ENERGY BUDGET AND ASPECTS OF ENERGY METABOLISM IN COMMON CARP, Cyprinus carpio.

By

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M.Sc (Fisheries) in
Fisheries Technology

A Thesis Submitted to the University of Stirling
for the degree of

Doctor of Philosophy

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February 1992

D E C L A R A T I O N

I hereby declare that the research work presented in this thesis was conducted by me and that this work has not been submitted for any other degree. All work referred to have been duly acknowledged.

Signed

Subhash C Chakraborty

DEDICATION

To my beloved Parents

ACKNOWLEDGEMENT

I would like to thank my Supervisor Dr Lindsay G Ross, for his advice, encouragement and supervision throughout the course of this research and during the writing up of this thesis. It is of no doubt that he has induced my interest in fish energetics. I also wish to thank my additional supervisor Dr. Barbara Ross for her keen interest and encouragement towards my research works during her stay in the Institute of Aquaculture. I remain eternally grateful for their patience and understanding during my moments of difficulty.

I would also like to register my gratitude to all members of staff and colleagues of the Institute, and in particular Mr. Richard McKinney, Mr. Allan Porter, Mrs. Elizabeth Stenhouse and her staff. In particular I wish to thank Mrs. Julia Farrington who helped to alliviate my stress time to time.

In addition I would like to thank my colleagues and friends at Stirling for their friendliness and helpful discussion.

I gratefully acknowledge the financial support of the Association of Commonwealth Scholarship Commission in the United Kingdom, and the Registrar, Bangladesh Agricultural University, Mymensingh, Bangladesh for granting me study leave from my lectureship.

Finally, I would like to thank my wife Sabitri, who helped me immeasurably throughout the course of my research and writing up of my thesis. Thanks are also due to my son, Shovan, for his realization of importance of my work and his helping in printing of this thesis. My little daughter, Shaswati, also encouraged me by her activities when I became bored with my works.

ABSTRACT

Aspects of the resting respiration rate, specific dynamic action (SDA) and components of the total energy budget of 55 - 80g common carp were studied in the laboratory.

The resting respiratory rate was monitored in computer operated metabolic chambers under different photoperiods. Common carp showed a crepuscular respiratory rhythm with peaks at dawn and dusk during a 12L : 12D photoperiod, with a mean oxygen consumption of 152 mg/kg/h. When acclimated to longer or shorter photoperiods respiration was also cyclic but with a lower mean respiratory rate. In continuous light or darkness respiratory rhythm was suppressed with no significant peakings.

In carp fed with three diets containing 20, 35 and 50% protein at a ration level of 0.40 to 1.00% body weight per day, SDA coefficient varied from 8.99 to 15.94% and was dependent on dietary protein but not on ration levels. SDA magnitude and post-feeding peak oxygen consumption varied significantly with both dietary protein content and total daily ration level. SDA duration was only related to ration size.

The pattern of food energy allocation between the major components of the energy budget varied with dietary protein content and ration levels. The energy lost as heat of metabolism was found to increase with dietary protein level and total ration. Energy lost as faeces 'F' varied from 19 - 24% of 'C' and did not appear to be related to either protein content or ration levels. Nitrogenous excretion increased with an increase of dietary protein but decreased with an increase of ration level in the diet. Regression equations were developed from the data to allow prediction of respiratory energy

loss 'R', faecal energy loss 'F' and energy lost through excretion 'U' from the food ingested 'C'.

Complete energy budget models compiled from experiments conducted over a 17 days period and using different diets did not successfully predict the actual growth. The energy budget balance was between 66.04% and 81.96%. Observed growth was less than predicted growth in every trial and it is suggested that this difference might have been due to short-term cyclic growth regulation and other minor experimental features. The data presented form the basis for the first reported study of total energy budgets in Cyprinus carpio.

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LIST OF ABBREVIATIONS

AE = Assimilation efficiency

APD = Apparent protein digestibility

C = Energy consumed

DE = Digestible energy

F = Faecal energy

FCR = Feed conversion ratio

ME = Metabolizable energy

NFE = Nitrogen free extract

NPU = Net protein utilization

P = Energy for growth

PER = Protein efficiency ratio

R = Energy for metabolism

R_a = Active metabolism

R_s = Standard metabolism

R_f = Feeding metabolism

SDA = Specific Dynamic Action

U = Energy of nitrogenous excretion

CHAPTER 1

General Introduction

The means of providing the energy essential for normal life processes, to compensate for losses caused by defaecation, excretion, respiration and to ensure the development and the growth of an organism is referred to collectively as metabolism (Steffens, 1989). For maintenance of necessary bodily functions, potential energy contained in the diet is converted into kinetic energy by means of digestion and oxidation. In this, the complex chemical compounds in food are broken down into simpler components with the simultaneous release of energy. The principle of conservation of energy remains valid throughout these physiological processes and there are close interrelationships between the metabolic reactions for releasing energy to support body functions, the replacement of spent materials, such as, enzymes, hormones etc., and the building of new tissues.

Over the years a series of concepts has been devised for the study of the overall energy exchanges in animals and man (Blaxter,1989). The study of "physiological energetics" - the rates of energy loss, gain and efficiencies of transformation as functional relations of the organism,

"bioenergetics"- exchange of energy within the cell, and "ecological energetics" - transfer of energy in different trophic levels, are closely related (Brett and Groves, 1979). The energy budget is a balance sheet of energy income set against energy expenditure (Brafield, 1985) and it is a careful accounting of the energy consumed in food, losses of energy from the body in faeces, heat produced by metabolism and the retention in, or secretion from, the body of energy represented by organic compounds (Blaxter, 1989). An energy budget is a powerful framework for identifying the most important aspects of the life of any fish (Soofiani and Hawkins, 1985). In a laboratory experiment a fish is considered as an open thermodynamic system exchanging energy with its surroundings in three ways - heat, work, and the potential energy of biochemical compounds (Brafield, 1985). According to the law of thermodynamics, the energy entering the fish body from outside equals the energy leaving the body plus the energy retained in the body as growth, i.e.

Energy input = energy output + energy in growth

In this case, energy input is in the form of food energy ingested (C), and the losses or output of energy are in metabolism (R), faeces (F) and non-faecal nitrogen excretion (U). Growth (P) results from a change (either increase or decrease) of energy, mass or length of body.

Generally, growth (P) is positive but in the case where a fish is losing weight, stored tissue energy is considered as entering the system. Thus, if the animal is not feeding the energy loss from tissue equals 'R + U', and F will be virtually zero.

The basic energy budget equation can be summarized by the equation developed by Petrusewicz and Macfadadyen, (1970):

$$C = P + R + F + U$$

After subtracting faecal energy (F) from food energy (C), only the energy for absorption and assimilation (P+ R + U), the digestible energy, remains. From this, the energy of nitrogenous wastes (U) in the form of ammonia (about 90%) is excreted through the gills leaving (P + R) as the metabolizable energy. Figure 1.1 shows these main energy transformations and losses in fish

a.

Food energy (C)

→faecal loss (F)

Assimilated Energy

→Nonfaecal loss (U)

Metabolize energy (ME)

→Heat increment (SDA)

Net Energy (NE)

Standard metabolism | Growth | Activity

Submaintenance feeding and food deprivation

b.

Food energy (C)

→Faecal loss (F)

Digestible energy

→Heat incremeant (SDA)

→Nonfaecal loss (U)

Metabolizable energy

Standard metabolism | Growth | Activity

c

Digestible (Assimilated) energy

Transamination | Deamination →SDA

deamination →nonfaecal loss

Metabolizable energy

Fig. 1.1. Bioenergetics scheme of food utilization. (a) after Niimi and Beamish, 1974; (b) revised version of (a); The biochemical basis for the revision is shown in (c).

Deamination of the ingested proteinaceous food may occur at either level of the bioenergetics scheme (Fig. 1.1c) depending on the nutritional composition of the food and the physiological state of the animal. Consequently, it is not appropriate to arbitrarily separate this portion of the bioenergetics scheme as was done in Fig 1.1a. The term 'metabolizable energy' used in a Fig 1.1b is not empirically equivalent to that in Fig 1.1a, the difference being the position of the heat increment. Metabolizable energy is probably the most accurate term in describing the energy remaining since it can either be anabolized or catabolized by the animal. The heat liberated as SDA is not normally utilized except perhaps in some tunas and sharks which maintain elevated body temperature (Carey et al., 1971). This further suggests that the heat increment should not be considered as a portion of metabolizable energy (Beamish et al., 1975)

Investigations of fish energetics have drawn insight and inspiration from studies of the energetics of agricultural systems, including the production of commodities such as meat, milk and eggs (Brody, 1945). It is not always easy to measure all of the energetic pathways operating concurrently in fish and components are frequently measured by difference. This is actually unsatisfactory in that the error involved in various measurements cannot be determined

because they are all incorporated in the remaining unestimated pathways.

A powerful technique for estimating the growth and food consumption rates of fish in natural populations or in laboratory conditions is bioenergetic modelling (Kitchell et al., 1974). This attempts to relate growth to predictor variables by using the principles of bioenergetics (Cui, 1987) and in these cases the energy entering the body of the fish equals the energy leaving the body plus the energy retained in the body as growth.

A detailed energy budget equation for fish was first proposed by Ivlev (1945), using the expression:

$$Q = Q' + Qr + Qt + Qv + Qw$$

where, Q = energy value of ingested food, Q' = energy value of materials laid down as growth, Qr = energy value of faecal and nitrogenous wastes, Qt = energy value of primary heat, Qv = energy value of external heat, Qw = energy value of internal work. Following this pioneering study, Winberg (1956), Warren and Davis (1967), Ursin (1967), Kerr (1971a, b, c) and Kitchell (1974, 1977) all developed different bioenergetics models for fish. Warren and Davis (1967) proposed various categories of uses and losses of ingested food energy and these were presented in a bioenergetic

scheme as follows:

$$C = F + U + SDA + R_s + R_a + G$$

where, R_s, R_a, G = standard, active metabolism and growth respectively.

Beamish et al. (1975) disagreed with this and proposed that the energy represented by SDA is lost before the energy is dissipated in excretion (Fig. 1.1). This modification does not however change the overall form of the equation.

Ursin's bioenergetic models which are based on the difference between anabolic and catabolic terms, have been developed for the rainbow trout, Salmo gairdneri (Sperber et al., 1977) and African catfish, Clarias lazera (Hogendoorn et al., 1983). But, Kerr's bioenergetics models are mainly based on Winberg's (1956) equation, where metabolism is divided into standard, spontaneous activity and foraging activity. In Kerr's models the temperature effect was not considered although he incorporated a relationship between maximum ration and potential maximum metabolic activity, with standard metabolism being independent of body weight.

Ricker (1971) described a complete energy budget for fish for a given period of time which was similar to that of Warren and Davis's equation and Elliott (1976b) applied it

for brown trout Salmo trutta in relation to body weight, water temperature and ration size.

The bioenergetics models of Kitchell et al.(1974) are also based on Warren and Davis's bioenergetics equation i.e.

$$G = C - (R + F + U)$$

where, R = respiration or metabolism (including standard, active or specific dynamic action). It was developed to describe populations of bluegill, Lepomis macrochirus and assumed that the growth (G) is obtained from the differences between energy consumed from the food (C) and the energy losses in the form of faeces (F), excretory losses (U) and respiration i.e. metabolism (R).

In 1977 Kitchell and his co-workers modified their earlier models in order to determine the specific growth rate of individual, non-reproducing yellow perch (Perca flavescens) and walleye (Stizostedion vetreum vetreum) and subdivided the energy components by submodelling. This approach has been developed for several species, such as the sea lamprey Petromyzon marinus (Kitchell and Breck, 1980), largemouth bass, Micropterus salmoides (Cochran and Rice, 1982; Rice and Cochran, 1984), lake trout, Salvelinus namaycush (Stewart et al., 1983) and pike, Esox spp. (Diana, 1983). In most cases, the rates of food consumption, body weight and temperature were considered as predictor variables, although some other predictor variables, such as dissolved

oxygen, unionised ammonia and seasonal variation have also been considered.

Comprehensive reviews of problems and difficulties in relation to various components of energy budgets in fish were produced by Brett and Groves (1979) and Fischer (1979). Braaten (1979) wrote a review on methodology and Elliot (1979) surveyed the energetics of some teleosts and derived growth models. Numerous attempts were made to compile energy budgets for fish in which at least some of the relevant factors were incorporated. Some of the more recent works on fish energetics are summarized in Table 1.1.

In some studies efforts have been made to measure all, or most, of the components and sub-components directly. Examples include the work of Warren (1971), Solomon and Brafield (1972) on perch, Perca fluviatilis and Elliott (1976b) on brown trout Salmo trutta. Although there are individual variations in fish species and differences in response towards food and other environmental conditions, an average energy budget for young carnivorous fish fed a ration well above maintenance was derived by Brett and Groves (1979) represented as

$$100I = 44\ M + 29\ G + 27\ E$$

Table 1.1 Energy budget studies for different fishes .

Species	Size (g)	Diet	Factors studied	Authors
Carnivorous fish			general energy budget study.	Winberg (1956)
Esox lucius Esox lucius Pike	250	Phoxinus	growth and maintenance assimilation efficiency and growth efficiency	Johnson (1966) Welch (1968)
Gasterosteus aculeatus Stickleback	1.0	Tubifex	energy transformation in a host-parasite system	Walkey and Meakins (1969)
Oncorhynchus kisutch O. kisutch coho salmon	1.2	fly larvae	temperature on energy & material utilization	Averett (1969) Warren (1971)
Perca fluviatilis Perch	12 -18.7	Gammarus	all components of energy budget continuously	Solomon and Brafield (1972)
Gadus morhua Atlantic cod	223	chopped fish	oxygen consumption, growth & metabolism	Edwards et al. (1972)
Lepomis macrochirus Bluegill sunfish	49	mayfly nymph	energy cost of food utilization	Pierce and Wissing (1974)
Histro histro Sargasum fish	1.0	shrimp	energy transformation from food to faeces	Smith (1973)
Ctenopharyngodon idella Grass carp	1200	Egeria	energy balance	Stanley (1974a)

Species	Size (g)	Diet	Factors studied	Authors
Sarotherodon mossambicus Tilapia			energy balance in relation to temp. and ration	Mironova (1976)
Salmo gairdneri Rainbow trout				Staples and Namura (1976)
Salmo trutta Brown trout	50.1	*Gammarus*	energy relation with body wt., temp. & ration	Elliott(1976b)
Rhinomugil corsula Mullet				Kutty (1981)
Gasterosteus aculeatus Stickleback				Wootton *et al.* (1980)
Crenimugil labrosus Thick-lipped mullet				Flowerdew and Grove (1980)
Oncorhynchus nerka Sockeye salmon				Brett (1983)
Sarotherodon mossambicus Tilapia		formulated and *Tubifex*	all components of energy budget using different diets	Musisi (1984)
Phoxinus phoxinus Minnow	1 - 5.5	*Enchytraes* sp.	different ration and temp on the bioenergetics & growth	Cui (1987)
Ctenopharyngodon idella Grass carp	9 - 17.0	formulated diets	energy allocation from ingested food	Carter and Brafield (1991)

where, I = rate of ingestion, M = metabolic rate, G = growth rate, E = total excretion rate and E is further divided into faecal = 20 and nonfaecal = 7

They also derived a general energy budget for young, well-fed herbivorous fish :

$$100I = 37M + 20G + 43E$$

where E is divided into faecal = 41 and non faecal = 2. All the values are expressed as percentages of total calories consumed (I).

Bioenergetics models are of particular interest in the study of the relationship between growth and environmental factors, to predict the rate of food consumption of fish in the laboratory or in nature and to assess the performance of different fish species under different environmental conditions (Cui, 1987).

Fish derive their energy by partial or complete oxidation of carbohydrates, fats and proteins ingested, following absorption from the diet or from breakdown of glycogen, fat or protein stored in the body. The quantitative requirement for any food depends largely on its composition (Hepher, 1988). The most efficient level of feeding and its utilization is attained only when the correct supply of energy and

essential nutrients are in the proportions required by the fish for maintenance, activity and growth (Steffens, 1989). The effects of the composition of the diet actually ingested on the quantitative physiological requirements must also be taken into account. According to the law of thermodynamics, the transformation of energy from one form to another always results in an increase in entropy, or the 'loss' of energy as heat. The transformation of food energy to net energy is carried out within the body stepwise through a series of catalytic processes with the minimum unavoidable heat loss. This loss in nutrition is known as 'heat increment' and is related to the biochemical oxidation processes, and so varies with different feed-stuffs. Fish are poikilotherms and their body temperature follows the ambient temperature. Metabolic heat produced is simply lost via the gills into the surrounding water, (Stevens and Sutterlin, 1976). This portion of the food energy or heat increment, should therefore be treated as an inevitable 'tax' on energy consumption (Hepher, 1988).

The fate of absorbed food in living systems is much more complicated than simple metabolic combustion for energy and deposition of matter in growth. The main reason for this is that many environmental factors strongly influence the biochemical state of the fish and its physiology. Secondly, the total heat produced bioenergetically during the catabolism of ingested proteins may differ from that

obtained by direct combustion of food, because protein nitrogen is not fully oxidized. Consequently, less net energy than expected may be determined by bomb calorimetry.

There are many reports on various aspects of energy metabolism in carp. Biological value as well as utilization of protein and energy supply in carp was studied by Ogino et al. (1973, 1976) and Kirchgessner et al.(1984). Steffens (1981) reviewed protein utilization of carp. Nijkamp et al. (1974) studied the retention of nitrogen, fat, ash, carbon and energy in growing carp. Huisman et al. (1979) studied the retention of energy, protein, fat and ash with growing carps of three different weight ranges at two temperatures using a commercial pelleted diet. No clear relationship was observed between the metabolism of crude protein and different carbohydrates (Svobodova et al. 1984). Digestible energy requirement at 10°C and 20°C were studied by Schwartz et al. (1984). Eckhardt et al. (1981) showed that casein and whites of eggs are unsuitable for growing carp but that defatted herring meal is the best protein source for standard rations for carp. Roth and Kirchgessner (1981) showed an optimum utilization of protein energy at 23.8°C for carp fed on diets containing 42% crude protein and 12% fat. Oxygen uptake, nitrogen and phosphate excretion in carp were determined by Hamada and Maeda (1983). Toshio et al. (1979) showed that carp utilise effectively both carbohydrate and lipid as a dietary energy source, and both

PER and NPU were significantly lower in the fish fed diets with DE content of less than 310 Kcal. They found maximum growth with 31% dietary protein.

Takeshi et al. (1984) determined the metabolizable and net energy (energy for maintenance and growth) for carp. The influence of weight and temperature on standard metabolism were studied by Beamish (1964). Hamada and Maeda (1983) measured oxygen uptake of carp fed with diet containing milk casein (40%), dextrin and soybean oil and found that oxygen consumption and SDA is proportional to ration size and protein content of the diet. Hamada and Ida (1973) fed common carp with natural and artificial diets and found that SDA peaked 3 - 4 hours after feeding, with second peak after 5 - 8 hours.

Methods of faecal collection, nutrient leaching and digestibility were studied by Smith (1971) and Windell et al. (1978). Ogino et al. (1973) and Kim (1974) determined the metabolic faecal nitrogen egested by common carp.

An apparatus was devised by Ogino et al. (1973) for measuring metabolic nitrogen and endogenous nitrogen excretion. Francoise (1974) reported that carp produce both ammonia and urea in the liver.

The total energy of metabolism 'R', lost as heat, can arise from three possible sources; resting metabolism, the activity of the fishes in water and feeding. These metabolic costs in the aquatic environment can be measured by indirect calorimetry by measuring oxygen consumption (Winberg, 1956, Davis & Warren, 1968; Brafield and Solomon, 1972; Braaten, 1979; Brett and Groves, 1979; Brafield, 1985). This method is based on the assumption that energy production in fish is an aerobic process and requires oxygen for oxidizing nutrients either from food or the fish's own tissues, although anaerobic metabolism may also exist in fish (Blazka, 1958). Depending on levels of activity of fishes, three metabolic rates can be distinguished (Fry, 1957; Brett, 1962; Kausch, 1972; Albrecht, 1974b; Braaten, 1979; Kutty, 1981): standard metabolism, routine metabolism and active metabolism. Standard metabolism is as near the base rate as normal experimental techniques will allow, with the fish quiescent and maintaining the minimum energy turnover for survival. The real standard rate is not often measured, because laboratory fish are rarely in this state. Beamish (1964) recognized routine metabolism, which includes standard metabolism plus normal spontaneous activity, Whereas active metabolism is due to forced activity of fish (Brafield, 1985) or maximum sustained activity (Brett, 1973). In addition, metabolism resulting from feeding (SDA) may also be observed (Brett and Groves, 1979). It is

necessary to differentiate between two states of active metabolism. Active metabolism level 1 relates to normal conditions of metabolism for a group of fish digesting food and engaged in free swimming motion. But active metabolic rate 2 is the highest level of metabolic activity with greatest swimming velocity, often to the point of exhaustion (Steffens, 1989).

Most attention has been given to determination of the resting rate, or minimum rate of energy expenditure to keep the organism alive, (Winberg, 1956) since this reflects the first and prime demand for food before any storage or growth can be achieved. This energy expenditure is referred to as the minimum maintenance metabolism and is determined in starving fish. By contrast, maintenance metabolism is approximately comparable to the standard or routine metabolism plus the energy cost (SDA) of processing the minimum food required to meet standard metabolism (Pandian, 1987). Studies have shown that it is difficult for fish to achieve zero motor activity. This unavoidable movement is included in the standard metabolism (Winberg, 1956, 1961; Brett, 1962). Thus, in the study of fish energetics, standard metabolic rate is the minimal maintenance or resting metabolic rate of an unfed fish below which physiological function is in some way impaired.

The difference between the standard metabolic rate and active metabolic rate is the metabolic scope within which the animal can function aerobically (Fry, 1947). This metabolic scope varies according to species and stage of development as well as being influenced by environmental variables (Priede, 1985).

Many authors have studied the oxygen consumption rate in fishes under different conditions (Winberg, 1956, Beamish, 1962; Brett, 1962; Kausch, 1969; Muir and Niimi, 1972; Hamada and Ida, 1973). Reviews of oxygen consumption by Winberg (1956) and Brett and Groves (1979) have shown that there are many factors affecting the standard metabolism in fish, the most important being the weight of fish, temperature, activity, pH, oxygen content in water, salinity and fish age. An average standard metabolic rate for fish of 89 +_34 (SD) mg O_2/kg/h with an extreme range of 26 to 229 mgO_2/kg/h is quoted from the "Biological Data Book" (Altman and Dittmer, 1974) by Brett and Groves (1979).

Some studies have shown that photoperiod is one important environmental factor affecting the rhythmic respiratory pattern in fish (Eriksson, 1973; Thorpe, 1978). A number of authors have shown that the resting metabolic rate in fishes during the day shows a natural variation with changing photoperiod (Winberg, 1956; Holliday and Blaxter,

1964; Brett and Zala, 1975; Ross and Mckinney, 1988). Together with temperature, the light period often regulates the cyclic and seasonal variations in growth (Hepher, 1988). Gross et al. (1965) stated that varying day length exerts a greater influence on fish growth than a constant day length which probably has an indirect effect involving regulation of metabolic activity by the endocrine system. Bjorklund (1958) found no relationship between daylength and growth, whereas two studies by Tryon, (1943) and Eisler, (1957) on growth of cutthroat and chinook salmon respectively showed a direct relationship when reared in dark or in light. For many fish species, including carp and trout, diurnal variations in activity have been reported, which are bimodal (Kausch, 1972).

The metabolic heat loss associated with the digestion and transformation of ingested food into metabolizable form is known as Specific Dynamic Action (SDA) and this has been reviewed by Brett and Groves, (1979) and Pandian (1987). Saunders (1963) was the first to study the effect of feeding on the metabolic rate of Atlantic cod, Gadus morhua using a "mass respirometer". Later, Warren and Davies (1967) presented the previously unpublished data of H. Sethi on Cichlisoma bimaculatum. In an elaborate study by Averett (1969) on coho salmon, Oncorhynchus kisutch under varying ration and temperature, it was found that SDA ranged from 4 to 45% of the ingested energy, with most

values occurring between 9 to 15%. Warren (1971) gave special attention to this higher SDA loss. He recognised that the upper values obtained by Averett (1969) were due in part to metabolic excitation accompanying feeding. Therefore, it is necessary to separate or minimize the energy loss of excitability and increased activities during experiments otherwise the partitioning of the energy becomes confused and SDA is unmeasureable.

From the review of Brett and Groves (1979) it appears that the metabolic loss attributable to SDA in fish appears to be 12 - 15% of the ingested food energy, when the ration levels are well above the maintenance. The compilation of data by Brett and Groves (1979) on the metabolic rates of feeding fish are mostly ration dependent. The relationship is either a linear function of metabolic rate increasing with ration, up to maximum intake as in salmon (Muir and Niimi, 1972) or a linear increase at lower ration levels tapering off at higher rations to form an upper plateau as in Cyprinus carpio (Huisman, 1974).

There have been some conflicting results from some authors on SDA, and several workers have questioned the validity of the conclusions based on early data (Jobling, 1981). For example, the effect of dietary composition on post prandial metabolic rates (Cho et al. 1976; Jobling and Davies, 1980; Schelles and Wissing, 1976) shows discrepancies which may

be explained on the basis of variations in digestibility between different formulations. Cho et al. (1976) in their experiments with rainbow trout showed that growth rate with a high protein diet (60% protein and 15% fat) was the same as that of trout fed a control diet (40% protein and 15% fat) even though those on the high protein diet ingested less energy. On a high fat diet (40% protein and 25% fat) growth rate was again higher than that obtained with the control diet although the rates of oxygen consumption of the two groups were similar which suggests that the dietary fat was deposited as tissue stores rather than promoting "tissue growth" in the form of protein/tissue synthesis.

A significant amount of energy supplied by food is lost as nitrogenous excretory products. For teleosts, Winberg (1956) reported a range of 3 - 5% of ingested energy, whereas, Elliot (1976b) reported a range of 4 - 12% in brown trout and this was temperature and ration dependent. Brett and Groves (1979) noted that the rate of excretion is affected by many factors including, dietary quantity and quality. In most teleost species, ammonia is the chief excretory product ranging from 60 - 98% of the total nitrogen excreted (Smith, 1929; Wood, 1958; Fromm, 1963; Elliot, 1976b). It is mainly excreted through the gills and a very small part is excreted in urine (Smith, 1929; Beamish and Thomas, 1984).

There is a linear relationship between nitrogen excretion and nitrogen absorption as was found in largemouth bass (Micropterus salmoides) by Savitz et al. (1977). Ingested nitrogen in fish is either passed out in faeces or after absorption either stored in growth materials or excreted. Therefore, the loss of energy through excretion (U) is of particular interest because the energy loss by ammonia excretion increases with increase of ingested energy (C). Early workers usually discounted this component in energy budgets (Brafield, 1985) but Brett and Groves (1979) in generalized budgets found 'U' values of about 7% of 'C' for carnivorous fish. Thus, it is clear that although modest it is significant and must be accounted for in compiling energy budgets (Brafield, 1985).

Faecal energy (F) loss represents the energy content of undigested foodstuffs and is difficult to separate from material sloughed from the gut wall and catabolized digestive enzymes which also appear as faeces (Beamish et al. 1975). The amount of faecal energy loss (F) is reported to vary from 2 to 31% of the consumed energy (Elliot, 1979) and an average value of 15% of the ingested energy was quoted by Winberg (1956). A more variable range (1.3% to 77.7%) was reported by Brett and Groves (1979) and Pandian and Vivekanandan (1985). In most cases the techniques for measuring this parameter are not perfect, leaching and dilution occur, and the waste

products are difficult to separate from the aquatic environment. A number of chemical indicators have been used in digestibility studies to avoid the necessity for total collection of faeces and the most commonly used of such indicators is chromic oxide (Hepher, 1988).

Assimilated or digestible energy represents the difference between food energy 'C' and that of faecal energy 'F'. In the bioenergetics scheme proposed by Warren and Davies (1967), assimilated energy is used rather than digestible energy. Digestible energy, expressed as a percentage of the ingested energy, is also termed absorption efficiency, which in carnivorous fish varies from 85% - 98% (Birkett, 1969; Iwata, 1970; Edwards et al. 1972; Beamish, 1972). For herbivores this varies in a wide range from 20% for grass carp, Ctenopharyngodon idella (Fischer, 1970) up to 80% for angel fish, Holocanthus bermudensis (Menzel, 1959). Absorption efficiency for fishes fed with prepared diets display a wide variation (Halver, 1972).

For determination of various levels of metabolism, many different respirometers and metabolic chambers have been developed and have been described in many publications (Mar, 1959; Blazka et al., 1960; Beamish, 1964; Brett, 1964, 1973; Hogendoorn et al., 1982; Pearson et al., 1984, Ross and McKinney, 1988). Fish fed with pelleted diets in metabolism chambers do not need to search or compete for

food in water. Therefore, the active metabolic rate in this case is considered as level 1. Thus, 'R', the total respiration for a fish or a group of fishes fed in metabolism chambers comprises the sum total of routine metabolism, heat increment or SDA and active metabolic level 1. The respirometers of the type devised by Solomon and Brafield (1972) are very useful for energy budget experiments in which routine metabolism can be monitored over a long period in a stress-free state. The energy budget may also successfully be determined in a relatively bigger metabolism chamber from which R, F, U and P can be measured successfully over a defined period of time. Fish in such metabolism chambers will show a lower level of activity allowing more metabolic scope for growth.

The primary concern in fish culture is to increase the weight of fish in the shortest possible time under economically acceptable conditions. To achieve this it is necessary to satisfy all of the physiological requirements of the animals as summarized by Brett (1979). Bioenergetic studies are of fundamental importance to the the advance of aquaculture. Manipulation of environmental factors in conjunction with improved diets holds great promise that gross conversion efficiencies of 50% energy and over can be achieved, at least for young fish (Brett and Groves, 1979).

The common carp, Cyprinus carpio has a global distribution, occurring in tropical as well as temperate regions and it is acclimatized to a variety of habitats and a wide range of environmental conditions (Jhingran and Pullin, 1985). On a worldwide basis, it is one of the most extensively cultivated fish species (Bardach et al., 1972; Jhingran, 1975). It has gained wide acceptance for intensive and extensive culture for the following reasons:

(1) It breeds naturally in confinement.
(2) It can spawn throughout the year.
(3) It has a fast growth rate.
(4) It is a hardy fish.
(5) It is resistant to crowding and disease.
(6) It can tolerate a wide range of environmental conditions.
(7) The species can be intensively cultured in cages, pens and small tanks.
(8) It is acceptable as food to the consuming public and is an excellent table fish in countries of the tropics and sub-tropics.

From the published information to date, it is clear that there has been only limited work on carp energetics. Therefore, an elaborate study of carp energetics would allow the development of a complete energy budget which may simultaneously serve the interests of practical fish

farming and lead to a better understanding of the relationship between metabolism and the various factors affecting it.

The aims of the present study were to investigate the resting metabolic rate of common carp using computer operated respirometers under normal and altered photoperiods in an effort to understand the variability of the reported respiratory data and its associated energy loss. A series of experiments was also conducted to determine the effect of dietary protein on the feeding metabolism of carp. Finally, the individual components of the energy budget for common carp fed with different protein diets were determined in experiments designed to measure all components simultaneously. From the interrelationship of the components C, R, F, U and P it was hoped that energy budget models could be developed which would describe the growth observed in these trials and act as a predictive model based on energetic principles.

CHAPTER 2

General Methods

Introduction: This chapter describes common methods used throughout the different parts of this study. More specific techniques are described in the relevant chapters.

2.1 Experimental fish and their maintenance

Disease-free Cyprinus carpio L. were obtained from Fison's Fish Farm, Monton and Fison Plc,Cedars Factory, Suffolk, England and stocked into the tropical aquarium of the Institute of Aquaculture at Stirling University. The fish used for the experiment were in the weight range 55 g to 80 g. The respirometer chambers were able to accommodate fish of up to 110g (approximately). However in trial runs, larger fish showed difficulty in taking feed pellets showing less interested in feeding. Therefore, the size range used was considered a compromise between fish being so small that they had room for significant locomotor activity and so large that they were restrained from feeding normally. A second stock of 60 fish of about 50g to 75 g weight was received from the same stock and reared in the aquarium of the Institute of Aquaculture to

be used for the third phase of the experiment (Chapter 5). Fishes in the holding system were fed once a day at 10.00 a.m. at subsistence ration (about 1% of body weight) with a commercial trout pellet (Ewos Ltd) of 2.5 mm size.

The fish used for measuring resting metabolic rate at different photoperiod were fed with the pelleted diet 2 days prior to being used in the respirometer. But those fish used in feeding experiment in the respirometers (chapter 4) or metabolism chambers (chapter 5) were given the same experimental diets for one week prior to use. In all cases no feeding was allowed for 2 days prior to fish being used in the respirometers or metabolism chambers.

2.2 The fish holding system

About 200 fish were maintained in 24 white opaque plastic aquaria each with a capacity of 60L set in a recirculating system in the Institute's tropical aquarium. Water in the recirculating system (Fig. 2.1) was pumped from a large sump tank (150L capacity) into two header tanks through 38 mm diameter PVC pipe. The water from each header tank was then distributed through a two-way ball valve into a 15 mm PVC pipe from where it was distributed to a total of 12 aquaria. The water flow in the system is shown in Figure 2.2. The outflow water from the plastic aquaria was drained through a common drain pipe into a

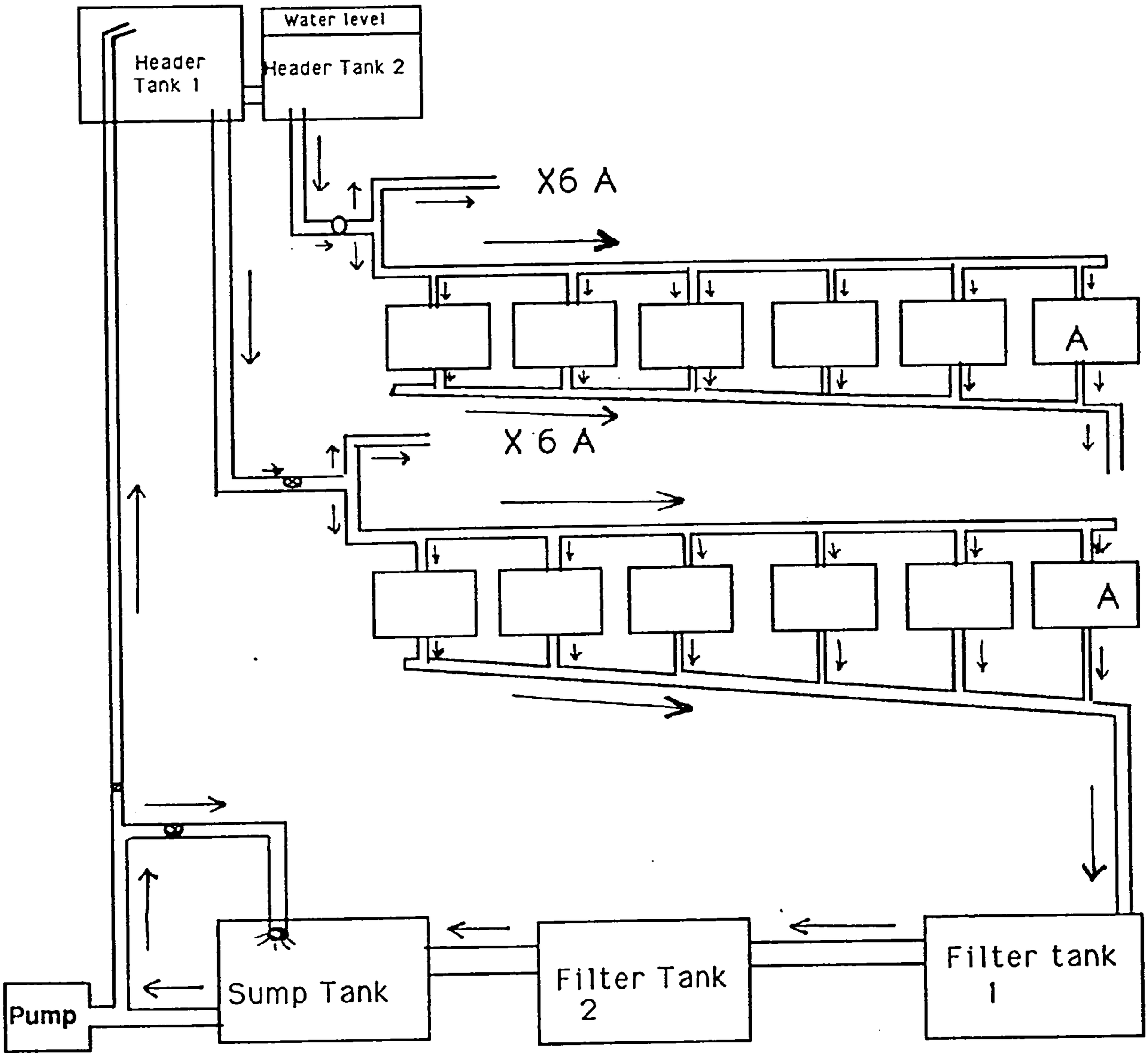


Fig. 2.1 Water recirculating system for holding fish in the tropical aquarium.

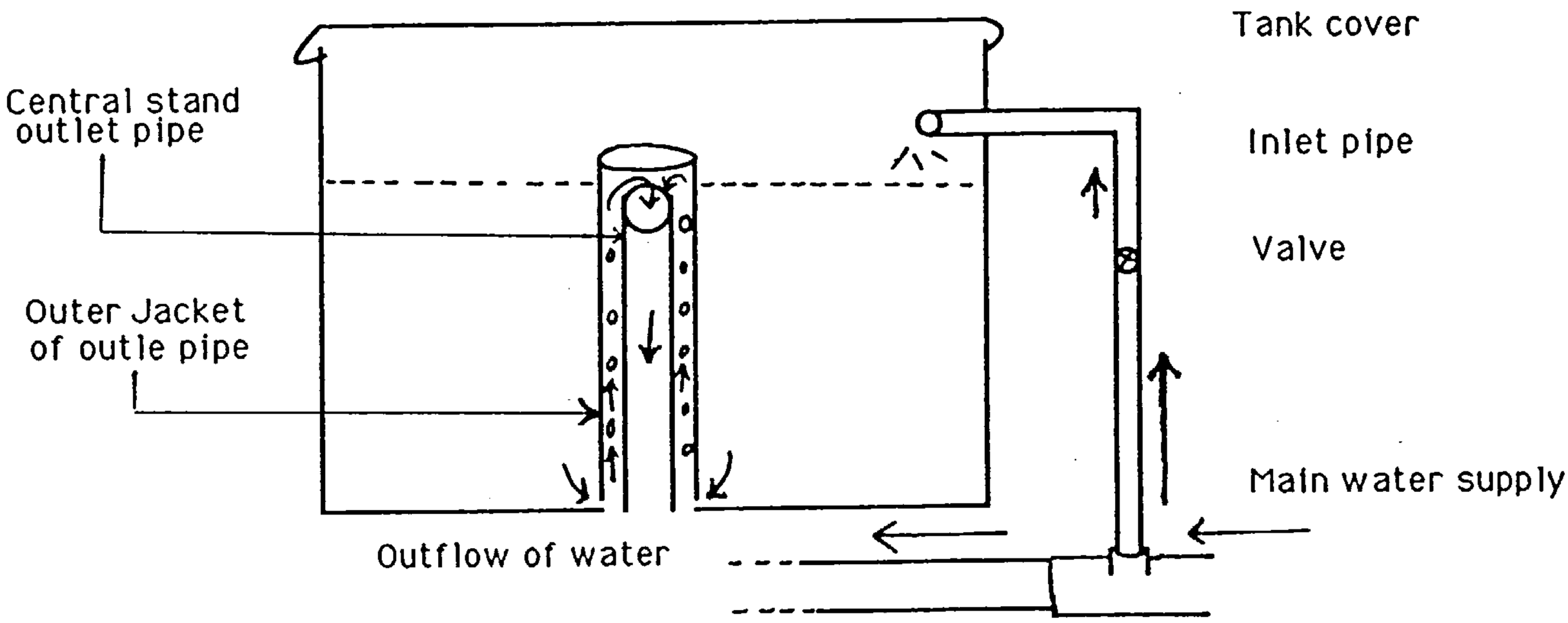


Fig. 2.2 Individual plastic aquarium for holding fish showing the direction of water flow .

filter tank situated at floor level containing numerous plastic rings which acted as biological filters. The water then passed into the sump tank through another filter tank for recirculation. The water temperature was maintained at 28.0 +_0.5^{o}C with aeration by an airstone in each aquarium Light intensity on surface water of fish tank was 25 lux

2.3 **Experimental Diets**

Three experimental diets containing 20, 35 and 50% protein were prepared using herring fish meal (Ewos Ltd), dextrin and corn starch (Sigma Chemicals Ltd) as the principal ingredients. In order to minimize the disintegration of feed ingredients into water, carboxy-methyl cellulose (CMC) (BDH Chemicals Ltd) was incorporated in the diet as binding material. To enhance the taste L - glutamic acid (Sigma Chemicals Ltd) was mixed in the diet and so make it appetizing to the fish. Chromic oxide (Cr_2O_3) was used in the diets for determination of apparent protein digestibility and to provide a uniform colour. The composition of the three diets are shown in Table 2.1.

To compensate for the shortfall in the essential amino acid profile in the 20% protein diet it was balanced by adding a supplementary amino acids mix (Table 2.2). The amount to be used was determined from the calculations based on the amino acid composition of fish meal (Ogino,

Table 2.1 Composition of experimental diets (dry weight basis).

	DIET		
Protein(%)	* 20%	35%	50%
Ingredients	g/Kg		
Fish meal	468.0	672.0	264.0
Corn starch	202.2	118.0	308.0
Dextrin	200.0	120.0	305.0
Vitamin mix	20.0	20.0	20.0
Mineral mix	20.0	20.0	20.0
Amino acid	23.3	----	----
Mono sodium glutamate (taste)	10.0	10.0	10.0
Binder (Carboxymethyl cellulose)	10.0	10.0	10.0
Marker (Chromic oxide)	5.0	5.0	5.0
Corn oil	41.5	25.0	58.0
	1000.0	1000.0	1000.0

* Supplementary Essential amino acids are added in this diet

Table 2.2 Supplemental amino acids mixed with 20% protein containing diet to balance the essential amino acid requirements for common carp

Amino acid	Amino acid added to the diet g/100g dry diet (20% dietary protein)
Arginine	0.25
Histidine	0.11
Isoleucine	0.06
Leucine	0.22
Lysine	0.65
Methionine	0.07
Phenylalanine	0.42
Threonine	0.52
tryptophan	0.03
Total	2.33

1980b; NRC, 1981). In order to economise on the use of amino acids, a total of 600g ingredients were mixed for the 20% protein content diet and 1 kg of ingredients for the other two.

The mineral premix used (Table 2.3) was formulated for trout by Tacon et al.(1982) and this has proven successful for tilapia and for common carp. A commercial vitamin mix supplied by Colborn - Dawes Nutrition Ltd was used with other ingredients in the diets (Table 2.4). Corn oil (Sigma Chemicals Ltd) was used to keep the lipid content nearly constant (about 8%) in the three diets.

After mixing the weighed dry ingredients for about 10 minutes in a Hobart industrial food mixture, corn oil was added slowly. The amount to be added was pre-determined to provide the required lipid content in the diet. Warm water was then added slowly into the mixes until the correct consistency was obtained for pellet formation. The mixture thus obtained was pressed through the extruder attachment of the Hobart and was broken into small pieces by hand as it emerged. The pellets were then dried overnight at 65°C and were packed in polythene bags and stored in a freezer at -20°C.

Proximate analysis of the individual diets was carried out to determine moisture, ash, crude protein, crude lipid

Table 2.3 Composition of mineral premix used in preparing diets.

Mineral	% inclusion level	Amount used as 2% per Kg diet (g)
Calcium orthophospate ($CaHPO_4.\ 2H_2O$)	72.77	14.554
Magnesium sulphate ($MgSO_4.\ 7H_2O$)	12.75	2.550
Sodium chloride (NaCl)	6.00	1.200
Potassium chloride (KCl)	5.00	1.000
Iron sulphate ($FeSO_4.\ 7H_2O$)	2.50	0.500
Zinc sulphate ($ZnSO_4.\ 7H_2O$)	0.55	0.110
Manganese sulphate ($MnSO_4.\ 4H_2O$)	0.25	0.050
Copper sulphate ($CuSO_4.\ 5H_2O$)	0.08	0.016
Calcium iodate ($CaIO_3.6H_2O$)	0.05	0.006
Chromic chloride ($CrCl_3.6H_2O$)	0.01	0.002
	100	20

Table 2.4 Composition of vitamin mix used in the diets

Vitamin	$mgKg^{-1}$
Vitamin K, menadione pyrimidinol bisulphate	900
Vitamin B_1, thiamine HCl	1200
Vitamin B_2, riboflavin	3000
Vitamin B_6, pyridoxine HCl	2000
Vitamin B_{12}, cyanocobalamin	2
Vitamin C, ascorbic acid	40000
Biotin, d -biotin	60
Nicotinic acid	20000
Pantothenic acid, ca d- pentothenate	4500
Folic acid	700
Ethoxyquin	10000
Vitamin A	250000IU/Kg
Vitamin D3	80000IU/Kg
Vitamin E	25000IU/Kg

and crude fibre contents. The results obtained are shown in Table 2.5. The analytical techniques are described in the following section.

The essential amino acid contents in the formulated diets (Table 2.6) was analysed by WELMET, the Edinburgh Protein Characterisation Facility, in the Department of Biochemistry, University of Edinburgh. An applied Biosystems 130A Microbe HPLC System was used to determine the essential amino acid content.

2.4 Analytical techniques

The proximate composition of samples were determined in triplicate, and sometimes quadruplicate, by the methods given in AOAC (1980), with certain modifications.

Moisture

Moisture content was determined from an accurately weighed sample by drying at 105°C for about 24 hours to a constant weight.

Table 2.5 Proximate analysis of experimental diets on dry weight basis.

Component	20%	35%	50%
Crude protein (%)	19.74	35.14	49.88
Crude lipid (%)	7.64	7.43	7.34
Moisture (%)	7.70	6.91	7.63
Ash (%)	7.15	9.68	12.29
Fibre	0.21	0.15	0.12
Chromic oxide (%)	0.50	0.50	0.50
N.F.E. (%)*	57.06	40.19	22.18
Total	100	100	100
** Energy value	17.57kJ/g	19.17 kJ/g	19.36 kJ/g

* N.F.E. = Nitrogen free extractives and calculated as

NFE = 100 - (% moisture + % crude protein + % crude lipid + % ash + % chromic oxide)

** Calculated by bomb calorimetry.

Table 2.6 Quantitative requirements of essential amino acids and essential amino acid content in 20, 35 and 50% protein content diet

Amino acid	*Essential requirement	50% protein content diet	35% protein content diet	20% protein content diet
Arginine	1.52	3.19	2.20	1.50
Histidine	0.56	1.10	0.79	0.53
Isoleucine	0.92	2.15	1.53	0.93
Leucine	1.64	3.57	2.48	1.61
Lysine	2.12	3.69	2.53	2.06
Methionine	0.64	1.38	0.89	0.60
Phenyl-alanine	1.16	1.79	1.28	1.14
Threonine	1.32	1.97	1.38	1.30
Tryptophan	0.24	0.51	0.36	0.22
Valine	1.16	2.88	2.00	1.15

* Values are expressed as grams per 100g of dry diet considering 40% dietary protein for maximum growth in common carp (Ogino, 1980b). Other values are also expressed as grams per 100g of dry diet.

Ash

The ash content was determined from a known weight of sample in a dry porcelain crucible by igniting in a muffle furnace at a temperature at 450°C for 12 hours.

Crude protein

Crude protein was determined using the micro-Kjeldahl method (Tecator, Kjeltec system, 1003 Distilling unit; Digestion system 40, 1016 Digestor) for measuring nitrogen and applying the empirical factor of 6.25 (Kjeldahl factor for animal protein) to the result to convert total nitrogen to percent protein which assumes that protein contains 16% nitrogen.

Crude lipid

Crude lipid was determined by extracting a known quantity of sample with analytical grade petroleum ether (B.P. 40 - 60°C) using the Soxhlet apparatus (Tecator, Soxtec system HT, 1043 Extraction Unit). The final percent crude lipid was obtained by evaporating the residual solvent in an oven for 1 hour at 105°C.

Crude fibre

Crude fibre was determined using the Tecator Fibretec system, (1020 Hot Extractor, 1047 hydrolysis unit).

Nitrogen Free Extract (NFE)

NFE was calculated by the difference method of Hastings (1976).

2.5 Bomb calorimetry

Brody (1945) summarized the principles of calorimetry, dating back to the early experiments of Lavoisier and Laplace in 1780. In direct calorimetry heat loss from an animal's body is measured directly. This technique has been considered unsatisfactory for studies on fish because of the relatively low heat production by fish and the high heat capacity of water in the experimental system (Brett and Groves, 1979). On the other hand, heat loss by vaporisation and radiation which complicate direct calorimetry in terrestrial animals does not occur in aquatic forms (Smith, 1971; Brett and Groves, 1979).

Indirect calorimetry, involving the measurement of gas exchange, was begun by Lavoisier. The energy equivalents for oxygen uptake (and carbon dioxide production) are

based on the heats of combustion of different energy substrates measured in a bomb calorimeter. In the construction of an energy budget, total energy intake is calculated from the energy value of feed and it is also necessary to know the energy content of the animal carcass and the faeces. Although chemical methods are sometimes used to determine the energy content of different substrates, these have been found to be unsatisfactory with biological samples involving oxidation by strong acids (Brafield and Llewellyn, 1982). Instead, the heat of combustion of a sample is usually measured with a bomb calorimeter.

The energy content of fish feed, faeces and fish carcass were determined by bomb calorimetry. A Gallenkamp Autobomb, Automatic Adiabatic Bomb Calorimeter was used in the present study. The material whose energy value was to be determined was homogenized and then dried to a constant weight at 70°C for 24 hours. A temperature lower than 70°C was to be avoided as from experience it is often insufficient to remove enough water which then resulted in the underestimation of energy value. However, a higher temperature was not used as this causes loss of volatile materials such as fats. At least three sample replicates were weighed and made into pellets which were then placed into a crucible and stored in a desiccator until bombed.

Before combustion of the samples in the bomb calorimeter the heat capacity of the machine was determined by burning a known quantity of analytical grade Benzoic acid which has an energy of 26.43kJ/g. The temperature rise in the calorimeter thermometer due to complete combustion of known quantities of Benzoic acid samples were primarily used in calculating the heat capacity of the machine. This value was then used in the determination of energy value of the samples.

Sample

Sample weight was chosen to give a total heat release of about 30,000J (7.500 cal). About 0.8 to 1.0g of feed samples and carcass samples were used in bomb calorimetry. A preliminary test was done with a small sample to determine the amount required. For faecal matter, the amount collected was less than that required for burning inside the bomb, therefore, it was mixed with a known quantity of Benzoic acid for burning. The actual increase of temperature in the calorimeter thermometer for burning of the Benzoic acid was then subtracted from the total increase of temperature due to burning of faeces plus Benzoic acid. The subtracted temperature was used for calculation of energy in the faecal sample. Samples used for burning were firmly compacted by pressing into a pillin order to prevent too rapid combustion which gives

false results because it usually causes a proportion of the sample to be blown out of the crucible without being burnt.

