protein nitrogen, indicating that such materials are readily absorbed from the gut. Unfortunately, there is a lack of information on the metabolic fate of such non-protein N substances as nucleic acids, especially any protein sparing effect they may have. In view of this, it was felt that correction of the NPU values for non-protein N may have led to other errors. Thus, as it is common practice in nutritional work to use Kjeldahl nitrogen as a measure of dietary protein content and degree of utilisation, this method was employed without modification.

The bacterial protein (Pruteen) proved to be a relatively good nitrogen source, implying that it could be used in large amounts in compounded rations as a replacement for fish meal. Results of some workers (Atack et al., 1979; Beck et al., 1978) would suggest that 25-50 % replacement of fish meal is possible without any depression of growth rate. Moreover, casein and whole egg protein can be used as partial or complete replacement of fish meal. For the future, more work is required on all the protein sources used here, especially to evaluate any long term problems associated with their

use. This might include such data as their effect on the long term health of the fish, on the palatability of the fish, which have fed on farm wastes, to the consumer and possible growth promoting effects of AA supplementation of these protein sources. Of course, conclusions drawn on the possible usefulness of all the proteins tested here are based solely on biological finding not on the economics of diet formulation which include such considerations as price and quantities available.

The use of growth rate as the only criterion for establishing protein quality can make the interpretation of data extremely hazardous. Animal requirements for dietary components other than AA are continously being perfected. The addition of natural protein sources to animal diets alters the overall mineral, vitamin, fatty acid, etc. profiles of these diets, and the effects of these differences cannot easily be excluded from the effects of the proteins under test. Acceptability and toxic components will also alter growth rates. Thus, methods evolved using weight gains only are the poorest possible choice.

Animal feeding trials may be more beneficial to the assessment of protein quality when either nitrogen flux is measured or growth results are used to support other results.

Animal feeding trials in general are of no use for determining how proteins serve to supply AA for maintenance or for productive purposes (Table 1Ø.1). The effects of

combining protein sources cannot be ascertained unless the combined foods are tested, because the results are not additive. Methods where nitrogen absorption is measured are useful for defining the quantity of protein needed for a physiological state and for defining alterations in protein brought about by processing or plant breeding variations. Growth assays can be used to detect treatment changes in a protein provided that the changes have an effect on the limiting AA or on digestibility (Table 1Ø.1). A measurement of protein digestibility can be included by incorporating analysis of faecal nitrogen. In addition, a bioassay procedure can usually detect toxic substances, antinutrients, or appetite inhibitors, thereby providing an additional safety test. The principles of the assays are simple and direct, and the calculations of results are relatively straightforward when compared with some of the predictors of quality based on AA analysis.

The disadvantages of bioassay methods:

Biological assays of protein quality such as PER and NPR are a function of the limiting AA in the test protein source. These tests neither provide any information about the identity of the limiting AA, nor take into account information about any of the other AA in the test protein.

Because protein quality measurements using animal assays

are made under conditions in which a single protein source is the limiting component of the diet, data from these assays do not represent the quality of a protein when it is eaten in a mixed-protein diet.

Table 10.1. Uses of Animal Feeding Methods in the Assessment of Protein Quality.(from Evans & Witty 1978).

Assay Function	BV	PER	NPR	NPU
1. Measures effectiveness of protein to supply AA for a given function	no[1]	no	no	no[1]
2. Aids in determining the optimum amount of protein needed	yes	no	no	yes
3. Compares changes in a protein	yes	yes[2]	yes[2]	yes
4. Aids in predicting how a protein will react when mixed	no	no	no	no

[1] Can be adapted to determine the availability of individual AA.

[2] Provided changes affect the limiting AA.

From the results of experiment 1 (Chapter 3), a strong correlation was found between PER, NPU, Digestibility and BV (Table 1Ø.2).

Table 1Ø.2. Comparison of four Bioassays.