Procedure

Two electrodes of the bomb are connected with nickel wire. The weighed, compact sample having dipped inside with a standard length of (about 9.0 cm) cotton thread was placed in a crucible held with a crucible support ring at the rear end of one electrode. The cotton thread was fitted to the firing wire with its free end. The bomb cap with the electrode was set inside the body of the bomb. The bomb cap was then hand tightened so that metal to metal contact was achieved between the bomb cap and body and the gaskets could not further be compressed. The bomb cavity was then filled with oxygen to a pressure of not more than 35 bar. When the test circuit was found to be intact, the firing button was pressed for 2-3 seconds for combustion of the sample inside the bomb. A sharp rise of mercury level in the calorimeter thermometer occurred and after 6-8 minutes, when no further increase of mercury level was found, the temperature was recorded. The difference between initial and final reading in the calorimeter thermometer and the heat capacity of the machine was then used to calculate the energy value (J/g) of the sample. The ash in the crucible from each sample was weighed separately to assure

the complete burning of the sample. The amounts of ash found from bomb calorimetry were observed to be similar to values recorded during proximate analysis. Energy values of samples were discarded if any significant variation of ash content were found as this may have been due to incomplete burning of the sample. In these experiments the energy value of the dried samples were not reported on an ash - free basis.

2.6 Dissolved oxygen

Oxygen consumption by fish in the individual respirometer chambers was measured by using an oxygen probe (YSI model 5739) and oxygen meter (model YSI 57) and calculated by the following equation

$$mgO_2/kg/h = \frac{(O_{2sat} - O_{2\ out}) \times \text{flow rate(l/h)} \times 1000}{\text{weight of fish (g)}}$$

where, O_{2sat} = D.O. in the reference chamber

O_{2out} = D.O. in the outlet of the respirometer chambers containing fish

The oxygen electrode used for measuring dissolved oxygen in water was calibrated using the Winklers' method (Stirling, 1985) at the start of each experimental run.

2.7 Ammonia

The phenol - hypochlorite method (Stirling, 1985) was used for measuring total ammonia in the water. Ammonia in water reacted with phenol and hypochlorite in alkaline solution to give indophenol blue. Sodium nitroprusside was used to intensify the blue colour at room temperature and the absorbance of standards and samples against a reagent blank were measured at 635 nm. Concentrations of ammonia in water samples were then calculated from the regression equation of the standards.

2.8 Ion exchange in resin

Since the respirometry was carried out in a recirculating system the ammonia produced in the system, which is toxic for fish life at a certain level, needed to be controlled.

The sodium forms of cation exchange resins (sulphonated polystyrene resins) commercially known as Dowex 50 - X8 were used to adsorb the ammonium ions from the water, releasing an equivalent amount of sodium ions.

The general column technique was applied for ion exchange purpose. In this operation the resin was held in a vertical column (6.8cm diameter and 45.0cm long) of opaque perspex through which flows the materials being treated

and through which regenerating solution and water washes were subsequently run. The bottom of the column was provided with a tap to control the outflow of regenerant and water-wash. As a solution moves down the column the ion exchange reaction is continually driven forward (i.e. downward). The 'bed volume' for the column could be simply determined from the formula $\pi r^2 l$, where r and l were the radius and length of the column. Before charging the column the resin was fully hydrated and part filled with water to maintain the resin in good condition.

Sodium chloride 5% (w/v) was used as a regenerant for the ion exchange resin. A flow rate of 0.1-0.12 bed volumes/minute was used and 890 ml of regenerant (NaCL) was applied for each 100 ml moist resin. After charging the resin it was conditioned by backwashing with deionised water.

The charged resin thus obtained was transferred with a little water from the column to a fine meshed bag of synthetic cloth. The resin was then set in an Eheim filter between two layers of synthetic wool. Care was taken that the resin was always moist. The lid of the filter was then fitted firmly and was installed for ion exchange in the sump tank of the respirometer.

2.9 Apparent protein digestibility (APD)

The apparent protein digestibility of protein in the fish feeds used in different experiments was determined using the method of Furukawa and Tsukahara (1966). Chromic oxide was estimated spectrophotometrically after digestion using perchloric acid and nitric acid. Then the percent chromic oxide values were used in the following equation to calculate the APD (%) :

APD (%) for protein =

$$100 - \left(100 \times \frac{Cr_2O_3 \text{ in diet (\%)}}{Cr_2O_3 \text{ in faeces(\%)}} \times \frac{\text{Protein in faeces (\%)}}{\text{protein in diet (\%)}}\right)$$

Chapter 3

The resting metabolism of carp, Cyprinus carpio and the effect of photoperiod.

3.1 Introduction

Knowledge of the physiological characteristics of fish is necessary in order to develop a scientific basis for their culture and management. Metabolic rate is of major importance because it reflects the energy expenditure of fish for resting, feeding and active metabolism, and consequently their growth requirements (Winberg, 1956). Depending on the activity of fishes three metabolic levels may be distinguished (Fry, 1957; Brett, 1962; Kausch, 1972; Albrecht, 1974b; Braaten, 1979; Brett and Groves, 1979, Brafield, 1985): standard, routine and active metabolism. According to Brett and Groves (1979) standard metabolism is the minimum rate of oxygen consumption expressed as mg of O_2/kg/h of an organism at rest in the post absorptive state and thermally acclimated; routine metabolism is the metabolic rate of an organism during normal spontaneous activity, and active metabolism is the rate of oxygen consumption during maximum sustained activity.

In fish, metabolic rate is primarily limited by the ability of the gills to extract oxygen from water (Priede, 1985). Both resting and basal metabolism have been used to denote the minimum metabolic rate accompanying the energy cost of maintenance. In practice, basal metabolism is expressed as the minimum energy cost when the animal is at rest in a thermoneutral environment in the post-absorptive condition (Brett, 1962). However, the possibility of obtaining absolute rest has frequently been questioned as fish are subject to excitement from experimental confinement. This has led to the measurement and use of standard metabolism (Brett, 1962). The definition of standard metabolic rate was clarified by Hill (1976). He stated that as fish are poikilotherms, they allow their body temperature to fluctuate over a wide range according to environmental circumstances. When such animals are post-absorptive and at rest, their metabolism at the standard rate for the prevailing body temperature. But for homoeotherms, there is a certain range of environmental temperature, termed the thermoneutral zone, in which metabolic rate turns out to be minimal and the rate in the resting, post-absorptive individual in the thermoneutral zone is termed the basal metabolic rate. In both cases, the "standard" measure of metabolic rate is a 'resting condition'. It is however, not a small challenge to persuade an experimental fish to rest completely. 'Rest' thus has a different meaning in different studies

of metabolism. Some workers apply the term 'routine metabolic rate' to reasonably quiet animals exhibiting only relatively small, spontaneous movements and reserve 'standard metabolic rate' for animals that have been coaxed to a truly minimal level. Thus, in the literature, many standard metabolic rates are in fact routine rates. When activity is truly minimal under standard conditions metabolic rate approximates that rate necessary for simple physiological maintenance of life.

The prime demand for food is to meet maintenance requirements before any energy storage or somatic growth. Thus, basal metabolism or resting metabolism has the highest priority (Brett and Groves, 1979). The determination of this physiological minimum has been given most attention among the various metabolic demands (Winberg, 1956) undoubtedly influenced by the considerable work on basal metabolism in homoeotherms (Brody 1945, Kleiber, 1961).

Metabolic rate is directly related to oxygen consumption and as in other animals is most often measured indirectly by the rate of oxygen consumption. It is generally agreed that this constitutes a suitable measure of the processes of energy conversion (Winberg, 1956). Subject to the proviso that no anaerobic processes of any consequence are taking place, a value of 13 - 14 J/mg O_2 may be assumed

(Winberg, 1956; Davis and Warren, 1968; Brafield and Solomon, 1972; Brett and Groves, 1979).

The rates of oxygen consumption of various fish under different conditions have been studied by many authors (Ege and Krogh, 1914; Baldwin, 1923; Clausen, 1936; Khalil, 1937; Oya and Kimata, 1938; Ivlev, 1939; Fry, 1947; Fry and Hart, 1948; Winberg and Khartova, 1953; Winberg, 1956; Brett, 1962; Beamish, 1964; Kausch, 1969; Averett, 1969; Elliot, 1969; Edwards et al. 1971; Muir and Niimi, 1972; Solomon and Brafield, 1972; Huisman, 1974; Brett, 1976b; and many others). The review work of Winberg (1956) on fish metabolism shows that the variability within the data presented by authors is frequently very large and a comparison between authors for the same species shows differences which sometimes cannot be interpreted. Many important factors such as water quality, acclimation time, space required for the experiment, level of stress and replication of experiments have often been overlooked resulting in reports of variable metabolic rate for the same fish (Winberg, 1956). There are many environmental factors effecting the oxygen consumption rate in fish and these has been summarized by Fry, (1957); Brett, (1962) and Brett and Groves (1979). Significant errors can also be introduced if the oxygen debt (due to intense muscular work) from earlier excitation is not fully replaced (Black, 1958b; Black et al., 1961). Since

fish do not have a definite basal metabolic rate like warm blooded animals, the experimental conditions need to be extremely carefully defined in order to produce reliable data. Thus, careful attention is needed for the determination of reproducible measures of metabolic rate in fish.

The oxygen consumption of fish has been measured by various authors in any one of the three basic methods, namely, use of hermetically sealed vessels (Ege and Krogh, 1914; Winberg and Khartova, 1953), the gas recirculation method based on Regnard method (Joylet and Regnard, 1877, Gardner, 1926) and flowing water method (Clausen, 1936, Oya and Kimata, 1938). In the flowing water method it is possible to measure the respiratory rate of a single specimen of fish of any size during the experiment provided that a constant environment and the immobility of the fish is maintained throughout. Most authors have determined oxygen consumption by Winkler's titration technique (Clausen 1933, 1936; Winberg, 1956; Ahmed and Magid, 1968; Huang, 1975; Beamish, 1964; Job, 1969) which is reliable and inexpensive, although instantaneous results are not easily possible. In recent years the oxygen electrode has gained wide acceptability (Diana, 1983; Kausch, 1969; Solomon and Brafield, 1972; Hamada and Maeda, 1973; Verheyen, 1985; Ross and Mckinney, 1988) due

to its accuracy and ability to give instantaneous readings.

Since the development of appropriate methods, respiratory rhythms in fish have been studied in many parts of the world (Muller, 1978). Physiological rhythms may coincide in time with a rhythmic phenomenon in the physical environment in such a way that there is a relationship between the two and a number of authors have shown that the metabolic rate in fishes varies regularly during the day (Spencer, 1939; Clausen, 1936; Winberg, 1956) and that the difference may be quite considerable.

There is a significant natural variation in the resting metabolic rate of some fishes during the day, related to changing photoperiod (Davies, 1962; Holliday and Blaxter, 1964; De Silva et al.,1986; Ross and Mckinney, 1988). Long term changes in the photoperiod are also known to influence respiratory rate in fishes (Withey and Saunders, 1973). A number of studies have shown that light is the main environmental variable affecting rhythmic patterns in fish (Eriksson, 1973; Swift, 1962, 1964; Muller, 1970; Byrne, 1971). However, the data are sometimes not as clear as in other animals either due to differences in measuring techniques (Eriksson, 1973) or to the size and overall design of the respirometer and whether flow is open or closed (Caulton, 1978). Since fish do not have a defined

basal metabolic rate like warm-blooded animals, quite a range of observations should be made to determine metabolic rate (Winberg, 1956) rather than the few measurements which have been made by many authors. Sometimes, the acclimation time has also been given little attention despite the fact that Fry (1957) pointed out its importance.

Carp, Cyprinus carpio is one of the principal cultured fish species in both tropical and sub-tropical water bodies. There has been a number of investigations of resting metabolism of common carp. Beamish, (1964) studied respiration of carp with special emphasis on standard oxygen consumption and Kausch, (1968), Hamada and Ida, (1973) and Huisman, (1974) also worked on standard metabolic rate in carp. Oya and Kimata, (1938) demonstrated the existence of well- developed diurnal variation in the metabolic rate of carp, Olifan (1940) studied the "rhythm of respiration" for carp larvae and Winberg and Khartova (1953) studied the metabolic rate of young carp during various hours of the day. However, these authors have reported a wide range of values for standard daily metabolic rate due to variations in techniques, equipment, and other undefined parameters. The effects of many different factors such as body weight, temperature, oxygen concentration, partial pressure, carbon dioxide etc. affecting the resting metabolism in carp has been

reported. However, there is no known report of the effect of changing photoperiod on resting metabolism in carp, Cyprinus carpio.

In view of this, the main objectives of this study were to observe and quantify the normal pattern of oxygen consumption, and hence resting metabolism during normal photoperiod and the effect of altered photoperiod on carp. These data were intended to provide an insight into natural rhythmicity, to give context to the variability in published data and would also be used as a baseline for studies of SDA and complete energy budgets.

3.2 Materials and methods

3.2.1 The acclimation system and fish stocks.

The acclimation system for the fish used in the present study was a part of the holding system for the experimental fishes which was described in detail in section 2.1.

Eighty Cyprinus carpio of mean body weight 70 (± 10.0)g were held in the water recirculating system at 28°C. The experimental fish were fed twice daily on a commercial pellet at their maintenance level of approximately 0.6% body weight (equivalent to 94 kJ DE/kg/day, according to Schwarz and Kirchgessner, 1984). The fish were not allowed to feed for two days before being placed in the respirometers.

3.2.2 Photoperiod acclimation

Two chambers, each consisting of 2 tanks from the main fish-holding system were light-sealed with thick black plastic sheets. Light and dark cycles were maintained by using one twenty-watt fluorescent tube in each compartment, set approximately 50 cm above the water surface and controlled by separate timers. Sixteen fishes,

eight in each tank, were acclimatized for four weeks to a single photoperiod before commencing trials in the respirometers. Light intensity in the respirometers had similar light intensity as in main system. The light : dark regimes used were :

(a) 24L : 0D - total light

(b) 18L : 6D - light On @ 0800 h ; Off @ 0200 h

(c) 12L : 12D - light On @ 0800 h ; Off @ 2000 h

(d) 6L : 18D - light On @ 0800 h ; Off @ 1400 h

(e) 0L : 24D - total dark

3.2.3 The respirometer system

The oxygen consumption, and hence resting metabolic rate, under different photoperiods was determined in a six channel automatic respirometer in which two channels were used for calibration of the system and four channels contained fish (Ross and Mckinney, 1988). In practice, channels 1 and 4 are used for recalibration of the system which occurs every 15 minutes. In each of the respirometer chambers, any blockage of the solenoid outlet valves due to scales etc. was prevented by setting a 1.5mm meshed polystyrene cone over the outlet. A computer-operated self cleaning routine was incorporated to dislodge any particles every 2 hours. The apparatus was checked properly with a test run before commencing each experimental run.

The respirometer was enclosed by thick black cloth to prevent external disturbance and the photoperiod was controlled by the computer. The respirometer chambers were provided with acrylic plates at each side which minimised fish movement. A water flow rate of about $15lh^{-1}$ and water temperature was maintained at $28^{o}C$ ($\pm 0.5^{o}C$) (Gallenkamp heater, thermostirer - 85) in the system. Air-saturated water was maintained throughout the experiment by two large air-stones placed in the header tank. In the sump tank the water was recirculated by an Eheim filter containing filter wool to reduce the particulate matter in the system. About one third of the water from the sump tank was replaced by fresh water of the same temperature every alternate day without disturbing the system. The pH and ammonia levels in the system were checked routinely by a digital pH meter (Russel 660 pH meter) and phenol-hypochlorite method (See section 2.6) respectively at both the start and the end of each experiment. After each experimental run the respirometer was thoroughly cleaned to minimize disease incidence.

Oxygen consumption was recorded by the microcomputer at five minute intervals in consecutive chambers, thus providing two readings per chamber per hour. The data were saved to disc for subsequent analyses.

3.2.4 Resting respiration in different photoperiods

Groups of four fish were taken randomly from the acclimation tanks in a plastic bucket and anaesthetized with benzocaine (Ross and Geddes, 1979). They were then blotted by tissue paper, weighed and placed in the respirometer chambers. The fish were allowed to acclimate to the new environment for the first 24 hours and they then remained in the chamber for a total of seven days without feeding. During this time the respirometer was monitored continuously. Between 12 and 20 naive fish were used in each photoperiod regime. In some cases the fish were unable to remain in the chambers for the full period due to accidental blockages. However, only the results from successful runs were processed further.

The results obtained from each experiment were initially logged as mg/kg/h. The data were reprocessed on a BBC-B microcomputer using a 52H smoother (Velleman and Hoaglin, 1980) to reveal respiratory peaks and troughs from the raw data. The minimum, mean and maximum daily oxygen consumption rates were then calculated for each 24 hour photoperiod. The oxygen consumption for all fish in each group were then expressed as hourly arithmetic means. Mean metabolic rates in different photoperiods were analysed by analysis of variance (Ryan et al., 1985). For peak values, the data were considered in three hourly blocks. The data

were depicted graphically using Cricket Graph 1.3 on a Macintosh SE microcomputer.

3.3 Results

Metabolic rate during normal photoperiod (12L : 12D)

Common carp acclimated to a normal photoperiod (12L : 12D) exhibited a notable variation in resting oxygen consumption rate during the 24 hour period. The respiratory pattern is cyclic with two significant peaks, one at the onset of light at 0800 hour followed by a decline during the light period and then another peak at about the onset of darkness. The mean oxygen consumption response of all fish treated at 12L : 12D is shown in Fig. 3.1a and a crepuscular rhythm is discernible, recognized by significantly higher ($p < 0.05$) oxygen uptake at about onset and offset of light. The percent frequency of mean respiratory peaks (the values considered as 15% above the mean) and troughs (the values 15% below the mean) over 24 hours observation also shows an increase and decrease during the onset of light and dark respectively (Fig. 3.2). The mean oxygen consumption rate over 24 hours was 152.45 mg/kg/h, ranged from 137.35 mg/kg/h at 0500 h to 171.71 mg/kg/h at 2000 h (Table 3.1). The mean oxygen consumptions during the 12 hours light period and 12 hours dark period were 152.94

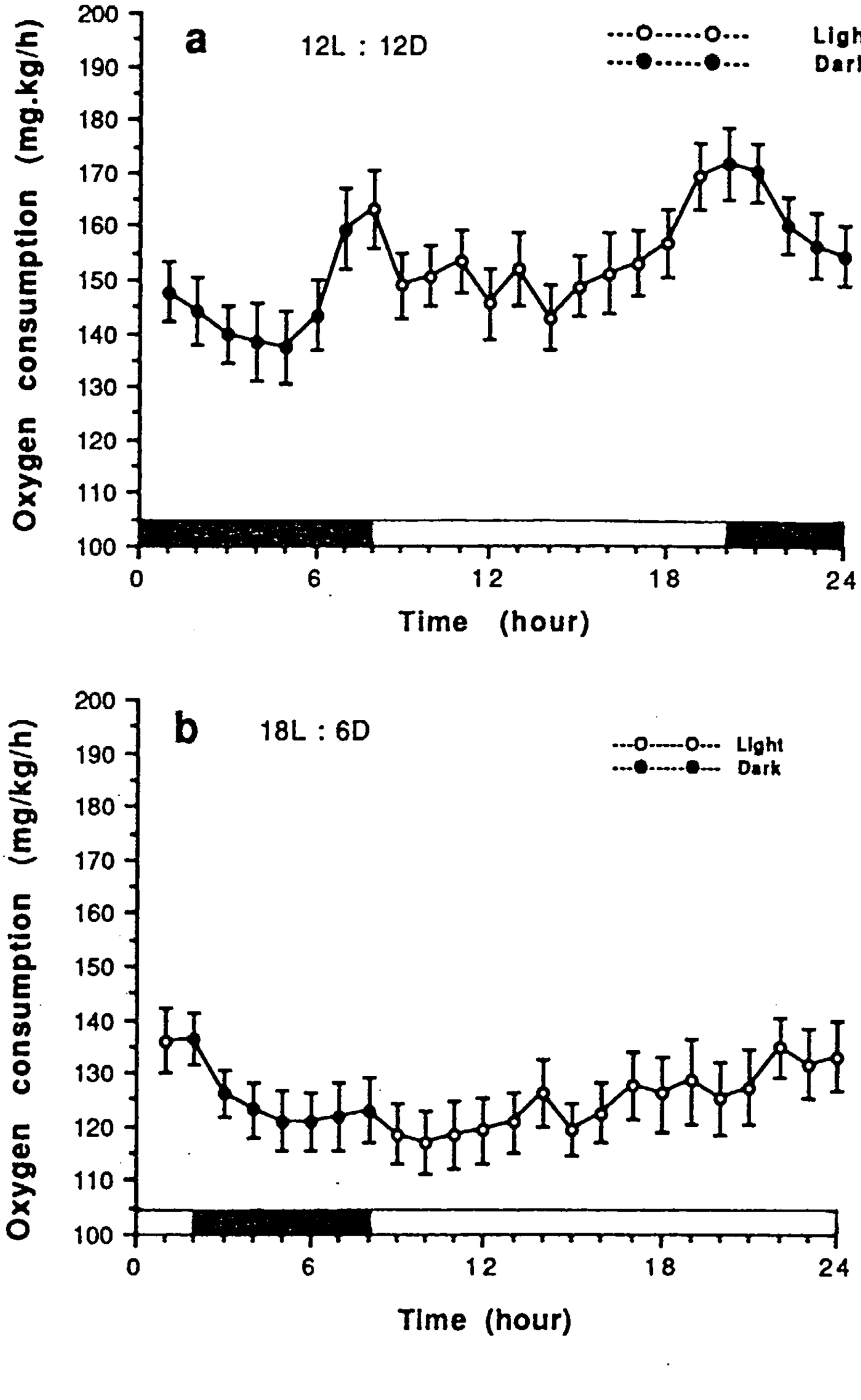
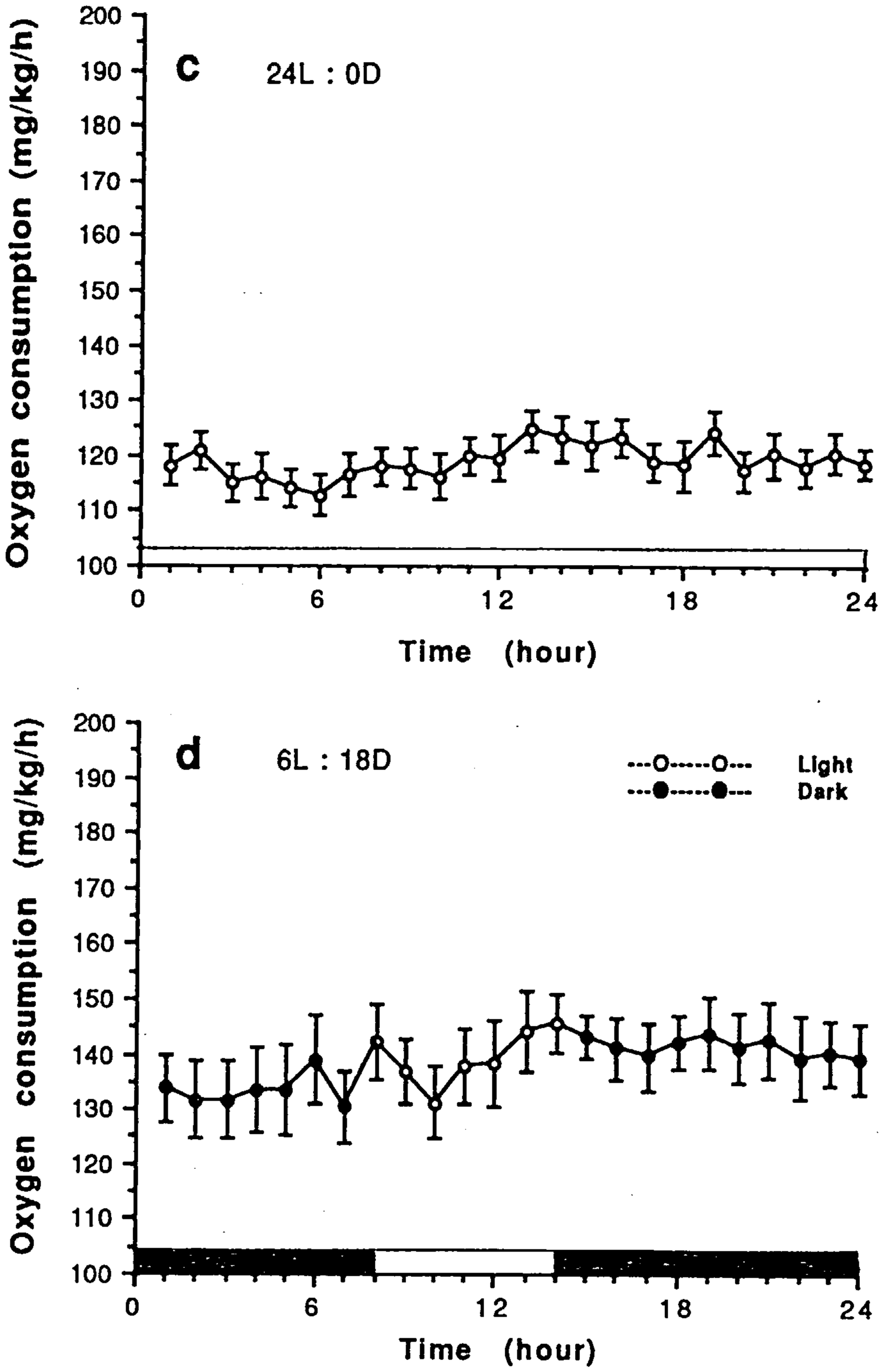
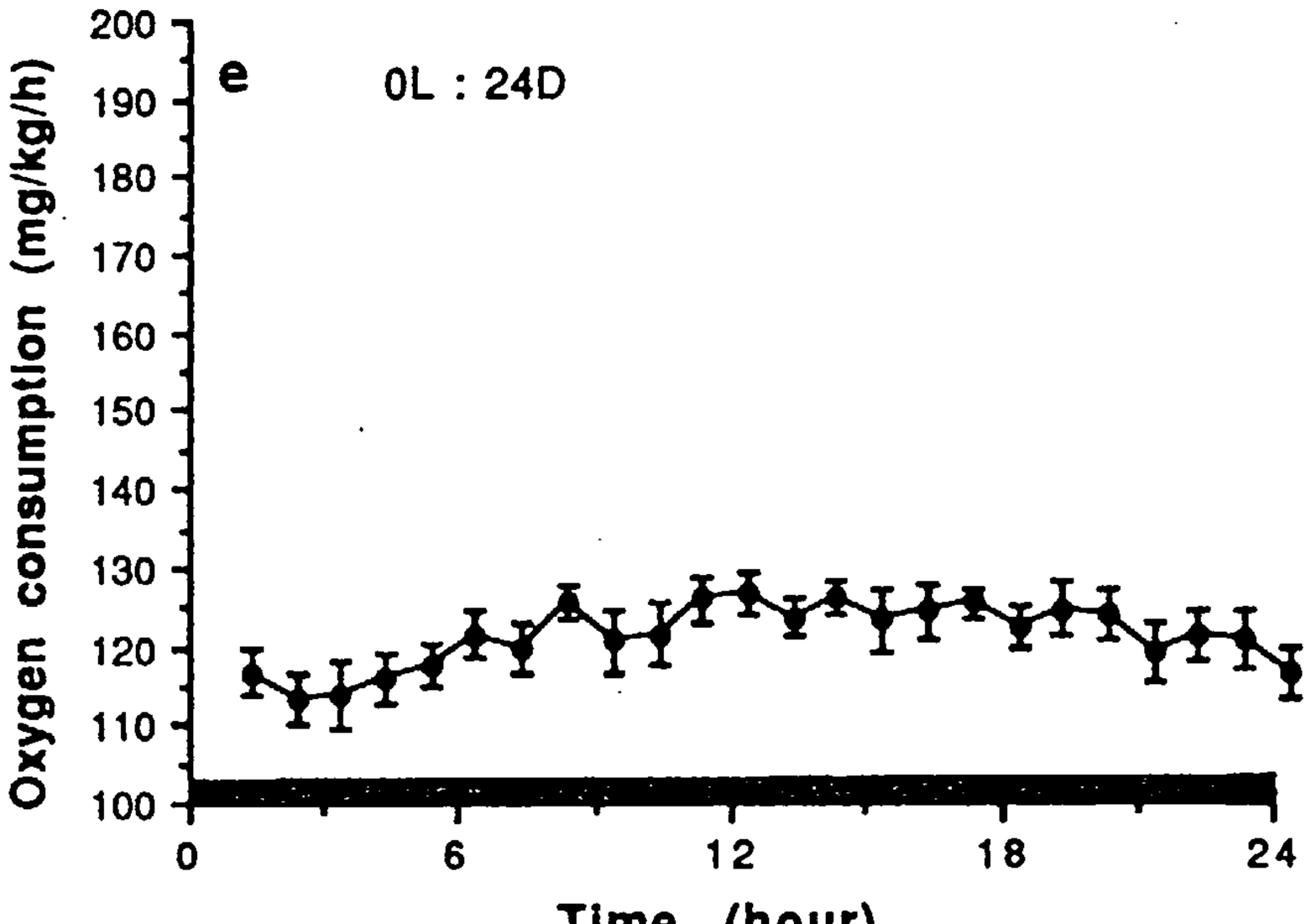


Fig. 3.1 (a - e). Mean metabolic rates of Cyprinus carpio in (a) 12L : 12D, (b) 18L : 6D, (c) 24L : 0D, (d) 6L : 18D and (e) 0L : 24D. The values are arithmetic means with vertical bars as +_ standard error of means.


c
24L : 0D
Oxygen consumption (mg/kg/h)
200
190
180
170
160
150
140
130
120
110
100
0
6
12
18
24
Time (hour)
d
6L : 18D
---O-----O--- Light
---●-----●--- Dark
Oxygen consumption (mg/kg/h)
200
190
180
170
160
150
140
130
120
110
100
0
6
12
18
24
Time (hour)

Continued..


e
0L : 24D
Oxygen consumption (mg/kg/h)
200
190
180
170
160
150
140
130
120
110
100
0
6
12
18
24
Time (hour)

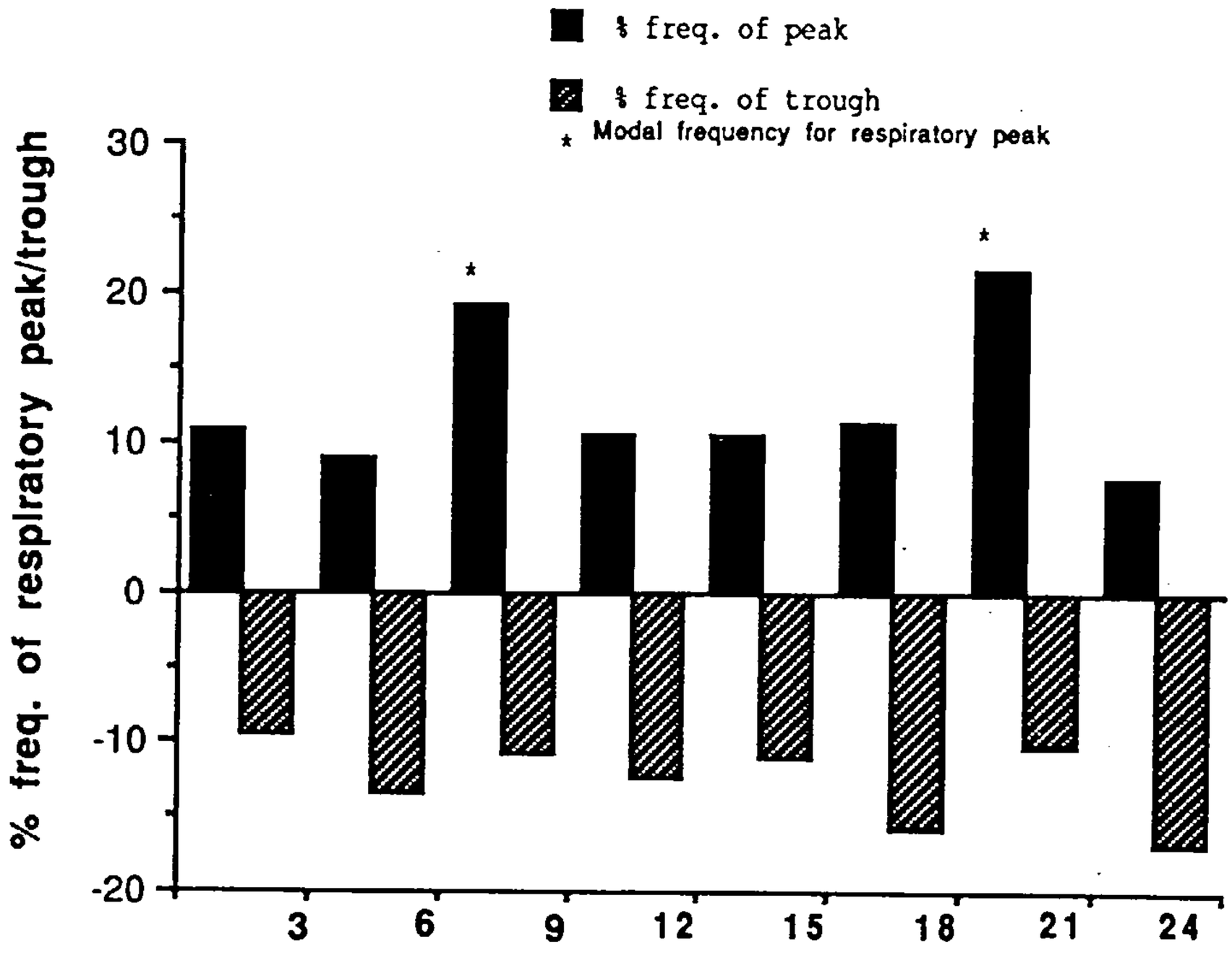


Fig. 3.2 Percent frequency of respiratory peaks and troughs in common carp during 24 hour period under 12L : 12D photoperiod.

mg/kg/h and 151.97 mg/kg/h respectively showing no significant difference.

Metabolic rate during 18L : 6D

The onset of light and dark also showed correspondingly higher oxygen consumption values in 18L : 6D experiments (Fig. 3.1b). The mean daily oxygen consumption was 125.19 mg/kg/h with a maximum value of 136.26 mg/kg/h at o200 h and a minimum of 116.96 mg/kg/h at 1000 h. The mean oxygen consumption during the light phase and dark phase were again very similar (Table 3.1).

Metabolic rate during 24L : 0D

The respiratory rhythmicity during total illumination was found to be very suppressed with a nearly steady state condition (Fig. 3.1c). In this case, the metabolic rate ranged from 113.26 mg/kg/h to 124.61 mg/kg/h with a mean daily value of 118.92 mg/kg/h.

Metabolic rate during 6L : 18D

Here, peaks in oxygen consumption values again occured during the onset of light and darkness in a pattern similar to that seen in the 12L : 12D experiments (Fig.

Table 3.1 Oxygen consumption during different photoperiod in carp, Cyprimus carpio, L.

Photoperiod	Oxygen consumption (mg/kg/h)					
L = Light D = Dark	Mean in 24 hrs (± SD)	Mean in Light (± SD)	Mean in Dark (± SD)	Maximum	Minimum	Difference (Max - Min
24L : 0D	118.92 (±3.09)	118.92 (±3.09)	-----	124.61 at 1300 hr	113.26 at 0600 hr	11.80
18L : 6D	125.19 (±5.78)	125.24 (±5.95)	125.04 (±5.70)	136.26 at 0200 hr	116.96 at 1000 hr	19.32
12L : 12D	152.45 (±9.70)	152.94 (±7.34)	151.97 (±11.92)	171.71 at 2000 hr	137.35 at 0500 hr	34.42
6L : 18D	138.46 (±4.63)	138.45 (±4.48)	138.46 (±4.80)	145.57 at 1400 hr	130.39 at 0700 hr	15.18
0L : 24D	119.21 (±3.97)	-----	119.21 (±3.97)	124.46 at 1200 hr	111.18 at 0200 hr	13.28

3.1d). The fish showed a range of respiratory rate from 130.39 mg/kg/h at 0700 h to 145.57 mg/kg/h at 1400 h having a mean value of 138.46 mg/kg/h (Table 3.1).

Metabolic rate during 0L : 24D

In continuous darkness the mean metabolic rate was 119.21 mg/kg/h ranging from 111.18 mg/kg/h to 124.46 mg/kg/h (Fig. 3.1e). In these conditions the daily trend was nearly flat with no sharp rise or fall.

The percent frequency of respiratory peaks at normal photoperiod (12L : 12D) was found significantly higher ($p < 0.05$) during the onset and offset of light than during other time of the day (Fig. 3.2). By contrast, the troughs during the same time were significantly lower ($p < 0.05$) during dawn and dusk.

Fig. 3.3 summarizes the daily mean metabolic rates in common carp in the different photoperiods. The daily mean metabolic rates in 24L : 0D and 0L : 24D were significantly different ($p > 0.05$) and lower than in the other photoperiods. The daily mean metabolic rate in 12L : 12D was found to be significantly higher ($p < 0.05$) than that in 18L : 6D and 6L : 18D which were also significantly different ($p < 0.05$) from each other.

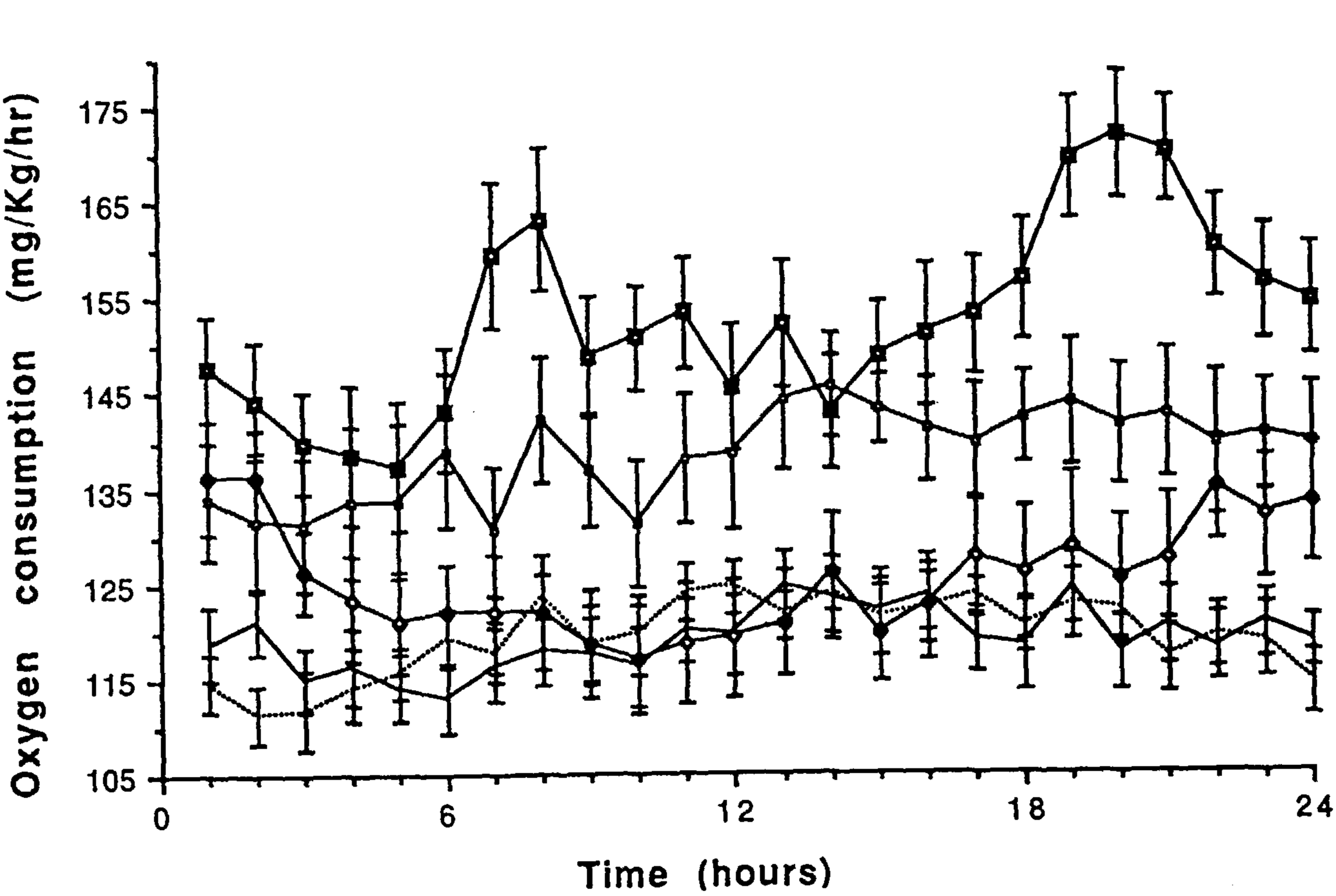


Fig. 3.3 Oxygen consumption during 24 hours in DL 0 : 24, 6 : 18, 12 : 12, 18 : 6, and 24 : 0 in common carp. Calculated values are hourly AM with vertical bars as +_ SEM during experiment.

From the above experiments it is seen that the mean daily oxygen consumption in different photoperiods - 12L : 12D, 18L : 6D, 24L : 0D, 6L : 18D and 0L : 24D reduced with either an increase or decrease in photoperiod and was maximum in the normal photoperiod (12L : 12D) (Fig. 3.4).

The differences in range of respiratory rate between the maximum and the minimum value was also reduced with either increase or decrease of photoperiod (Fig. 3.5).

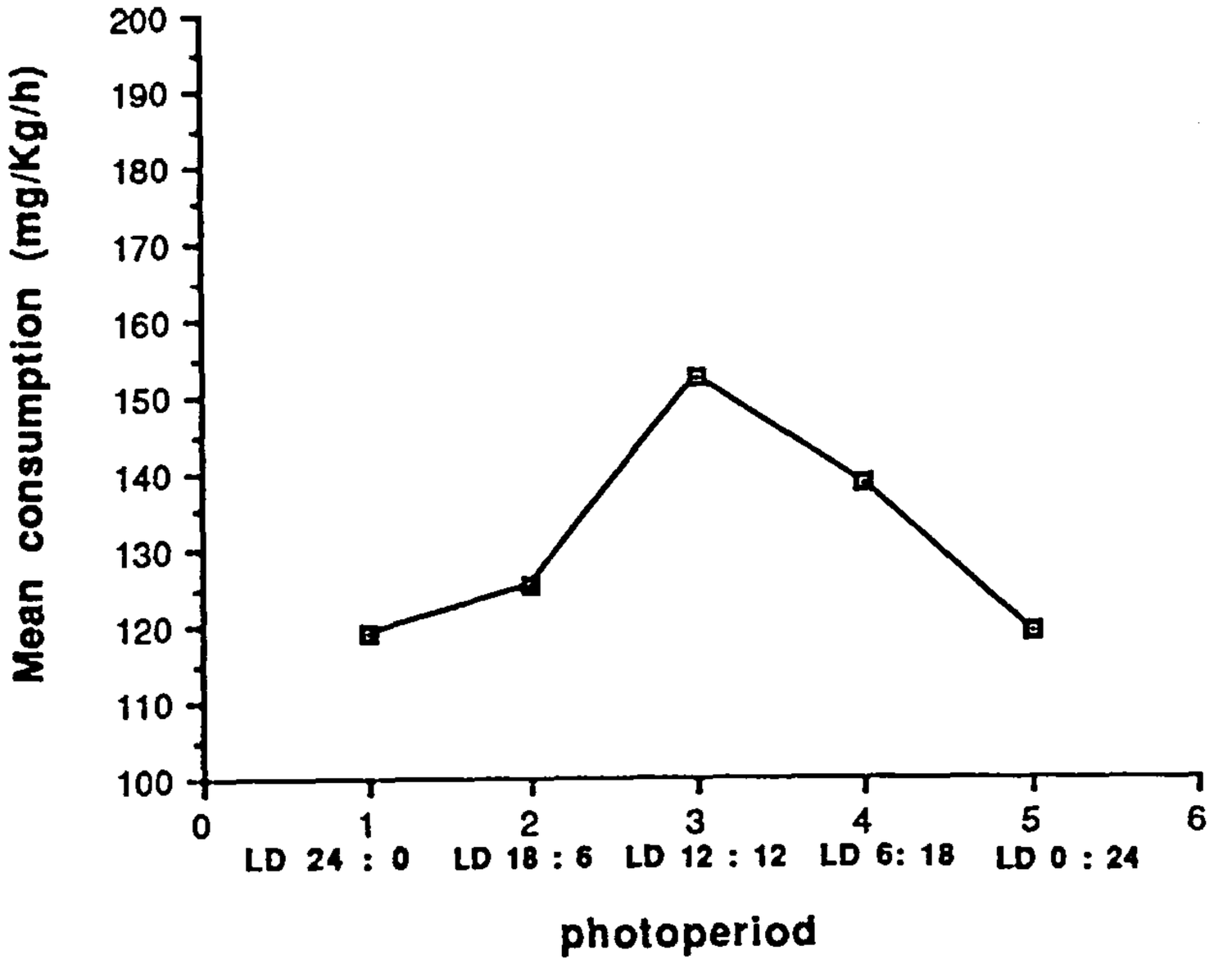


Fig. 3.4 Mean oxygen consumption of C. carpio over a 24 hr period in different photoperiods.

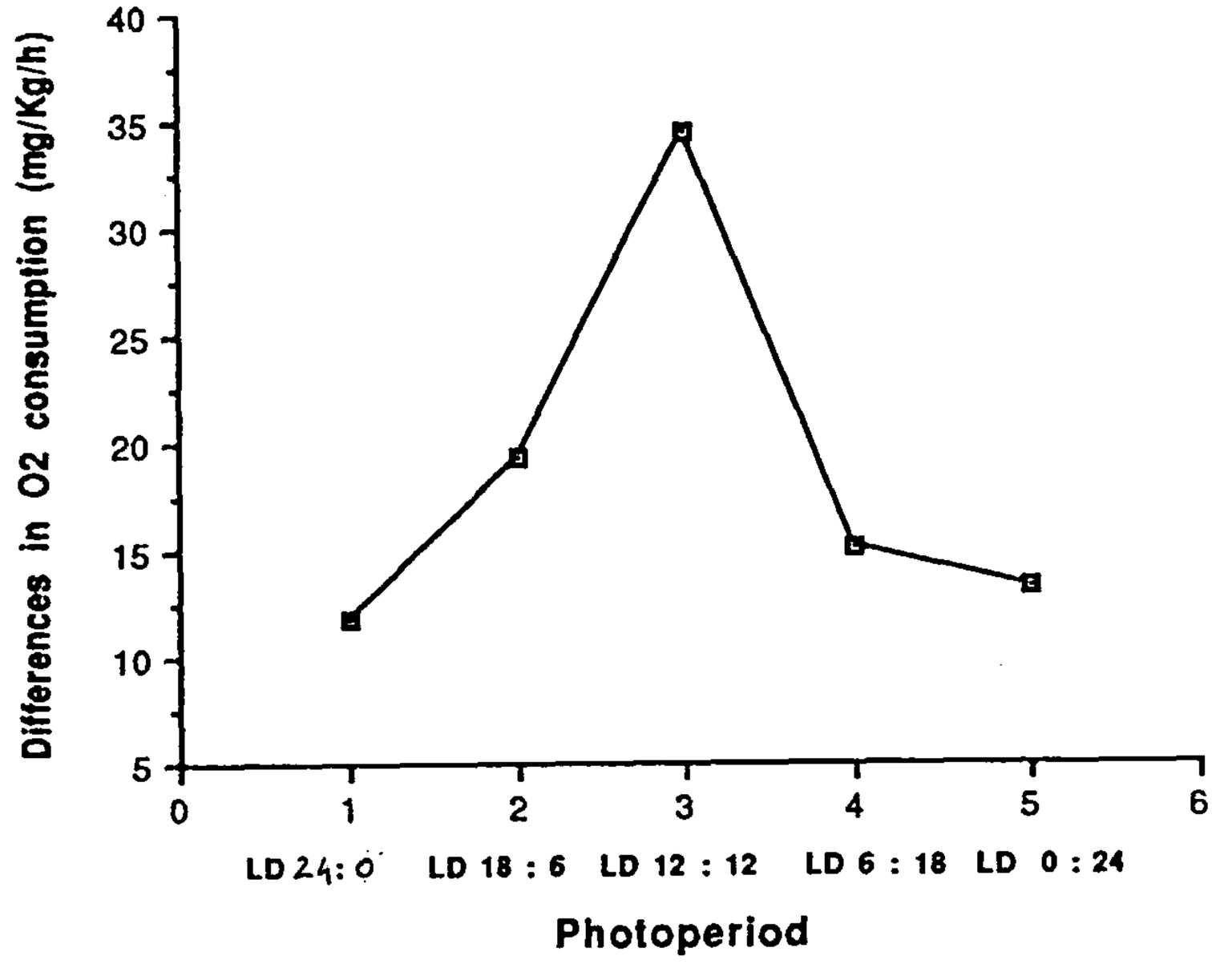


Fig. 3.5 Mean differences (maximum - minimum) in oxygen consumption of C. carpio during 24 hr period in different photoperiods.

3.4 Discussion

The variation in metabolic rate during the day in Cyprinus carpio is evident in this work (Fig. 3.1a) and under a normal photoperiod (12L : 12D) the common carp showed two respiratory peaks during the onset of light and darkness. Many authors have described a single daily peak in metabolic rate (Hoar, 1956, in gold fish, Carassius auratus; Hirata, 1973, in Salmo salar; Swift, 1962 in Salmo trutta; Winberg and Khartova, 1953 and Hamada and Maeda, 1983, in Cyprinus carpio; Ross and Mckinney, 1988 in Oreochromis niloticus). Some have reported a crepuscular pattern (Eriksson, 1973, in Salmo salar and Salmo trutta; Marais, 1978, in Liza dumurelli; Huang, 1975, in Cyprinus carpio; Nagarajan and Gopal, 1983 in Sarotherodon mossambicus; Kumari and Nair, 1979 in Noemocheilus triangularis). This work has clearly shown a crepuscular rhythm in carp held under a normal photoperiod.

The final data were expressed as the mean of hourly blocks extracted from the original raw data. No notable overall change in respiratory rate was observed over the long period of observation suggesting that the respirometers used were appropriate for such studies (Gnaiger, 1983) and that sufficient replicates were used. In processing

the data any exceptionally high values (very few) which might have been been due to the motor activity of the fish inside the respirometer chambers were ignored.

Water quality has a large influence on fish and their growth (Hepher, 1988). Excessive low or high pH beyond the range of 6.5 - 9.0 can lower the growth rate and the extreme values can be detrimental (Swingle, 1961; Alabaster and Lloyd, 1980). In this study the pH range was within an acceptable range, temperature did not vary beyond 28°C (±0.5°C) and oxygen concentration was kept at about 7.8 mg/l in the system. Chiba (1965) found that at less than 3 mg/l dissolved oxygen growth rate, feeding rate and oxygen consumption rate are decreased. Since ammonia is the major excretory product in fish the un-ionised form of ammonia has a toxic effect on fish. In this study the accumulated ammonia in the system was far below the toxic level because a part of water (about one third) was changed every alternate days during the experimental runs.

In this study, fish did not always respond equally and this variation in oxygen consumption was due to individual physiological variation. A large number of replications over a long period of observation minimizes this type of error. In addition, the condition of fish inside the respirometer chambers were intended to be stress free

except for that arising due to confinement which was inevitable. Similar reports with goldfish (Carassius auratus) have shown that individuals differ from one another in total activity throughout the entire cycle so that one goldfish might be described as sluggish and another as hyperactive (Spencer, 1939)

The results of this study with Cyprinus carpio in normal photoperiod supports some of the previous works (Olifan, 1940; Huang, 1975). In contrast, some authors found a single daily peak (Oya and Kimata, 1938; Hamada and Ida, 1973; Hamada and Maeda, 1983) or no rhythmicity at all in the carp (Spencer, 1939). Earlier, Clausen (1936) demonstrated a daily periodicity in the largemouth bass with morning and late afternoon maxima in oxygen consumption. In the black bullhead, low activity during daytime alternated with increased activity at night.

Similar variations in metabolic rate have been reported for other species of fishes by different authors. Varying reports on Oreochromis niloticus (Crepuscular rhythm in yolk-sac larvae and single peak at dawn in 12 mm fry by De Silva et al., 1986; crepuscular by 5 - 25g fish by Nagarajan and Gopal, 1983: single daily peak for 100g fish by Ross and Mckinney, 1988) suggest that the cause may be due to differences in measuring techniques, acclimation period length of observation for experiment, water quality and

level of stress in the respirometer. Alternatively, the growth stage may be important, thus the bimodal activity rhythm found in sockeye salmon Oncorhynchus nerka immediately after hatching changes to unimodal, light active 10 - 14 days after hatching and this is maintained for 11 months (Byrne, 1971). Similarly, Spencer (1939) recorded a clear change in rhythmic activity pattern in carp of different age groups.

It can be seen from Table 3.1 that the mean metabolic rate in the light was not much different from that recorded during the dark period. Similar observations were reported by other authors (Winberg and Khartova, 1953; Krivobok, 1953; Yablonskia, 1951; Karzinkin, 1952). Obviously, this does not apply to cases where, the light by stimulating the activity of fish can increase the metabolic rate indirectly. Hourly peaks, in which the oxygen consumption rates are much higher (15% and more) than the mean values, and hourly troughs, in which the oxygen consumption rate is much less (less than 15%) than the mean value, could be seen in the raw data. When expressed as percent frequency a higher frequency of peaks and lower frequency of troughs were noted during the onset of light and darkness at 0800h and 2000h (Fig. 3.2). This, again, supports the crepuscular pattern of rhythmicity in common carp during normal photoperiod (12L : 12D).

Table 3.2 lists the reported values for oxygen consumption in carp and a large variation is evident. It is clear that there are some correlation between respiratory rates and fish weight used at different temperatures in addition to other undefined factors.

When held under an extended light regime (18L : 6D) the oxygen consumption rate of carp was also rhythmic. There was an increase in rate of oxygen consumption during onset of darkness followed by a decrease and a further increase towards the onset of light (Fig. 3.1b) and the mean oxygen consumption became reduced (Table 3.1).

In a reduced light regime (6L : 18D) the mean daily oxygen consumption was also reduced (Fig.3.1d) with very little difference in mean oxygen uptake in the light and in the darkness. Similar results were observed by Roberts (1961) who concluded that changes between short and long daily photoperiods altered levels of oxygen uptake in sunfish, Lepomis gibbosus, and crucian carp, Carassius carassius. The same was found in rainbow trout Salmo gairdneri which spawn in the autumn (Evans et al., 1962).

Although a respiratory rhythm was evident under 18L : 6D and 6L : 18D photoperiods the increase in metabolic rate during the onset of light and darkness was less clear than in 12L : 12D. This indicates that the rhythm is

Table 3.2. List of different values for oxygen consumption in Cyprinus carpio obtained from different sources.

Fish weight (g)	Respiratory rate ($mgO_2/kg/h$)	Sources
100g	43 at 11°C	Khalil (1937)
24.7 - 51.6 mg	0.5 - 0.65 at 20°C cu.mm/mg/h	Winberg and Khartova (1953)
24.7g	150 at 20°C	Streltsova (1953)
----	204	Ivlev (1945)
----	107 at 20°C	Saunders (1963)
146g	48 at 20°C	Beamish (1964)
100g	104 at 30°C	Beamish (1964)
134g	117.3 at 35°C	Beamish (1964)
10 ± 5g	80 at 10°C	Kausch (1969)
10 ± 5g	136 at 15°C	Kausch (1969)
10 ± 5g	214 at 20°C	Kausch (1969)
31 - 47g	48 at 17°C	Huisman (1974)
2 - 16g	83 at 23°C	Huisman (1974)
54g	*111 - 129 at 25 ±1°C	Hamada and Ida (1973)
318g	*173 at 25 ± 1°C	Hamada and Maeda(1983)
581g	*50 at 25 ± 1°C	Hamada and Maeda (1983)
70 ± 10g	152 at 28°C	This paper

* Converted value from ml/hr to mg/kg/hr.

endogenous in origin, related to the changing dark-light cycle. As in this study, the respiration rates of long-photoperiod fish, lepomis gibbosus kept in 15 hour light were significantly lower than rates of fish on the 9 hour day at all temperatures above 10°C (Roberts, 1964). However, he also noted that once gonadal development begins, physiological sensitivity to the length of the daily photoperiod is lost until the completion of breeding.

In the 18L : 6D and 6L : 18D experiments, the mean reduction of metabolic rates may be influenced by the level and duration of acclimation (Brett, 1962). Sufficient acclimation to a new environment is a very important factor in the physiology of fishes (Fry, 1971). The acclimation period to altered photoperiods used for fish in this study was 3 weeks and this was considered sufficient to allow full photoperiod acclimation in the range of homeostasis relating to adaptation (Roberts, 1961).

When carp were held in total illumination (24L : 0D) or total darkness (0L : 24D) the overall respiratory rate was reduced and had almost no rhythmic fluctuation over the full experimental period. This suppression is probably due to long term acclimation in continuous light or dark (Brett and Southerland, 1970) during which the normal

physiological (hormonal) response is lost. Hutchison and Konl (1971) showed that the oxygen consumption of tropical toads, Bufo marinus in total light or darkness showed no persistent rhythm when animals were maintained at LL or DD for many days although distinctive cycles were found under photoperiod of LD 8 : 16, 12 : 12 and 16 : 8. Nagarajan and Gopal (1983) exposed Oreochromis mossambicus to constant darkness and found that they consumed comparatively less oxygen. However, the normal bimodal rhythm of oxygen consumption continued in both the constant light conditions (LL) with advanced peaks, and dark (DD) conditions with delayed peaks. Diurnal rhythms in goldfish (Carassius auratus) under continuous light served to reduce the total activity and at the same time distributed more evenly over 24 hours (Spencer, 1939). In this context it should be noted that physiological adaptations to environmental change are influenced by the acclimation limits of adjustment of a state with a new environment (Prosser, 1955)

Overall, it is clear that daily mean metabolic rate decreases from that in the normal photoperiod (12L : 12D) when there is an increase or decrease of light or darkness (Fig. 3.2). It is also evident that the differences between maximum and minimum daily mean oxygen consumption in each of the photoperiods decreases from normal photoperiod towards an increase or decrease in light or

darkness (Fig. 3.3). This indicates that the longer or the shorter the photoperiod the less is the metabolic fluctuation. Nagarajan and Gopal (1983) showed that the mean level of oxygen consumption and its range of variation is high when O. mossumbicus were exposed to longer photoperiods.

Standard metabolism varies greatly for an animal according to age, nutrition,season of the year, endocrine balance and other factors (Prosser, 1955). It is well known that experiments in the laboratory under controlled conditions allow investigation of the effect of environmental factors on daily rhythmic patterns of oxygen consumption (Schwassmann, 1971). Most physiological activities are directly controlled by the endocrine system in fish. It has been shown that environmental manipulation of the dark-light cycle has a stimulating effect on production of growth hormone in sockeye salmon (Brett and Southerland, 1970) and the effect of light on the growth of fish may be mediated by hormones such as somatotropin produced in the pituitary, or thyroxine produced in the thyroid which are involved in metabolism and growth (Gorbman, 1969). Swift (1955) has shown that seasonal variation in thyroid gland activity of 3 year-old Salmo trutta can be related to day length. In common carp photoperiodism is probably involved although less is in ovarian maturation (Davies et al., 1986)

Mean thyroxine and triiodothyroxine in blood plasma in Salmo gairdneri showed variation linked with day-length, and was minimum in a long day period (Osborn et al., 1978). Some workers have shown that photoperiod may also change the daily serum cortisol pattern in fish (Noeske and Speiler, 1983; Redgate, 1974, in Cyprinus carpio; Singley and Chavin, 1975 , Fryer, 1975 and Peter et al., 1978 , in goldfish; Garcia and Meier, 1973, and Srivastava and Meir, 1972 in gulf killifish).

A diurnal rhythm in phototactic behaviour was reported by Kawamoto and Konishi (1955) for Girella punctata but it was not noticeable in Mugil cephalus. Jones (1956) reported that minnows were active during daylight but that this pattern was reversed if their tank contained a hollow brick where they could hide from the light. When cover was provided, they were very active around sunrise and sunset. It was later shown that the activity of Salmo trutta changed from day-active during winter to night-active in summer (Muller, 1969) and a similar change from light activity in winter to summer dark activity was described in Cottus poecilopus, from the Arctic (Andreasson, 1969).

There is an interaction between temperature and photoperiod and feeding rate which affects growth (Hepher, 1988). Experiments have shown that fish growth is affected by the length of the daily period of light more than by

temperature. The growth of yellow perch (Perca flavescens) at two temperatures of 16 and 22°C and two light periods of 8 and 16 hour varied more significantly with the photoperiod than that with temperature (Huh et al., 1976) and Gross et al.(1965) working with green sunfish (Lepomis cyanellus) concluded that photoperiod influences growth through increasing conversion efficiency and also possibly through stimulating food consumption. Kadmon et al. (1985) obtained similar results with sea bream (Sparus aurata) exposed to a long photoperiod. Brown (1946b) found that specific growth rates of brown trout (Salmo trutta) were significantly lower with 12 or 18h of light per day than with 6 h at a constant teperature of 11.5°C. Both Anderson (1959) and Bjorklund (1958) showed no relationship between daylength and growth but significantly higher growth was observed in the light-reared chinook salmon fingerlings than in dark-reared groups (Eisler, 1957). Coho salmon (Oncorhynchus kisutch) reared under delayed photoperiods grew about five times as fast in sea water as did those exposed to the natural photoperiod (Clarke and Shelbourn, 1986; Brauer, 1982) but the same authors found that accelerated photoperiod did not advance smolting of underyearling coho (Clarke and Shelbourn, 1986). Studies with mature goldfish indicated that greater growth in darkness was attributed to lack of activity (Bjorklund, 1958).

Some correlation has been observed between endocrine rhythms in fish and other rhythms, such as daily activity pattern, as has been suggested for mammals (Ashcoff, 1978, Moore - Ede et al., 1982). Sexual cycle, in terms of gonadal development in trout was shown to be positively influenced by light (Hoover and Hubbard, 1937). There appears to be photoperiodic control for prolactin release since there is a circadian rhythm of circulating prolactin levels in different vertebrates, such as rats (Butcher et al., 1972; Freeman and Neill, 1972; Neill, 1972; Kizer et al., 1975). In teleosts, there have also been reports showing daily changes in circulating prolactin levels in Carassius auratus (Leatherland and Mckeown, 1973; Spieler and Meier, 1976), Fundulus grandis and Mugil cephalus (Spieler, 1975). Leatherland et al. (1974) showed that plasma levels of prolactin and growth hormone in Oncorhynchus nerka exhibited a marked circadian rhythm. Forbes et al. (1975) and Bourne and Tucker (1975) reported that a long photoperiod results in an increase of serum prolactin in sheep and calves respectively. Longer photoperiods and higher temperatures caused pituitary prolactin release of the goldfish, Carassius auratus (Mckeown and Peter, 1976) where serum prolactin changed in a circadian rhythm and the rhythm was modified depending on the photoperiod length.

Continued exposure of fish to darkness for over 20 days resulted in lower gonadal development (Yadav and Diana, 1982). Significant daily fluctuations in serum gonadotrophin (GTH) levels were detected in mature female goldfish (Hontella and Peter, 1978) subjected to different photoperiod. Studies of the relationship between thyroid hormone and oxygen consumption have given conflicting results (Hoar, 1957). A number of recent studies have shown that thyroid hormones can stimulate the somatic growth of certain fish species (Narayansingh and Eales, 1975b; Donaldson et al., 1979; Higgs et al., 1979; Fagerlund et al., 1980; Lam, 1980) probably by the stimulation of growth hormone release from the pituitary (Leatherland and Hyder, 1975; Higgs et al., 1976). Although some authors have reported that thyroid hormones stimulate oxygen consumption related to protein synthesis (Millard et al., 1979) otner have noted no such effects (Gorbman, 1969; Leatherland, 1982). However, removal of pituitary gland from teleost fishes results in a decrease in the rate of oxygen consumption (Hanson and Stanley, 1970; Chan and Woo, 1980a, b). Different experiments with serotonin in Fundulus grandis (Fingerman, 1975), Adrenocorticotropic Hormone (ACTH) in Carasssius auratus (Singley and Chavin, 1975) and blood cortisol (Chavin, 1973) have shown that there is a close correlation of hormonal activities with different physiological

activities including metabolic change which are influenced by change of photoperiod.

A reciprocal photoperiod regime in postsmolt Atlantic salmon (Salmo salar) showed significantly lower standard rates of oxygen consumption than those in a simulated natural photoperiod regime when both were tested in total darkness in sea water in late summer (Withey and Sounders, 1973).

In fishes, light has varying effects on daily activity patterns (Richardson, 1952; Steven, 1959; Davis, 1962; Chaston, 1968; Muller, 1978). There exists a correlation between the daily activity rhythm and the feeding cycle in fishes (Hirata and Kobayashi, 1956; Hirata, 1957). Under normal conditions a definite activity pattern can be seen in trout (Salmo trutta) which are most active at period dusk and dawn; whereas under constant condition the activity becomes more random and higher in constant darkness than in continuous illumina- tion. Reversing the light conditions results in a changed activity (Chaston, 1968, 1969).

From the above discussion it is clear that there is also a hormonally controlled relationship between physiological and metabolic processes and the changing photoperiod.

The initial demand for food is to meet the maintenance requirement (Brett and Groves, 1979; Pandian, 1987) but food energy drawn in excess of maintenance requirements may then be used by the animal for other activities, including growth (Fry, 1971). Indirect calorimetry is widely used for calculating the heat lost by a fish in which the total amount of oxygen consumed over the period under consideration by the fish is multiplied by an energy equivalent (Q_{ox}) to produce an estimate of energy (Brafield, 1985). For starving fish, respiring its own tissue for maintenance metabolism, a Q_{ox} i.e. energy equivalent of 13.56 J/mg oxygen (Brett and Groves, 1979) has been used.

In this study the daily mean metabolic expenditure of 70 (10+_)g C. carpio for resting metabolism under a normal photoperiod was 152.45 mg/kg/h X 24 = 3659 mg/kg/day which is equivalent to 49.62 kJ/kg/day. This energy is extracted first from any food energy ingested by the fish. This energy expenditure in other photoperiods of 18L : 6D, 6L : 18D, 24L : 0D and 0D : 24L were 40.74, 45.06, 38.70 and 38.79 kJ/kg/day respectively.

Therefore, these data are of particular interest in the study of carp energetics. The average daily energy consumption for resting metabolism in normal and varying photoperiod have been described. This provides a firm

baseline for studies of SDA and total energy budgets. The data are also of direct relevence for successful carp aquaculture systems where oxygen concentrations may be limiting.

CHAPTER 4

SDA AND THE EFFECT OF PROTEIN ON THE FEEDING METABOLISM OF CARP

4.1 Introduction

When a previously fasting animal consumes food, its metabolic neat production increases for a period of time above the level represented by basal or standard metabolism even though all other conditions are kept constant. This increase in heat production associated with the ingestion of food is given several names. In human physiology it is termed Specific Dynamic Effect of food and was first recognised by Lavoisier and Laplace (1780) and was later demonstrated in homeothermic animals. Rubner (1902) showed the effect in the dog and he coined the term Specific Dynamic Effect (SDE) to denote the increase in metabolic rate following food intake. He made the very general statement that SDE was the waste heat produced by reactions necessary to support the physiological process of the body. The German word for Effect' was mistranslated into English as 'Action' and thus the abbreviation SDA (Specific Dynamic Action) arose (Maynard and Loosli, 1969).

The increase in metabolic rate after feeding is also

variously known as the calorigenic effect of food, heat increment of feeding, thermal energy or thermogenic effect (Brody, 1945). However, SDA is discussed by various authors under different names, for example 'the calorigenic effect' by Kleiber, 1961; Garrow, 1974; 'the stimulating effect of food on metabolism' by Hamada and Ida, 1973; 'apparent specific dynamic action' by Beamish, 1974; 'the entropic tax paid during food conversion' (Ware, 1975), 'the energy cost of food utilization' (Mishrigi and Kubo, 1976), 'enhancement of metabolism after food intake' (Yarzhombek et al., 1983) 'the inescapable cost of feeding' (Brafield, 1985), 'the heat increment of feeding' (Cho and Koushik, 1985) and 'relative specific dynamic action' (Gaffney and Diehl, 1986)

In poikilothermic species it has been observed as a post prandial increase in the rate of oxygen consumption although few attempts have been made to determine this heat increment by direct calorimetry (Smith, et al., 1978, Davies, 1966). Following food ingestion the oxygen consumption rate in fish increases and then gradually declines back to its resting level (Fig. 4). There are three principal components of the SDA effect, the total magnitude, the maximum increased level or peak level and the duration (Jobling and Davies, 1980). All of these features have been shown to be dependant on several

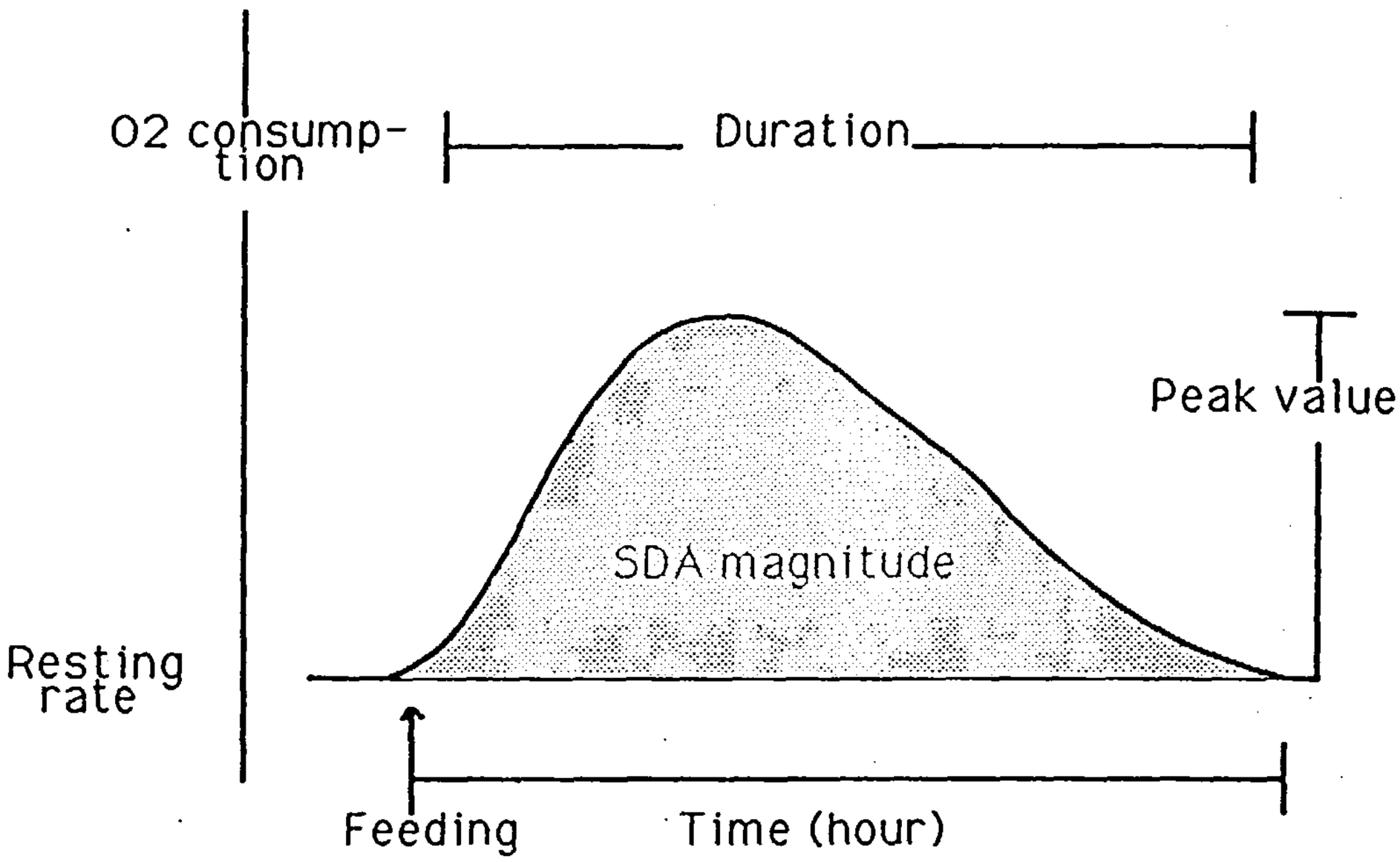


Fig. 4. Schematic description of feed-related elevation of the metabolic rate (Jobling, 1981c). The dotted area indicates the overall level of oxygen consumption.

factors including the weight of fish, fish density, meal size, diet composition and temperature (Muir and Niimi, 1972; Beamish, 1974; Caulton, 1978 ; Smith et al., 1978b; Vahl and Davenport, 1979; Brett and Groves, 1979; Jobling and Davies, 1980; Jobling, 1981; Fletcher, 1984; Medland and Beamish, 1985).

The 'peak level' is described as the highest level of oxygen consumption above the resting or pre-feeding, level attained within the 'duration' of the increased oxygen consumption. Whereas, the 'duration' is the time (h) during which the oxygen consumption by the fed fish is above the resting metabolism (Fig. 4).

The 'magnitude' of the specific dynamic action (SDA) is defined as the sum of excess metabolic heat production induced by the ingested meal, integrated from the time metabolism first rises to when it falls back to the base, or resting level.

The SDA magnitude, which is expressed as mgO_2 (or converted into kJ or kcal) is the total amount of oxygen consumed, or energy expended, by the fish in the SDA response. The magnitude of SDA can be determined by plotting the curve of increased oxygen consumption after feeding against time until it subsides to the pre-feeding rates and then integrating the area beneath the curve. A

common way of expressing SDA is as a percentage of the total caloric value of the food ingested (Hill, 1976).

Data on SDA in fish from the literature are presented in Table 4.1 which shows quite a large range of variation, suggesting that SDA is a complex effect. Hepher (1988) noted that this increase in oxygen consumption is variously associated with the extra energy required for transportation of food in the alimentary tract, its digestion, absorption and post absorption metabolic processes related to the ingested food.

Many workers have reported that the SDA magnitude increases either linearly in relation to meal size (Jobling and Davies, 1980) or in an exponential relationship (Averett, 1969, quoted from Tandler and Beamish, 1979). SDA is also affected by diet composition (Tandler and Beamish, 1980; Hamada and Maeda, 1983) and has been found to be positively correlated with protein content in the diet.

The chemistry of SDA is not completely understood but it is assumed that the post prandial energy liberated is due largely to the result of the deamination of amino acids (Beamish et al. 1975). They concluded that the absorbed amino acid nutrients during digestion are deaminated or transaminated mainly in the liver, followed by storage or

utilisation, while the liberated NH- groups may be excreted or retained for amino acid synthesis. The liver thus appears to be the primary if not the only site of heat production (Buttery and Annison, 1973) Although heat production is most pronounced with protein (Krebs, 1964) a lesser amount is released following ingestion of carbohydrate and fat.

It is difficult to separate the energy lost to SDA in digestion, absorption, transportation and deposition of food materials. Hence it is sometimes termed "apparent specific dynamic action" (Beamish, 1974) which incorporates both the mechanical and biochemical aspects of feeding. The mechanical aspect here may include grasping of food, chewing, swallowing, and peristalsis (Tandler and Beamish, 1979, 1981), although Jobling and Davies (1980) considered peristalsis to have a negligible effects.

Specific dynamic action in fish is not well understood (Beamish, 1974; Smith, et al., 1978) and caution has been suggested in the application of observations made in other animals (e.g. mammals, birds etc) to explain the phenomenon in fish (Cowey, 1980). As a result of ammonotelism, fish have a comparatively lower SDA (Smith et al.,1978) than that of ureotelic and uricotelic animals and thus need more energy to be derived from protein

(Cowey, 1980).

SDA magnitude in fish varies greatly (Table 4.1) approaching nearly 30% of the gross energy of ingested food at its maximum (Kleiber, 1961). It is smaller in herbivores than for omnivorous fish consuming diets of equivalent energy (Weatherly, 1976). This increased metabolic rate is different from the calorigenic effect of such hormones adrenaline and thyroxine or such drugs as dinitrophenol which raise the metabolic activities of the body cell (Brody, 1945). Brody also agreed that contraction of gut muscle makes only a small contribution to the increase of metabolic rates. Smith (1982) claimed that SDA is not related to the synthesis of enzymes, digestive fluids and mucus as they are present in the cells before intake of food. A completely opposite view was supported by Pierce and Wissing (1974). Brody (1945) was of the opinion that SDA is caused by catabolism in generating ATP which involves the oxidation of protein, carbohydrate and lipid thereby explaining why SDA is also found with a non-protein diet.

Jobling (1983) obtained sufficient data to suggest a close relationship between thyroid hormone secretion, protein synthesis, metabolic rates and other evidence giving clues to the involvement of these factors in SDA and he suggested that the 'protein synthesis / growth (Ashworth,

Table 4.1. Reported values of SDA as a proportion of food energy in fish. values in brackets are means.

Species	Food type	SDA Coeff.(%)	Remarks	Sources
Salmo charki		13.0 - 46.0		Broksen et al., (1968)
Coho salmon		9.0 - 15.0		Averett (1969)
Salvelinus fontinalis		28.2		Kerr (1971)
Kuhlia sandvicensis	Tuna flesh	16.0 - 19.0	estimated energy of food	Muir and Niimi, (1972)
Histrio histrio	Shrimp	15.2 - 36.2 (23.7)	SDA/C higher in bigger fish	Smith, (1973)
Micropterus salmoides	Emerald shiner	14.2 +_4.2	Values from various sizes at swimming speeds.	Beamish, (1974)
Lepomis macrochirus	Mayfly larvae	4.8 - 24.4 (12.7)		Pierce and Wissing, (1974)
L. macrochirus	pelleted feed	7.5 - 32.3 (14.9)	No significant differences for diets of varying composition	Schalles and Wissing, (1976)
Salmo gairdneri	Pelleted diet	8.0 - 12.0 (9.7)	Influenced by dietary composition	Cho et al., (1976b)

contd...

Species	Food type	SDA coeff.(%)	Remarks	Sources
Oncorhynchus rhodurus	chopped fish	9.5 - 25.9 (16.9)		Miura et al., (1976)
Tilapia rendalli	Macrophytes	4.9 - 13.8	SDA/C increased with temperature	Caulton, (1978)
Salmo gairdneri	Pelleted diet	1.56 (ME)	Direct calorimetry	Smith et al., (1978b)
Salmo salar	Pelleted diet	2.48 (ME)	Direct calorimetry	smith et al., (1978b)
Blennius pholis	Mussel flesh	7.5 - 11.9 (9.7)		Vahl and Davenport (1979)
Salmo gairdneri	Pelleted diet	9.0 - 15.0		Cho and Slinger, (1980)
Crenimugil labrosus	Pelleted diets	5.1 - 23.6		Flowerdew and Grove, (1980)
Pleuronectes platessa	Fish Paste	10.0 - 18.0 (13.1)	varies with dietary composition but indi fferent to temp.	Jobling and Davies, (1980)
Micropterus salmoides	Pelleted diets	5.1 - 17.5	SDA related to prot ein level in the diet	Tandler and Beamish, (1980)

contd...

Species	Food type	SDA coeff.	Remarks	Sources
Odontibutis obscura	gold fish	9.7 +_2.9	measured at 15-30°C	Machida (1981)
Lepomis macrochirus	gold fish	3.3- 29.6	measured at 20-30°C	
Micropterus salmoides	gold fish	5.0 - 37.0 (25.1)	measuerd at 30°C	
Rhodeus rhombeus	tubificid worm	4.7 - 22.2	measured at 30°C	Machida (1981)
Rhodeus lanceoratus	tubificid worm	9.8 - 20.9	measured at 30°C	
Eleotris oxycephala	gold fish	5.0 - 15.3	measuresd at 20-30°C	
Cyprinus carpio	Pelleted diet		Linear relation betw-een SDA and ration	Hamada and Maeda, (1983)
Gadus morhua	Pelleted diet	2.91 - 19.92	measured at 7 - 18°C	Soofiani & Hawkins (1982)
Cyprinus carpio	Pelleted diet	16.0		Yarzhombek et al., (1983)
Salmo gairdneri	Pelleted diet	10.0		Yarzhombek et al., (1983)
Coregonus schinizi	Artemia	28.7	Fish at larval stage	Dabrowski and Kaushik, (1984)

Species	Food type	SDA coeff.	Remarks	Sources
			to food intake	
Salmo gairdneri	Pelleted diet	8.5 - 33.7		Medland and Beanish, (1985)
Pomadasys commersonni	Clam	8.1 - 15.2		du Preez et al., (1986)

1969) offered the best basis for furtner research into SDA for protein synthesis and metabolic rates. It has been shown that post-prandial metabolic rates and rates of growth are correlated, suggesting that the increase in metabolic rate following feeding is connected with tissue synthesis (Brook and Ashworth, 1972; Alver and Brook, 1978; Kreiger, 1978; Jobling 1981b,1983).

In the study of specific dynamic action it is customary to express SDA in metabolic terms (energetics term) as a percentage of the food intake, the SDA coefficient (Smith et al., 1978). Generally, specific dynamic action (SDA) in fish is measured by indirect calorimetry (Beamish, 1974) as the post-prandial oxygen consumption which can then be converted into units of energy using appropriate oxy-calorific equivalents (Brett and Groves, 1979). Estimates of this metabolic expenditure associated with feeding are usually obtained from laboratory experiments using different types of respirometers (Soofiani and Hawkins, 1985). By using suitable types of respirometers where the motor activity of fish inside the respirometer chambers may be controlled as much as possible (Ross and Mckinney, 1988), the increased oxygen consumption for a fed fish with a defined ration may be measured accurately over a defined period of time. The values may then be applied to fish in the field for comparison, provided the same kind of food intake is known. The SDA co-efficients

thus obtained are important in fish culture where they must be taken into consideration when constructing energy budgets involved in feeding and growth during holding and on-growing (Solomon and Brafield, 1972, Pierce and Wissing, 1974).

Particularly limited work has been reported on SDA in common carp, notable exceptions being the measurements made by Krayukhin (1962), Kausch, (1969), Hamada and Ida (1973), Huisman (1974), Hamada and Maeda (1983), Yarzhombek (1983) most of which are cited in chapter 1.

The aim of this study was to investigate SDA in common carp, Cyprinus carpio and to observe the effect of different dietary protein levels and daily feed intake levels upon the post prandial stimulation of oxygen consumption.

4.2 Materials and Methods

4.2.1 Experimental animal and husbandry

Cyprinus carpio L. in the weight range 63.50 - 83.00g and average total length of 14-17cm were held in the tropical aquarium of the Institute of Aquaculture, University of Stirling. The stock was received pathogen-free and was held in white plastic aquaria of about 60l capacity maintained at 28°C (+0.5°C) in an aerated water recirculating system (see chapter 2). Each of the aquaria held 8 to 10 fish. The fish were fed on sinking-type commercial fish pellets (42% protein content) (Ewos-Baker Ltd) once daily between 9.00 - 10.00 hours at the rate of about 1% body weight (wet wt.) to serve as a maintenance ration. A 12L : 12D photoperiod was maintained in the system with 8.00 to 20.00 h as the light period.

4.2.2 The Respirometer

There are many different types of both closed and flow-through respirometry systems, but for this study, the oxygen consumption of Cyprinus carpio was measured using a microcomputer operated six channel respirometer of a flow-through type (Ross and Mckinney, 1988) as was used in the previous experiment (see chapter 3). The respirometer was cleaned thoroughly with clean water after each run and with

a bleach-water mixture at every 4th or 5th run in order to prevent any excess bacterial growth in the system or diseases in the fish.

Water quality in the recirculating system was maintained by controlling the major water quality criteria such as ammonia, pH, temperature. Accumulation of any metabolic wastes produced by the fish in the water degrades the quality and eventually may reach levels potentially harmful to the fish. Like other teleost fish, common carp is ammoniotelic and produces ammonia as its main (about 80 - 85%) excretory product. Therefore, to prevent the levels of ammonia rising in the water, 'Dowex' (Dow Chemicals Ltd), an ion exchange resin was used in a Eheim filter installed in the sump tank for filtering the water. The principle is the exchange of ammonia ions from the solution and replacing them with sodium ions in water (see Section 2.8). Ammonia in the water was tested at the start and at the end of each experimental run using the phenol-hypochlorite method as described by Stirling (1985) (see Section 2.6) to ensure that the concentration of ammonia had not produced levels harmful to fish. The pH of the water samples was measured regularly using a Phillips PW 9409 pH meter. Moreover, during each experimental run nearly one third of water in the sump tank was replaced on alternate days with fresh tap water of the same temperature.

4.2.3 **Experimental diets**

Three experimental diets containing about 20, 35 and 50% protein were used in this study and their composition, formulation and proximate analysis have been described in detail in Section 2.2.

4.2.4 **Experimental procedures**

The experimental procedure followed in this study was described in detail in Section 3.2.3 and the oxygen consumption by fish in the respirometer chambers was determined by the computer as in the previous experiment and was represented as mg/kg/h at every 30 minutes interval.

Experimental fish in holding tanks were not allowed to feed for two days before putting them in the respirometer.

Four experimental fish (acclimatized at 12L : 12D) for each run were removed from the holding tank and sedated in a bucket containing Benzocaine, Ethyl-p-amino benzoate (Ross and Geddes, 1979). Within one minute the fish was weighed to 0.1g on a top loading balance after blotting off excess water and were placed in the respirometer chambers. The lids were then replaced and wing nuts tightened, the flow of water through the chambers was restored and air bubbles

were removed from inside the chambers. Finally, data recording was started and left overnight so as to allow the fish to adjust to their new environment.

To avoid additional stresses incurred with force feeding, the fish were fed in the chambers. The fish were not allowed to feed for the first 2 days in the chambers and during that time the resting rate in unfed and undisturbed conditions for each fish was determined both to serve as the base line for oxygen consumption of fed fish when determining the SDA magnitude and to confirm the crepuscular variation in oxygen consumption.

A total ration of 1% of body weight, consisting of 0.5% at each meal, was pre-weighed and fed to the fish in the respirometer chambers via the rubber bung in the lid. Just before feeding the water flow rate was reduced and pellets were introduced via the feeding port. The number and weight of uneaten pellets was recorded and uneaten pellets were then removed carefully with a plastic tube with minimal disturbance to the fish. The rubber bung was then replaced and normal water flow restarted. The first feeding was usually at 1000 h in the morning and the second one at about 1200h noon. Feeding continued usually for 5 to 7 days depending on the response of fish in the chambers. After each full-feeding (@ 1% ration) a further 24 hrs were

allowed to record the effect of feeding otherwise they were fed every day in the morning.

Sham-feeding experiments were also conducted, in which a similar procedure was used as for true fed runs, but using no ration. This allowed an estimation of the rise in oxygen consumption due to the fishes behavioural activity at the time of feeding.

The pellets offered were taken by the majority of the fish within a few minutes but a mixed response was observed for some of the fish which either regurgitated the pellets or sometimes spat them out of the mouth. Some fish did not respond at all towards the feed even by the seventh day or at the end of the feeding trial. The oxygen consumption data from these fishes with irregular behaviour were discarded. A total of 7 experimental runs was done with 20% protein content diet among which only 5 were considered to be successful; out of 8 experimental runs with 35% protein content diet 6 were successful. Similarly, five experimental runs were successful out of eight conducted with the 50% protein content diet. The food ration consumed by the fish in the respirometer varied from 0.40% to 1.00% of the body weight

At the end of each run when the pre-feeding oxygen consumption level had been re-attained, the water flow to the

respiration chambers was stopped, the chamber lids unscrewed and the fish removed and returned to a separate holding tank. Tnese fish were not reused.

The oxygen consumption due to SDA was determined from a graph of oxygen consumption against time. The area between the curve so obtained and the curve representing the resting rate considered to be due to SDA. Resting rate was calculated on the basis of the mean of oxygen consumption from the first two unfed days. Individual resting rate was used for determination of each SDA parameter (peak value, duration and magnitude) from each fish fed on a defined ration. Finally, the recalculated mean resting rate for each ration size was compared with the mean SDA rate in order to develop models for SDA and to show the relationship between dietary protein and other SDA aspects.

Data processing was by correlation, regression and analysis of variance and was carried out using MINITAB (Ryan et al., 1985). All graphics and regression line-fit was done by Macintosh SE using Cricket graph 1.3.

4.2.5. Digestibility of diets

Since it was not possible to collect the faeces from the respirometers, a separate trial using fish of a similar weight was carried out and faeces were collected to measure

the apparent protein digestibility of feeds. For this purpose a group of 6 fish was kept in the holding tank with about 40l water. After three days of starvation, fish in this tank were fed to satiation using the experimental diets. Faecal material produced was collected by siphoning and these were pooled and dried. A second feed was given the next day and faecal matter was again collected, pooled and dried. During collection the tank was inspected every half an hour, so that materials collected had not been in the water for more than 30 minutes. Regular sampling reduced losses of solubles from the faeces into the water. The dried samples were analysed to determine the apparent protein digestibility by the method of Furukowa and Tsukahara (1966) using chromic oxide as a marker in the feed.

4.3 Results

4.3.1 Water quality maintenance

Water samples from the sump tank and header tank were found to have very similar concentrations of ammonia during each experimental run indicating the recirculating system to be well mixed. The total ammonia concentrations in the water found during each experimental run were spread more or less evenly increase in the range of 0.021 to 0.250 mgl^{-1}. The level of ammonia showed no relationship to the amount of feed given during the run but was correlated with continuous changes in the ion exchange efficiency of the resin as it became exhausted. Recharging of resin prevented a significant increase of toxic ammonia in the system.

The pH of the water was found to be 7.55 (+_0.30) and so the proportion of unionised ammonia was calculated using the expression:

$$\% \text{ unionised ammonia} = 100/(1 + \text{antilog } (pKa - pH))$$

where pKa is the negative logarithm of unionised constant which is 9.15 at 28°C (Stirling, 1985). Thus, unionised ammonia was found to be 16.80% of the total

ammonia, and thus the maximum concentration of unionised (toxic) ammonia was 0.042 $mg\Gamma^{1}$.

The temperature of the water in the system was controlled at 28 +_0.5°C.

4.3.2 Acclimation to the respirometer

Although some fish showed an increased rate of oxygen consumption after introduction to the respirometer, most of the fishes became stable within 12 to 16 hours. Oxygen consumption throughout the initial 48 hour period was found to vary between individual fish, but this variation was small.

Peaks of respiratory activity were found to occur during the onset of light and dark and these details of resting rates in common carp inside the respirometer chambers have been described in chapter 3.

In the confined respirometer chambers fish did not respond well in the first day of feeding and usually only took a small ration. On the second day of feeding a mixed response was revealed. Fish either became accustomed to feeding in the chamber and ate normally but with reduced ration, or they would not feed at all. Some fish regurgitated the feed. A few fish showed an intermediate

response and ate : very small quantities of feed. Results obtained from fish which did not feed inside the chambers were discarded.

4.3.3 Sham feeding

There was no significant effect of sham feeding on the respiratory activity of common carp due to the feeding procedures and handling stress (Figure 4.1). The observed oxygen consumption subsequent to sham feeding was seen to be accommodated within the range of variation for fish at rest. Any increase in oxygen consumption was principally due to excitement of fish and lasted for only 1-2 hours.

4.3.4 The general post-feeding response to oxygen consumption

In general, oxygen consumption gradually increased above the prefeeding or resting level shortly after feeding and continued for several hours having one or more peak values followed by a fall to the resting level. Fig. 4.2 shows an example of the post-prandial mean oxygen uptake by fish over the mean resting rate with different dietary protein levels and a 1% ration level. Significant increase ($p < 0.05$) in oxygen consumption is seen with an increase of protein in the diet.

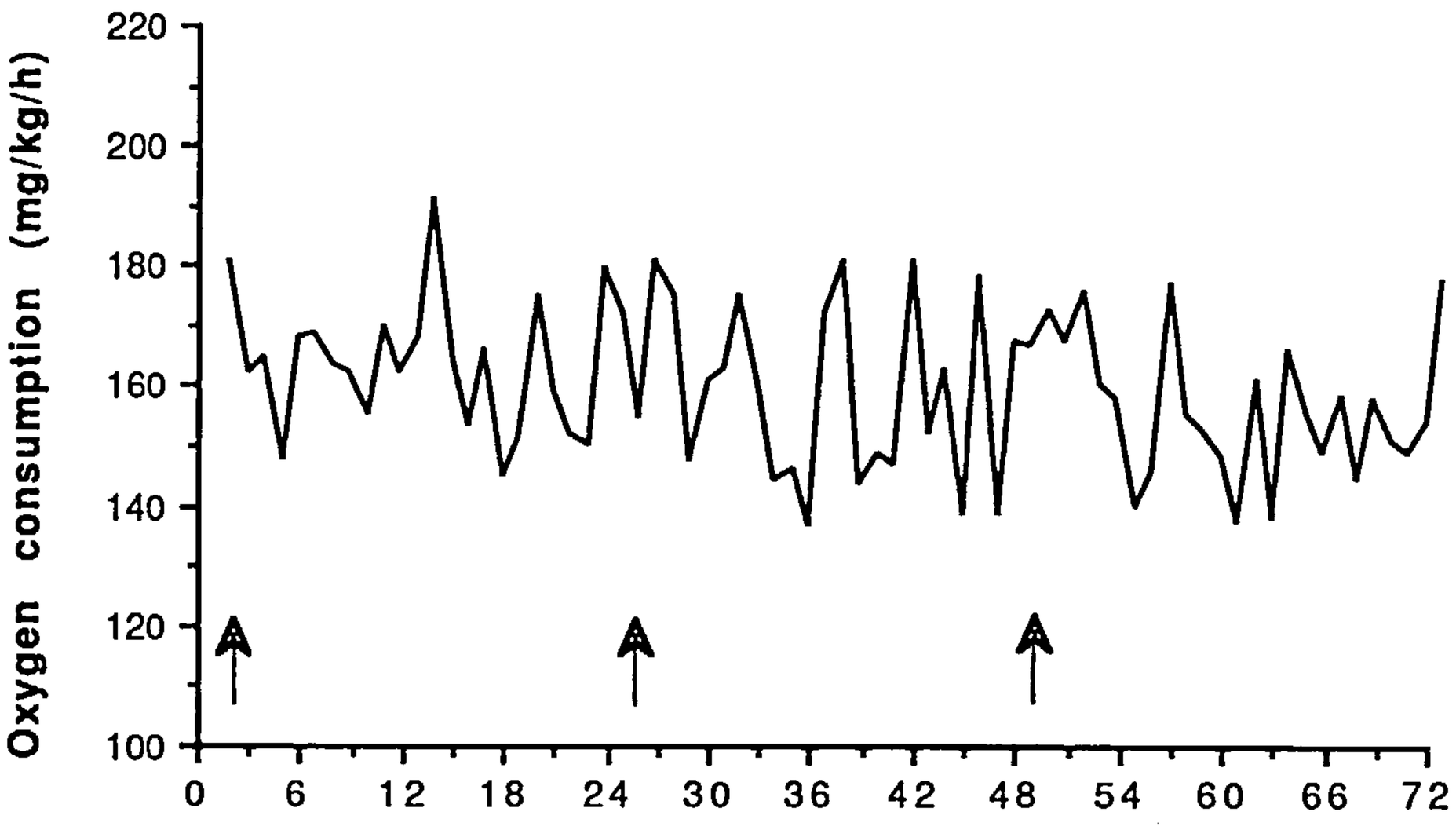


Fig. 4.1 Typical sham feeding response in Cyprinus carpio of 63 - 83g body wt.

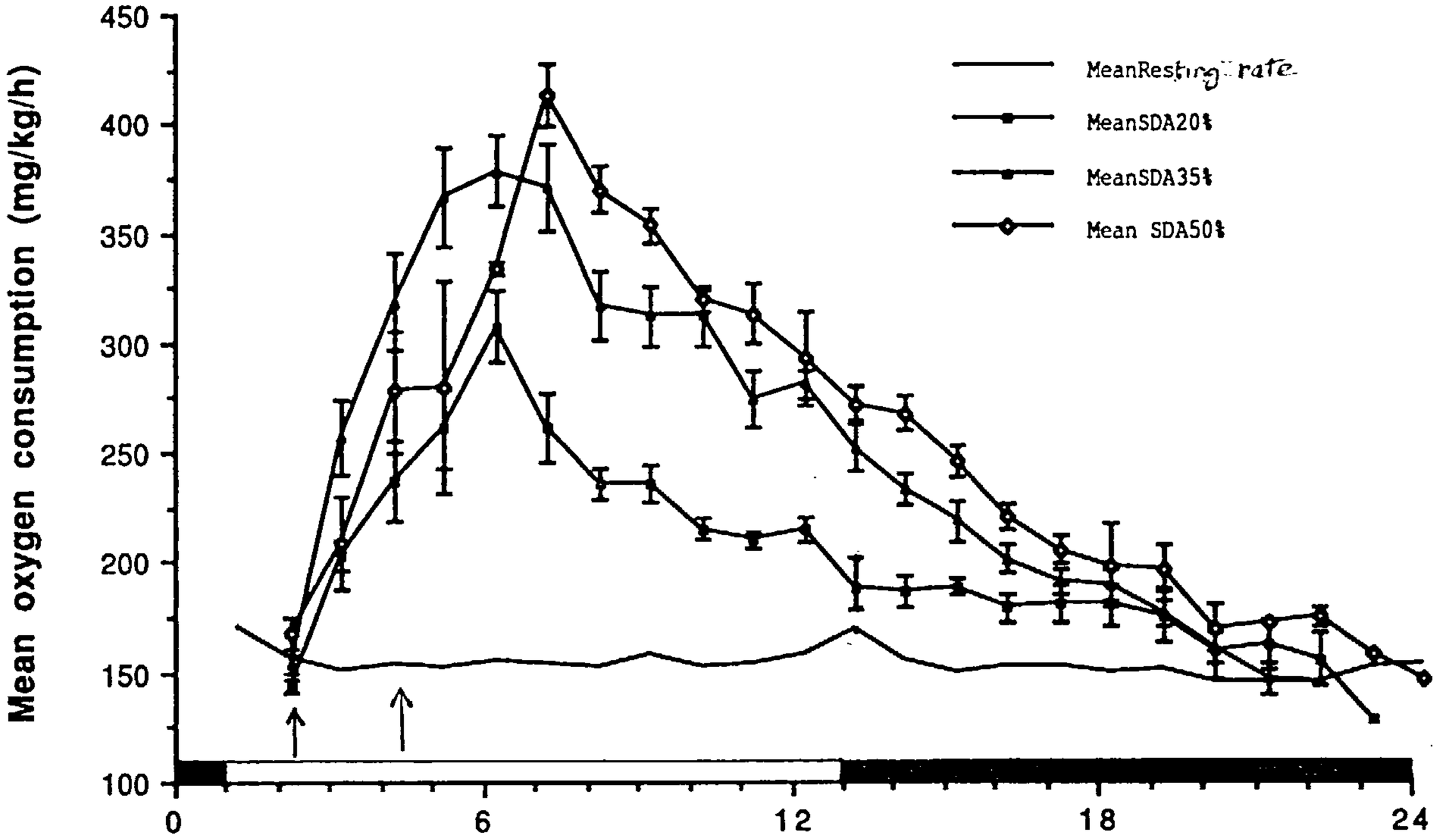


Fig. 4.2 Features of SDA of Cyprinus carpio fed on 20%, 35% and 50% dietary protein at 1.00% ration level (typical). Values are arithmetic means during experimental period. Vertical bars are the +_ SEM. Arrow indicates time of feeding.

4.3.5 Response to feeding with different protein content diets

Figs. 4.3a - y show the post-feeding increase of oxygen consumption against the mean prefeeding resting rate at different rations (% body weight) when fed a 20% protein content diet. The principal features of the SDA response to a 20% protein content diet are shown in Table 4.1.