Parameters examined	r
PER: NPU	- Ø.793
PER: Digestibility	- Ø.624
PER: BV	- Ø.8Ø9
NPU: Digestibility	Ø.962
NPU: BV	Ø.999
Digestibility: BV	Ø.954

From the results of experiments 3 and 4 (Chapter 4 & 5), it was found that the urea concentration in the blood of fish is not sensitive to dietary protein quality because the nitrogenous end-products of protein metabolism consist of 9Ø% ammonia, the rest being urea and uric acid. Thus in contrast to mammals the urea method for evaluation of protein quality is possibly not suitable for fish. However, there was a positive correlation (r= Ø.89) between plasma urea and protein content in the diets of carp.

From the results of experiments 3 & 4 it was decided to

investigate the effect of protein quality on ammonia production of carp (Experiment 4, Chapter 6). This study indicates that peak ammonia excretion rate by carp may be used as the basis for comparing the relative efficiency of dietary protein utilization.

The advantages of this method are that it is less expensive and requires less time than assays based on fish feeding.

Further studies are needed, however, to clarify the effects of other dietary components of test diets or of maintenance diet and test protein load.

The results of the enzymatic method for measuring protein digestibility (Experiment 5, Chapter 7) showed a strong correlation between in vivo and in vitro protein digestibility (r= Ø.99). This method is simple, less expensive, needs a short time, less materials and simple equipment. Further studies are needed to measure AA released by the in vitro method to predict the EAAI and BV and correlate the results with corresponding in vivo parameters. In vitro protein digestibility can supply data on AA availability.

It is generally agreed that the methods of last resort for the biological evaluation of proteins are assays in vivo. Animal tests are expensive and time- and material-consuming and yield valid information only in the hands of the expert. Even so, the results are open to a wide range of interpretations. Animal assays are generally too slow to follow the different steps of a new

industrial operation or to guide a genetic screening. For these reasons *in vitro* methods, either chemical or enzymatic, are here to stay, in spite of all their imperfections. When used with discrimination they are of great help to the nutritionist and represent an indispensable complement to the animal assays.

Simulation of the physico-chemical conditions in the digestive tract of a fish during *in vitro* digestion tests leads to a good correlation with *in vivo* results.

The development of *in vitro* methods for the nutritional evaluation of proteins has made significant strides from the early attempts based solely on the determination of total nitrogen. Nevertheless, results obtained with some laboratory procedures give values which bear little relation to protein quality, as measured by several established animal tests. Other *in vitro* methods may appear useful; yet the occasional discrepancies introduce an uncertainty that is disconcerting.

Inconsistencies between animal tests and *in vitro* methods may arise from several sources.

1. Proteins of the same type obtained from different sources may vary in nutritional value, and batches tested *in vitro* are frequently not representative of the protein fed.

2. *In vitro* methods may not show the biological effect of AA antagonism or balance, although this is apparently considered in the integrated AA indices.

3. Only some *in vitro* methods take into consideration

the "availability" of EAA (Sheffner et al., 1956); most do not.

4. Feeding tests are sometimes affected by factors other than protein, including bacterial contamination of the diets; toxins, such as gossypol in cottonseed meal; and goitrogens.

Neither animal tests nor in vitro methods measure the nutritional quality of proteins under many stress conditions. It is not yet known whether AA requirements vary with such situations as infection, trauma, and anxiety; or whether AA patterns needed by the body are the same for maintenance, growth, and protein replenishment after deficiency and diseases.

The nonprotein components of the diet also influence the utilization of protein: variation may occur due to differences in total energy intake as well as in the supply of vitamins and minerals. These factors cannot be measured in vitro. What is evaluated is the relative quality of proteins under comparable conditions of use.

The degree of accuracy required for the assessment of protein quality will depend on the manner in which the results are applied. For certain purposes nitrogen data or solubility characteristics will suffice. For example a chemical score or integrated AAI may be useful to determine the suitability of protein concentrates for use in supplementing the food supply of undernourished populations. To estimate the effects of processing upon digestibility or AA availability, microbiological and

enzymatic procedures are generally necessary; the FDNB procedure for available lysine may also be useful for this purpose.