The post prandial peak oxygen consumption with 20% dietary protein increased significantly ($p < 0.05$; $r = 0.838$) with the increase of energy intake in the diet (Fig. 4.4a). The peak oxygen consumption value ranged from 195 $mgO_2/kg/h$ to 358 $mgO_2/kg/h$ (Table 4.2). A similar, significant, relationship was observed with SDA duration ($p < 0.05$; $r = 0.748$), SDA magnitude ($p < 0.05$; $r = 0.937$) and with energy intake (Fig. 4.4b,c) but the SDA coefficient was found to be not significantly related ($p > 0.05$; $r = 0.219$) to energy intake (Fig. 4.4d). SDA duration varied from 8 to 20 h and the SDA magnitude ranged from 323 $mgO_2/kg/h$ to 1506 $mgO_2/kg/h$ depending in energy intake. SDA coefficients ranged from 6.6 to 12.06% having a mean value of 8.99% (Table 4.2). There was no significant correlation between the time taken to reach to peak with energy intake ($p > 0.05$, $r = 0.356$) (Fig. 4.4e). The time to reach maximum varied within 1 to 5 hours (Table 4.1) and the percent increase of post-feeding

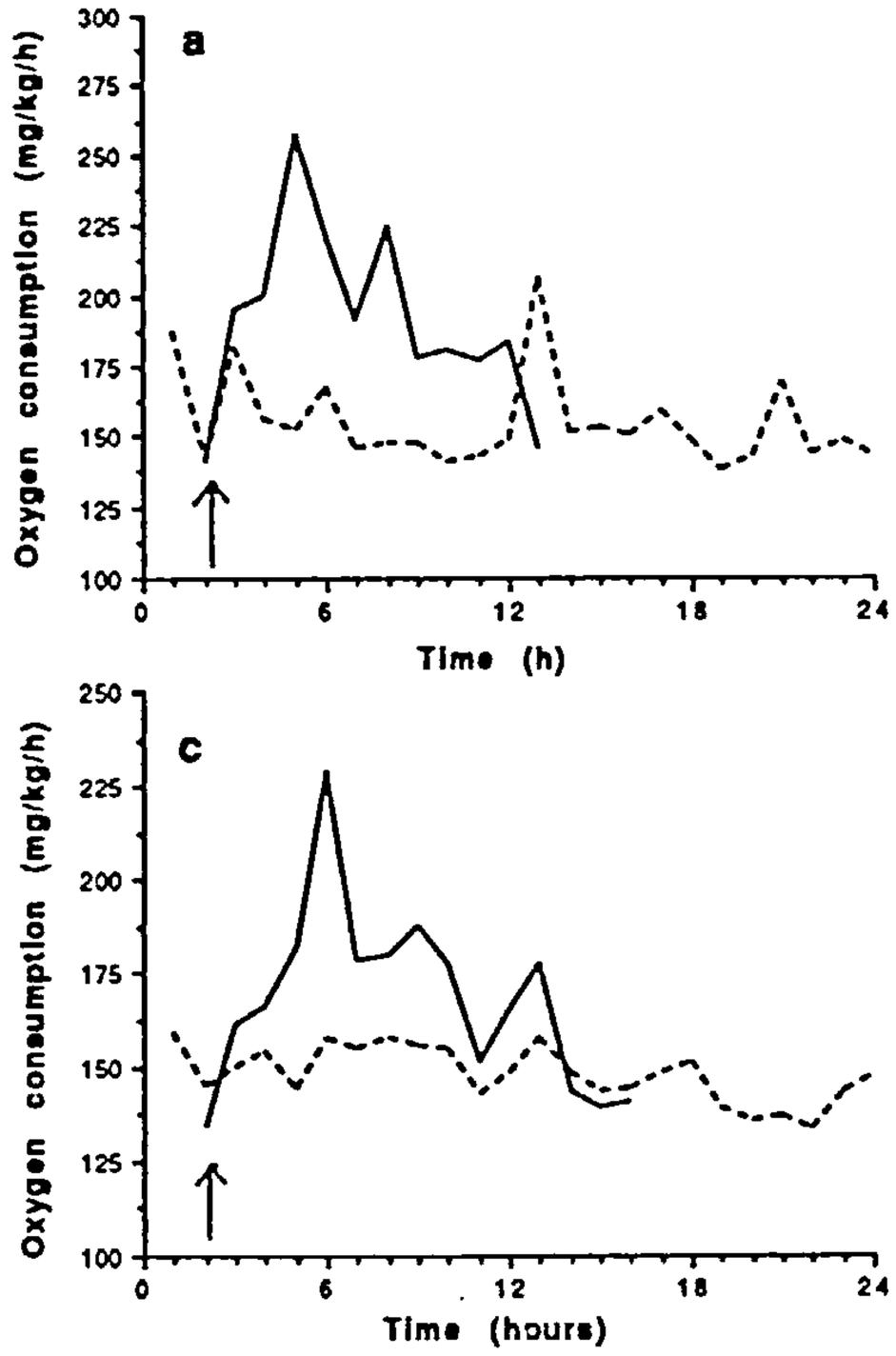
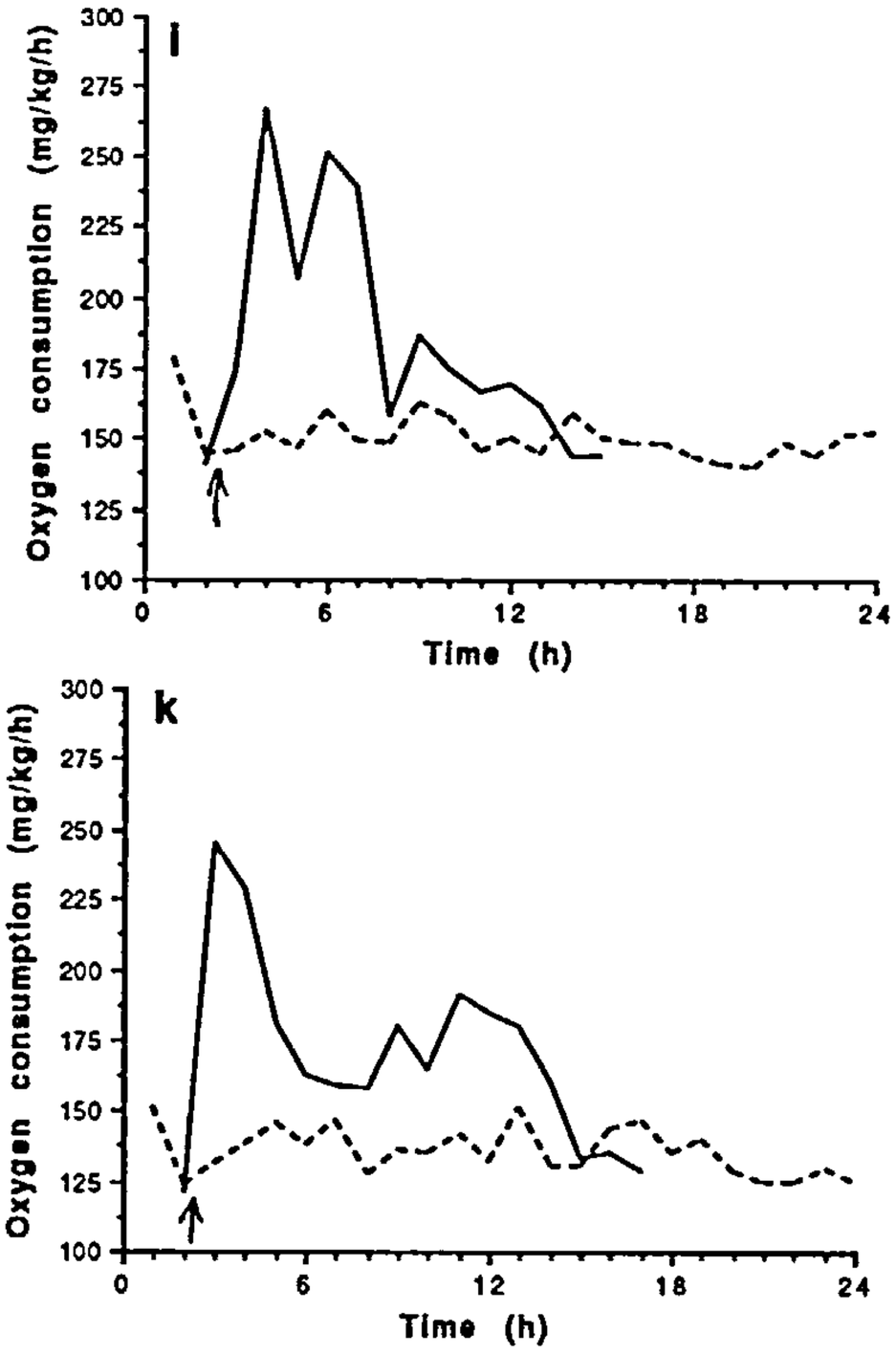
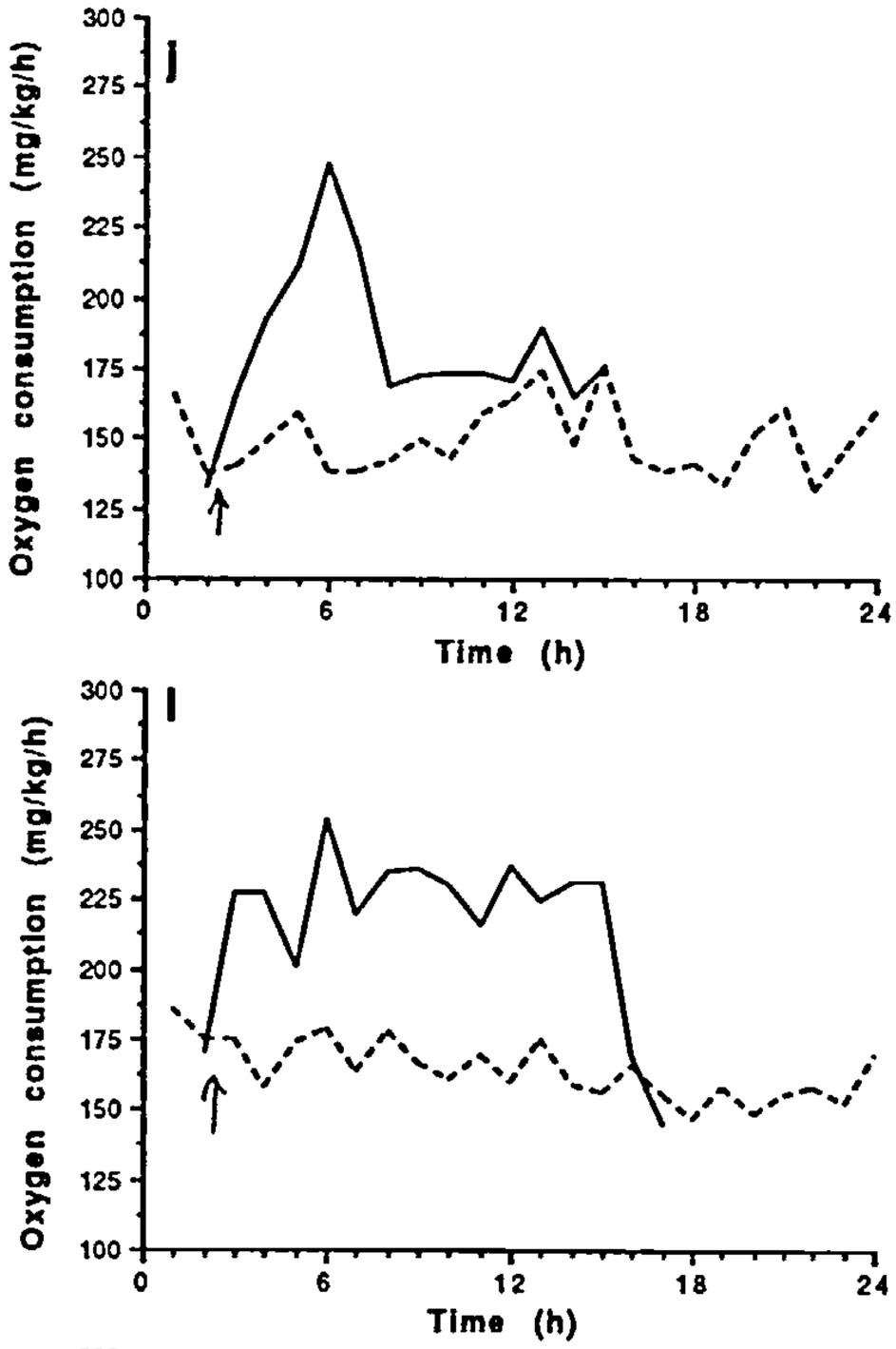
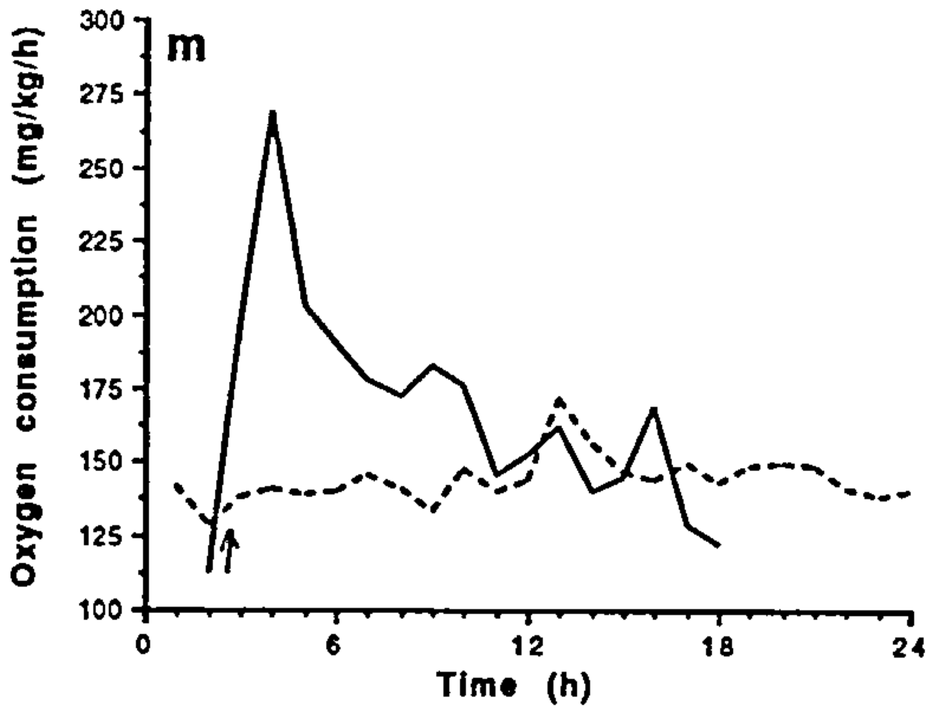
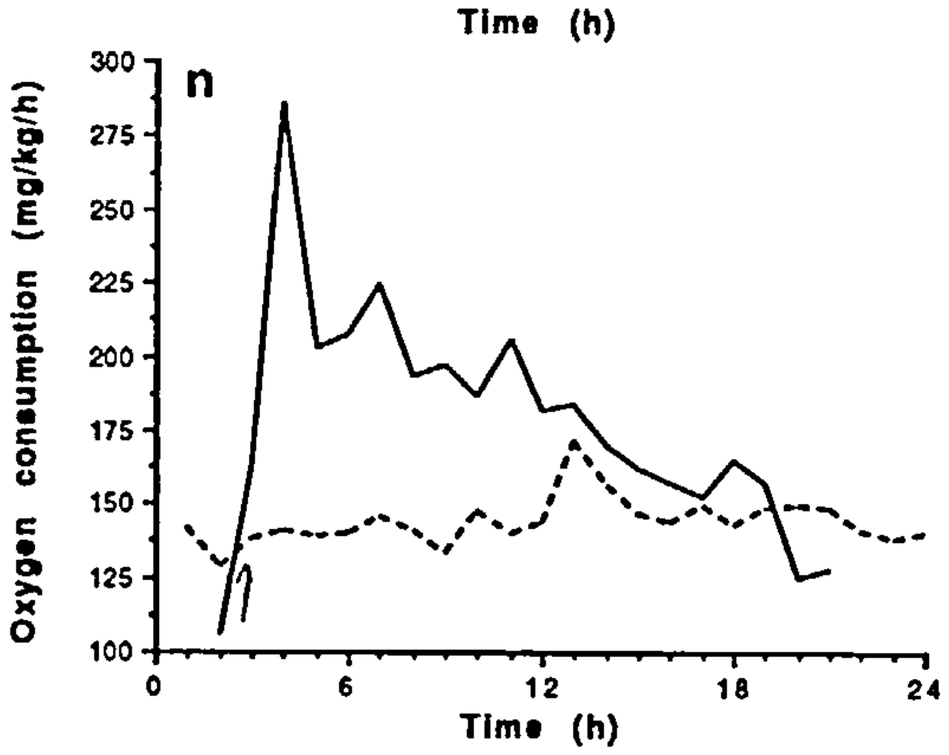


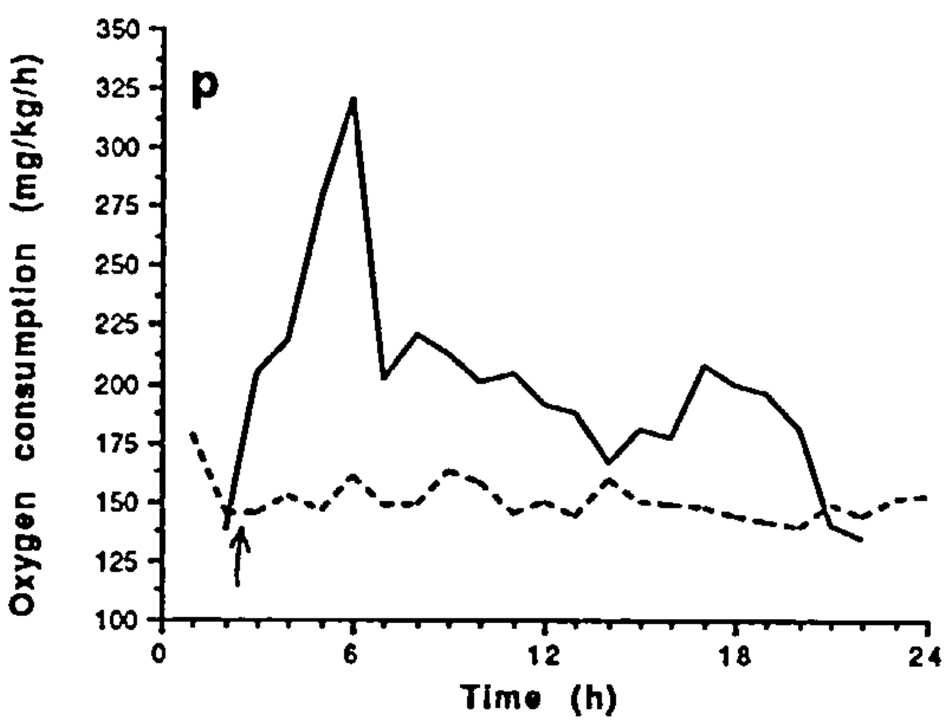
Fig. 4.3 (a - y). Post-prandial oxygen consumption (solid line) and resting metabolic rate (dotted line) of Cyprinus carpio subsequent to being fed on 20% protein content diet at (i) 0.40% body wt. ration (a - c); (ii) 0.50% body wt. ration (d - k); (iii) 0.75% body wt. ration (l - n); (iv) 1.00% body wt. ration (p - y). Arrow indicates time of feeding.

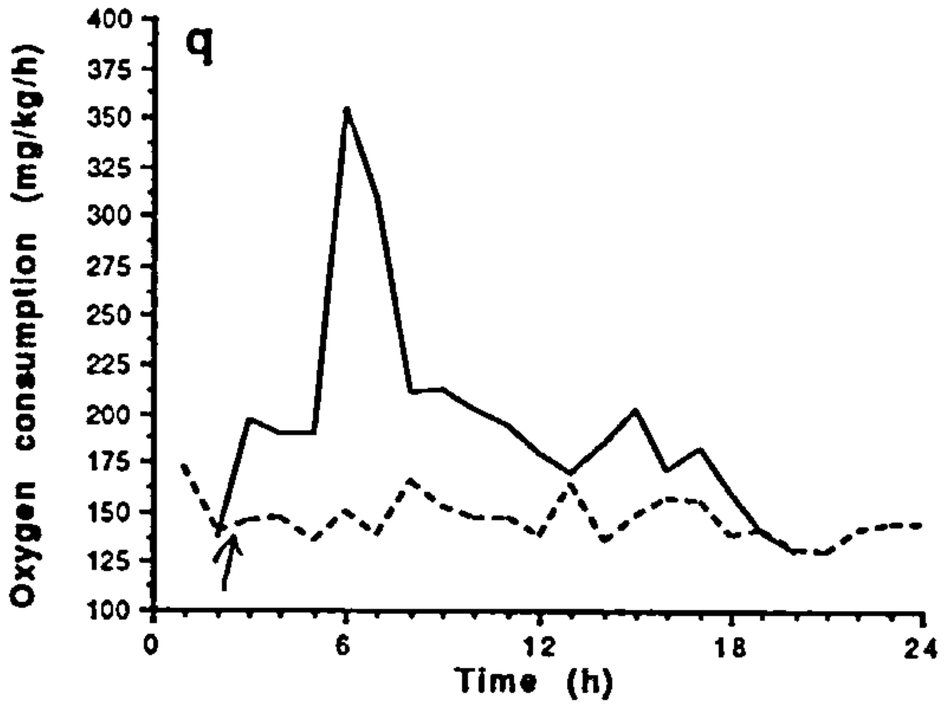

k
Oxygen consumption (mg/kg/h)
300
275
250
225
200
175
150
125
100
0
6
12
18
24
Time (h)


l
Oxygen consumption (mg/kg/h)
300
275
250
225
200
175
150
125
100
0
6
12
18
24
Time (h)

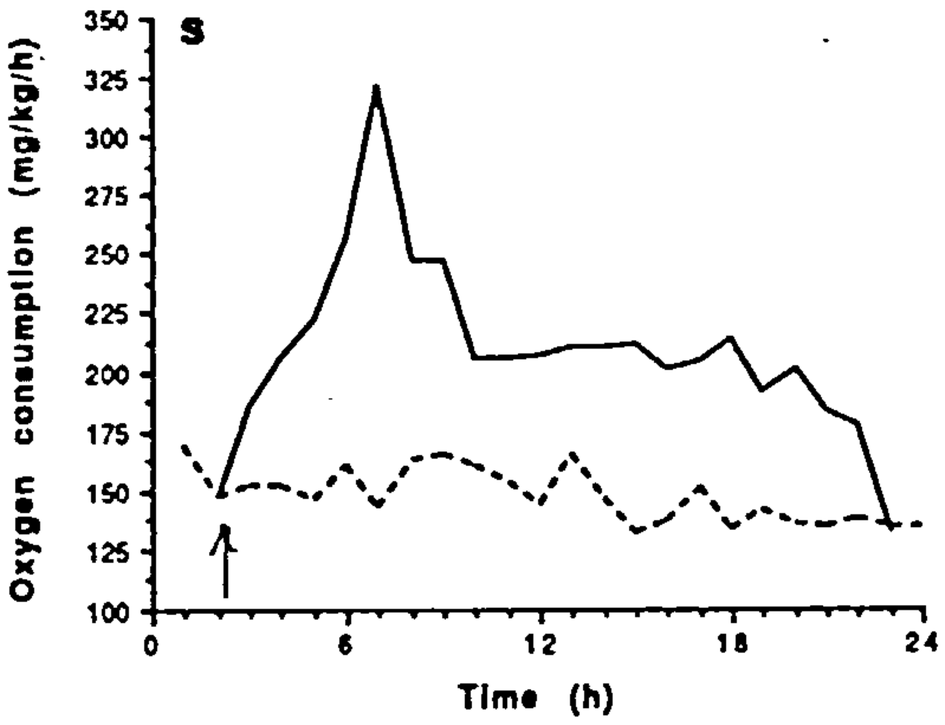

m
Oxygen consumption (mg/kg/h)
300
275
250
225
200
175
150
125
100
0
6
12
18
24
Time (h)


n
Oxygen consumption (mg/kg/h)
300
275
250
225
200
175
150
125
100
0
6
12
18
24
Time (h)


p
Oxygen consumption (mg/kg/h)
350
325
300
275
250
225
200
175
150
125
100
0
6
12
18
24
Time (h)


q
Oxygen consumption (mg/kg/h)
400
375
350
325
300
275
250
225
200
175
150
125
100
0
6
12
18
24
Time (h)

continued...


s
Oxygen consumption (mg/kg/h)
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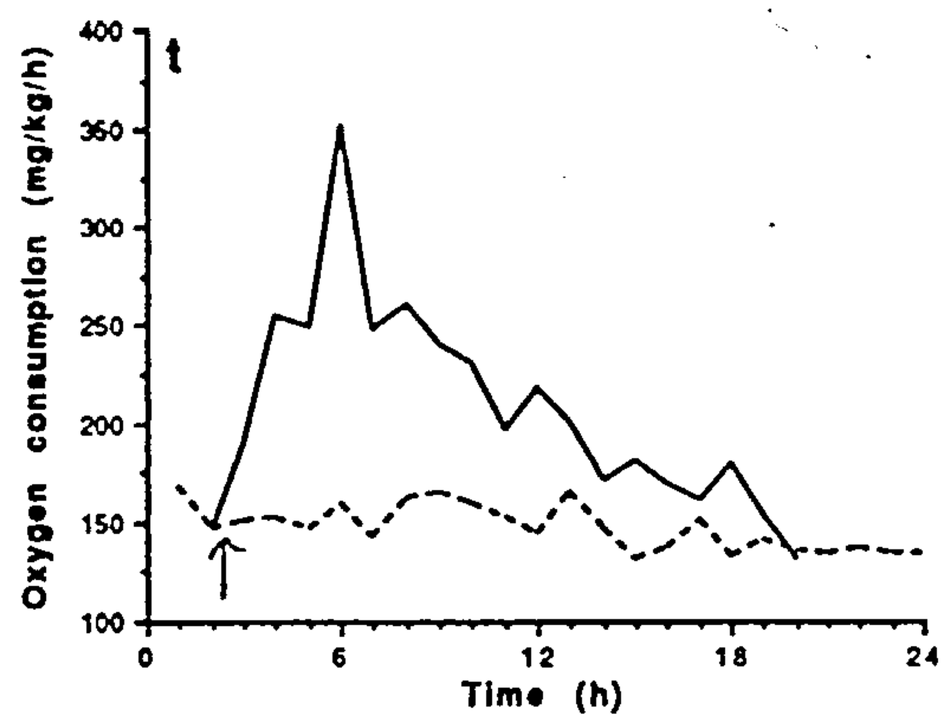
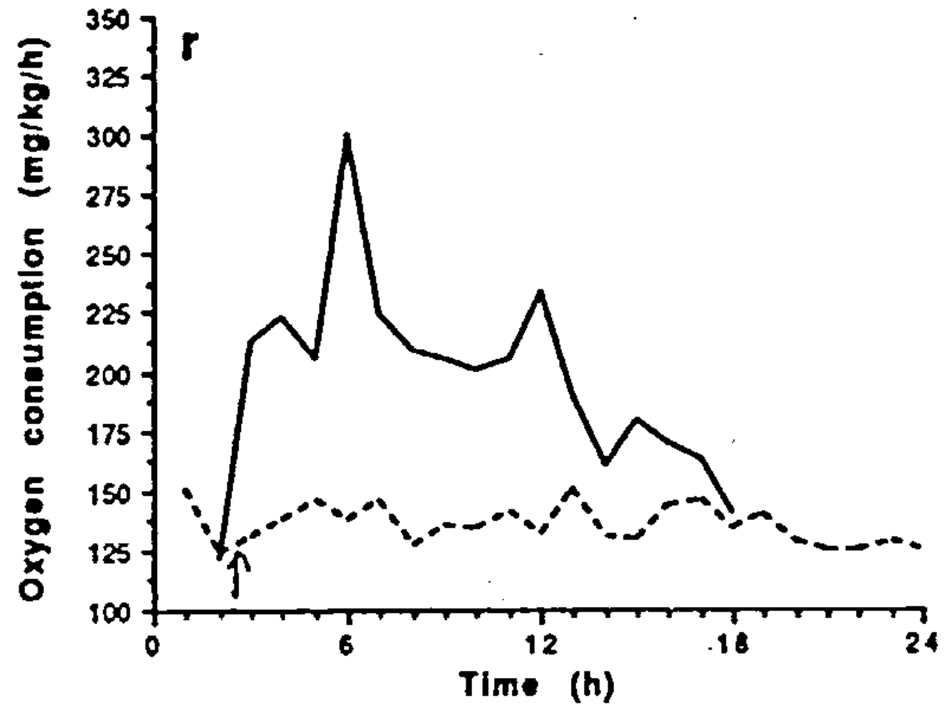
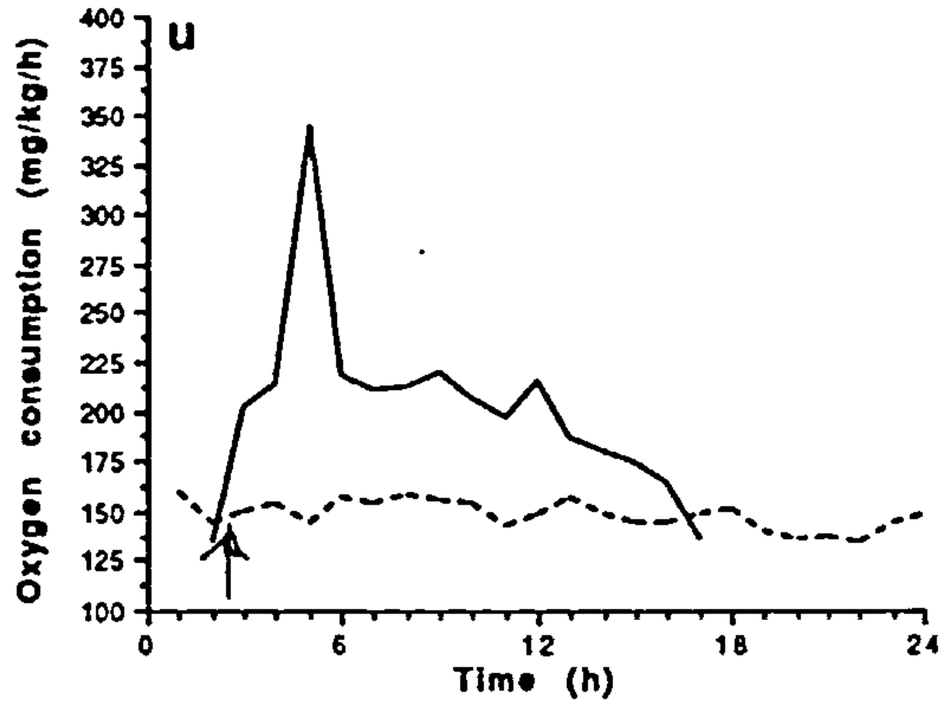
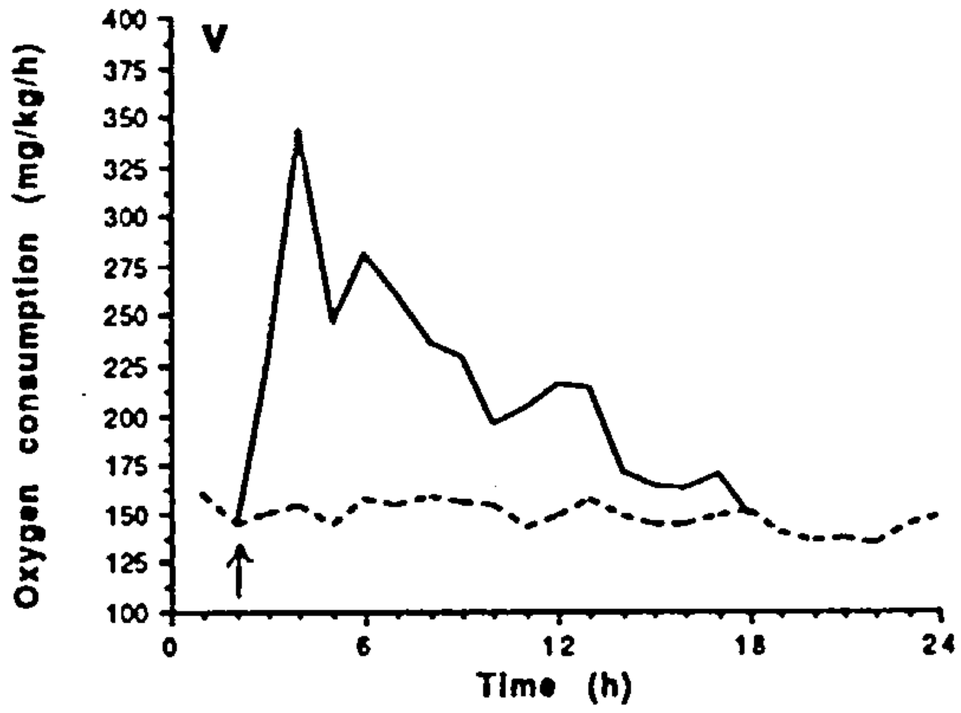
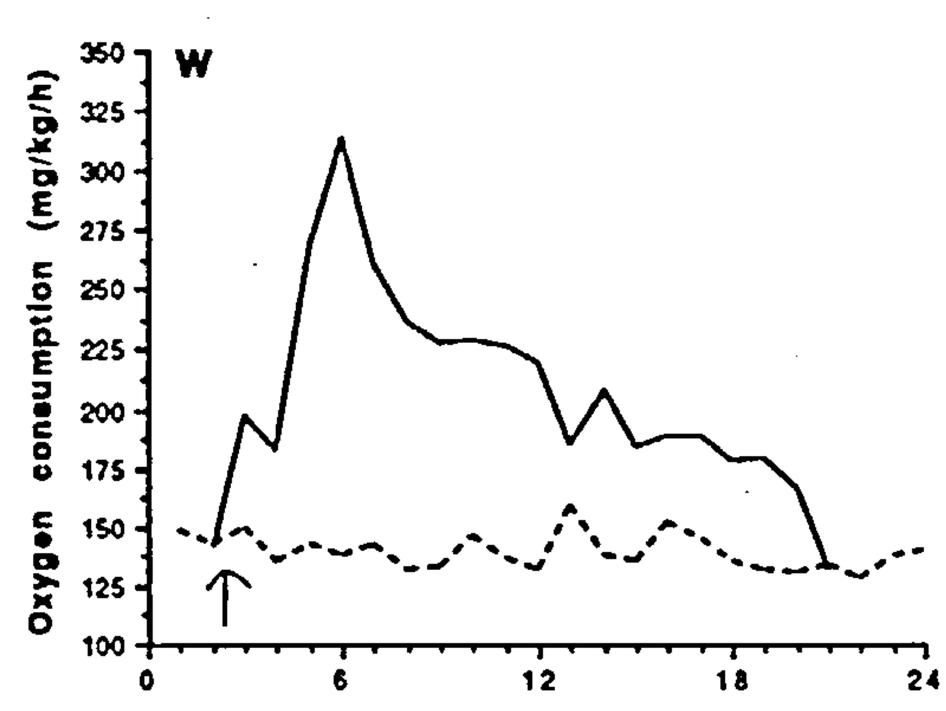
325
300
275
250
225
200
175
150
125
100
0
6
12
18
24
Time (h)

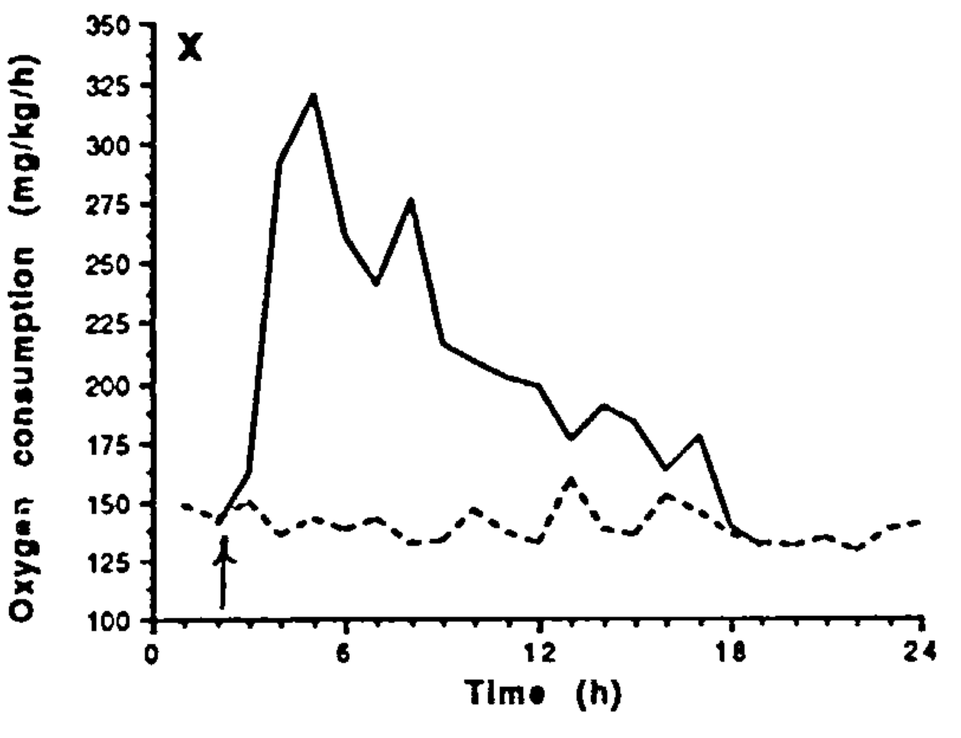

t
Oxygen consumption (mg/kg/h)
400
350
300
250
200
150
100
0
6
12
18
24
Time (h)


r
Oxygen consumption (mg/kg/h)
350
325
300
275
250
225
200
175
150
125
100
0
6
12
18
24
Time (h)


u
Oxygen consumption (mg/kg/h)
400
375
350
325
300
275
250
225
200
175
150
125
100
0
6
12
18
24
Time (h)


v
(mg/kg/h)
400
375
350
325
300
275
250
225
200
175
150
125
100
0
6
12
18
24
Time (h)


W
Oxygen consumption (mg/kg/h)
350
325
300
275
250
225
200
175
150
125
100
0
6
12
18
24
Time (h)


x
Oxygen consumption (mg/kg/h)
350
325
300
275
250
225
200
175
150
125
100
0
6
12
18
24
Time (h)

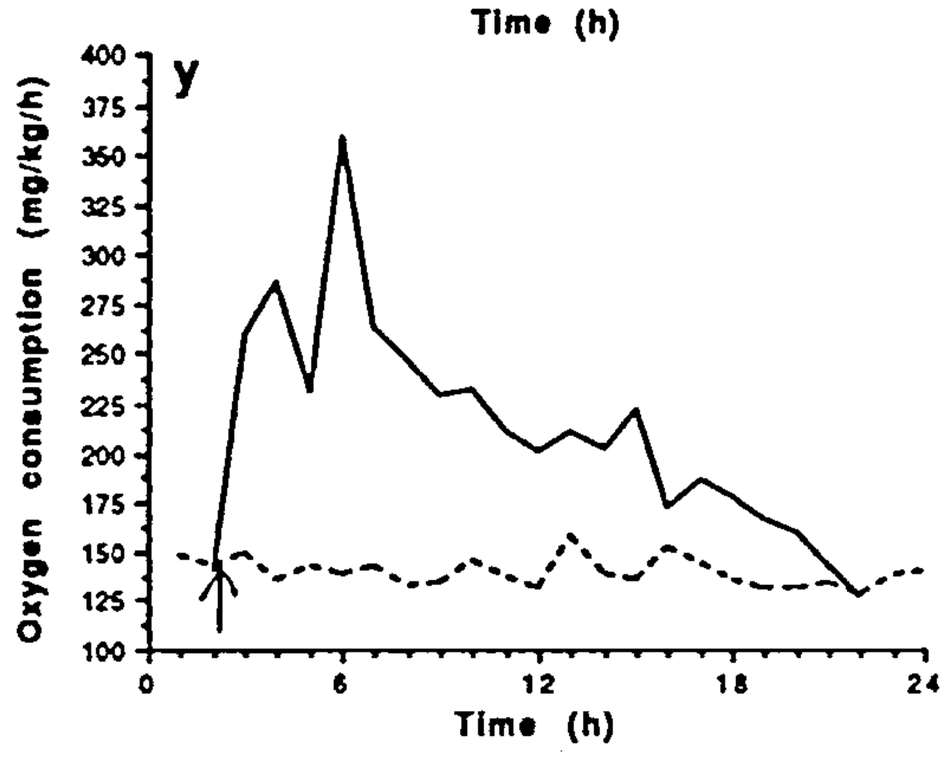

y
Oxygen consumption (mg/kg/h)
400
375
350
325
300
275
250
225
200
175
150
125
100
0
6
12
18
24
Time (h)

Table 4.2 The main features of SDA in common carp, Cyprinus carpio in response to 20% protein diet.

Fish wt (g)	Ration (% body weight)	Minimum resting (mgO_2/kg/h)	Peak (mgO_2/Kg/h)	Peak (Over % resting rate)	Time to reach peak(h)	Duration (h)	SDA magnitude (mgO_2)	Energy intake (kJ)	SDA magnitude (mgO_2/kg)	SDA Coefficient (%)
67.50	0.40	141.04	257.65	77.34	5	8	31.79	4.744	470.96	9.61
69.10	0.40	123.38	195.40	53.59	1	10	26.88	4.856	389.00	7.83
68.30	0.40	133.46	228.57	66.64	4	13	22.09	4.800	323.43	6.60
67.50	0.50	141.04	280.55	79.79	2	17	50.28	5.929	744.88	12.06
67.50	0.50	141.04	268.06	71.78	2	16	42.09	5.929	623.55	10.65
67.00	0.50	121.93	214.16	45.96	1	14	36.27	5.885	541.34	8.83
67.00	0.50	121.93	271.58	101.17	3	13	33.77	5.885	504.02	8.23
68.60	0.50	133.18	246.38	75.75	4	11	40.34	6.026	588.90	9.68
71.20	0.50	139.37	266.92	75.02	2	11	35.05	6.254	492.27	8.05
70.50	0.50	127.45	256.57	86.58	4	12	28.94	6.193	410.49	6.71
70.30	0.50	125.10	244.84	77.02	2	12	34.81	6.176	495.16	8.09
68.50	0.75	133.18	366.95	126.96	5	16	65.94	9.026	962.62	10.35
71.20	0.75	127.80	268.85	91.61	2	15	51.97	9.382	729.91	7.94
71.20	0.75	127.80	285.95	103.79	2	17	49.83	9.382	699.85	7.61
71.20	1.00	139.37	319.99	100.00	4	19	68.88	12.510	967.41	7.89
70.50	1.00	130.09	355.21	137.01	4	16	66.65	12.386	945.39	7.77
70.30	1.00	125.10	300.42	118.02	4	15	72.95	12.351	1037.69	8.50
72.50	1.00	131.77	321.57	128.46	5	20	95.91	12.738	1322.89	10.79
72.50	1.00	131.77	351.93	119.70	4	17	82.81	12.738	1143.58	9.32
68.30	1.00	133.46	344.65	156.30	3	14	65.78	12.000	963.10	7.86
68.30	1.00	133.46	343.56	123.34	2	15	73.37	12.000	1074.23	8.77
74.50	1.00	126.80	313.29	126.75	3	18	99.43	13.089	1334.63	10.89
74.50	1.00	126.80	320.50	131.97	4	17	95.07	13.089	1276.10	10.41
74.50	1.00	126.80	358.18	159.25	4	19	112.16	13.089	1357.85	11.08
Mean X										8.99(±1.45)

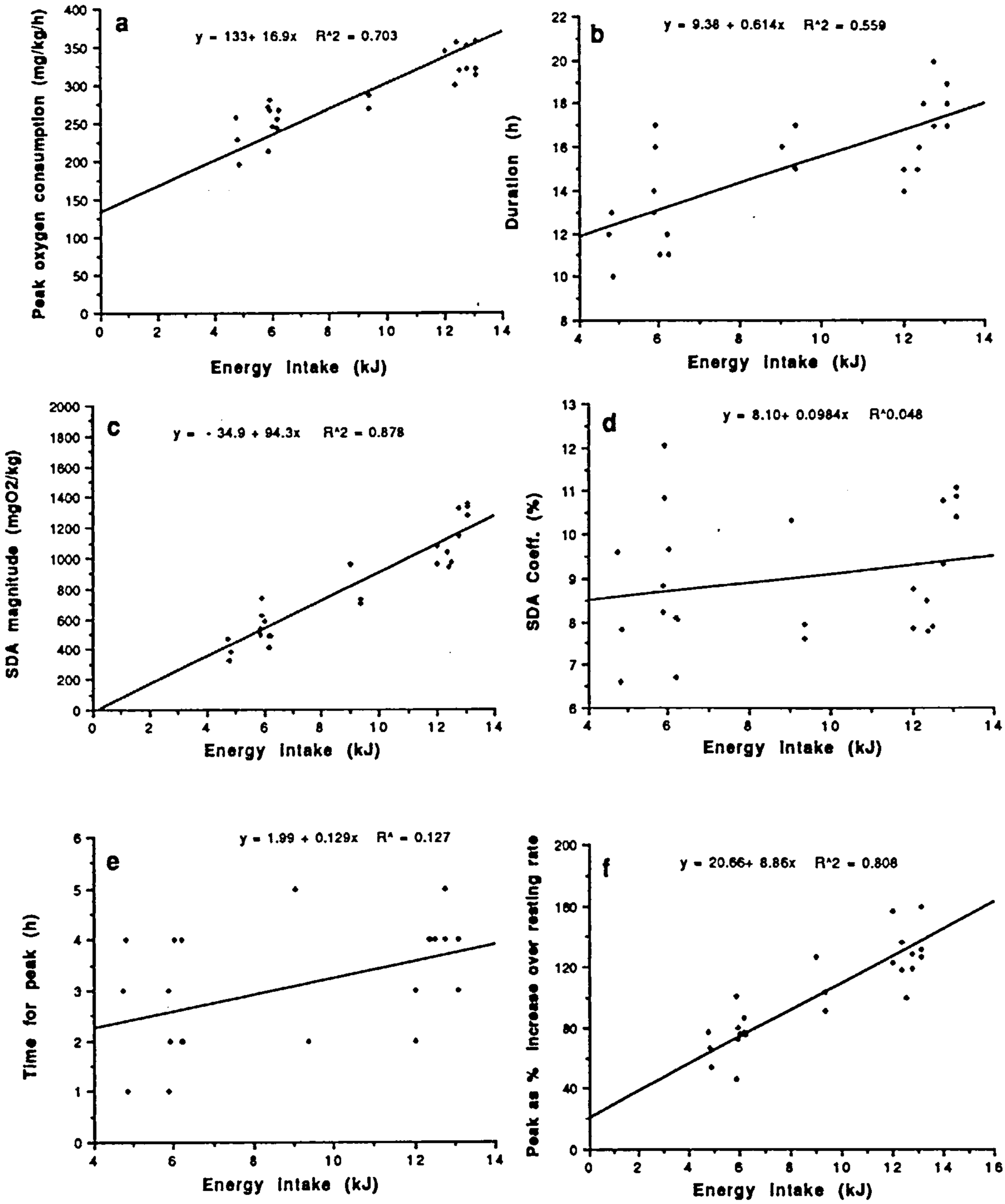


Fig. 4.4 (a - f). Relationship between the aspects of SDA in response to 20% protein content diet. The regression equation is in the simple form $y = a + bx$. R^2 means the square of correlation coefficient.

oxygen consumption over resting was significantly positively correlated ($p < 0.05$, $r = 0.898$) with a range of 45.96% to 159.25% as was expected (Fig. 4.4f).

No significant relationship between the fish weight and SDA coefficient was found ($p > 0.05$. $r = 0.189$) (Fig. 4.5). Similarly, there was no significant relationship between SDA coefficient and different ration level ($p > 0.05$; corr. coeff. 0.223 and SDA coeff. = 8.0551 + 1.277 ration) (Table 4.2, Fig. 4.12a).

Figures 4.6 (a - y) show the increases of respiratory activity of individual common carp after feeding on different ration levels of the 35% protein diet. In these trials, oxygen consumption reached a greater peak level followed by a decrease to the resting levels. The major features of the SDA response to feeding of 35% protein diet at different ration levels by common carp are summarized in Table 4.3.

The maximum value of oxygen consumption and SDA magnitude in different trials were found to be directly dependant on the ingested energy ($p < 0.05$) having correlation coefficients of 0.873 and 0.904 respectively (Fig. 4.7a, c). In these trials the peak oxygen consumption ranged from 244.91 mg/kg/h with energy of 0.4% ration diet to 430.40 mg/kg/h with energy of 1.0% ration diet and the SDA

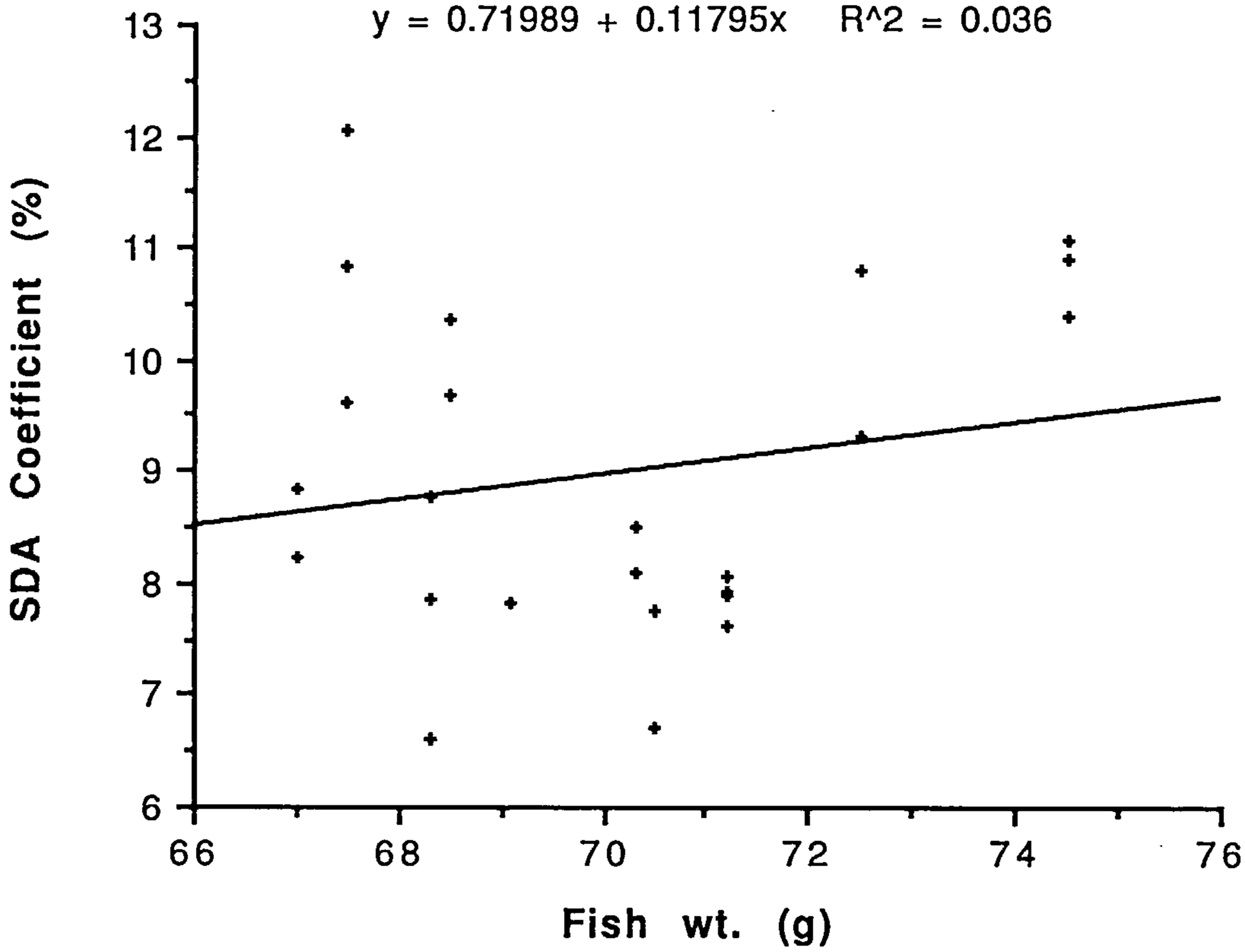


Fig. 4.5 Relationship between SDA coefficient and fish weight of Cyprinus carpio fed with 20% dietary protein at different ration levels (0.40% to 1.00% body wt.).

Table 4.3 The main features of SDA in common carp, Cyprinus carpio in response to 35% protein diet.

Fish wt (g)	Ration (% body weight)	Minimum resting ($mgO_2/Kg/h$)	Peak ($mgO_2/Kg/h$)	Peak (over % resting rate)	Time to reach peak(h)	Duration (h)	SDA magnitude mgO_2/fish)	Energy intake (kJ)	SDA magnitude (mgO_2/kg)	SDA Coefficient (%)
75.60	0.40	136.94	244.91	79.51	3	16	52.37	5.797	692.72	12.71
60.50	0.40	131.36	246.23	70.20	4	12	44.85	4.639	741.32	13.60
60.50	0.40	131.36	254.62	95.32	5	13	50.74	4.639	838.67	14.18
63.50	0.40	130.36	257.11	91.97	2	12	42.67	4.869	671.98	14.18
63.50	0.40	130.36	260.23	94.30	2	12	44.64	4.869	702.99	12.90
79.50	0.40	136.63	259.75	83.84	5	18	57.61	6.096	724.65	12.09
63.50	0.50	130.36	345.66	155.89	5	16	60.41	6.086	951.33	13.86
79.30	0.50	141.87	276.08	80.26	2	18	74.98	7.600	945.50	13.94
79.50	0.50	136.63	321.60	119.41	5	17	68.62	7.620	863.14	12.75
73.00	0.50	138.07	308.13	123.16	2	13	56.67	6.997	776.30	11.40
73.50	0.50	147.23	258.69	75.70	3	13	56.70	7.044	771.42	11.41
70.00	0.50	145.12	253.31	41.40	4	14	55.70	6.709	795.71	11.59
73.50	0.75	147.23	348.62	106.50	7	15	86.55	10.567	1177.56	11.56
78.50	0.75	130.66	278.25	155.46	5	16	101.59	11.286	1294.14	12.64
73.00	1.00	138.07	336.26	184.02	3	17	144.93	13.994	1985.34	14.58
77.00	1.00	125.09	435.25	182.95	4	17	141.48	14.760	1837.40	13.49
78.50	1.00	139.31	402.45	132.25	4	19	133.41	15.048	1699.49	12.48
78.50	1.00	139.31	409.66	162.35	5	17	141.56	15.048	1803.33	13.24
65.50	1.00	134.77	430.40	183.10	3	18	137.03	12.556	2092.06	15.36
65.50	1.00	134.77	404.08	183.76	4	18	135.33	12.556	2066.10	15.17
65.50	1.00	134.77	402.90	190.10	5	17	144.47	12.556	2205.64	16.20
70.00	1.00	145.12	427.50	161.05	5	16	151.89	13.419	2169.85	15.93
70.00	1.00	145.12	364.17	194.43	2	16	139.13	13.419	1987.57	14.59
78.50	1.00	130.66	403.00	181.48	2	17	147.37	15.048	1877.32	13.78
Mean, $\bar{X}$										13.40 ($\pm$1.38)

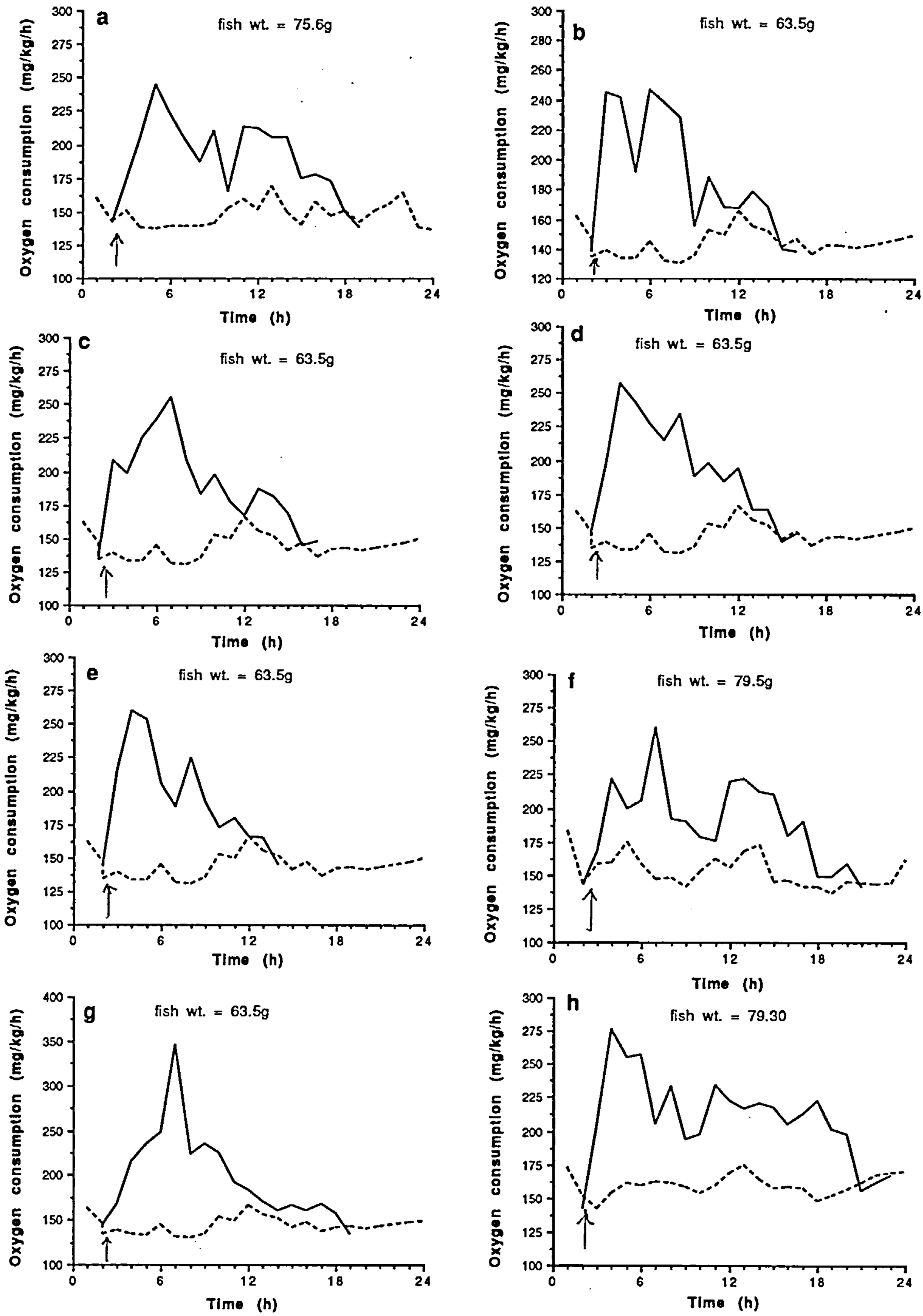
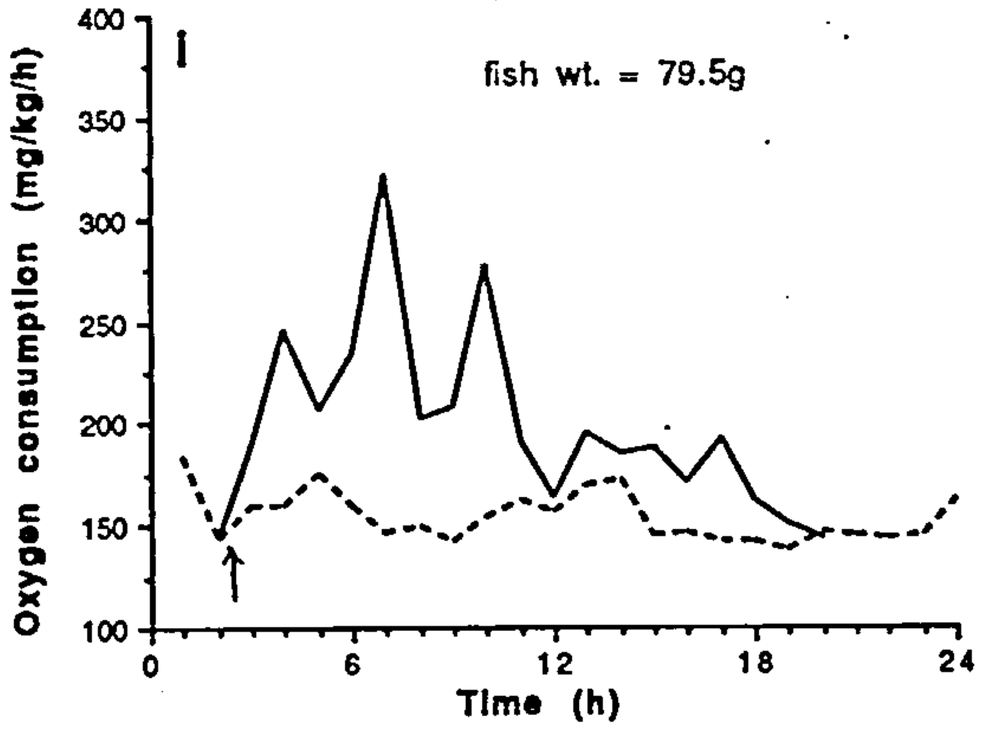
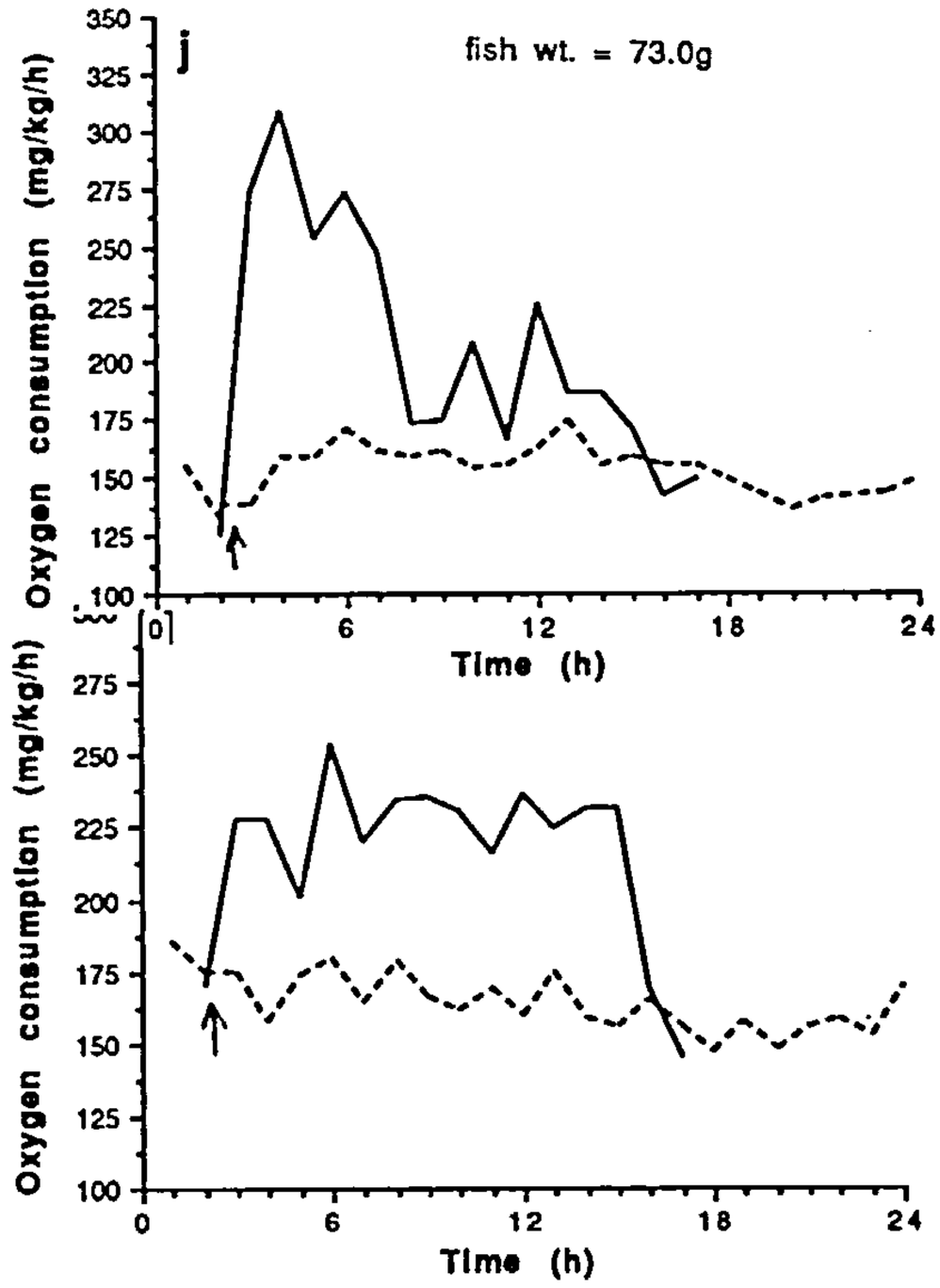
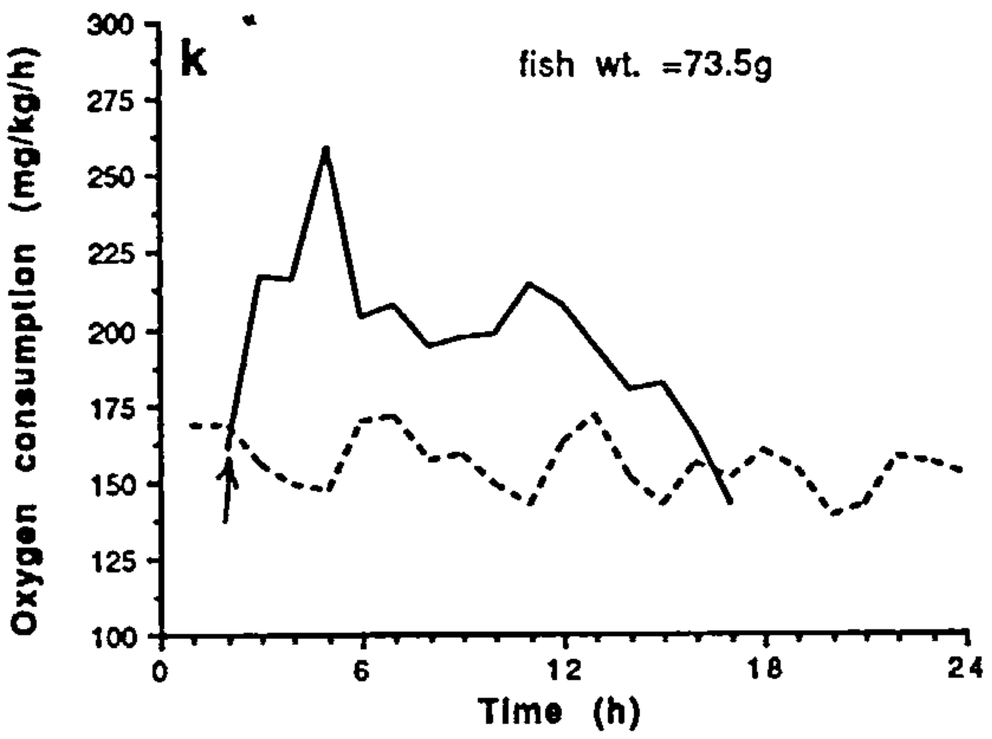
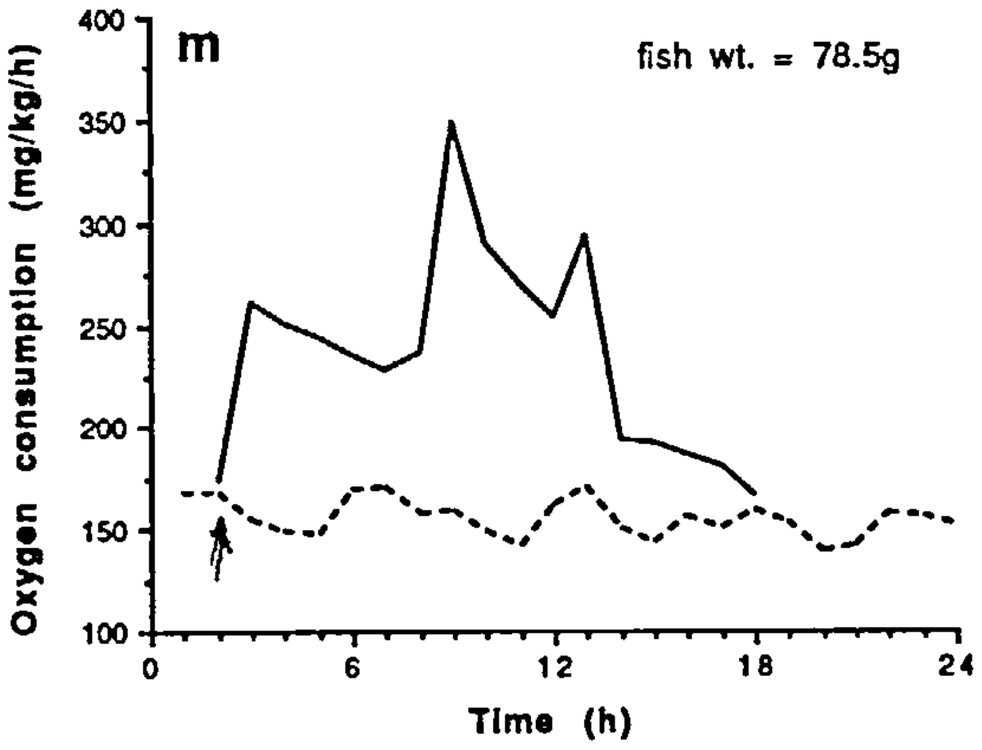


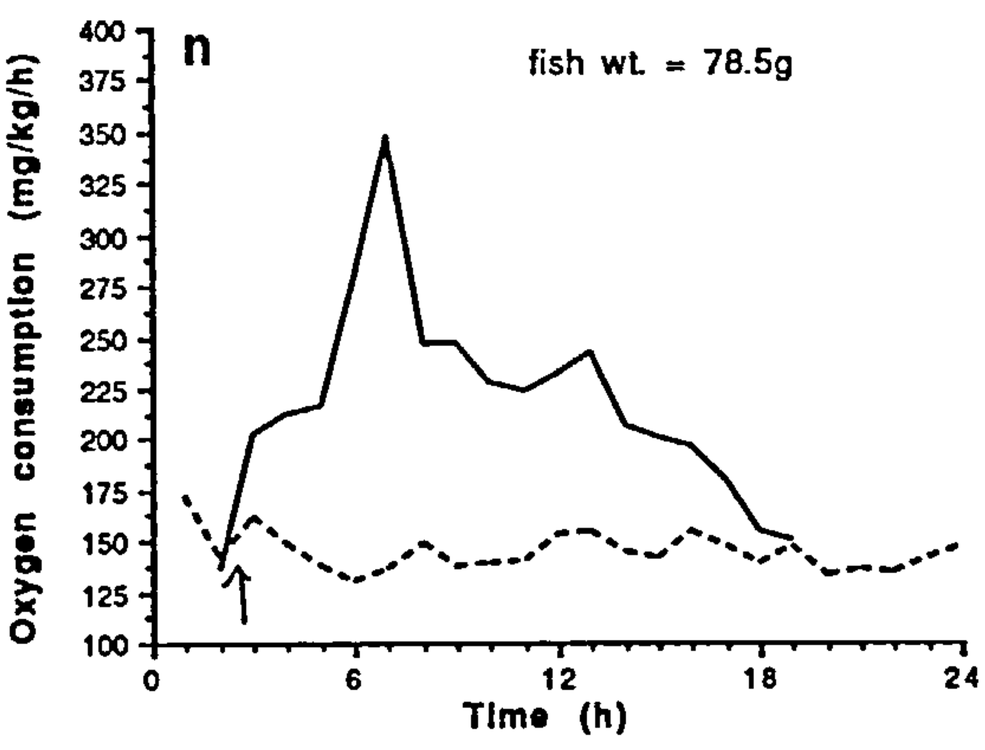
Fig. 4.6 (a - y). Post-prandial oxygen consumption of Cyprinus carpio subsequent to being fed on 35% protein content diet at (i) 0.40% body wt. ration (a - f); (ii) 0.50% body wt. ration (g - l); (iii) 0.75% body wt. ration (m & n) and (iv) 1.00% body wt. ration (p - y). Arrow indicates time of feeding (10.00 hr in the morning).

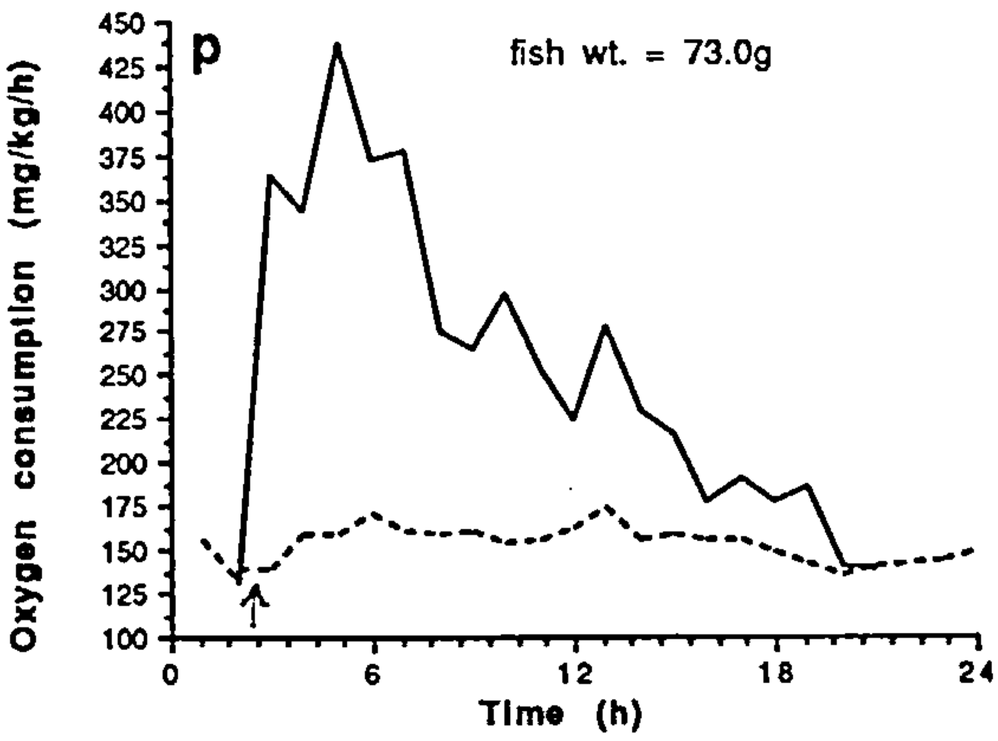

i
fish wt. = 79.5g
Oxygen consumption (mg/kg/h)
Time (h)

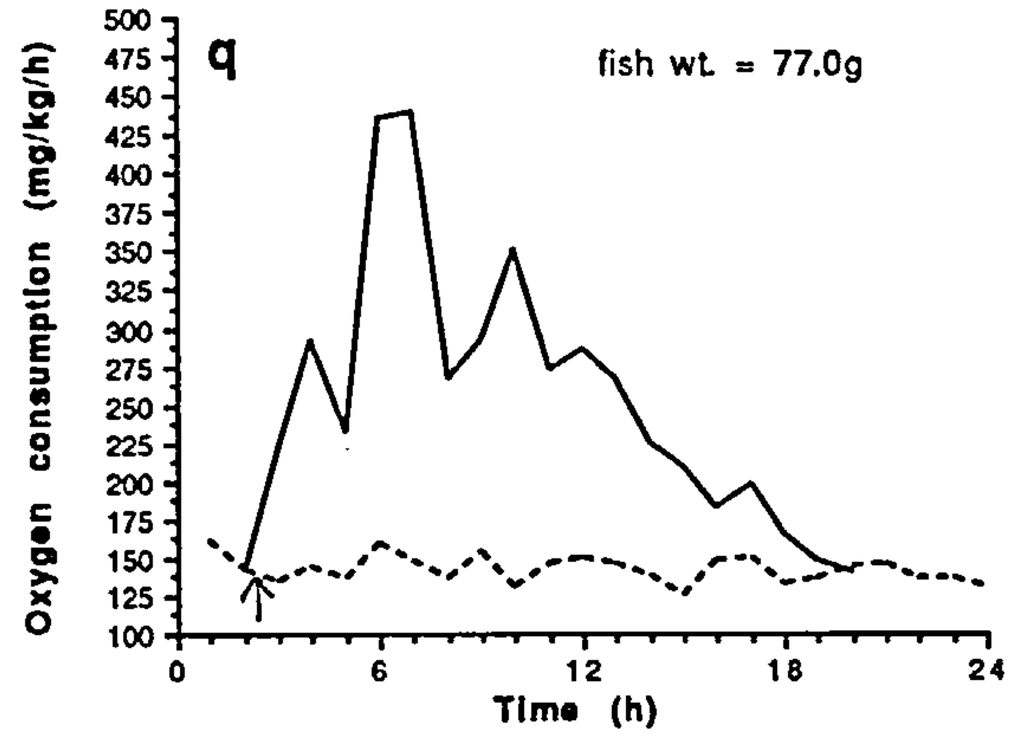

j
fish wt. = 73.0g
Oxygen consumption (mg/kg/h)
Time (h)


k
fish wt. =73.5g
Oxygen consumption (mg/kg/h)
Time (h)

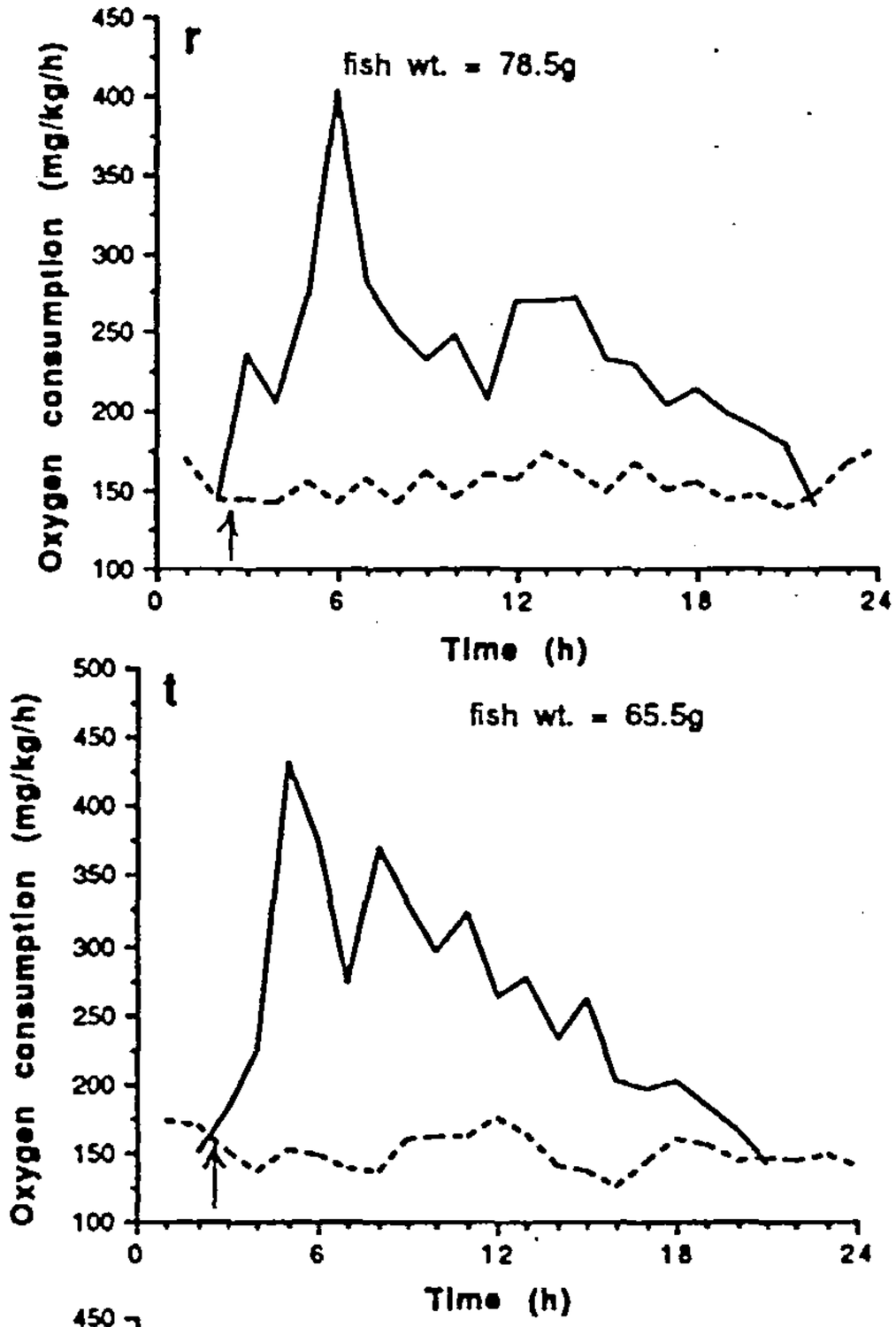

m
fish wt. = 78.5g
Oxygen consumption (mg/kg/h)
Time (h)

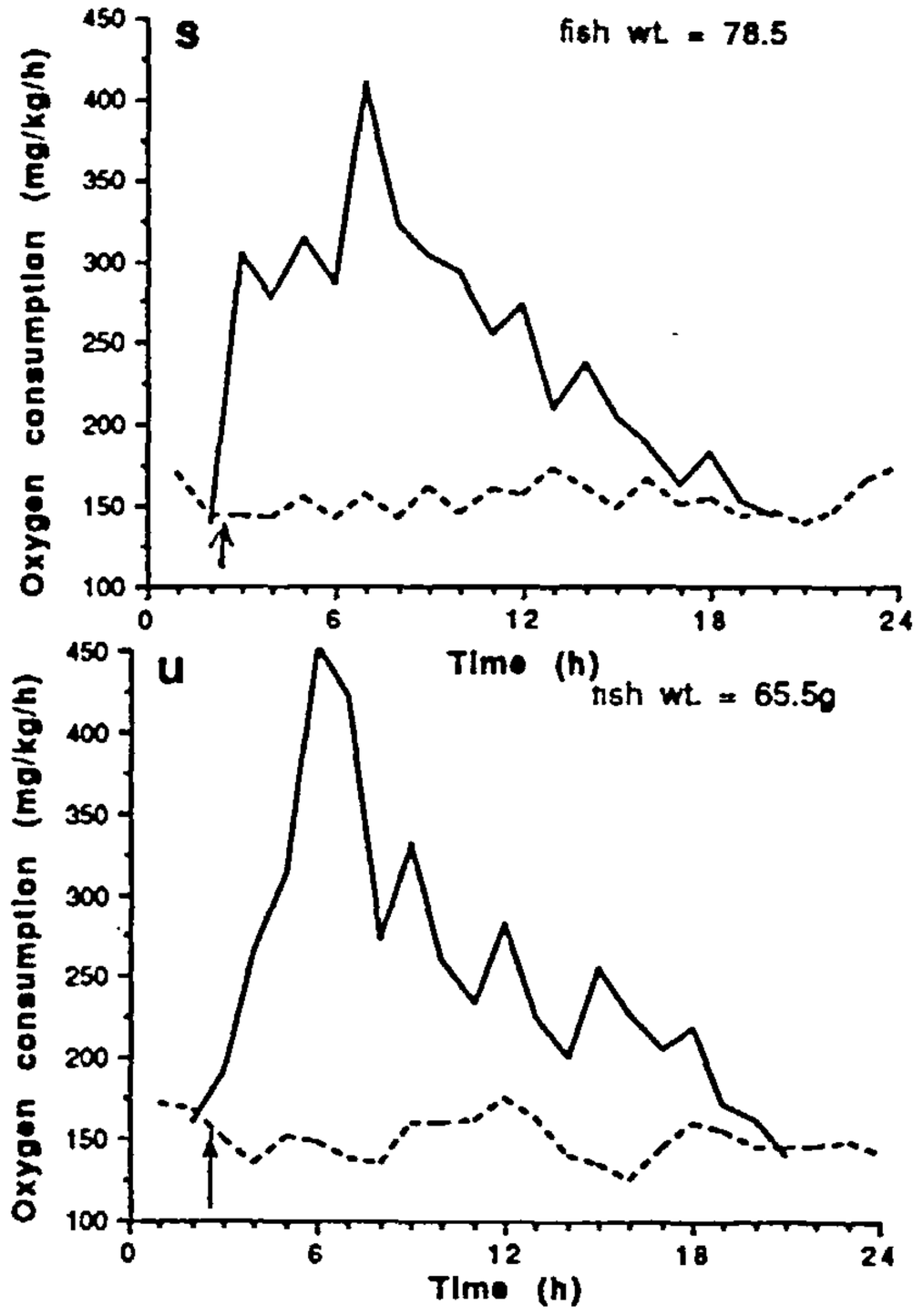

n
fish wt. = 78.5g
Oxygen consumption (mg/kg/h)
Time (h)


p
fish wt. = 73.0g
Oxygen consumption (mg/kg/h)
Time (h)


q
fish wt. = 77.0g
Oxygen consumption (mg/kg/h)
Time (h)

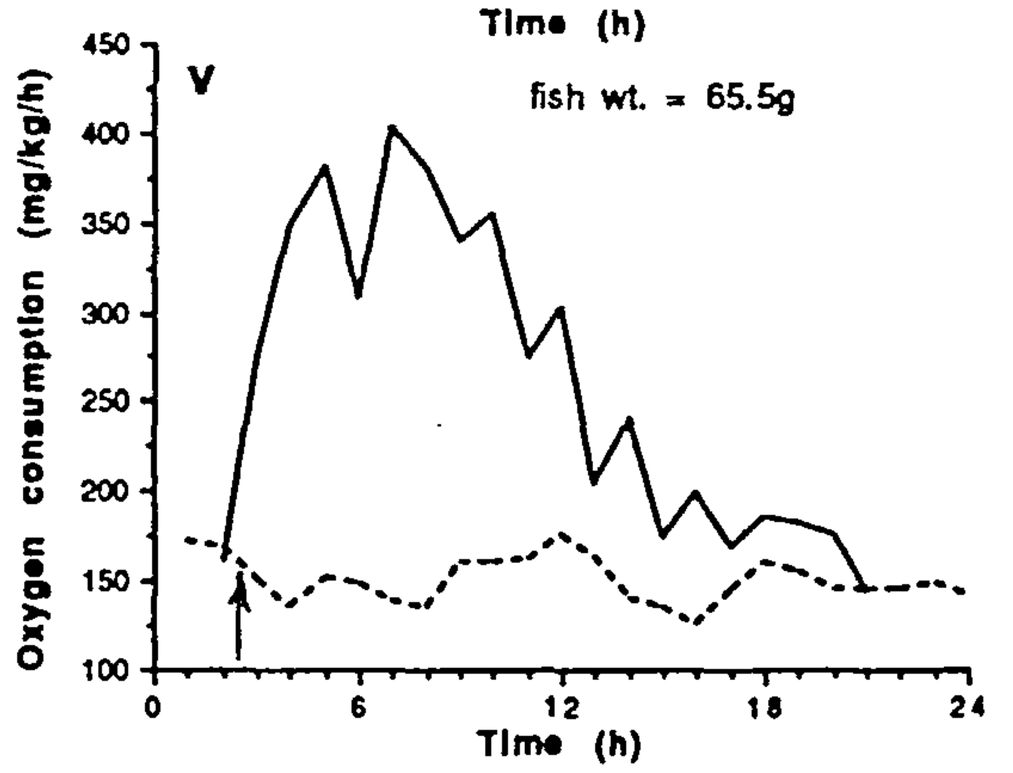
continued...

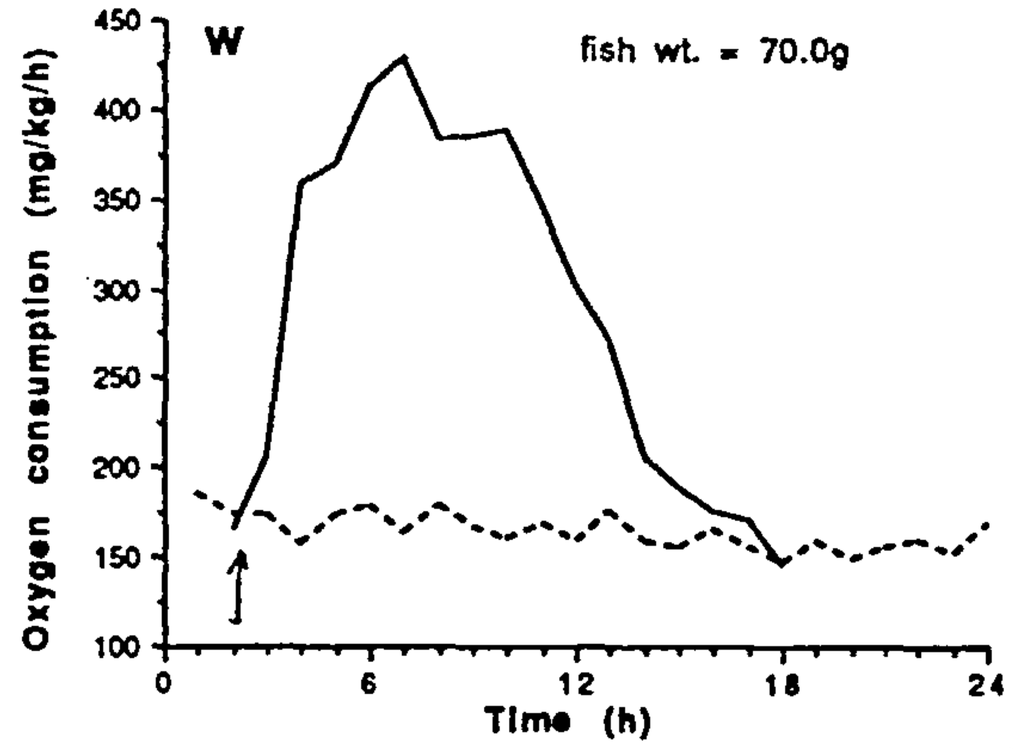

r
fish wt. = 78.5g
Oxygen consumption (mg/kg/h)
Time (h)

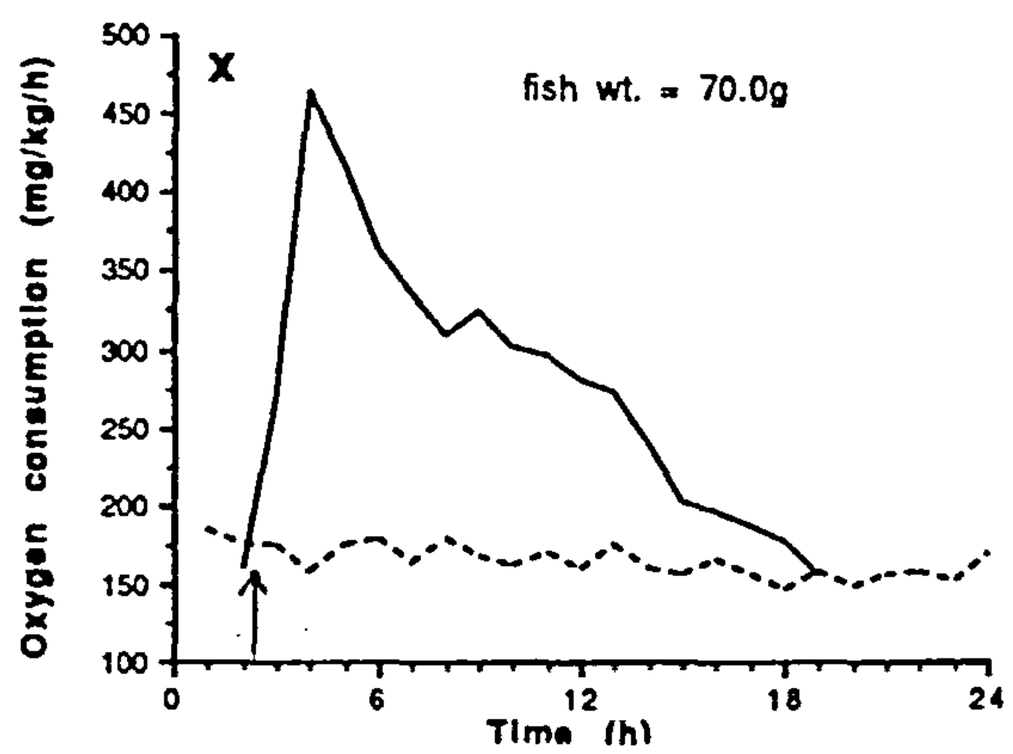

s
fish wt. = 78.5
Oxygen consumption (mg/kg/h)
Time (h)

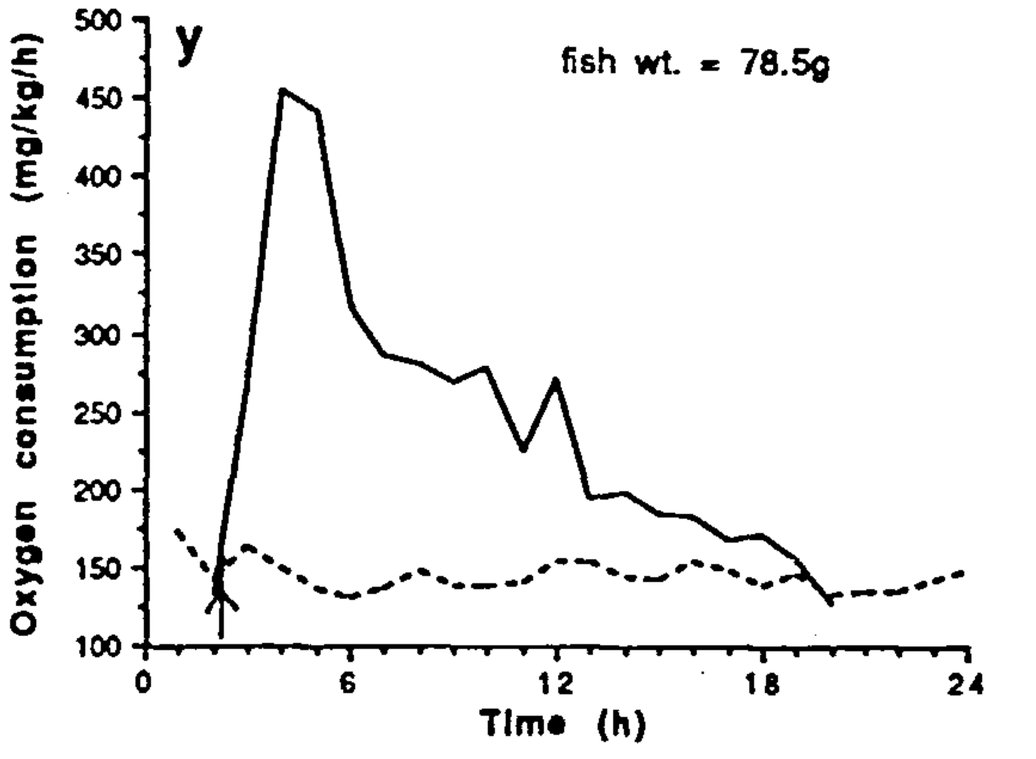
t
fish wt. = 65.5g
Oxygen consumption (mg/kg/h)
Time (h)

u
fish wt. = 65.5g
Oxygen consumption (mg/kg/h)
Time (h)


v
fish wt. = 65.5g
Oxygen consumption (mg/kg/h)
Time (h)


w
fish wt. = 70.0g
Oxygen consumption (mg/kg/h)
Time (h)


x
fish wt. = 70.0g
Oxygen consumption (mg/kg/h)
Time (h)


y
fish wt. = 78.5g
Oxygen consumption (mg/kg/h)
Time (h)

magnitude similarly ranged from 693 mgO_2/kg to 2206 mgO_2/kg (Table 4.3).

The SDA duration ranged from 12 to 19 h and although this was significantly correlated to energy intake ($p < 0.05$; $r = 0.713$), it is probably better considered as an irregular response towards the energy intake (Fig. 4.7b, Table 4.3).

From figure 4.7d it is evident that there exists no significant relationship ($p < 0.05$; corr. coeff. -0.004 between SDA coefficient (ranged from 11.40 to 16.25) and energy intake. The time required to reach the peak value ranged from 2 to 7 hours (Table 4.3) and was not significantly correlated ($p > 0.05$; corr. coeff. 0.182) with energy ingestion (Fig. 4.7e). However, percent increase of oxygen consumption over resting rate (70.20% to 183.76%) showed a significant increase ($p < 0.05$; 0.817) with an increase of energy in the diet (Fig. 4.7f; Table 4.3). No significant ($p > 0.05$; corr. coeff. 0.362) relationship was found between the weight of fish and SDA coefficient with a change of energy intake (Fig. 4.8) with 35% dietary protein.

Figure 4.9(a-y) shows the response of respiratory activity to feeding 50% protein content diet at different ration levels (0.4% to 1.0% body weight). There was some variabilty in response with clear correlation between the

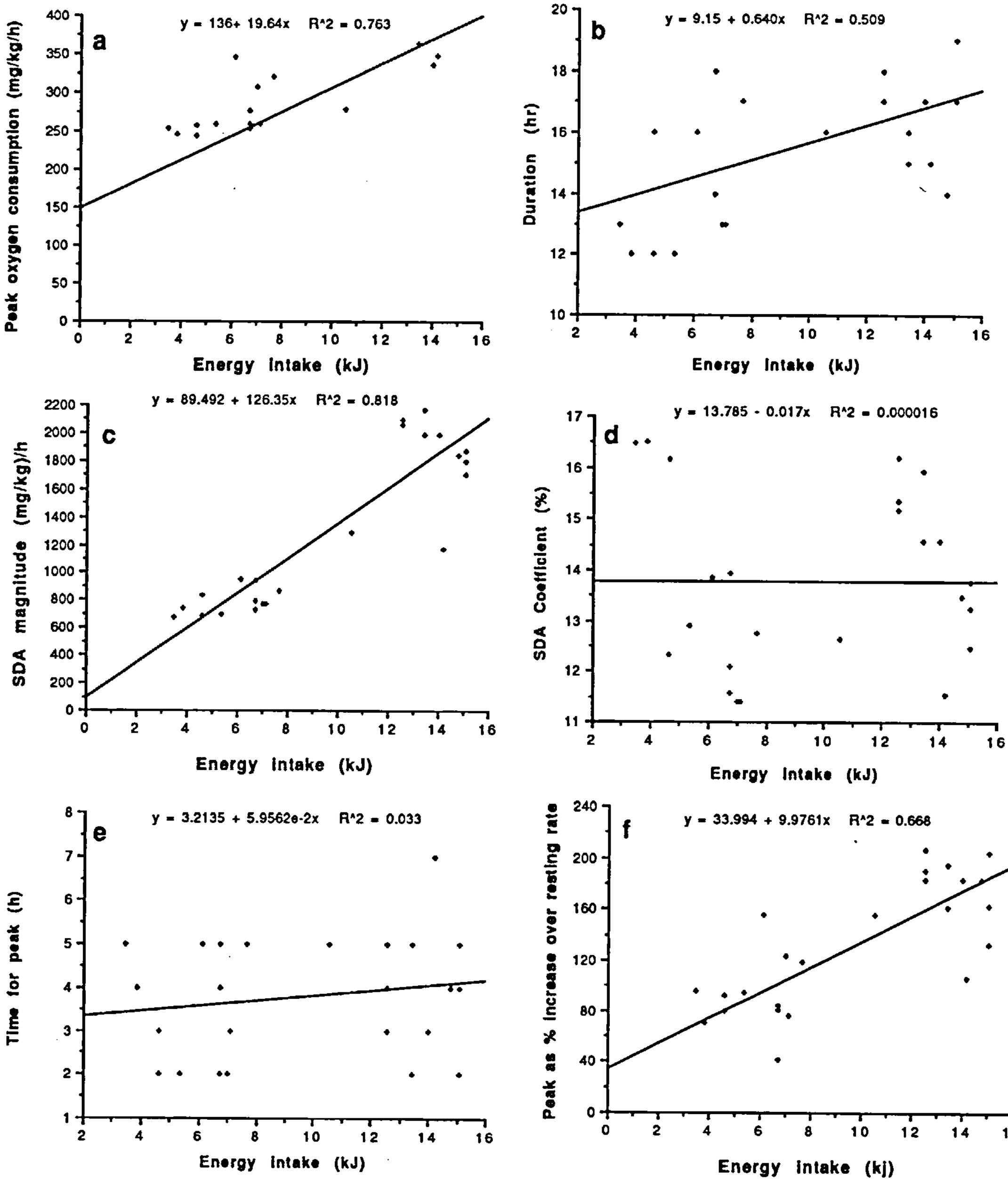


Fig. 4.7 (a - f). Relationship between the aspects of SDA of Cyprinus carpio fed on 35% protein content diet. The regression equation is in the simple form $y = a + bx$. R^2 is the square of correlation coefficient.

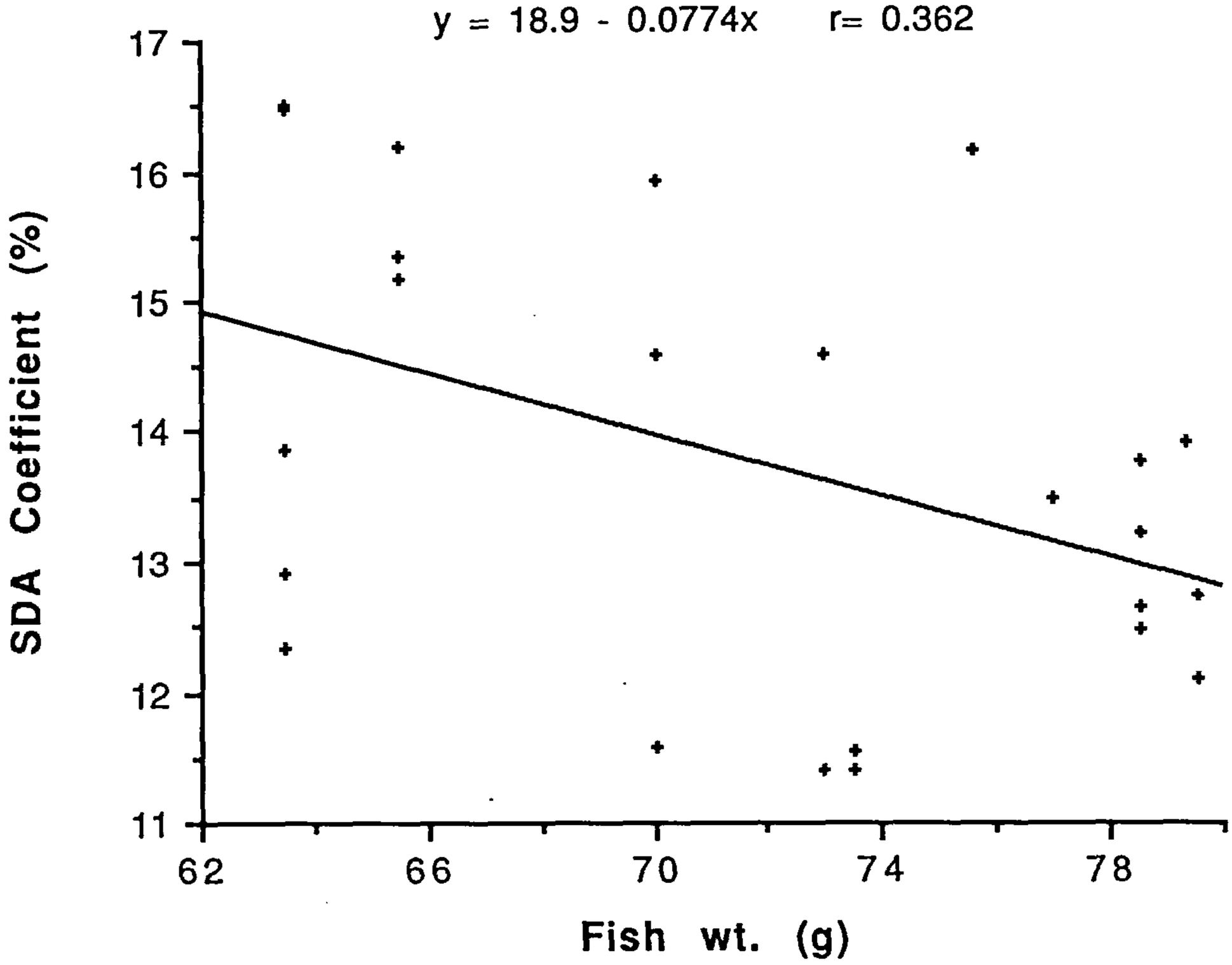


Fig. 4.8 Relationship between the body weight of fish and SDA coefficient in Cyprinus carpio fed with 35% dietary protein at different energy levels, The regression equation is in the form y = a + bx. R^2 is the square of correlation coefficient.