In vitro methods are extremely useful under circumstances for which they are appropriate. When used with discretion, they are an important complement to animal tests for the nutritional evaluation of protein quality. The in vitro methods for protein evaluation must ultimately be checked in comparative tests with the animal species for which the proteins are intended. However, animal feeding trials are expensive and laborious, and they are quite unsuitable for routine use. For such applications as quality control in food manufacture, for monitoring the commercial development of new protein foods, and for rapid screening of novel protein sources, the in vitro procedures have much to offer, and they will clearly increase in importance in the future. They must be used with discrimination, but properly used they are becoming an indispensable adjunct to animal assay.

From the results of experiment 6 & 7, it was found that TFAA at zero time was higher than at 6, 24 and 48h after force-feeding. This was perhaps because the fish were starved only 48h before the experiments started, in contrast to Nose (1973), whose fish were starved 96 h before the experiment was started. Nose found that the AA concentration at zero time was lower than at all times up to 7 days after feeding.

The PEAAI as demonstrated at 24 h in experiments 6 & 7 could be used as an index for protein quality evaluation in fish. The high value (97.22) for EAAI for sunflower (diet 6) at 24h may be due to high availability of AA or rapid protein depletion when the protein intake is insufficient.

The PEAAI for diets 7, 8, 9 and 1Ø (Table 8.26) was lower than the corresponding value at lower protein concentration (ca 15%), meaning that EAAI was decreased with the increase of protein concentration in the diet.

Increasing the protein concentration in carp diets has no effect on PAA patterns. It was initially expected that a greater increase in the concentrations of circulating AA would be seen corresponding to the increasing amount of protein fed, and also to see a more rapid removal of the circulating EAA in high protein concentrations. However, these changes did not occur. Perhaps if one could measure the concentrations of AA in portal blood after feeding, one could observe a graded response of the absorbed AA to increasing dietary protein. It appears that the liver must exert a major control function in regulating the circulating concentrations of FAA after a meal, because the circulating concentrations of both the TFEAA were not similar, regardless of the level of protein fed in this experiment. This apparent regulatory effect of the liver would appear to function in assuring that the extrahepatic tissues could make

maximum utilization of dietary AA for protein synthesis. The absence of any apparent effect of the protein concentrations on circulating AA patterns appears to indicate that there is adequate or excess energy in all of the test diets to facilitate similar AA utilization. Differences in availability of AA in dietary protein may be reflected in blood AA concentration. Digestion of protein may be a limiting factor and the rate of absorption of individual AA by the intestine is affected by the presence of other AA.

The rationale behind AA scoring is both simple and logical since the role of dietary protein is to provide AA and nitrogen for the synthesis of body protein and other metabolically important nitrogenous metabolites. Protein (nitrogen) and AA requirements are thus the logical yardsticks by which to measure protein quality, and precise knowledge about these requirements is the basis for evaluating the nutritional significance of dietary protein quality.

Two major factors, however, make application of AA scoring more difficult than the apparently simple and logical underlying rationale would suggest: (1) There is as yet no universal agreement on the "requirement" or standard to be used , and (2) the mere consideration of AA composition of a protein after hydrolysis does not necessary mean that AAs are all of equal nutritional bioavailability. These factors are considerd in more detail in the following discussion of the advantages and

disadvantages of using AA scoring.

Knowledge of the AA content of a protein supplies information not only on the "limiting" AA but also on the other AAs. The latter information may be more important than (or at least as important as) that concerning the limiting AA. For example, an understanding of the advantages of AA complementation that are realized by mixing two food proteins, such as wheat and soy, requires knowledge of the lysine and sulphur AA composition of the two protein sources. Thus, a potentially more complete description of the nutritional value of a protein might be obtained from a knowledge of its AA composition.

In addition, there are practical advantages in using AA composition data (Table 1Ø.3) for meeting requirements; less time is required, costs are smaller than those involved in the conduct of bioassays and there is allowance for some understanding of the nature of the foodstuffs.

Disadvantages of AA scoring.