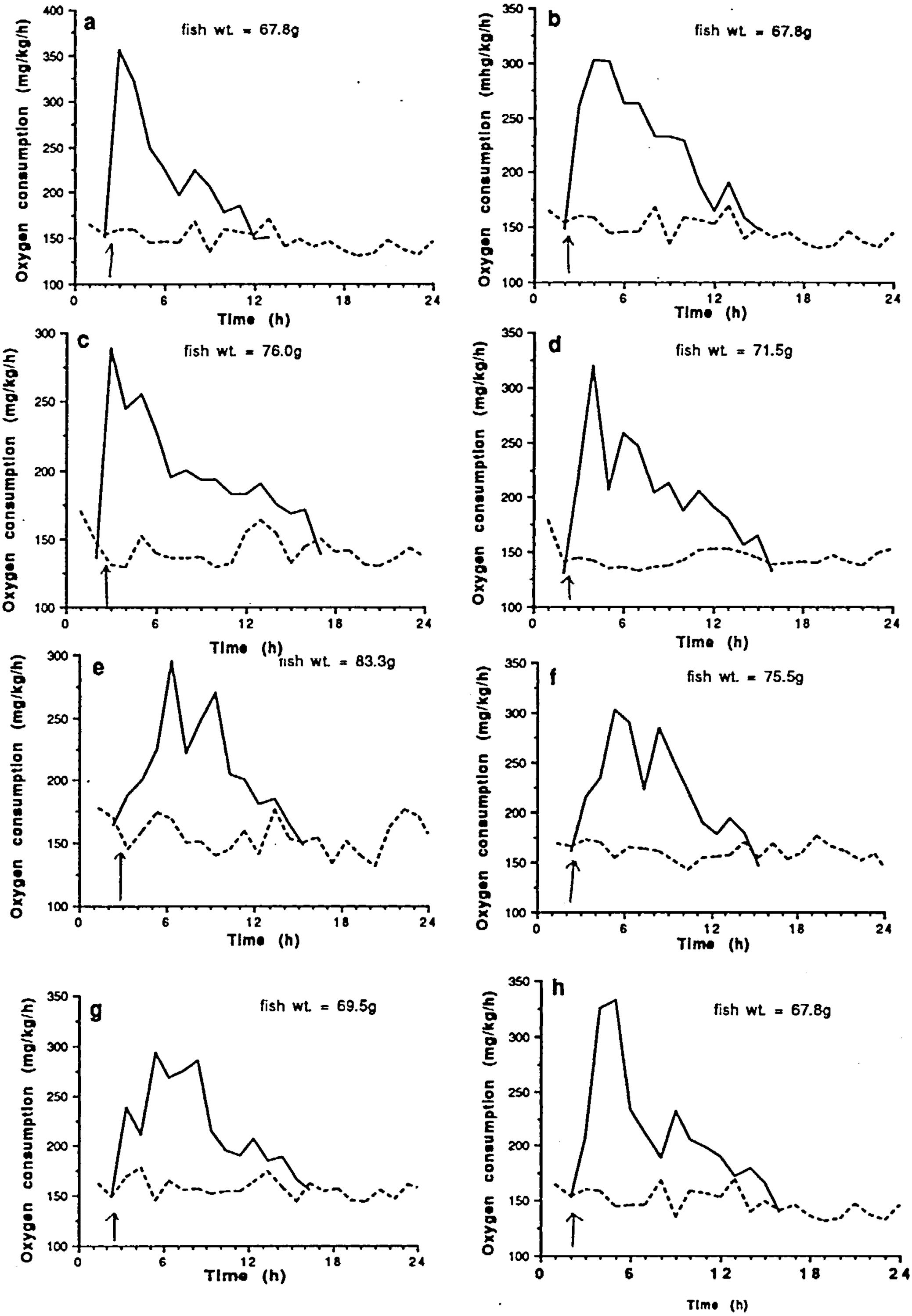
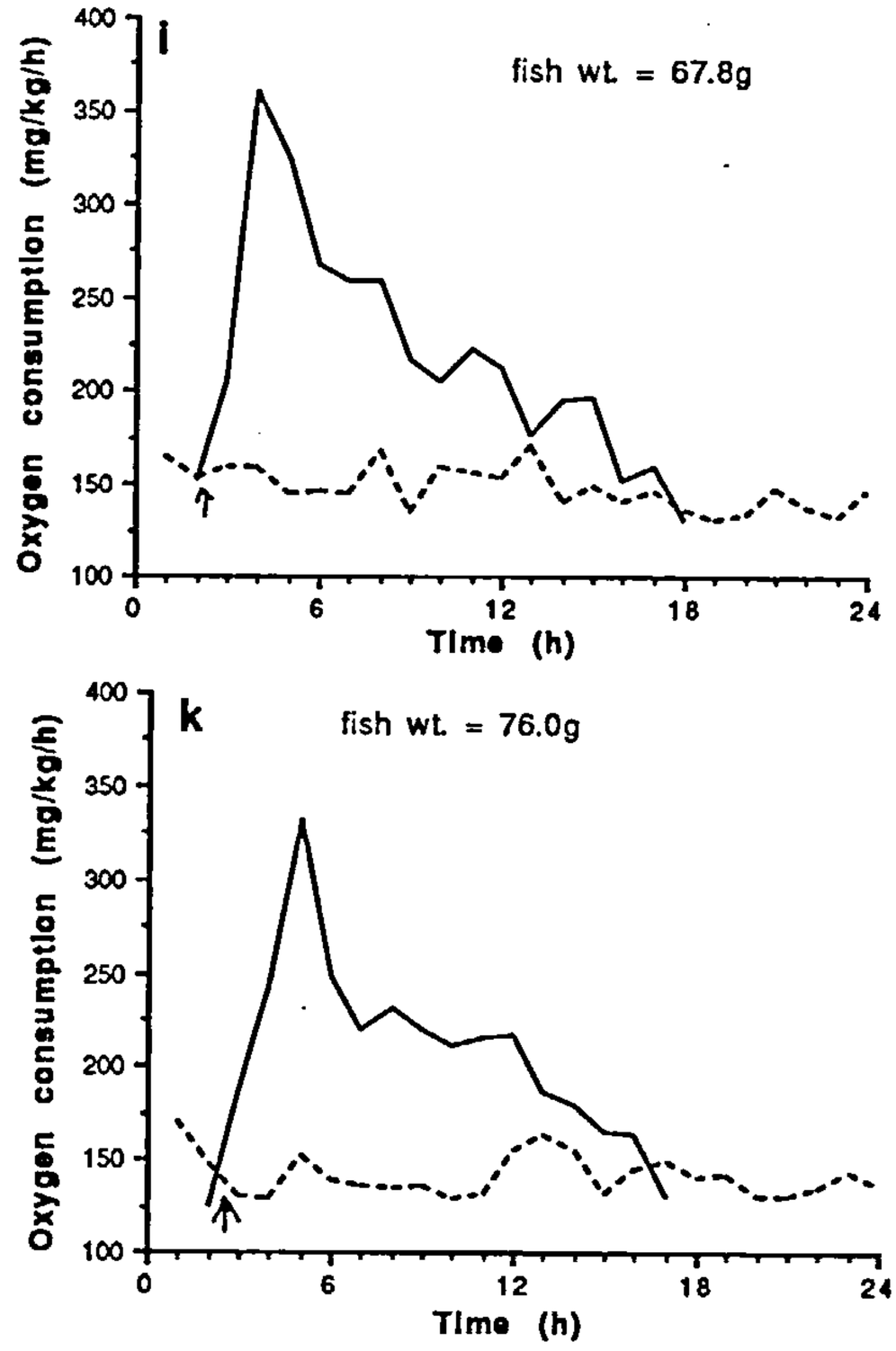
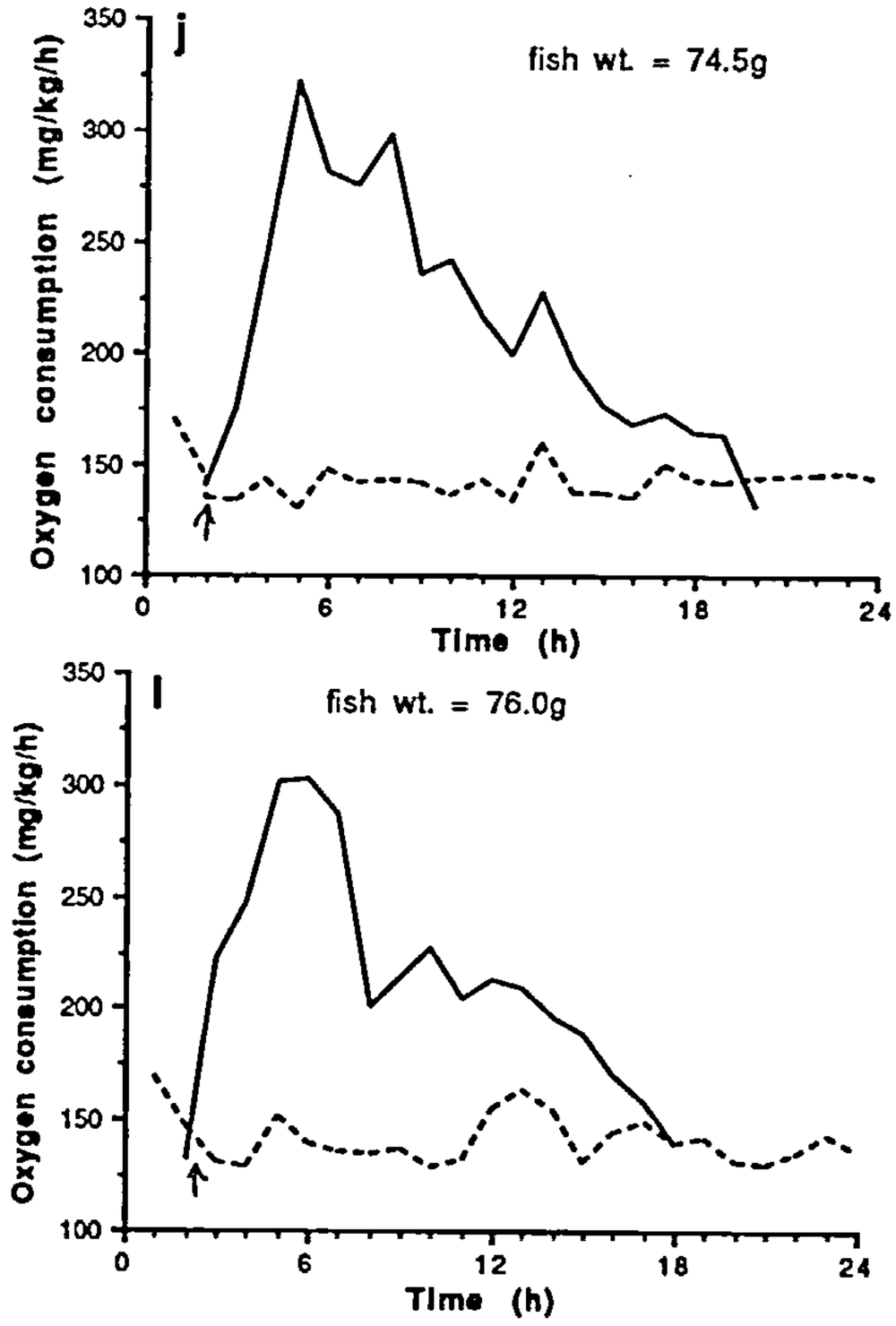


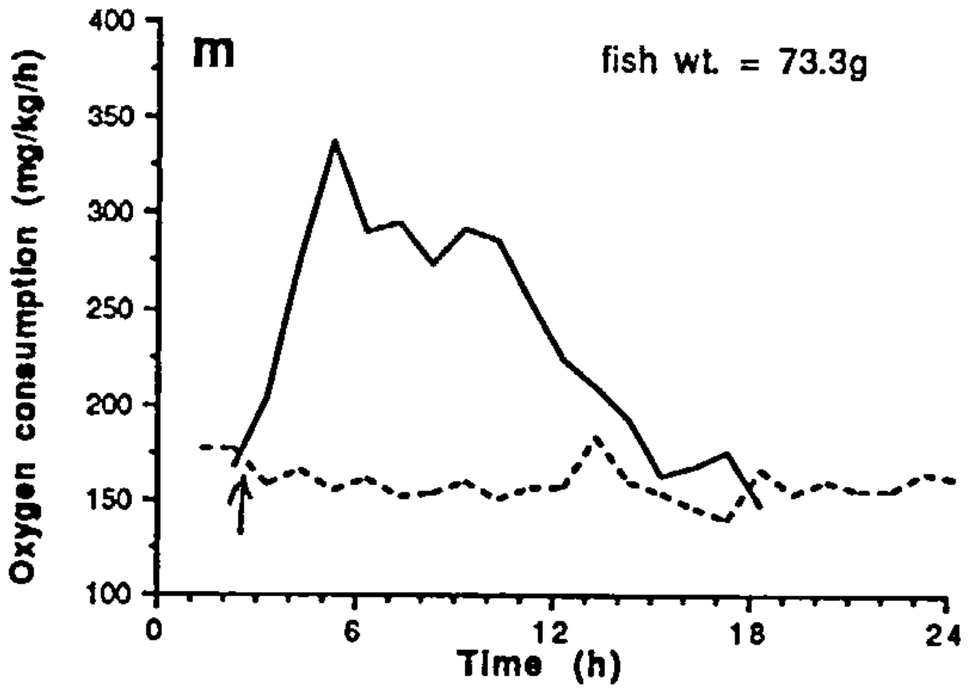
Fig. 4.9 (a - y). Post-prandial oxygen consumption (solid line) against resting rate (dotted line) of Cyprinus carpio subsequent to being fed on 50% protein content diet at (i) 0.40% body wt. ration (a - g); (ii) 0.50% body wt. ration (h - t); (iii) 0.75% body wt. ration (u & v) and (iv) 1.00% body wt. ration (w - y). Arrow indicates time of feeding (10.00 hr in the morning) and the time "0" starts at 8.00hr in the morning.

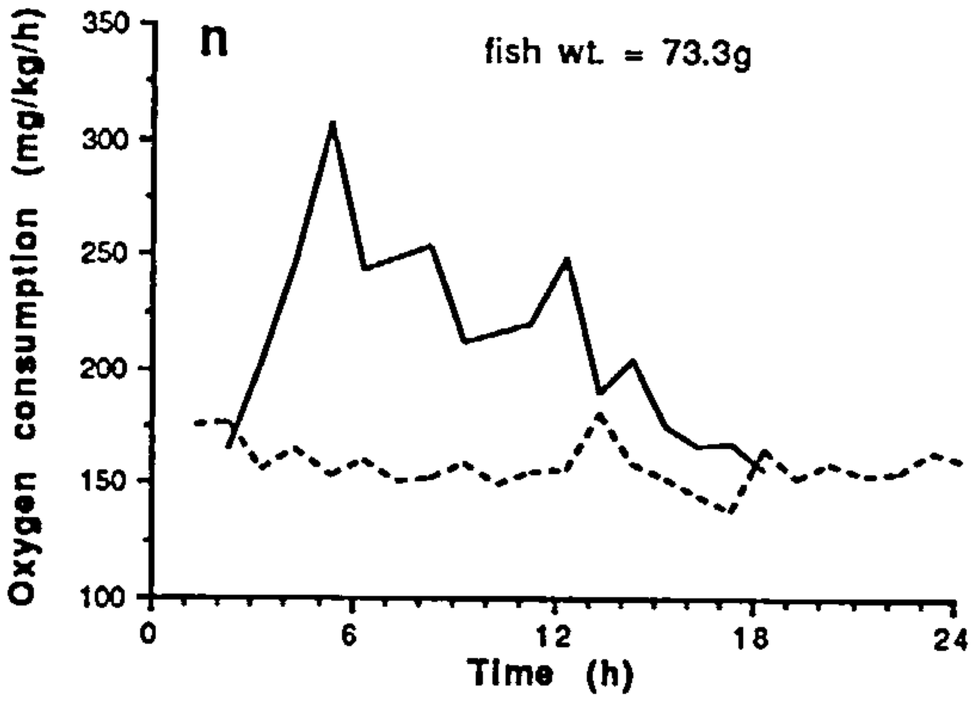

i
fish wt. = 67.8g
Oxygen consumption (mg/kg/h)
Time (h)

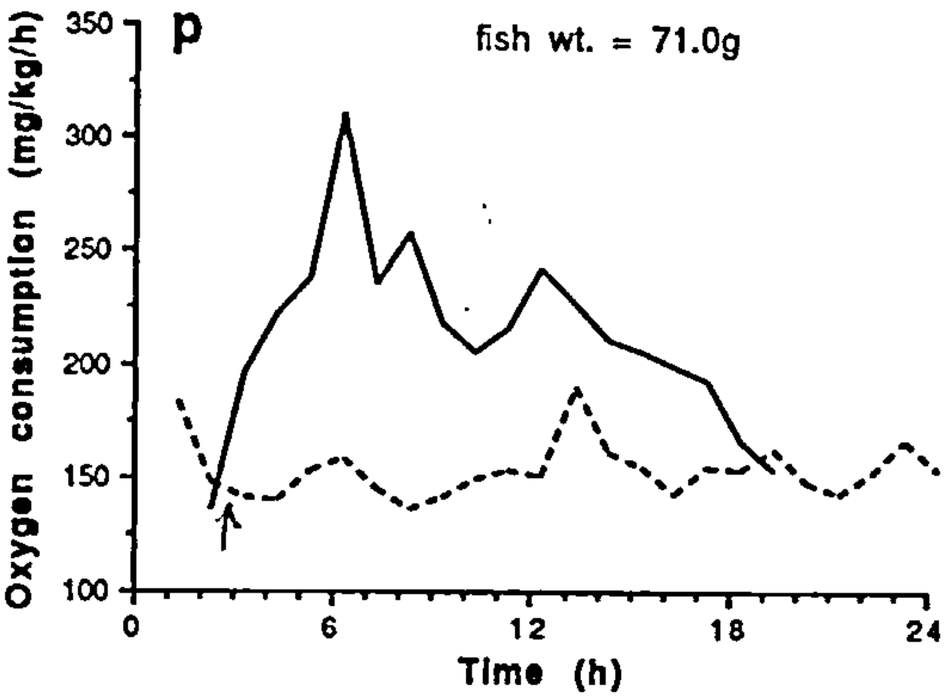

j
fish wt. = 74.5g
Oxygen consumption (mg/kg/h)
Time (h)

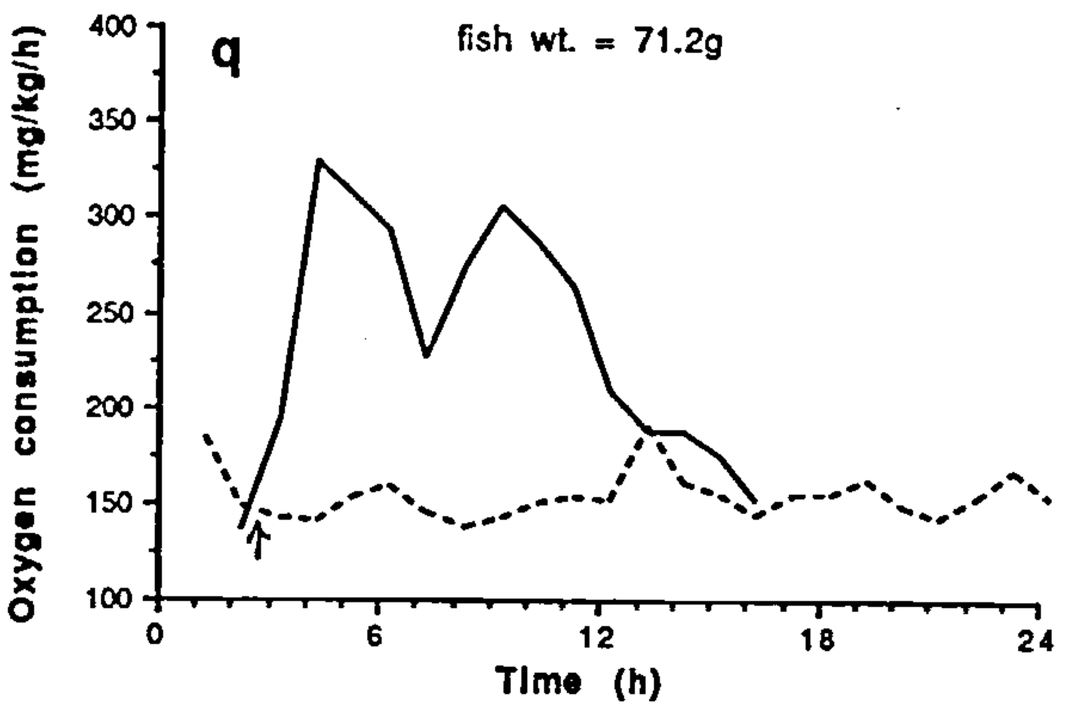
k
fish wt. = 76.0g
Oxygen consumption (mg/kg/h)
Time (h)

l
fish wt. = 76.0g
Oxygen consumption (mg/kg/h)
Time (h)

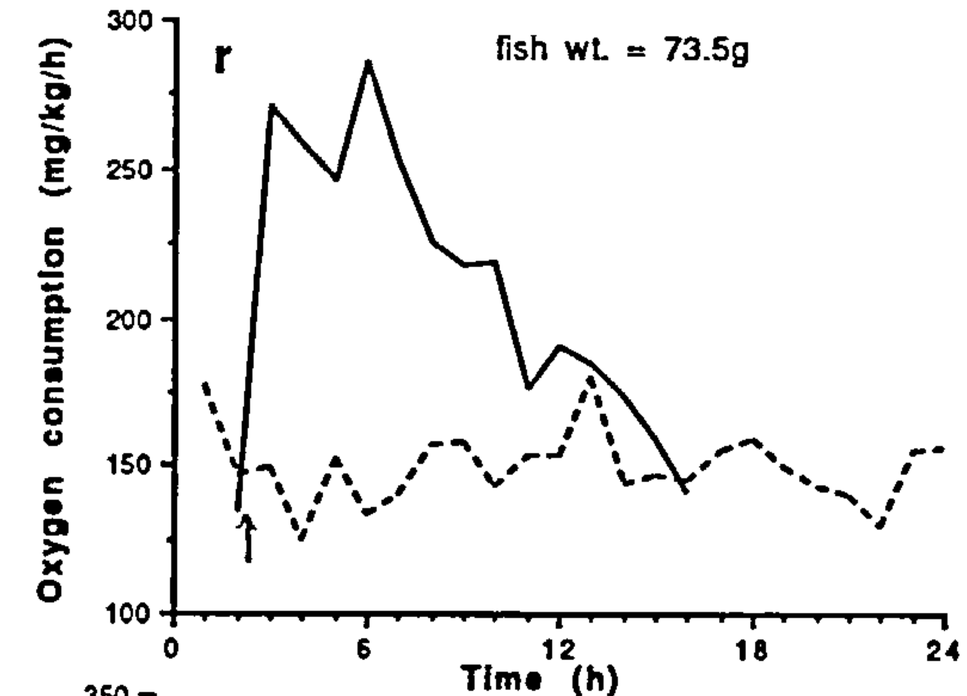

m
fish wt. = 73.3g
Oxygen consumption (mg/kg/h)
Time (h)

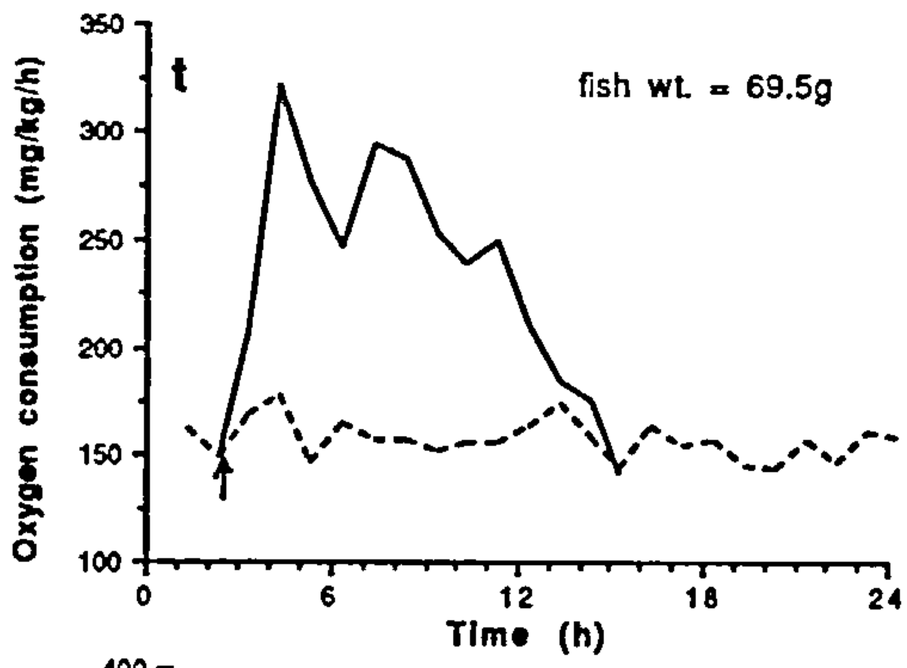

n
fish wt. = 73.3g
Oxygen consumption (mg/kg/h)
Time (h)

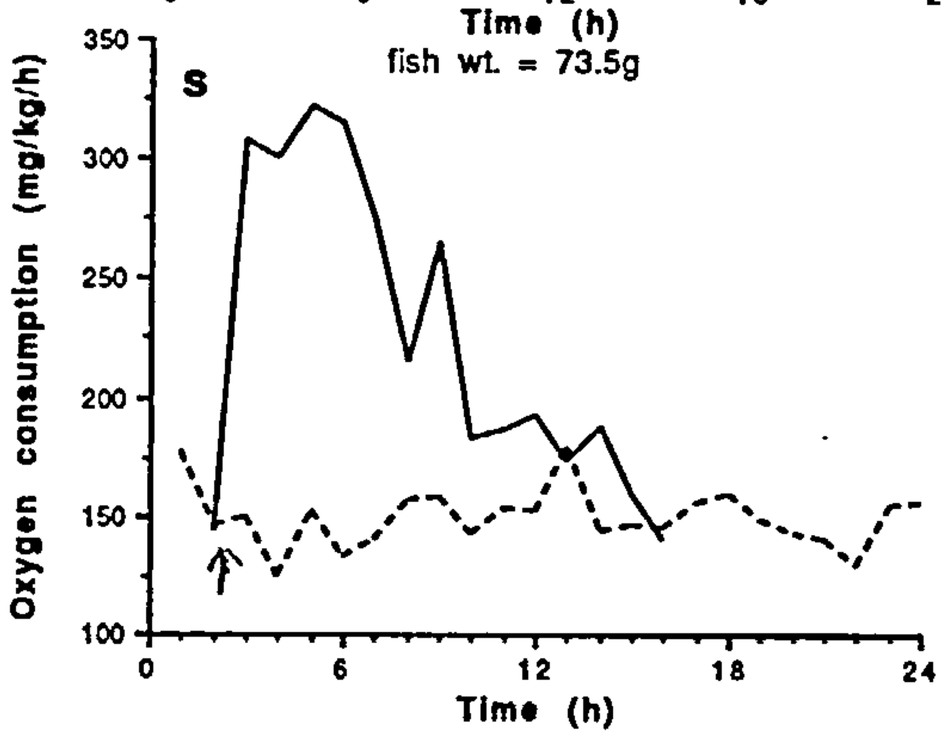

p
fish wt. = 71.0g
Oxygen consumption (mg/kg/h)
Time (h)

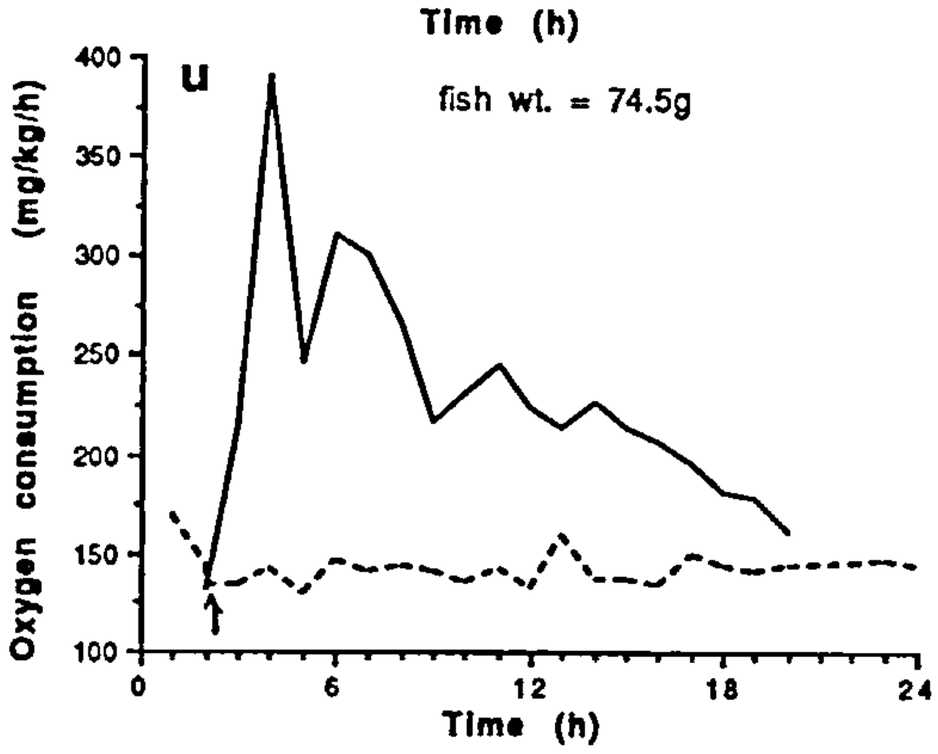

q
fish wt. = 71.2g
Oxygen consumption (mg/kg/h)
Time (h)

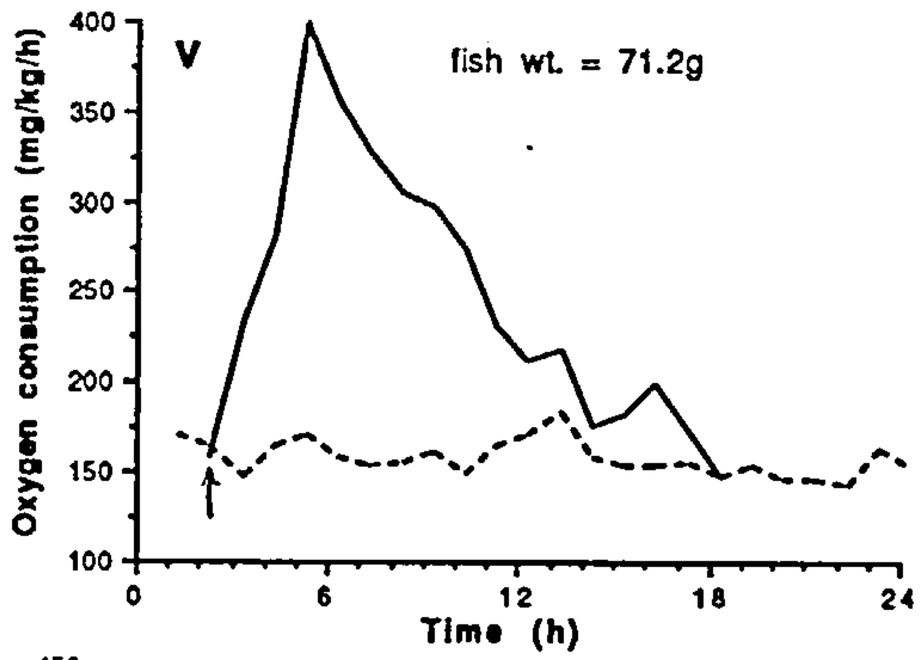
continued...

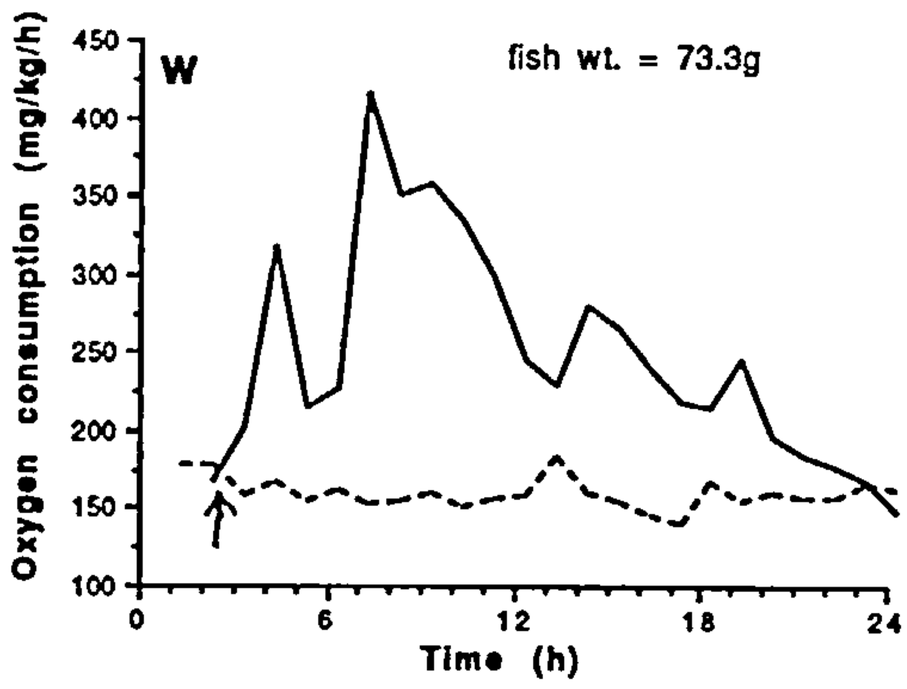

r
fish wt. = 73.5g
Oxygen consumption (mg/kg/h)
Time (h)

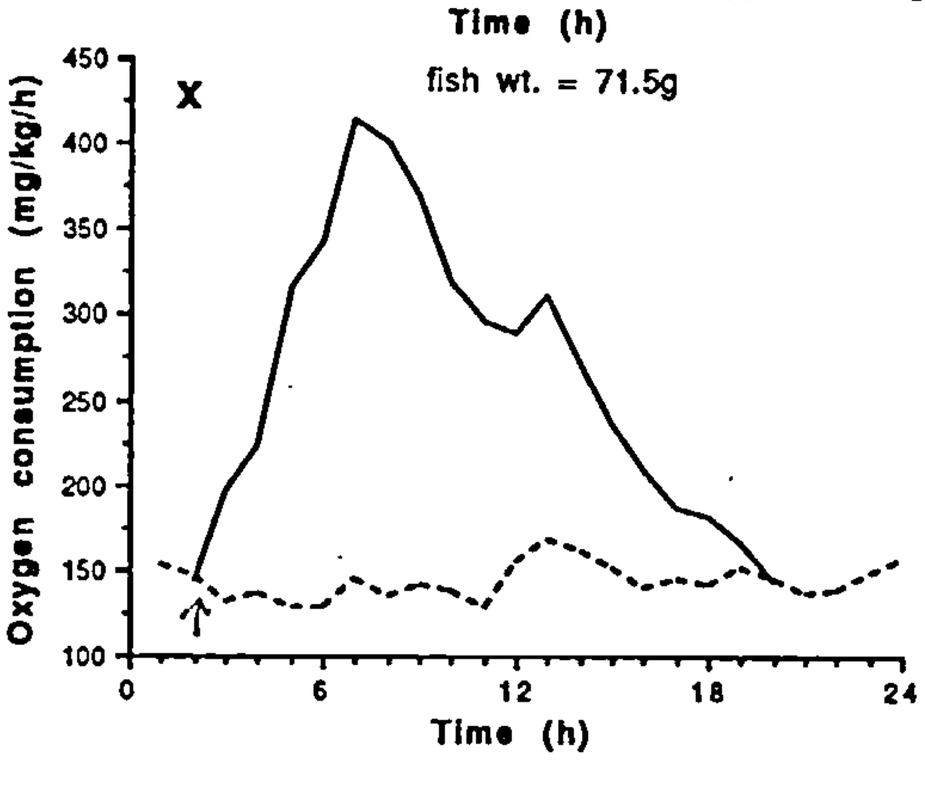

t
fish wt. = 69.5g
Oxygen consumption (mg/kg/h)
Time (h)

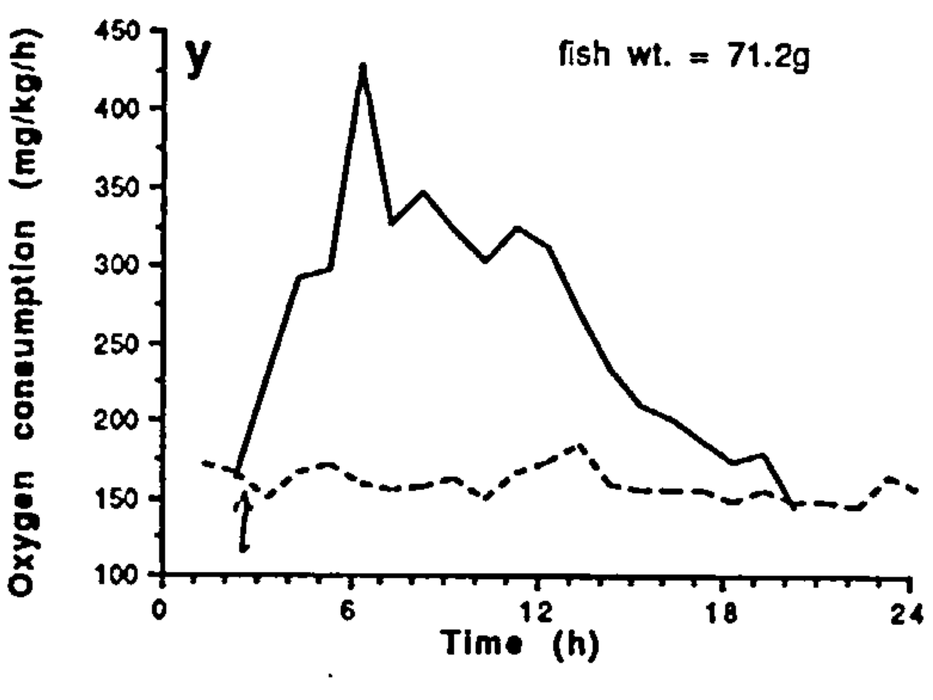

s
fish wt. = 73.5g
Oxygen consumption (mg/kg/h)
Time (h)


u
fish wt. = 74.5g
Oxygen consumption (mg/kg/h)
Time (h)


v
fish wt. = 71.2g
Oxygen consumption (mg/kg/h)
Time (h)


w
fish wt. = 73.3g
Oxygen consumption (mg/kg/h)
Time (h)


x
fish wt. = 71.5g
Oxygen consumption (mg/kg/h)
Time (h)


y
fish wt. = 71.2g
Oxygen consumption (mg/kg/h)
Time (h)

ration levels and the consequent SDA magnitude, duration and peak of the response. The principal SDA features with 50% dietary proteins at different ration levels are reported in Table 4.4.

As with the 20% and 35% protein diets the maximum (peak) oxygen consumption, SDA duration and SDA magnitude were significantly positively correlated ($r = 0.776$, 0.822 and 0.847 respectively, $p < 0.05$) with an increase of energy intake (Fig. 4.10a, b, c respectively). The peak oxygen consumption was found to be 288 mg/kg/h with a 0.4% body weight ration, increasing to 420 mg/kg/h with a 1.0% ration. The duration of SDA ranged from 10 to 21 hours and SDA magnitude ranged from 866 mgO_2/kg (at 0.4% ration level) to 2300 mgO_2/kg/h (at 1.0% ration level) (Table 4.4). No significant relationship ($p > 0.05$; corr. coeff. - 0.389) was observed between SDA coefficient (12.85 to 19.46%) and energy intake (Fig. 4.10d). The time to reach the peak value with 50% dietary protein ranged from 1 to 5 hours and was not significantly related ($p > 0.5$, corr. coeff. 0.534) to ingested energy (Fig. 4.10e). Once again, however, the percent increase over resting rate with energy intake ranged from 97% to 204% (Table 4.4) and was significantly correlated ($p < 0.05$; corr. coeff. 0.824) with ingested energy. (Fig. 4.10f). There was no significant relationship ($p > 0.05$, $r = 0.130$ and 0.084

Table 4.4 The main features of SDA in common carp, Cyprinus carpio in response to 50% protein diet

Fish wt (g)	Ration (% body weight)	Minimum resting (mgO_2/Kg/h)	Peak (mgO_2/Kg/h)	Peak (% resting rate)	Time to reach Peak(h)	Duration (h)	SDA magnitude mgO_2/fish	Energy intake (kJ)	SDA magnitude (mgO_2/kg)	SDA Coefficient (%)
67.80	0.40	132.11	355.18	137.94	1	10	58.73	5.250	866.22	15.43
67.80	0.40	132.11	302.44	97.26	2	12	65.99	5.250	973.30	17.40
76.00	0.40	128.58	288.24	138.92	1	14	69.08	5.885	908.94	16.40
71.50	0.40	132.22	319.06	124.48	2	13	63.95	5.529	894.40	16.26
83.30	0.40	129.22	290.16	76.28	4	12	59.56	6.450	715.00	12.85
75.50	0.40	120.17	297.34	99.82	3	10	63.85	5.846	845.69	16.24
69.50	0.40	138.00	287.54	80.53	3	13	60.24	5.382	866.76	15.32
67.80	0.50	132.11	331.96	109.01	3	13	66.40	6.563	979.35	14.24
67.80	0.50	132.11	359.48	142.48	2	15	82.90	6.563	1228.14	17.36
74.50	0.50	130.28	321.53	134.51	3	17	101.10	7.211	1357.04	19.46
76.00	0.50	128.58	330.69	118.60	3	14	89.93	7.357	1183.28	16.86
76.00	0.50	128.58	302.44	116.97	4	15	94.19	7.357	1239.34	17.65
73.30	0.50	122.72	329.35	139.23	3	16	95.66	7.095	1305.04	18.41
73.30	0.50	122.72	301.42	104.11	3	15	69.38	7.095	946.52	13.35
71.00	0.50	130.37	304.57	100.22	4	16	82.66	6.872	1164.22	16.58
71.00	0.50	130.37	322.09	131.78	5	13	90.32	6.872	1272.11	18.12
73.50	0.50	125.05	285.36	107.06	4	13	67.47	7.114	917.95	13.16
73.50	0.50	125.05	321.96	111.27	3	13	84.44	7.114	1148.84	16.48
69.50	0.50	138.00	315.97	83.66	2	12	71.16	6.727	1023.88	14.69
74.50	0.75	130.28	389.80	192.37	2	18	126.24	10.817	1694.49	15.77
71.20	0.75	135.67	392.20	139.14	3	15	103.99	10.338	1460.53	13.97
73.30	1.00	122.72	407.81	203.80	5	21	158.91	14.190	2166.57	14.46
71.50	1.00	126.59	413.16	156.36	5	17	164.43	13.842	2299.72	16.38
71.20	1.00	135.67	420.75	177.59	4	17	156.04	13.842	2191.15	15.61

15.94(+1.68)

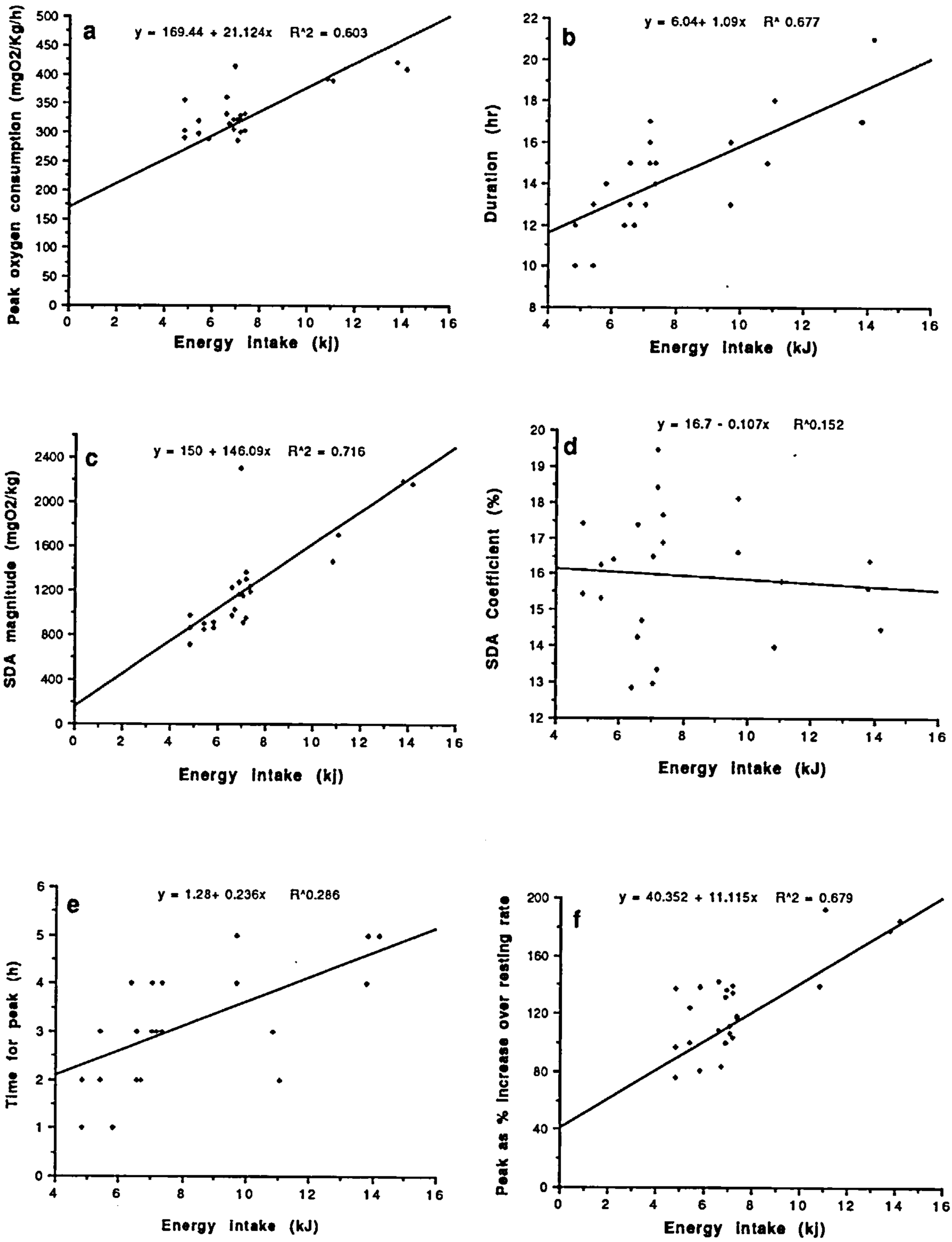


Fig. 4.10 (a - f) Relationship between energy intake from 50% protein diet and (a) peak oxygen consumption, (b) SDA duration, (c) SDA magnitude, (d) SDA coefficient, (e) time to reach peak and (f) peak as % increase over resting rate in Cyprinus carpio. The regression equation is in the form $y = a + bx$. R^2 is the square of correlation coefficient.

respectivly) between the fish weight and SDA coefficient or SDA magnitude (Fig. 4.11).

The lack of a significant relationship between the SDA coefficient and fish weight was probably a result of the use of fish in narrow weight range in all these experiments. No significant relationship ($p > 0.05$) was observed between SDA coefficient and ration level in Cyprinus carpio fed on the three protein-content diets (Fig. 4.12)

Figure 4.13 shows the significant ($p < 0.05$) increase in SDA coefficient with dietary protein content.

Based upon one way-analysis of Variance (ANOVA), a summary of the significant differences ($p < 0.05$) peak values, magnitude and duration between sham feeding and fish fed at different ration levels with 20, 35 and 50% dietary protein is given in Table 4.5. The energy intake values described in previous graphs have been converted from the rations used in different diets. There were significant differences ($p < 0.05$) in peak values observed between all the feeding levels of 0.40%, 0.50%, 0.75% and 1.00% except with 20% and 35% proteins at 0.40%, 0.50% and 0.75% ration level and with 35% and 50% protein at 1.00% ration level. Significant differences ($p < 0.05$) were shown between SDA magnitude in all the treatment groups except

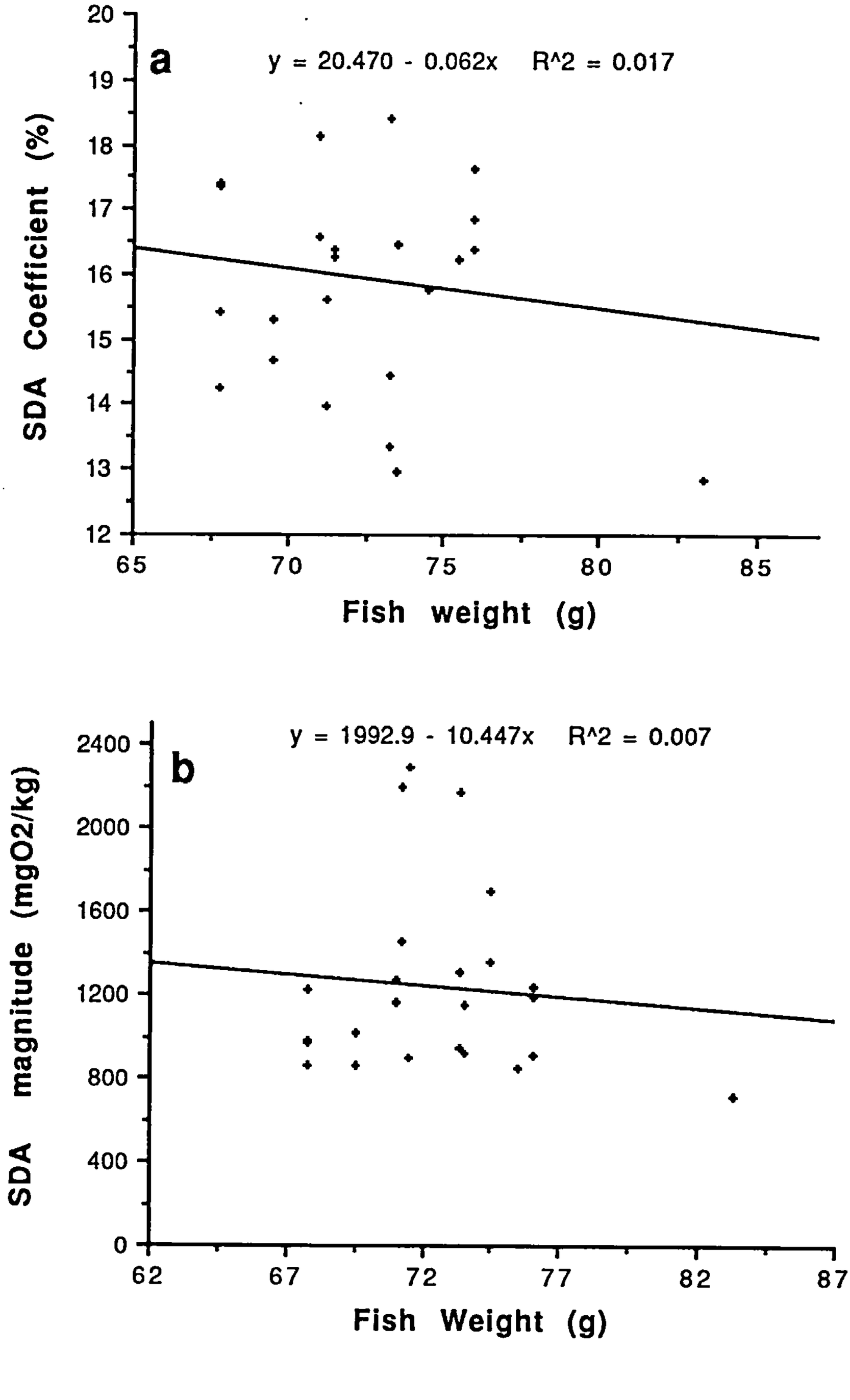


Fig. 4.11 Relationship between the fish weight with (a) SDA coefficient and (b) SDA magnitude in Cyprinus carpio. The regression equation is in the form $y = a + bx$. R^2 is the square of correlation coefficient.