The general weaknesses of scoring systems are the following :

(1) AA composition does not reveal protein digestibility or AA availability.

(2) The utilization of a specific single protein, when consumed in mixed diets, is affected not only by the concentration of the limiting AA of the specific single protein source, but also by those of the of EAA present

Table 1Ø.3. Uses of Chemical methods in the Assessment of Protein Quality (from Evans & Witty, 1978).

Assay function	AA analysis	Chemical score	EAAI	In vitro digestion
1. Measures effectiveness of protein to supply AA for a given function	no	yes	yes	yes
2. Aids in determining the amount of protein needed	yes	yes	yes	yes
3. Compares change in a protein	yes [1]	yes [1]	yes [1]	yes
4. Aids in predicting how a protein will react when mixed	yes	yes	yes	yes

[1] When changes alter AA profile rather than availability

in high amounts in the other protein sources consumed at the same time, and by the amount of non-protein nitrogen. A score for a single protein source, based on the single most limiting AA, does not reflect these considerations.

The amount of a nonlimiting AA (eg. lysine in meat products) may be much more important in relation to the value of a specific protein food in the diet as a whole than the nominal limiting AA.

(3) While not strictly a protein quality consideration, the presence of antinutrients or toxic materials is not revealed by AA analysis. Such materials can affect biological response. However, their occurrence in animal protein sources is rarely (if ever) observed.

(4) Because of AA recycling, especially when lysine is the limiting AA, there may be large discrepancies between score and biological response for low-quality protein.

(5) AA composition data are not always accurate and sufficiently reproducible; a similar observation, however, can also be made for fish bioassays.

(6) Disagreement exists on the appropriate AA reference pattern to use. Also, the reference pattern may be different for populations of different ages.

As far as the use of metabolic indices of protein quality is concerned, further development of the techniques is needed. The normal physiological variability in enzyme activities needs to be assessed to accurately determine whether or not the differences encountered can be attributed to alterations in protein quality. So, the results of experiments 8, 9 and 1Ø indicate a good adaptation of proteolytic activity to changes in the qualitative composition of the diets. Enzyme activities in the intestine are a function of diet, filling of the gut, temperature, water chemistry, pH, age of the fish and exogenous enzymes (Hofer, 1979). For proper interpretation, proteolytic activity should be calculated in each part of the gut on the basis of the actual pH in each segment. Deficient reabsorption extends the time at which enzymes are active leading to continuous loss of body proteins. Combined with low utilization of dietary proteins and high enzyme production, the increased loss of enzyme proteins might actually contribute to the difficulties encounterd when rearing fish with artificial diets.

If the absorption process, reflected by the concentration of AA in the blood, were studied in connection with enzyme secretion and digestion in the successive parts of the intestine, it might be possible to draw a significant conclusion.

Proposal for Further Research.

1. Bioassays for protein quality.

The evaluation of methods for nutritive values of protein in fish has been insufficiently studied thus far. Any such study would examine the applicability to carp or other fish of the method developed by Bender and Miller (1953) and Miller & Bender (1955) for the measurement of NPU in rats by carcass analysis, and estimate the nutritive values of several kinds of new protein sources. It is suggested that further studies with fish be made and NPU, PER, BV, digestibility and nitrogen balance measured. Also the relationship between total proteolytic activity in whole gut and protein sources should be examined; and the relationship between total proteolytic activity in whole gut and the protein digestibility be investigated.

2. Ammonia production as Index for Protein Quality.

The relationship between protein sources and their nutritive parameters in the above experiment and ammonia excretion at various intervals after feeding these diets should be made.

3. In Vitro Methods for Protein Quality.

High quality proteins are those that are well digested and contain all the EAA necessary for protein synthesis in vivo and in a ratio that supports good growth.

Since protein synthesis requires that all the AA be available at the same time and as the AAs are liberated from protein *in vivo* only after hydrolysis by the digestive enzymes, it would seem that a measure of the amount of EAA released by the digestive enzymes *in vitro* should give a good estimate of protein quality.

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