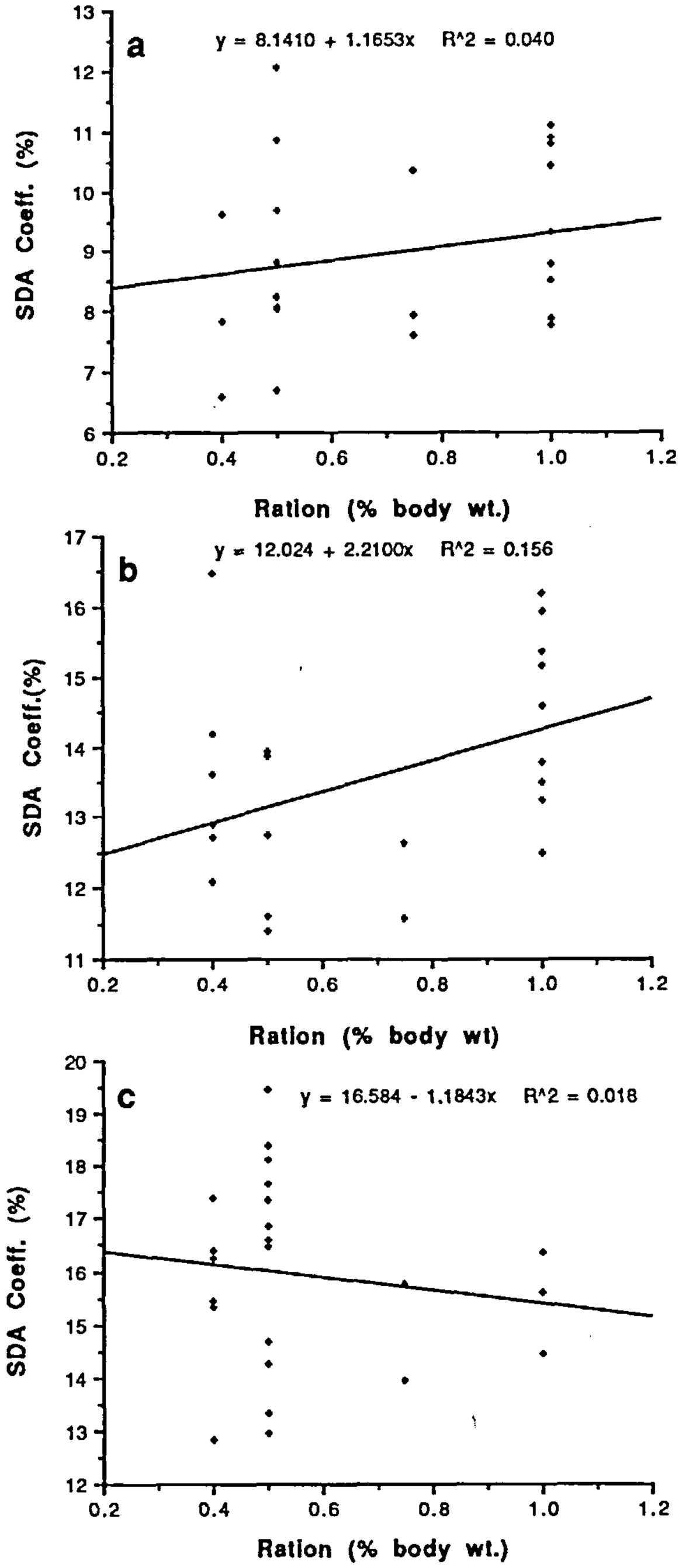


Fig. 4.12 Relationship between SDA coefficient and ration level in Cyprinus carpio fed on (a) 20% dietary protein, (b) 35% dietary protein and (c) 50% dietary protein. Lines fitted with regression as $y = a + bx$. R^2 = square of correlation coefficient.

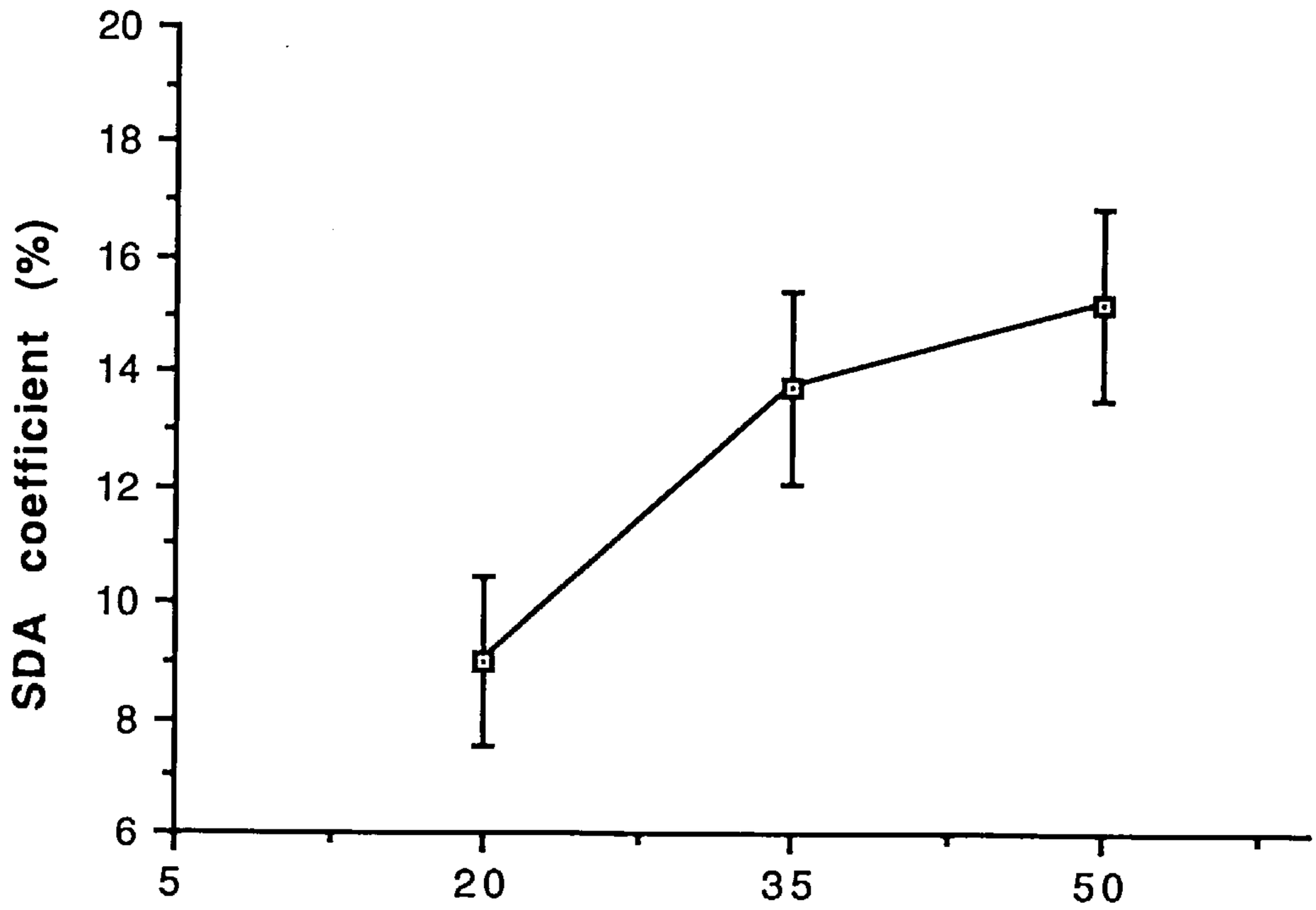


Fig. 4.13 Relationship between SDA coefficient and dietary protein. The values are the arithmetic means with vertical bars for standard deviation, (n = 24).

Table 4.5 Summary of the significant differences in SDA between sham feeding and fish fed at different ration levels with 20, 35 and 50% dietary protein. (n.s = not significant; * = $p < 0.05$).

a) **Peak value**

	Protein	Sham	20	35	50
@ 0.4% ration	20	*	--	n.s	*
	35	*	--	--	*
	50	*	--	--	--
@ 0.5% ration	20	*	--	n.s	*
	35	*	--	--	*
	50	*	--	--	--
@ 0.75% ration	20	*	--	n.s	n.s
	35	*	--	--	n.s
	50	*	--	--	--
@ 1.00% ration	20	*	--	*	*
	35	*	--	--	n.s
	50	*	--	--	--

b) **Magnitude**

@ 0.4% ration	20	*	--	*	*
	35	*	--	--	*
	50	*	--	--	--
@ 0.5% ration	20	*	--	*	*
	35		--	--	*
	50		--	--	--
@ 0.75% ration	20	*	--	*	*
	35	*	--	--	n.s
	50	*	--	--	--
@ 1.00% ration	20	*	--	*	*
	35	*	--	--	*
	50	*	--	--	--

c) **Duration**

@ 0.4% ration	20	*	--	n.s	n.s
	35	*	--	--	n.s
	50	*	--	--	--
@ 0.5% ration	20	*	--	n.s	n.s
	35	*	--	--	n.s
	50	*	--	--	--
@ 0.75% ration	20	*	--	n.s	n.s
	35	*	--	--	n.s
	50	*	--	--	--
@ 1.00% ration	20	*	--	n.s	n.s
	35	*	--	--	n.s
	50	*	--	--	--

with 35% and 50% dietary protein at 0.75% ration level. But except for the sham feeding with other dietary proteins the duration of SDA at different ration levels was not significantly ($p > 0.05$) different.

A summary of the significant differences obtained from one-way analysis of variance (ANOVA) in SDA magnitude between sham feeding and fish fed different dietary protein at ration levels of 0.4, 0.5, 0.75 and 1.0% are shown in Table 4.6. with all diets of dietary protein content the SDA magnitudes were significantly different ($p <0.05$) from one another with the single exception of the 0.50% and 75% rations at 35% protein and all these showed a significant increase of SDA magnitude with an increase of protein in the diet.

The SDA duration showed no significant variation ($P >0.05$) with 0.4 and 0.5% ration level and 0.75 and 1.00% ration level when fish fed with 20% dietary protein (Table 4.6). At 35% protein content diet no significant differences ($p > 0.05$) were seen between ration levels except with sham feeding ($p > 0.05$). Mixed responses were observed with 50% dietary protein, where all the treatments showed significant differences ($P < 0.05$) except between 0.5 and 0.75%, and 0.75 and 1.00% ration levels ($p > 0.05$) (Table 4.6).

Table 4.6 Summary of the significant differences in SDA between sham feeding and fish fed with different protein diets at 0.40. 0.50. 0.75 & 1.00% ration levels. (n.s = not significant; * = $p < 0.05$).

	Ration	sham	0.40	0.50	0.75	1.00
a) Magnitude						
@ 20% protein diet	0.40	*	--	*	*	*
	0.50	*	--	--	*	*
	0.75	*	--	--	--	*
	1.00	*	--	--	--	--
@ 35% protein diet	0.40	*	--	*	*	*
	0.50	*	--	--	n.s	*
	0.75	*	--	--	--	*
	1.00	*	--	--	--	--
@ 50% protein diet	0.40	*	--	*	*	*
	0.50	*	--	--	*	*
	0.75	*	--	--	--	*
	1.00	*	--	--	--	--
b) Duration						
@ 20% protein diet	0.40	*	--	n.s	*	*
	0.50	*	--	--	*	*
	0.75	*	--	--	--	n.s
	1.00	*	--	--	--	--
@ 35% protein diet	0.40	*	--	n.s	n.s	n.s
	0.50	*	--	--	n.s	n.s
	0.75	*	--	--	--	n.s
	1.00	*	--	--	--	--
@ 50% protein diet	0.40	*	--	*	*	*
	0.50	*	--	--	n.s	*
	0.75	*	--	--	--	n.s
	1.00	*	--	--	--	--
c) Peak						
@ 20% protein diet	0.40	*	--	n.s	*	*
	0.50	*	--	--	*	*
	0.75	*	--	--	--	*
	1.00	*	--	--	--	--
@ 35% protein diet	0.40	*	--	n.s	n.s	*
	0.50	*	--	--	n.s	*
	0.75	*	--	--	--	*
	1.00	*	--	--	--	--
@ 50% protein diet	0.40	*	--	n.s	*	*
	0.50	*	--	--	*	*
	0.75	*	--	--	--	*
	1.00	*	--	--	--	--

Feeding 0.4% and 0.5% ration diet produced no significant variation (P >0.05) in peak values when fish fed with 20% protein diet (Table 4.6). All other values were, however, significantly different (p <0.05). Similar observations among different treatments were seen with 50% protein diet. The 35% dietary protein the treatments, namely, 0.4 and 0.5%; 0.4 and 0.75%; and 0.5 and 0.75% showed no significant relationship (p > 0.05) but the others showed a significant (p <0.05) relationship among them.

Overall, duration and peak give mixed significant differences and there is no major trend here. However, magnitude is significantly (p < 0.05) different in almost every case and there is a strong trend.

The variations in SDA magnitude with changing protein levels in the diet at different ration levels are shown in Fig. 4.14 (a - d). The magnitude of SDA was found to be directly related with the change of protein in diet and increased with increase of protein content in the diet. The regression equations for these relationships are :

Magnitude = 102.33 + 15.75 protein, for 0.4% ration diet

Magnitude = 152.68 + 19.90 protein, for 0.5% ration diet

Magnitude = 293.55 + 26.00 protein, for 0.75% ration diet

Magnitude = 523.92 + 35.84 protein, for 1.00% ration diet

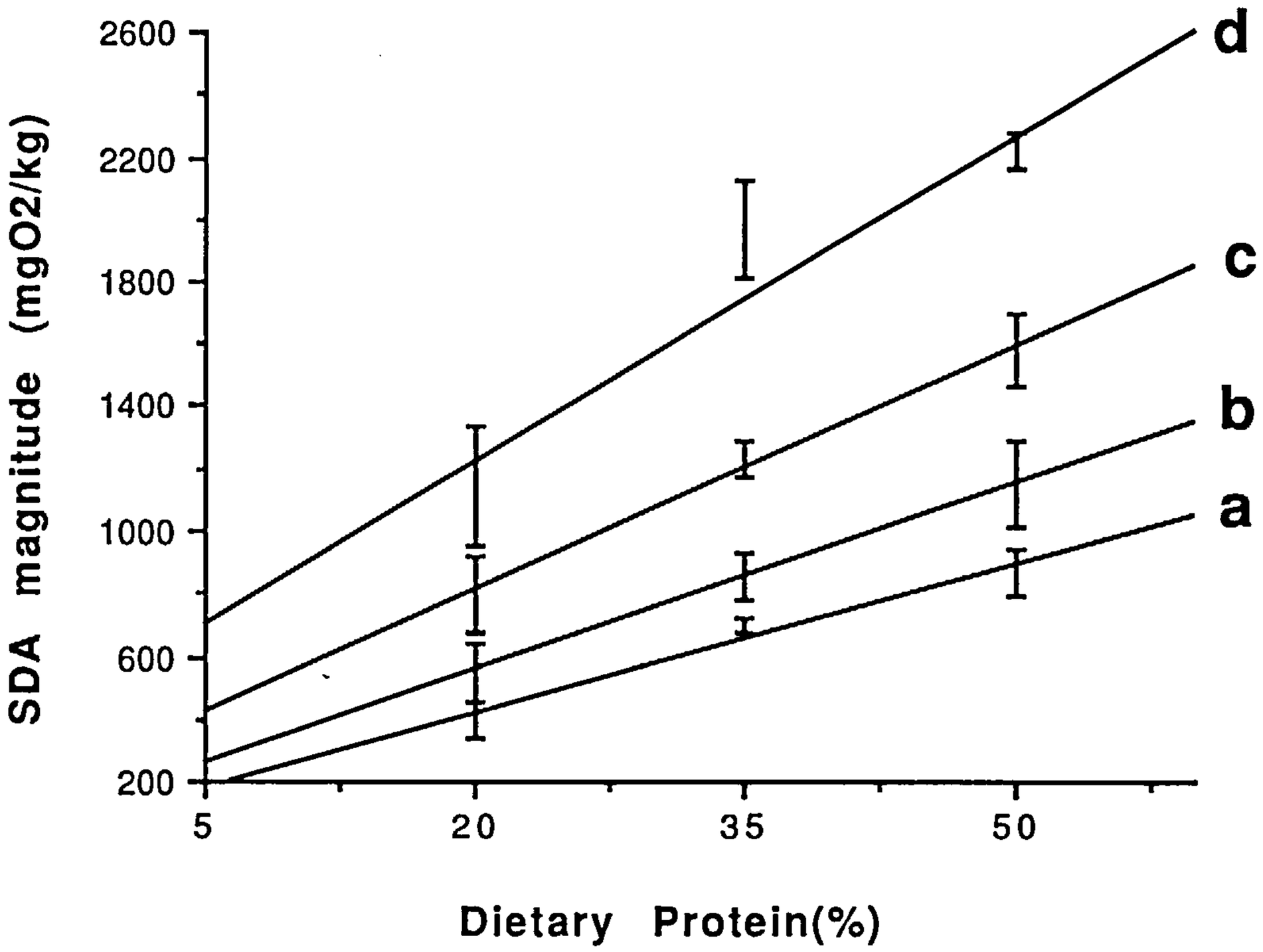


Fig. 4.14 (a - d) Variations in magnitude of SDA in common carp, Cyprinus carpio fed on different protein diets at different ration levels of 0.40%, 0.50, 0.75 and 1.00% in a, b, c, and d respectively. The vertical bars are standard deviation. Line fitted by regression equation (n = 24).

The variation in duration of SDA with changing dietary protein was not significant at any ration levels (Fig. 4.15a - d) and therefore no regressions were calculated.

Variations in peak values with the change of dietary proteins at different ration levels are shown in Figs. 4.16(a - d). It is clear that an increase of protein in the diet results in a corresponding increase in the peak oxygen consumption at different ration level. The relationship between the peak value and different protein diet is given by the regression lines:

Peak = 174.45 + 2.57 protein, for 0.4% ration diet,

Peak = 216.41 + 2.09 protein, for 0.5% ration diet,

Peak = 239.52 + 2.79 protein, for 0.75% ration diet

Peak = 290.79 + 2.79 protein, for 1.00% ration diet

By combining all of the data used to produce the preceding regression equations, the following general regression models were derived to express the relationship of major SDA aspects as follows:

$$\text{Magnitude} = -872 + 24.6\,P + 1725\,R \quad \ldots\ldots\ldots\ldots (1)$$

$$\text{Peak} = 84.0 + 2.73\,P + 215\,R \quad \ldots\ldots\ldots\ldots (2)$$

$$\text{SDA Coeff.} = 5.58 + 0.222\,P - 1.07\,R \quad \ldots\ldots\ldots (3)$$

Where, P = dietary protein (%), R = ration level (%)

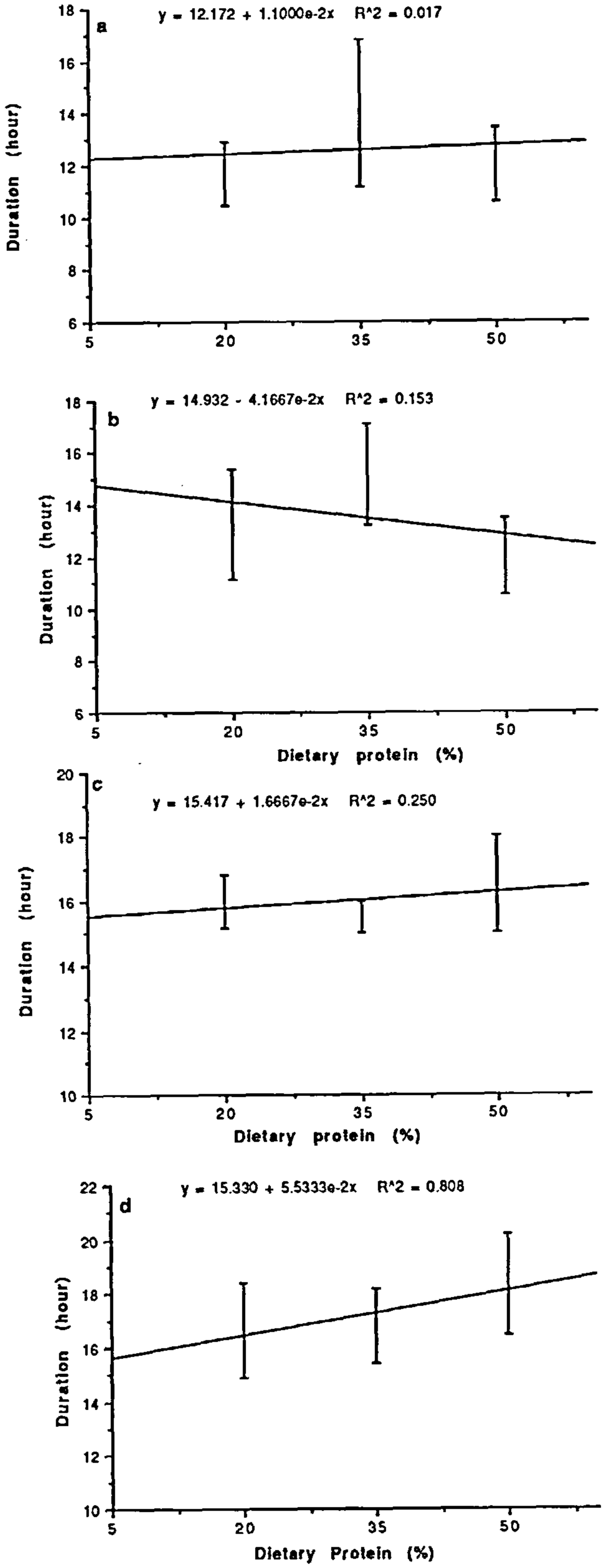


Fig. 4.15 (a - d) Variations in SDA duration in Cyprinus carpio fed on different dietary proteins at different ration levels of 0.40, 0.50, 0.75 and 1.00% in a, b, c, & d respectively. The vertical bars are standard deviation. Line fitted by regression equation (n = 24).

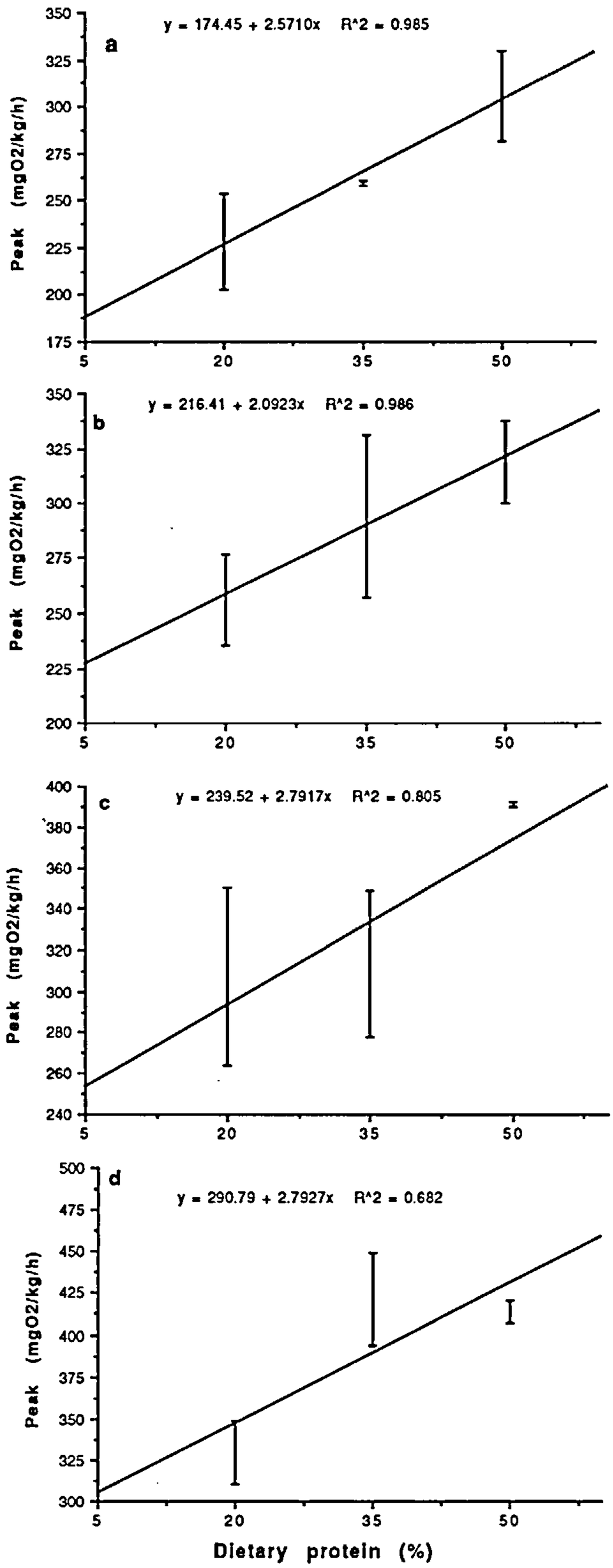


Fig. 4.16 (a - d) Variations in peak oxygen consumption in Cyprinus carpio fed on different dietary proteins at different ration levels of 0.40, 0.50, 0.75 and 1.00% in a, b, c, & d respectively. The vertical bars are standard deviation. Line fitted by regression equation. (n = 24).

The apparent protein digestibility (APD) based on faecal analysis for the marker chromic oxide, was 72.79 (+_1.41), 81.89 (+_0.53) and 83.58 (+_0.54)% for the 20, 35 and 50% protein diets respectively (Table 4.7). There was, thus a significant ($p < 0.05$) increase of apparent protein digestibility with the increase of dietary protein. This may be due to higher carbohydrate and lower protein in 20% protein diet causing rapid passing of material through the alimentary canal, carrying with it some proteins (Tunison, 1943, 1944). Alternatively, reduction of proteolytic enzyme-activity may be caused by carbohydrate in the diets effectively reducing enzyme induction (Falge, et al., 1978).

Table 4.7 Apparent protein digestibility (APD) from different dietary protein(20, 35 and 50% protein) in common carp, Cyprinus carpio

Protein in diet (%)	Protein in faeces (%)	Chromic oxide in diet (%)	Chromic oxide in faeces (%)	APD (%)	APD (%) (mean)
	13.73	0.50	1.21	71.26	
19.74	13.83	0.50	1.30	73.05	72.79
	13.93	0.50	1.36	74.06	
	15.53	0.50	1.26	82.46	
35.14	15.41	0.50	1.18	81.42	81.89
	15.49	0.50	1.21	81.78	
	21.12	0.50	1.29	83.59	
49.88	21.39	0.50	1.35	84.11	83.58
	20.98	0.50	1.24	83.04	

4.4 DISCUSSION

Measurement of oxygen consumption rate attributable to SDA requires determination of pre-feeding and post-feeding metabolic rates in the same apparatus. A large variety of either open (Morooka, 1966; Tamura and Morooka, 1973; Staples and Nomura, 1976) or closed respirometers (Brett, 1964, 1973; Solomon and Brafield, 1972; Ross and Mckinney, 1988) have been used for measurement of SDA in fish. The present study used the computer controlled apparatus described by Ross and Mckinney (1988) for respirometry studies.

Resting metabolism, SDA and costs of locomotor activity in fish are all accommodated within the cost of metabolic capacity (Brett and Groves, 1979; Flowerdew and Groves, 1980; Calow, 1985; Cho and Kaushik, 1985). However, resting rate can not be controlled but the other two can be regulated (Priede, 1985) by using different types of respirometer. Some respirometry systems are designed to account for motor activity (Beamish, 1974) and the fish is set in the chamber to swim at a specific rate against a constant water flow. However, there is the problem that SDA responses will be masked by general oxygen consumption. The system used in the present work was designed to minimize motor activity and therefore the oxygen

consumption response can be considered to be due to SDA assuming that the fish were stress free.

The surroundings of the experimental animal in any respirometry studies should be kept natural as far as possible (Solomon and Brafield, 1972). For example, the measurement of the metabolic rate requires the oxygen concentration in the respiration chamber to be over 50 to 60% of air saturation (Fry, 1957; Dahlberg et al., 1968; Machida, 1981). In this work, the respirometry system had the ability to maintain an air saturation of virtually 100% in the header tank and consequently in the respirometer chambers. The size range of the fish used was optimum for the dimensions of the respiration chamber and the siting of the respirometer in the laboratory, control of photoperiod (12L : 12D) and commencement of every experimental run at about same time of day (6.30 p.m) minimized any potential differences due to metabolic fluctuation (Tandler and Beamish, 1979). The flow-through design of the respirometers also allowed the fish to become habitat - acclimated for sufficient time to overcome any initial handling stress (Caulton, 1978; Ross and Ross, 1983).

Many workers (Caulton, 1978; Solomon and Brafield, 1972; Ross and Ross, 1983) have remarked upon the significance of handling stress to respiration and that this effect may

continue for up to 36 hours, varying with different fishes. Thus, high oxygen uptake rate caused by handling subsided to steady level within 3 to 4 hrs (Machida, 1981), 3 to 5 hrs in Atlantic cod, Gadus morhua (Saunders, 1963), 5 to 9 hrs in roach, bleak and gudgeon (Mann, 1965), 2 to 3 hrs in 10-g jack mackeral, Trachurus japonicus (Azeta and Kimura, 1971), 14 to 18 hrs in largemouth bass (Beamish, 1974)

Common carp is a hardy fish and tolerant of adverse conditions (Jhingran and Pullin, 1985). In this study with common carp the handling stress produced an elevation in oxygen consumption of 56 to 123% of the resting level but this was found to have subsided after 10 to 14 hours in the closed respirometers. A comparatively greater increase in respiratory rate in Oreochromis niloticus (150% to 300%) caused by handling stress at the beginning of experiment was observed by Ross and Ross (1983). In this study, following the stress period, data on resting rate were collected. Some fishes showed intermittent and restless motor activity inside the respirometer chambers from the beginning which continued for a significantly long period. These fishes also refused to eat in the chambers and the data from these animals were ultimately discarded.

It was assumed that the fish used for the study of SDA were at rest before feeding. After 14 to 15 hours in the respirometer chambers data collection for the resting rate

was started and after 2 days, they were allowed to be fed, assuming that the post-feeding respiratory response was exclusively due to SDA response.

The respirometer rig was a complex system where considerable problems could arise due to failure of any one component part. Despite this the system was nearly trouble free during these trials. The only exception was the fixation of six new solenoid valves during the experiments. Regular, thorough, cleaning of the system between runs and back flushing prevented diseases and any detritus on fish scales from blocking the solenoid valves.

Oxygen consumption due to SDA was measured as an increase above the resting rate where the resting rate has been found to have a crepuscular pattern i.e. increases at dawn and dusk. This pattern has been reported for carp and trout by Kausch (1968, 1972). Many authors have depicted the resting rate through the day as a straight line (Hamada and Maeda, 1983) from the time of feeding to the subsequent minimum value. In this study the individual resting curve for each fish was used allowing for variations over the 24 hour period. The increased post feeding oxygen consumption over this curve was used to estimate SDA magnitude.

The data obtained in this study in some cases showed considerable variation or unexpected values. It was considered that this was due to excitement or possibly due to some individual residual motor activity in the respirometer chambers.

In the sham fed fish there was no significant increase in oxygen consumption with resting rate due to the feeding procedure (Fig. 4.2) but after feeding oxygen uptake increased and then gradually declined back to its resting level (Figs. 4.3, 4.6 and 4.9). Similar observations of increased post-prandial metabolic rate in fish have been made by many authors (Krayukhin, 1962, Chiba, 1965 in carp; Mann, 1968 in trout; Kausch, 1969 in carp; Muir and Niimi, 1972, in aholehole; Beamish, 1974, in large mouth bass; Schalles and Wissing, 1976, in bluegill; Caulton, 1978, in tilapia; Smith et al., 1978b, in salmonids; Vahl and Davenport, 1979, in blennies; Brett and Groves, 1979; Jobling and Davies, 1980, in plaice; Tandler and Beamish, 1979, 1980 and 1981 in largemouth bass; Cho, 1982 in rainbow trout; Hamada and Maeda, 1983, in carp; Medland and Beamish, 1985 in rainbow trout; Le Grow and Beamish, 1986 in rainbow trout; Ross et al. unpublished data, on tilapia and so on).

The question which has intrigued investigators for many years is why heat production should increase after food. To

account for SDA, several general theories have been postulated, beginning with those of Voit (1901), Zuntz and otners (1901) in the nineteenth century. Of these, one of the oldest which is still valid is that of Rubner (1902). He showed that SDA was due to the waste heat produced by reactions necessary to support the physiological processes of the body. This statement is sufficiently general and can accommodate a wide variety of modifications.

Mitchell (1962) considered various theories and their subsequent modifications and concluded that attempts to determine the cause of SDA have been largely unsuccessful and ephemeral because they have assumed or implied that there is only one cause that dominates others. In fact, the causes are many, some undoubted real while others are highly speculative in nature.

The feeding related increase of metabolic rate is caused for a number of reasons which were summarised by Bondi (1987):

(1) heat arises from the work of digestion, i.e. from mastication of the food and its propulsion through the digestive tract, (2) the work of nutrient metabolism, where energy is liberated by oxidative reactions occurring inside the tissue is never fully utilized to the benefit of the animal, and a part of it is lost as heat as a result of incomplete transfer of energy, (3) heat production also

results from increased muscular activity due to metabolism of nutrients.

Blaxter (1989) discussed the heat arising in general from the absorption of nutrients. He noted that the amino acids and small peptides, which are the product of digestion of protein, are absorbed by active transport processes involving carrier proteins. On absorption, polypeptides are hydrolysed by dipeptidases present within the epithelial cells of the gut mucosa and these increase markedly in concentration as the amount of protein supplied by the diet is increased.

Similar active transport processes coupled to an energy demanding sodium ion movement apply to carbohydrate absorption. Glucose from the breakdown of starch materials is absorbed by both active transport and diffusion processes. The heat production which accompanies active absorption arises from the ATP - dependant sodium transport component (Smith and Ellory, 1970; Buclon, 1974); it is the enthalpy change associated with oxidation which leads to ATP formation - the ultimate source of heat. There is a direct proportionality between sodium movement in one direction and amino acid or sugar in the other. Lipid digestion involves partial hydrolysis to fatty acids, monoglycerides and bile salts with the hydrophobic, fat-soluble components in the interior which are absorbed passively in the cells.

Protein are specifically synthesized for the purpose of lipid transport from the mucosa; it is not simply a non-specifically adsorbed protein. Again an energy cost is involved in the synthesis of triglyceride from the mono-glyceride and longer chain fatty acids. Fatty acids with a carbon chain-length of less than 14 are absorbed actively by a sodium-dependent process (Blaxter, 1989).

Thus, quite apart from the energy needed to synthesize the digestive enzymes, to enable the entero-hepatic circulation of the bile, and to synthesize and secrete the gastrointes-tinal hormones, the processes of digestion and absorption impose additional costs. These are broadly proportional to the amount of food ingested and thus contribute to the heat increment per unit of food. In addition, oxygen must be supplied for the oxidation of the absorbed nutrients.

Some authors prefer to use the term "apparent SDA". The view is that SDA is considered to represent the metabolic expenditures resulting from the intermediate biochemical reactions incident to the nutritive process. However, the difficulties in separately identifying the expenditure of energy required for digestion, absorption, transportation and deposition of nutrients from that for the physical or mechanical aspects of feeding have led to the application of a less precise term, "apparent SDA" (Beamish, 1974; Elliot, 1976). This apparent SDA in fish has been equated

to the post-absorptive elevation in oxygen consumption by some authors such as, Averett, 1969; Muir and Niimi, 1972; Beamish, 1974; Pierce and Wissing (1974). In the study by Tandler and Beamish (1979) the apparent SDA was divided into mechanical SDA, to the oxygen uptake associated with the energy cost for physical processing of a meal and biochemical SDA, the energy utilized by anabolic and catabolic processes associated with the ingestion of a standard diet. In the present study the overall post prandial metabolic increase is simply termed, SDA.

Figs. 4.3, 4.6 and 4.9 show the responses of oxygen consumption to different protein content diets and it is obvious that the increase of metabolic rate after feeding shows a quite large variation. This variation may be due to several factors, of which natural individual variation is important as with all biological systems. Therefore, small differences in body weight, condition, sex, age and even the individuality of the fish may affect the result. This variation in the data was partly overcome by substantial replication of trials.

The post-prandial oxygen consumption increased and then gradually declined to the resting level and three common phenomena (Fig. 4), the maximum or peak level of increase, the duration of the SDA effect and its magnitude were revealed in (Figs. 4.3, 4.2)

various fish by different authors (Jobling, 1981; Tandler and Beamish, 1979, 1981; Soofiani and Hawkins, 1982; Medland and Beamish, 1985).

These features of the SDA response were found to be dependent on the protein content of the diet and the energy intake at different ration level (Table 4.2 - 4.4).

An increase in magnitude in relation to meal size has been shown by Edwards et al., (1972) in Atlantic cod, gadus Morhua L.; Hamada and Ida (1973) in Cyprinus carpio, carassius auratus, Acheilognatus lanceolate; Schalles and Wissing (1976) in bluegill, Lepomis macrochirus; Caulton (1978) in Tilapia rendalli; Vahl and Davenport (1979) in blenny, Blennius pholis; Jobling and Davies (1980) in plaice, Pleuoronectes platessa ; Machida (1981) in Eleotris oxycephala, Lepomis macrochirus, Micropterus salmoides, Rhodeus rhombeus and Rhodeus lanceoratus; Spencer (1984) in Oreochromis spilurus. The increase of SDA magnitude with increased ration size in these cases may be interpreted by the ingestion of respective energy in rations which takes much more energy to digest, absorb and assimilate. In most cases the relationship was found to be linear. Hamada and Ida(1973) found the increase of metabolic rate in Carassius auratus, Cyprinus carpio, Acheilognatus lanceolate, and Tridentiger obscurus due to SDA is in proportion to the amount of food consumed. Hamada and Maeda (1983) working

with common carp, Cyprinus carpio showed that SDA magnitude was proportional to the ration size and is affected by the compostion of the diet and was much higher for a high protein diet.

However, Tandler and Beamish (1979) working with largemouth bass, Micropterus salmoides showed that the apparent SDA was exponentially related to ration size as was recorded for coho salmon, Oncorhynchus kisutch (Walbaum) by Averett (1969). By contrast, a linear relationship in apparent SDA was reported in largemouth bass maintained at a controlled level of activity and fed emerald shiners (Beamish, 1974) and in swimming sockeye salmon, Oncorhynhus nerka fed a formulated diet (Brett, 1976).

The relationship between SDA coefficient and the weight of the fish in this study was not found to be significant ($p > 0.05$) (Table 4.2, Fig. 4.5) when fed any of the diets. The narrow weight range used (from 67.0 to 74.5g) was probably of help in minimizing this effect. A similar result was reported for largemouth bass, Micropterus salmoides (59.0 to 74.4g body weight) by Beamish (1974) where apparent SDA expressed in energy units as a percentage of ration did not differ significantly with weight of fish. In contrast, Smith (1973) observed a direct relationship between relative apparent SDA and length of the sargassum fish (Histrio histrio) fed a natural diet of shrimps. A decline

in SDA was observed with increasing body weight in largemouth bass (Tandler and Beamish, 1981) and the rate of this decline increased directly with the level of energy ingested.

The time (h) to reach the peak oxygen consumption level in this study varied from 1 to 7 hours after feeding and was not significantly ($p > 0.05$) affected by energy intake and protein content of the diet ingested (Table 4.2, 4.3 and 4.4). In the study of Tandler and Beamish (1981) with largemouth bass, Micropterus salmoides L. fed with a dry pelleted diet (35.8% protein) the maximum oxygen uptake rate was reached within 2 hours of feeding. But Muir and Niimi (1972) working with 44-g aholehole with ration sizes of 2.3% and 4.5% and that maximum oxygen uptake rate occured at about 10 to 12 hours after feeding at 23°C. Beamish (1974) observed that oxygen consumption in largemouth bass, (Micropterus salmoides) attributable to the feeding procedure increased to a maximum within about 2.0 h (range 1.0 - 3.5 h) and thereafter declined to prefeeding level within 6 hours. It was also observed that the time required to reach maximum oxygen consumption after a meal varied from a mean (+_SD) of 6.0 +_3.3 h at 2% body weight/day to 13.3 +_3.3 h at 8%. Jobling and Davies (1980) noted that the peak level of oxygen consumption in plaice, Pleuronectes platessa was reached before satiation and

tney concluded that the processes producing the SDA effect are limited by cellular metabolism.

Hamada and Ida (1973) reported two peaks in 10.2g common carp, one 3 - 4 h after feeding and the other 5 - 8h after feeding. The peak value was found dependant to the amount of food intake. Hamada and Maeda (1983) recognised a general pattern for peak oxygen consumption in common carp. They found that when the ration size was small, the peaks of oxygen uptake appeared earlier, sometimes in 45 minutes and diminished gradually. As the ration size increased, a large peak appeared over 1 to 10 hours after feeding and fluctuated in a complex manner.

The time taken to reach the peak may vary with the ration size and feeding conditions (Beamish, 1974; Jobling and Davies, 1980) or may not (Muir and Niimi, 1972; Vahl and Davenport,1979) but is generally achieved within 12h of the ingestion of the meal. Vahl and Davenport (1979) noted that the height of the peak was independent of the amount of food supplied although this may have been a consequence of the small range of ration sizes fed. Jobling and Davies (1980) reported that the height of the peak increases with increasing ration up to a maximum level, which was achieved below the maximal feed intake.

Machida (1981) showed that the time to reach peak metabolic rate differed variously with the ration size and species. His results indicated that post-feeding oxygen uptake rate of the active swimmers reached its maximum rather soon as compared with the demersal fish. These differences in timings of the maximum metabolic rate with species may be due to differences of the digestive system. He also suggested that perhaps the shape of the SDA curve is influenced by the structure and functions of the digestive organs which are directly correlated to food habit and the mode of life of the fish.

Ross et al. (unpublished data) recorded a range of peak times in Oreochromis niloticus and noted a range from 3.7 to 21.4 h with a linear increase at different ration level (0.5% to 3.5% body weight), but the range from 3 to 7 h at different protein levels (5 to 41% protein level) showed no clear correlation. In aholehole, (Kuhlia sandvicensis) for both levels of rations of 2.3 and 4.5% body weight required longer time (10 - 12h) for reaching peak oxygen consumption after feeding showing no relationship (Muir and Niimi, 1972).

A clear correlation between the duration of elevated rates of oxygen uptake over resting rate and the energy intake from different rations and protein content of the diet can be seen in Tables 4.2, Fig. 4.4b; Table 4.3, and Fig.

4.7b; Table 4.4, Fig. 4.10b. A similar correlation between the duration of the response and and meal size was observed in bass fed emerald shiners (Beamish, 1974) and in Plaice, Pleuronectes platessa L. (Jobling and Davies, 1980). The most straight forward explanation for all these data is that larger meals take a longer time to digest.

The duration of elevated metabolic rate is highly variable between fish species and under different experimental conditions. Thus, oxygen consumption was elevated for 42 h following a ration of 2.3% body weight and for 60 h after 4.5% ration diet in aholenole, Kuhlia sandvicensis (Muir and Niimi, 1972). A range from 8 - 18 h was recorded in thick lipped mullet, Crenimugil labrosus (Flowerdew and Grove, 1980). Oxygen consumption for 1 - 6g of Oncorhynchus kisutch returned to prefeeding levels in less than 24 h (often 6 h), even when ration was of the order of 10% body weight (Averett, 1969). In this case the fish accepted a second meal during the period of elevated oxygen consumption but aholehole seldom did this. Therefore, the differences in duration of the elevated oxygen consumption may be related to ration quality, species and to fish size. Presumably this duration is a reflection of biochemical reaction rates.

Tandler and Beamish (1981) showed that the length of time (12 to 76h) that the rate of oxygen uptake remained

elevated was positively related to energy ingested and negatively related to body weight of bass (Micropterus salmoides L.). Soofiani and Hawkins (1982) obtained similar results for juvenile cod, Gadus morhua.

Hamada and Maeda (1983) recorded the duration of specific dynamic action in common carp ranging from 14 to 18 hours at 25°C. In this case, as the ration size increased, the peak rate was maintained for longer and showed an overall increase over the first ten hours. The oxygen uptake diminished thereafter until the minimum level, although in many cases the oxygen uptake increased again after attaining to this minimum level.

Le Cgrow and Beamish (1986) working with rainbow trout, Salmo gairdneri indicated that dietary protein level does not significantly influence the duration of elevated metabolism, which is consistent with Schalles and Wissing's (1976) data on spontaneously active blue Cgill. In contrast, elevated oxygen consumption by largemouth bass following feeding at high ration levels has been correlated with time recorded for the absorption of protein (Beamish, 1974). Thus, at lower ration sizes, the absorption of protein required more time than was necessary for oxygen consumption to return to pre-feeding levels. After ingestion of 2% body weight meal/day by a bass of 91g, protein absorbed in slightly less than 46 h whereas, elevated levels of oxygen

uptake with apparent SDA continued for only 21h and absorption of protein was approximately 85% complete within the time required for the elevation of oxygen consumption after a meal of 2% ration to return to prefeeding levels. But for 85% absorption of protein at 8% ration took only 30h.

SDA is regarded as a post-absorptive phenomenon (Brody, 1945) which appears to be highly variable in fishes. In experiments on Atlantic cod (Gadus morhua) fed large meals of herring, oxygen consumption remained elevated for about 7 days at 10°C and 4 - 5 days at 15°C (Saunders, 1963). Schalles and Wissing (1976) in their studies found that the duration of the increase in metabolic rate subsequent to feeding was most probably influenced by the digestion rate of the diet where the data implied that dry food pellets are digested more slowly than a natural food such as mayfly nymph (Pierce and Wissing, 1974). A similar response has been observed in the rainbow trout (Salmo gairdneri) by Windell and Norris, (1969).

To enable comparison of the feeding response with different diets at different ration levels, the peak was calculated as a percent increase over resting rate (Table 4.2, for 20% dietary protein; Table 4.3, for 35% dietary protein and Table 4.4, for 50% dietary protein). In all cases, it was found that the peak as percent resting increased linearly

($p < 0.05$) with increase of ration size (Figs. 4.6f, 4.7g and 4.8g). This is due to the increase of energy in the diet and supports the findings of many of the authors described earlier.

The SDA magnitude was clearly related to both energy intake from varying ration size and protein content in the diets (Tables 4.2, 4.3 and 4.4; Figs. 4.4c, 4.7c, 4.10c). In these cases, with the increase of energy intake the SDA magnitude increased significantly ($P < 0.05$) with different levels of dietary protein. A linear increase in apparent SDA was reported in largemouth bass, maintained at a controlled level of activity and fed emerald shiners, Notropis atheronoides Rafinesque (Beamish, 1974) and in swimming sockeye salmon, Oncorhynchus nerka, fed on a formulated diet (Brett, 1976). In largemouth bass, the SDA magnitude increased linearly with the protein content of the diet (Tandler and Beamish, 1980).

The value of SDA coefficient enables a general comparison to be made among fish species and diets and reported values are summarised in Table 4.1. There is a large variation in the values in different fish depending on the experimental conditions, type of food used.

Le Grow and Beamish (1986) noted an SDA coefficient of 15.3% and 18% with 48% and 60% protein diet in rainbow

trout, whereas Beamish (1974) reported a value of 14.2% when largemouth bass were fed on emerald shiners, Notropis atherinoides, (55% protein), and in the same species, Tandler and Beamish (1980) obtained an SDA coefficient of 15.8% when fed on 50% protein pelleted diet. Muir and Niimi (1972) observed SDA values of 16 - 19% of the ingested energy for two specimens of aholehole, Kuhlia sandvicencis, fed on tuna flesh (= 65% protein).

In the present study, the SDA coefficient was found to increase with the protein content of the diet. The mean values were 8.99% (from 6.60 to 12.06%), 13.76% (from 11.40 - 16.52%) and 15.95% (from 12.85 to 19.46%) with 20, 35 and 50% protein content diets respectively (Table 4.2, 4.3 and 4.4) but there was no significant ($p < 0.05$) relationship with changing energy intake resulting from different ration levels and weight of the fish (Fig. 4.4d, 4.5; 4.7d, 4.8; 4.10d and 4.11).

Similar observations were made by Beamish (1974) in largemouth bass. He considered that the relationship between SDA and meal size or fish weight was due to the metabolic rate and growth plasticity of fish, which can be adjusted to a large range of temperatures and conditions in nature. These adjustments may involve a complex interaction between components of resting and active metabolism and the food energy supply and may imply "underlying feedback or

homeostatic mechanisms operating on the dynamic energy system" (Paloneimo and Dickie, 1966). Similar effects were noted in Oreochromis niloticus by Ross et al. (unpublished data) where the SDA coefficient at different ration levels ranged from 4.61 to 5.31% showing no significant relationship with ration level and weight of fish.

Cho et al.(1976) found an increase in the SDA from 9 to 12% of the gross energy ingested associated with an elevation of dietary protein from 40 to 60% in meals of rainbow trout. By contrast, Schalles and Wissing (1976) noted that percent protein in the diet had no significant effect on mean oxygen uptake after feeding or the estimates of energy cost of feed utilization in bluegill sunfish Lepomis macnrochirus fed with formulated diets containing 24 to 45% dietary protein. However, in the study of Machida (1981) RSDA (SDA against routine rate) values expressed as percent of ingested energy varied among species and within species, and ranged from 7.3 to 10.8% for carnivorous fish, except for juvenile largemouth bass. This seems to be some relationship with the developing function of the digestive organs which changes with the body size, resulting in different SDA in young, juvenile or adult fish. The estimates of RSDA in Machida's study on young largemouth bass were slightly lower than those in Beamish's experiment due to the use of "routine" metabolic rate in the estimation of RSDA.

Varying ration level in the diet had no significant effect on the SDA coefficients (Fig. 4.12) throughout the experiments. The values for SDA coefficients of 8.99% (with 20% protein diet), 13.8% (with 35% protein diet) and 15.95% (with 50% protein diet) of the ingested energy are well within the limited range of 5% to 20% suggested by Priede (1985). Yarzhombek et al. (1983) obtained the SDA coefficient of 16% using pelleted diets with C. carpio.

Since SDA is related to digestion and absorption, nutrient digestibility is very important. The apparent protein digestibility for protein in the diets ranged from 72.79 to 83.58% (Table 4.7) suggesting that the diets were of a good quality. The lower SDA coefficients reported in the literature, such as the value of 2% in sea water and 5% in fresh water for Anguilla anguilla (Knights, 1985) could possibly be due to two factors. Either, with a well balanced diet made from high quality ingredients the cost of digestion and catabolism are much lower resulting in an energetically effective utilization of the diet, or the quality of the diet is so poor that few nutrients are released and absorbed from it. This can be confirmed by measuring the digestibility of the feed by the fish. By contrast, artificially high SDA values may result from a less favourable amino acid balance, greater costs of mastication for different forms of diet, smaller rations producing much more 'mechanical' cost (Jobling, 1981)

which produce a higher cost of processing, and so on. Knights (1985) suggested that elevated water quality criteria, such as ammonia, produced in experimental systems could lead to hypermetabolism. In the present study the error due to ammonia was reduced by using the ion exchanger and by regular changing of water in the system.

Since SDA is a chiefly post-absorptive process the variation in the SDA coefficient with different fish may be influenced by several factors. A well-balanced diet with high quality ingredients has a higher digestibility (Hepher, 1988) and shows lower values for SDA coefficient. The real differences in dietary physiology between stomachless and stomached fish with differences of activity of post-absorptive enzyme systems for protein (Hepher, 1988 pp. 44,, Noaillac - Depeyre and Gas, 1973, Ferraris et al., 1986), carbohydrate (Furuichi and Yone, 1982b; Morita et al., 1982), enzyme activity on the type of diets (natural, pelleted etc.) (Schlottke, 1938, quoted from Steffens, 1989; Kitamakido and Tachino 1960a, b; Bulnheim, 1974), digestion rate (Rozin and Mayer, 1961; Barrington, 1957) and digestibility. The carp is a stomachless fish and its protein digestion relies solely on tryptic enzymes rather than acidic or pepsinogen secretion as in stomached fish. This strategy may have a high energy cost for food processing.

Brooke and Ashworth (1972) measured the post-prandial increase of metabolic rate and rates of growth in malnourished children and found a highly significant relationship between the increase in metabolic rate and growth. This added support to the theory that post prandial increases in metabolic rate represent the 'energy cost of growth'. Kreiger (1978) reported a significant difference in post-prandial metabolic responses between rapid and slow growing rats after food deprivation. Thus, there is evidence that the increase in post-prandial metabolic rate can be correlated with tissue synthesis in the resting animal.

Supportive evidence for the 'energy costs of growth' theory can be summarized as follows:

(1) post-prandial metabolic rate varies with dietary composition and diets most favourable to growth induce the greatest effect;
(2) the greatest post-prandial increase in metabolic rate is found with rapidly growing fish;
(3) following feeding, the increase of metabolic rate is greatest under conditions most favourable to growth.

During starvation of fish, stored pools of free amino acids and fat are being used for maintenance (Shimeno, 1974). When the stores of amino acids and fat are exhausted after a long starvation, somatic tissue will be catabolised. When

refeeding starts these stores are replaced before any growth takes place (Hepher, 1988). The energy cost of producing the complex somatic tissue is probably more than the cost of producing amino acids and fats. If the main factor in SDA was growth, it may be expected that during replacement of stores after starvation or food deprivation the SDA magnitude would be higher than when previously fed and the synthesis of somatic tissue was taking place. This study shows no significant difference in the SDA magnitude of feeds subsequent to feeding or fasting which suggests that there is no metabolic differences between tissue synthesis and growth. Jobling (1981, 1983b and 1983c) opposed the case for SDA being a growth-related effect which is associated with a short-term increase in protein synthesis and turnover following feeding. The "energy cost of growth" theory as suggested by Brody (1945) seems to be contradictory to the traditional view. Some authors have shown SDA to consist of two distinct peaks (Hamada and Ida, 1973; Flowerdew and Grove, 1980; Fullarton, 1988). Fullarton (1988) postulated that the first peak was due to catabolism and deamination whereas the second one could be due to growth. No clear double peaks in SDA were noted in the present study.

Three main causes were for SDA were suggested by Cho and Kaushik (1985)

(a) Digestion and absorption of the feed

(b) Transformation and interconversion of tne feed substrates and their retensions in tne body tissues.

(c) production and excretion of metabolic wastes.

Jobling and Davies (1980) have shown that mechanical costs of digestion and absorption are not part of SDA and state that SDA is a post-absorptive effect. When constructing an energy budget it is customary to consider SDA as an 'energy cost of feeding' and as a protein related phenomenon. Krebs (1964) attributed SDA to the wasting of some of the energy of amino acid degradation and the energy needed for urea synthesis. However, Lusk (1931) showed an increase in metabolic rate following the ingestion of carbohydrate and fat, and Garrow and Hawes (1972) showed that the increased oxygen consumption is not closely related to urea production. However, the increase in metabolic rate is related to ammonia production in tilapia (Mckinney, 1991). Brett and Zala (1975) showed that there was no significant stimulation of urea production associated with feeding because fish are mainly ammoniotelic. Undoubtedly, some part of the SDA can be attributed to the cost of active transport of tne product of digestion across tne intestinal mucosa, but, as an SDA effect has been demonstrated in dogs when amino acids were injected intravenously (Wilhelmj and Bollman, 1928) tne SDA is probably best considered as a post-absorptive effect. In teleost fishes, most of nitrogenous waste is excreted as ammonia (about 80%, Smith,

1929) which is energetically relatively cheap to produce. It is excreted passively and so is unlikely to be a major component of SDA.

The second factor described by Cho and Kaushik (1985) is possibly the major contributor to SDA. The macromolecules from different substrate must be hydrolysed into utilizable nutrients and thus, amino acids must be deaminated. Increase of dietary protein in the diet causes increased SDA (described earlier), so giving emphasis to the theory that protein deamination is a major cause of SDA. The magnitude of the SDA is not only determined by the amount of protein but also by the type and quality of the amino acids ingested. Although protein appears to be the major determinant of SDA, it may not be the only factor, and to test this hypothesis Jobling and Davies (1980) plotted the SDA against the digested protein energy and concluded that the overall magnitude appears to be determined both by the protein content and to a lesser extent, the energy content of the other constituents of fat and carbohydrate. So, the catabolism of carbohydrates and lipid to form ATP accounts for the SDA observed subsequent to feeding a non-protein meal.

SDA is an important part of the energy budget of fishes, and of relevance in fish culture. If we consider that SDA is due to mastication, peristalsis of digestive tract,

digestion and absorption, deamination, catabolism and waste product synthesis then attempts should be made to minimise the effect. On the other hand, if the energy cost for SDA was to be considered as an energy cost of growth then the feed and feeding regimes should be designed to maximise growth regardless of the SDA magnitude.

Although the defined ration and protein level in the diet having a major influence on SDA, the other components of the diets e.g. carbohydrate and lipid may also have some effect. Moreover, the change in quality of the protein in the diet may also affect the accuracy of the model. Higher protein levels may change (increase) the SDA due to the cost of processing of excess protein. Change of fish weight may have an effect due to the variable growth rate. Although in this study the ration size was restricted to only about 1% of the body weight, the amount of the ration needed to satiate larger or smaller fish is different.

From the above discussion it is clear that SDA is the result of many processes. Differences of opinion over the causes of SDA in the literature are due to authors working on different species under different conditions. As a physiological rule each species has its own physiological differences, and perhaps the basis of SDA is same but the importance of these factors varies from species to species. The models developed for SDA are of importance for

aquaculturists at least in two aspects. Firstly, the post feeding oxygen requirements in an intensive culture system can be calculated and, where the ration and protein levels are known, this information can be used to ensure an adequate oxygen content in water. Secondly, in energy budgeting the amount of energy wasted or expended as "SDA" can be considered.

CHAPTER V

ENERGY COMPONENTS AND ENERGY BUDGET

5.1 Introduction

Most early studies of energetics were restricted to the transfer of energy from one trophic level to another (Winberg, 1970; Odum, 1971). Since about 1940, however, studies on physiological energetics or animal bioenergetics developed and these centred on the rates of energy expenditure, the losses, gains and efficiencies of energy transformation as functional relationships of the whole organism (Musisi, 1984).

The energy budget is a balance sheet of energy input (food intake) against energy expenditure. Some authors have developed an energy flow diagram showing the main steps that the consumed energy follows through the body and the paths of energy expenditure or distribution (Davies, 1964, Beamish et al., 1975).

The main components of an energy budget for fish, expressed in its simplest form are:

$$C = R + F + U + P$$

or

$$P = C - (R + F + U)$$

where, C = consumption, the energy content of the food eaten,

R = respiration or total metabolism, the net loss of energy as heat,

F = Energy lost in faeces,

U = Excretion, the energy lost in nitrogenous excretion

P = Production or energy in growth materials,

The major terms in this regard were defined by Petrusewicz and MacFadyen (1971).

Where the fish is not growing but losing weight, the tissue energy loss is considered as entering the system. Thus, if the animal is not feeding the energy loss from tissue equals 'R + U'. The manner in which the dietary energy is partitioned into various components in terms of animal utilization is shown in chapter 1 (Fig. 1.1).

In laboratory studies, the energy income (C) to the budget is relatively easy to determine because a measured quantity of known energy content of live or artificial diet is fed to fish under confined conditions. But it is

important to know the amount of total energy of food (C) practically consumed and of faeces (F) produced. Sometimes the leaching of nutrients from pellets into water, regurgitation of pellets, uneaten food, consideration of non-energy food (which is not utilized in bioenergetic consideration) and so on may cause an overestimation of C. In this aspect, it may be claimed that an accurate measurement of C and F in a long term energy budget gives the absorbed and assimilated energy by the fish (Brafield, 1985).

Generally, in investigating fish respiration 'R' for bioenergetic purposes, one should necessarily be aware of the measured type of metabolism and it is difficult to measure all the components of 'R' separately. The first component, routine metabolism (see chapter 1), is the metabolic rate during normal spontaneous activity of fish (Brett and Groves, 1979). The second component 'R_a' is the energy lost as heat due to motor activity for seeking food, maintaining position and interaction and the third component 'R_f' is the heat of nutrient metabolism or heat increment or Specific Dynamic Action (SDA) due to heat released by the chemical reactions related to the processing of ingested food. The total heat production (R) can be estimated indirectly by measuring oxygen utilized in the respiratory process. Ammonia is a chief nitrogenous excretory product (U), and under some circumstances (as

in starvation), a small amount of urea may be produced (Elliott, 1976a) together with some other nitrogenous substances, like, amino acids, uric acid and creatine which may be ignored.

Numerous attempts have been made to compile complete energy budgets for fish species. The studies of different aspects of energy budgets for different fishes by different authors have been summarized in chapter 1 (Table 1.1). Among the more recent works are the studies on grass carp (Ctenopharyngodon idella) by Carter and Brafield (1991) in which the energy allocation at different planes of nutrition was studied and the overall balance of energy during long term experimental periods (for a month) were presented for fish fed on four diets varying in high protein, high carbohydrate, high lipid and dried duckweed Lemna spp. or starved. The energy balance of young tilapia (Tilapia mossambica) under different temperature conditions and at a different feeding level was measured by Mironova (1976) who showed that the temperature most favourable for growth changes with the age of the fish and favourable (faster) growth occurs at an earlier age.

Staples and Nomura (1976) studied the energy budget of Salmo gairdneri in ponds at constant temperature and found that total metabolic expenditure 'R' increased with increasing body size and feeding level.

Elliott (1976b) studied the energetics of feeding, metabolism and growth of brown trout (Salmo trutta L.) and showed that the weight of fish and temperature affected all the components C, F, R, U and B (growth or loss of weight), and equations were developed to estimate these components for trout on maximum rations.

Fischer (1978) reviewed the literature on fish energetics and pointed out the selected problems of fish bioenergetics. He noted that in spite of the importance of the energy value of food ration, more important still is its digestibility which is the result of quality and quantity of food, physiological peculiarities of fish and the environmental conditions. He also considered that the biochemical role of dietary carbohydrate varies with species and that carbohydrates participate to a very low extent in growth 'P' but as they are readily combustible this allows the utilization of proteins and lipids for growth.

The energy budget of tilapia (Sarotherodon mossambicus) was studied by Musisi (1984) who compared the growth rates of fish fed different diets and found maximum growth (P) with a high protein and fat diet fed ad libitum over 90 days of observation. The highest metabolism (R) was recorded with fish fed on fat and protein diet. She

compiled complete energy budgets for individual fish which showed the balance of the budgets of 73.8% - 108.8%.

The effect of ration, temperature and body size on C, F and U of a cyprinid, Phoxinus phoxinus was studied by Cui (1987) and he noted that the maximum food consumption was related to body weight and temperature and the absorption efficiency increased with increasing ration. On average 6.5% of food energy was seen to be lost in 'F' and 5.1% in 'U' by minnows feeding on white worm, Enchytraeus spp. He also developed a bioenergetics growth model for Phoxinus phoxinus, which gave good predictions of the form of the growth - temperature relationship, but failed to predict the growth - ration relationship well.

There has been great variability in the techniques used to compile the data for published energy budgets. Variations in food quality and quantity, feeding rules, respiratory rate measurements, fish size and age and other factors, have meant that the compilation of a comprehensive table of energy budgets may not reveal much of significant value. Nevertheless, Brett and Groves (1979) attempted to synthesize the data existing at the time to make the following general energy budget for young fish feeding well (figures are expressed as the percentage of energy of food intake 'C').

For herbivores : 100C = 20P + 37R + 2U + 41F and

for carnivores : 100C = 29P + 44R + 7U + 20F.

These equations clearly show the lower feed digestibility efficiencies (higher percentage of 'F') in herbivores, the relatively small amounts of nitrogenous waste (U) and higher value for 'R' than 'P'. In considering the basic energy budget equation (C = P + R + F + U), by subtracting 'F' from 'C' the digestible energy (the energy absorbed and assimilated by the fish) can be calculated (P + R + U) from which the energy in nitrogenous waste 'U' is excreted leaving 'P + R' as an indicator of the metabolizable energy.

The energy value of 'C', 'F' and 'P' may be accurately determined by direct combustion of dry samples in the bomb calorimeter. Whereas,the total energy lost as heat (R) can be estimated indirectly by measuring oxygen utilized in the respiratory process and then by multiplying by an appropriate oxycalorific coefficient (Q_{ox}) which is a conversion factor for the energy released from different substrates (protein, fat and carbohydrate). Thus, for proteins, the following equation (Brafield and Llewellyn, 1982) represents the production of ammonia (as ammonia is the most common and chief excretory product in teleosts) from the respiration of 100g protein:

$(4.42\ C, 7.00\ H, 1.44\ O, 1.14\ N) + 4.6\ O_2 \longrightarrow 1.14\ NH_3 + 4.42\ CO_2 + 1.79\ H_2O)$

where, C, H, O, N denotes carbon, hydrogen, oxygen and nitrogen mol respectively. It is assumed that a typical protein is composed of the following proportion $C_{53\%}$, $H_{7\%}$, $O_{23\%}$, $N_{16\%}$ (Kleiber, 1975). Sulphur has been ignored here. (Kleiber, 1975). Therefore, considering the atomic weights of 12, 1, 16 and 14 respectively the number of moles of different elements become 53/12 = 4.42; 7/1 = 7; 23/16 = 1.44 and 16/14 = 1.14.

In this example, 1967 kJ energy is lost as heat (as it is the energy value of 100g protein (2364 kJ) minus that of the 1.14 mol of ammonia (397 kJ)) in the complete combustion of 1 mol of protein by 4.6 mol oxygen. Thus, the Q_{ox} for protein is (1967/147.2) = 13.36 J per mg oxygen respired. For calculation of 'R' by applying Q_{ox}, it is assumed that all the energy liberated by respiration is ultimately lost as heat aerobically. The other parameters C, P, U and F, reflect potential energy as chemical bonds. During growth the energy in chemical bonds is retained as chemical energy (Musisi, 1984).

From the feed, fish generally respire a complex mixture of substrate (protein, fat and carbohydrate). Therefore a composite Q_{ox} needs to be used to calculate the precise

result. For starving fish respiring their own tissue, a Q_{ox} value of 13.56 J per mg oxygen consumed is suggested (Brett and Groves, 1979).

Nitrogen in the diet of a fish will either appear in faeces or be absorbed in growth materials, or be excreted via the gills and urine. The chief energy lost in excretion (U) by teleosts is in the form of ammonia which is lost principally through the gills. The rate of nitrogen excretion is affected by different factors e.g. food quality and quantity (Elliott, 1976a, Brett and Groves, 1979). Nitrogen excretion increases linearly as nitrogen absorption increases and the relationship between nitrogen absorption and nitrogen retention is also linear (Savitz et al., 1977). An interesting aspect of nitrogen metabolism is the increase in nitrogenous excretion following a meal and this closely parallels the SDA effect. In an energy budget the energy lost in nitrogenous excretion (U) is the smallest component. Nevertheless, it is significant (Brett and Groves, 1979) when compared with 'C', and account must be taken of it when compiling the energy budget. There are two basic methods for determining U. In the first method the values for oxygen consumption to respire protein in $mgO_2/kg/h$ are multiplied by an energy value of 2.70 J/mgO_2 (Brafield and Solomon, 1972) or 2.59 J/mgO_2 consumed (Elliott and Davison, 1975) to obtain the estimate of energy lost in excretion (U).

In tne second method, the total excreted ammonia (both NH_3 and NH_4^+) in aqueous solution is measured. Then 'U' is calculated from the value of total ammonia nitrogen liberated multiplied by the energy value of ammonia which is equivalent to 24.83 J per [illegible] mg ammonia (5.94 cal/mg) excreted (Elliot and Davison, 1975). Since the nitrogenous excretion in teleosts is with few exceptions, mainly ammonia, the energy loss in other form of nitrogen,e.g. urea, may be ignored in the energy budget.

Brett (1962) discussed the problems associated with and compiled a list of terms used in fish respirometry. The most recent reviews on physiological energetics of fish were those of Brett and Grove (1979), Brafield (1985) and Priede (1985). The application of laboratory studies of fish energetics to field studies were reviewed by Warren and Davies (1967). They modified the energy budget equation of Ivlev (1945) with more clear definitions of terms and represented as the over-all energy budget of a fish for any given time. In addition, they made laboratory studies to relate between food consumption (C) and growth (P) of sculpins (Cottus perplexus). The results of laboratory experiments on growth efficiency were applied to the natural situation by Kerr (1971a, b, c) and he produced a mathematical model for growth efficiency and metabolism which gave precise predictions against observed data.

There have been numerous studies on the relationship between dietary energy and protein content on growth of carp (Ogino, et al., 1976; Takeuchi et al., 1979b; Steffens, 1964a; Schwartz et al., 1983 ; Murai et al., 1985; Zeitler et al., 1983, 1984; Gongnet et al., 1987; Watanabe, et al., 1987; Eckhardt et al., 1983, Kirchgessner et al., 1984). However, there have been no studies to date investigating energy partitioning of the consumed food to describe a complete energy budget for carp, Cyprinus carpio considering the relationship with different dietary protein and ration sizes.

The purpose of this study was to conduct a series of experiments in metabolism chambers to investigate the interrelationship between energy pathways of the simple bioenergetic model by using common carp, Cyprinus carpio. L. as a test animal. The results from different trials were used to establish an energy budget model with different protein diets and to show the relationship between the energy budget components under varying feeding regimes. These total energy budgets were also used to verify the results from the resting respiration (Chapter 3) and SDA experiments (Chapter 4).

5.2 Materials and Methods

5.2.1 Experimental fish

Common carps (Cyprinus carpio L.) of 65.0 +_8.0g used in the experiments were held in a recirculating system in the tropical aquarium of the Institute of Aquaculture (described in section 2). The maintenance of the fish and acclimatization with experimental diets during the pre-experimental period was described in section 2.

5.2.2 Water quality

Poor water quality or even pronounced fluctuations in water quality, may cause a significant decrease in appetite, growth and food conversion efficiency (Smart, 1981). Therefore, ammonia (total nitrogen), oxygen, pH and temperature of the water were measured routinely, twice weekly during the acclimatization and experimental period in the rearing system. The methods used were described in chapter 2.

5.2.3 Experimental system

The experimental system (Fig. 5.1) was set up in an undisturbed corner of a small aquarium of the Institute of

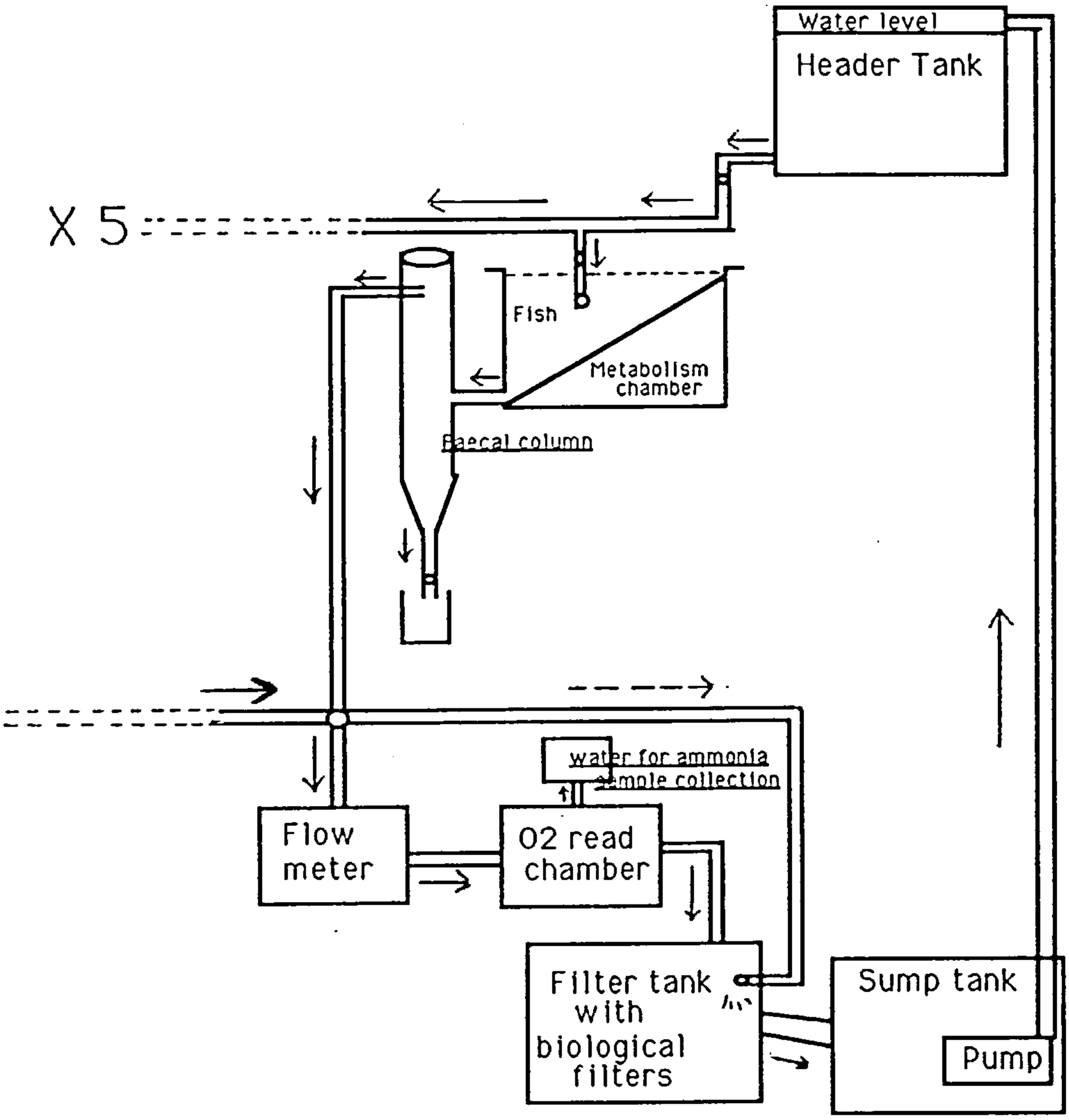


Fig. 5 Schematic diagram of the metabolism chambers used for Cyprinus carpio.

Aquaculture so as to minimize disturbances. In order to simultaneously measure respiratory rate (R), faecal loss (F), nitrogenous excretion (U) and growth, a flow-through water recirculating system was constructed comprising five metabolic chambers. The following components of the system were specially constructed for the purpose.

Metabolism chambers

Each metabolism chamber consisted of a transparent, acrylic rectangular tank (38.5 cm X 21.5 cm X 24.2 cm) with a capacity of 20 litres. The tank was partitioned internally by setting a perspex sheet sloping diagonally and made water tight by silicon glue so that no water could transfer between the two sections. At the top corner, a small gap was provided as a means of filling the lower chamber with water. Thus, the capacity of the upper, active, chamber became 10 litre of water. The outlet of the metabolism chamber was set at the bottom corner of the slope and was connected with the inlet of the faecal column by a small (10.0 cm long) rubber tube. Each metabolism chamber had a plastic lid to prevent the fish from jumping out

Faecal column

Each faecal column (Fig. 5) consisted of a PVC pipe of 7.6 cm diameter and 42.0 cm long with an inlet and outlet

made from plastic tubes (12.0 mm diameter) set at a distance of 29.0 and 5.0 cm from the top respectively. At the bottom of the column a 8.0 cm diameter plastic funnel was fitted with small screws and silicon glue. The slope of the funnel was about 70^{o} so that the faecal matters entering the pipe would settle to the bottom of the funnel. The bottom of the funnel was fitted with a stoppered rubber tube. The upper outlet of the faecal column was connected to a three-way valve by a rubber tube (10 mm diameter).

Respiration (R) and Excretion (U) measuring chamber

For determination of oxygen consumption and excretion by the fish in the different metabolism chambers, effluent water from the faecal column was allowed to pass through a gap flow meter and then through a cuvette housing an oxygen probe (YSI model 5739) which was connected to an oxygen meter (YSI model 57) (see chapter 3). The cuvette was set on a magnetic stirrer. A rubber bung was fitted to the top of the cuvette through which a 0.5 mm bore hypodermic needle was passed. A short piece of 0.5 mm bore rubber tube was fitted to the needle and this reached near the bottom of the cuvette. A 5.0 ml syringe was then used to withdraw water samples from inside the cuvette for the measurement of total ammonia content in the water.

5.2.4 Working principle of the system

Water from the sump tank was pumped into the header tank through a PVC pipe. From the header tank water was allowed to flow through a two-way valve into a common distributing pipe from where each of the metabolic chambers received water through another two-way ball valve. The lower part of the metabolism chambers was also filled with water in order to maintain the balance of the water pressure on the separating-perspex sheet although the lower portion was not an actual part of the system. The flow rate in each of the metabolic chambers was maintained manually using the ball valves.

The water level required in the metabolism chambers was marked by a black line so that any change of water level in the chamber could be identified immediately. There was a small outlet on the rear of each chamber, situated just above the marked lines so that any accidental overflow of water could escape through a common outlet leading into the filtration tank. Water entered the chambers at a controlled flow rate of 40l/hr and eventually passed into the faecal column. Because of the slope of the internal partition, faecal matter also passed into the faecal column where, because of the lower velocity, it settled down to the bottom of the column, the supernatant water escaping via the three-way valve system. This three-way valve

allowed the effluent water to be directed either towards the cuvette via a flow meter for determination of oxygen concentration and ammonia excretion and then into the outlet leading to the filtration tank, or directly into another the filtration tank when no measurement took place.

The water flow from the metabolism chambers was regulated by the three-way valves in such a way that when water from any one of the chambers was allowed to pass through the cuvette, the water flow from the remaining four was directed into the filtration tank. Thus, by using these valves, all chambers could be sampled successfully.

Water from the filtration tank flowed into the sump tank through a 5.0 cm diameter PVC pipe. The filtration tank was provided with biological filters (numerous plastic rings for supporting microbe and other suspended materials). The temperature in the system was maintained at 28^{o} C +_ 0.5^{o} C by three water heaters (NIMROD, thermostatic heater) set in the sump tank and two large air stones were installed in the header tank in order to maintain the air saturaion. About one third of the water was replaced every third day by fresh clean water to help maintain the water quality in the system. During the experimental period a 12L : 12D photoperiod was maintained in the laboratory by using three 20w fluorescent tubes set at a distance of about 1 meter above the system and controlled by a timer.

5.2.5 Experimental Procedure

The system was first run without fish in the metabolism chambers for four days in order to stabilise the new environment. From the five metabolic chambers the middle one, chamber no. 3, was used as a reference chamber to measure the oxygen and ammonia in the system water, the other four being used for fish.

No feed was given to the acclimatized fish in the holding tanks for 24h before starting an experiment. Four randomly selected experimental fish were sedated using Benzocaine, Ethyl-p-amino benzoate (Ross and Geddes, 1979), blotted, dry, and were then weighed individually and placed in metabolism chamber. Each chamber was then covered by its lid. The water flow in the system was adjusted based on a test run so that oxygen concentration at the outlet of the metabolism chamber was within the safe level for fish even at their peak consumption. The fish were then allowed to adiust to their new surroundings overnight.

After the fish were established in the new system the actual recording of oxygen consumption and excretion from the metabolic chambers was started. Oxygen consumption values were directly recorded from the oxygen meter and ammonia excretion was measured by taking water samples from the cuvette (for methodology see Chapter 2). Chambers 1, 2,

4 and 5 contained fish and chamber 3 was used as a reference.

During the first two unfed days, the routine metabolic rate in the unfed state was determined. From the third day, food containing either 20%, 35% or 50% protein (see Chapter 2) was given at the rate of 1%, 2% or 3% of their body weight respectively. It was noted that the carp could only consume about 3% of their body weight at most and this can be considered as the ad libitum level.

The feeding of fish started at 1000 h: every day. Three to four pellets at a time were given to the fish which they engulfed promptly (usually, they swallowed the pellet immediately after it entered the water). Sometimes the pellet was regurgitated and was then swallowed by other fishes in the chamber. Each feeding was continued until the fish no longer responded positively to the pellets. Feeding of fish continued hourly according to their ration size. No feed pellets were found unutilized by the fish in the chamber. Thus, the food given can be considered to be an accurate estimate of 'C' (consumption). It was found that the maximum ration (3%/day) could be achieved by 1600 hr in the evening. Fish were grown in these chambers for a total of 18 days.

Since the experiment needed a 24 h observation and sampling

over long periods (18 days), in both the replicates, it was not practically possible to continue observation and recordings throughout the trial. Instead, the 24h monitoring experiments were conducted with intermittent gaps. The oxygen consumption, ammonia excretion and faecal collection were recorded on 11 different days over the whole experimental period. The normal feeding of fish continued regularly on a daily basis so as to produce good growth.

Each experiment with two replicates were conducted with diets containing 20, 35 and 50% protein at 1, 2, and 3% of the body wt. of fish/day in each treatment group. Two replicates each of a total of 48 fish were used in 12 experiments with 4 fish in each treatment. Oxygen consumption, faecal production and ammonia excretion rate were recorded on days - 1, 3. 5, 7, 9, 10, 12, 14, and 16 for monitoring data for each replication. Table 5.1 (replicate's mean) shows a synopsis of the experiments conducted.

5.2.6 Measurement of major parameters

Oxygen consumption

The oxygen consumption in the metabolism chambers was obtained from the following equation :

Table 5.1 Food consumption of Cyprinus carpio with different protein diets and ration levels.

Expt. No.	Initial mean wt. per tank (g)	Feeding regime	Ingestion(C) (g/tank/day)	Energy in ingested food (kJ/day)
20% Protein diet	1	251.64 X=62.91 (+8.21)	Unfed	----	-----
	2	253.43 X=63.35 (+5.95)	@ 1% body wt.	2.53	44.45
	3	251.12 X=62.78 (+4.19)	@ 2% body wt.	5.02	88.19
	4	226.47 X=56.63 (+4.90)	@ 3% body wt.	6.79	119.87
35% Protein diet	5	236.25 X=50.06 (+7.27)	Unfed	----	----
	6	282.58 X=70.64 (+10.89)	@ 1% body wt.	2.83	54.14
	7	266.42 X=66.60 (+9.05)	@ 2% body wt.	5.33	102.13
	8	257.42 X=64.36 (+5.11)	@ 3% body wt	7.72	148.03
50% Protein diet	9	248.00 X=62.09 (+3.18)	Unfed	------	-----
	10	244.80 X=61.20 (+9.26)	@ 1% body wt.	2.45	47.44
	11	255.10 X=63.78 (+5.11)	@ 2% body wt.	5.10	98.74
	12	253.00 X=63.25 (+10.02)	@ 3% body wt.	7.59	146.96

Oxygen consumption (mg/kg/hr) = [(Y - X) x F x 1000] / W

Where, X = Oxygen conc.(mg/l) at outlet of metabolism chamber

Y = Oxygen conc. (mg/l) at outlet of reference chamber

F = Flow rate (L/hr) of water through the metabolism chamber

W = Weight of fish (g) in the chamber

Faecal materials

Any faecal material which settled in the trap before feeding on the days of monitoring was discarded by flushing the column. The faecal material from every feeding day was collected the following morning. During collection, the inlet rubber connection of the column was clipped to temporarily stop the flow from the metabolism chamber and the column was detached from the connection. The water from the faecal column was collected through the stoppered outlet into two glass beakers so that all suspended faecal matter together with settlings at the bottom of the column were retained. The column was reset quickly onto the metabolism chamber with its normal water flow. The faecal material in the collected water was recovered by immediate centrifugation and the solids from the centrifuge tubes

were poured into a pre-weighed glass petri dish for subsequent drying in a hot air oven at 70° C. The dried and weighed sample of the faeces from every collection day were kept in labelled separate glass vials for subsequent analysis for protein and energy content (see Chapter 2).

Apparent protein digestibility

Protein digestibility of the faeces from the experimental diets were measured using an inert marker method. Chromic oxide was used as marker in the experimental diets (Furukawa and Tsukahara, 1966) . In this method chromic oxide in faeces was determined spectrophotometrically by the following formula:

$$X = (Y - 0.0032) / 0.2089$$

where, X = Chromic oxide content (mg/100 ml)

Y = Optical density, and then

% Chromic oxide in sample = (X/A) x 100

where, A = amount of sample (in mg)

Digestibility was considered as the apparent digestibility co-efficient according to the following formulae:

Apparent Digestibility (%) = 100 -[100 x (C1/FC1 x FN1/N1)]

Where, C1 = indicator (chromic oxide) content of test diet(%)

N1 = nutrient content of the test diet (%)

FC1= indicator content of faeces of fish fed the diet

FN1= nutrient content of faeces of fish fed the diet.

Nitrogenous excretion (U)

In the present study the nitrogenous excretion in the form of ammonia was measured as total ammonia in the flowing water by the method of Stirling(1985) in which ammonia reacts with phenol and hypochlorite in alkaline solution to give indophenol blue. Sodium nitroprusside was used to intensify the blue colour at room temperature. The method has been described in chapter 2. The results were expressed as mg/kg/hr by applying the following equation :

Ammonia excretion (mg/kg/hr) = (B - A) X F X (1000/W)

where,B = ammonia in the reference chamber

A = ammonia in the water from the metabolic chamber

F = flow rate (l/hr)

W = weight of fish in the metabolic chamber

The excretory losses measured in the form of ammonia were tnen converted into heat energy by using the conversion factor (energy equivalent) of 24.83 J/mg (5.94 cal /mg) for ammonia nitrogen assuming that protein contains 16% nitrogen (Elliot and Davison, 1975).

Growth (P)

Growth in this study was measured by change in wet weight or dry weight during the experimental period. For this purpose, the initial body weight of fish and final body weight after the experiment were recorded. The fish were then sacrificed by piercing them through the brain and the carcasses were oven-dried at 105° C for 24 hours. The dried fish were weighed and ground homogeneously (Moulinex Blender mill - 2) for subsequent analysis of proximate composition and energy content.

The following simple growth evaluation criteria were then calculated:

i) Growth rate: The change in weight during the experimental period expressed as percentage

$$\text{i.e. } [\ (W_t - W_o)/W_t\ ] \times 100$$

where, W_t = final weight of fish (g),

W_o = initial weight of fish (g).

ii) Feed conversion ratio (FCR): The ratio between the weight of the food consumed and the weight gain of the fish.

$$FCR = (C \times t) / (W_t - W_o)$$

where, C = Total food intake (g)

t = no. of days of feeding (= 16)

iii) Protein efficiency ratio (PER) : The ratio between the weight gain of fish and the amount of protein consumed:

$$PER = (W_t - W_o) / P,$$

where, P = crude protein eaten (g)

5.3 RESULTS

5.3.1 Ingested energy, C.

The ingested energy (kJ/day) from different protein content diets used at different feeding regimes in each of the replicates of 12 experiments is shown in Table 5.1.

5.3.2 Oxygen consumption and SDA

The oxygen consumption of fish fed a 20% protein diet at 1, 2 and 3% body weight per day (experiment 2, 3, 4) are shown in Figs. 5.1 and 5.2. Fig 5.1, a to i, shows the SDA effect from the diet at 1% ration on each measurement day. As the pattern of SDA was similar up to the end of the experiment Fig 5.2 summarizes the mean SDA response for the three ration levels used and the daily data are not shown for 2% and 3% rations.

It can be seen from the figures that metabolic rate increased to a certain level after the ingestion of food (shown by an arrow mark). This continued for several hours and then decreased to the routine rate after about 11 to

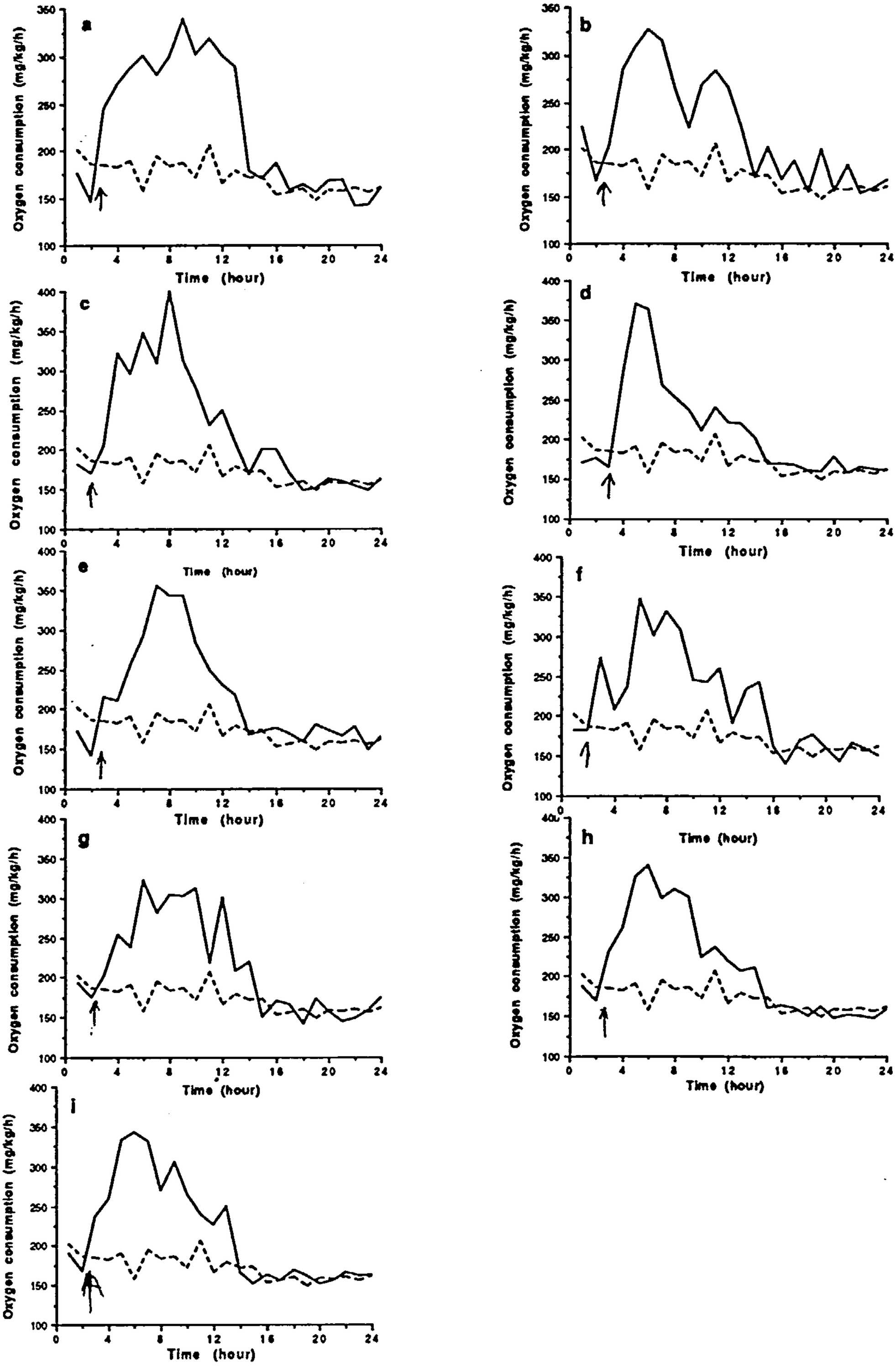


Fig. 5.1 (a - i) Change of oxygen consumption in Cyprinus carpio fed with 20% protein diet at 1% ration level. (a - i) shows changes over routine rate on day 1, 3, 5, 7, 9, 10, 12, 14 and 16, respectively. Values are hourly arithmetic mean n = 9. Legend : dotted line ---- routine rate, solid line ____ total respiration over routine rate Arrows are time of feeding.

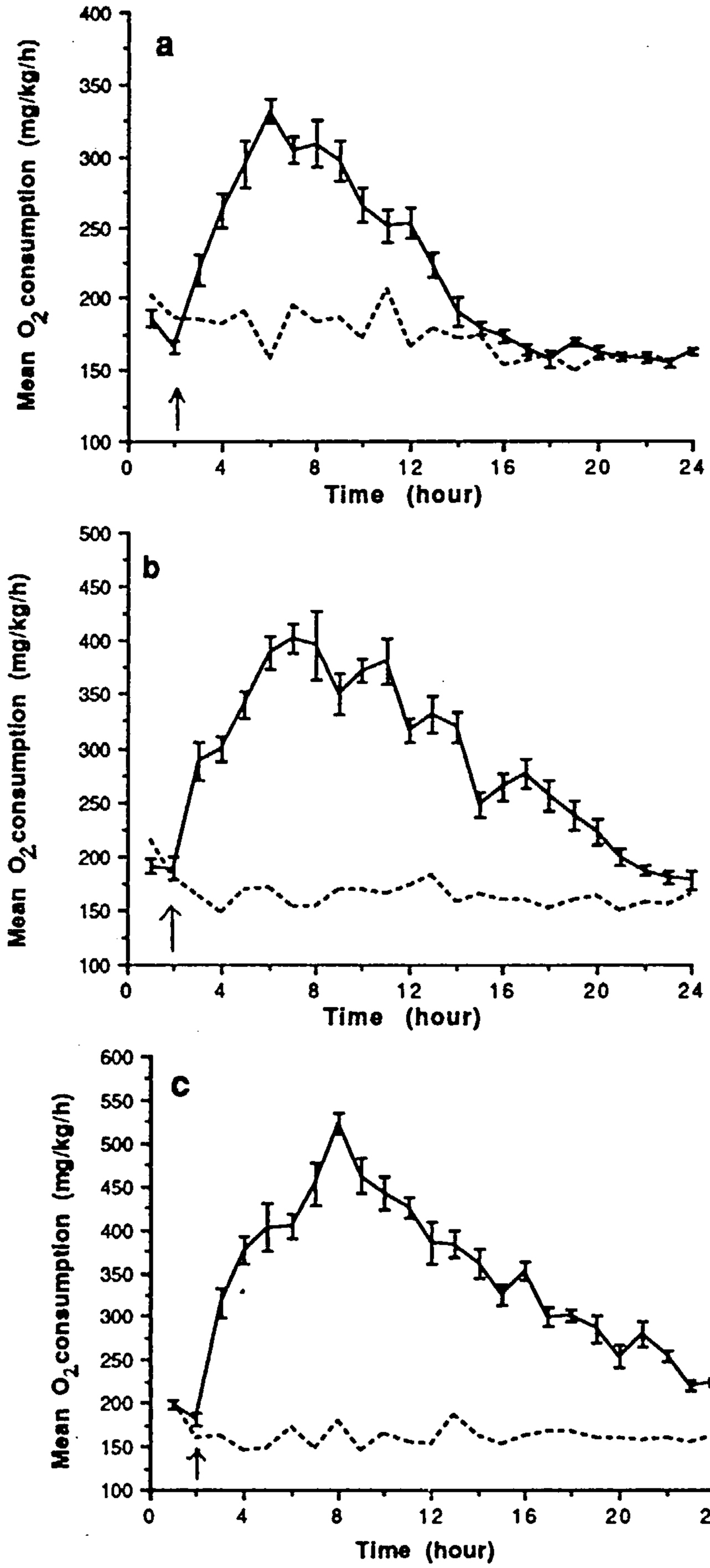


Fig. 5.2 (a - c) Change of hourly mean oxygen consumption in Cyprinus carpio fed with 20% protein diet at (a) 1% ration level, (b) 2% ration level and (c) 3% ration level. Values are hourly arithmetic mean ± SEM, n = 9. Legend : as in Fig. 5.1.

22+ hours showing no further effect of feeding on respiration.

The mean oxygen consumption and SDA effect after feeding fish with the 35% protein diet is shown in fig. 5.3 (experiments 6, 7, and 8). A much higher oxygen consumption was noted with a longer duration than that found with the 20% protein diet. The effects of feeding the 50% protein diet (experiments 10, 11 and 12) are shown in fig. 5.4. A correspondingly higher increase in oxygen consumption with increase of ration levels can be seen than those observed with 20 and 35% protein content diets. At the same time the duration of increased respiratory rate above the routine level also increased.

The mean unfed routine metabolic rates measured in all experiments varied from 153.31 to 171.12 mg/kg/h and these values were not significantly different ($P > 0.05$) (Table 5.2).

The peak value of the SDA response (Table 5.2) increased significantly ($p < 0.05$) with higher ration levels (Fig. 5.5a) and protein content (Fig. 5.5b) in each diet. The peak values ranged from 348.71 to 648.81 mg/kg/h and increased with ration level and dietary protein level. From these peak values, the percent increase of respiration over routine rate due to feeding the different diets were

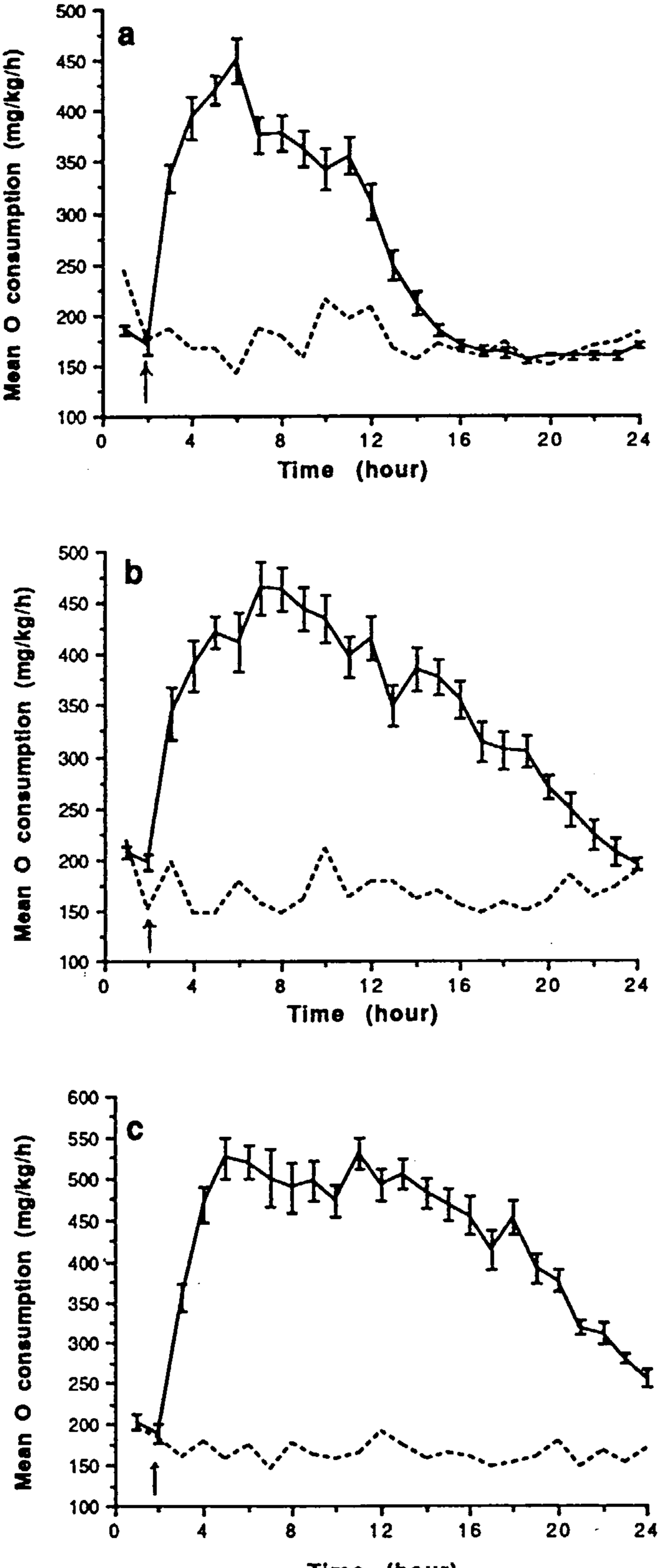


Fig. 5.3 Change of hourly mean oxygen consumption in Cyprinus carpio fed with 35% protein diet (a) fish fed at 1% ration level, (b) fish fed at 2% ration level and (c) fish fed at 3% ration level. Values are hourly arithmetic mean ± SEM, n = 9. Legend : as in Fig. 5.1. Arrow indicates the time of feeding.

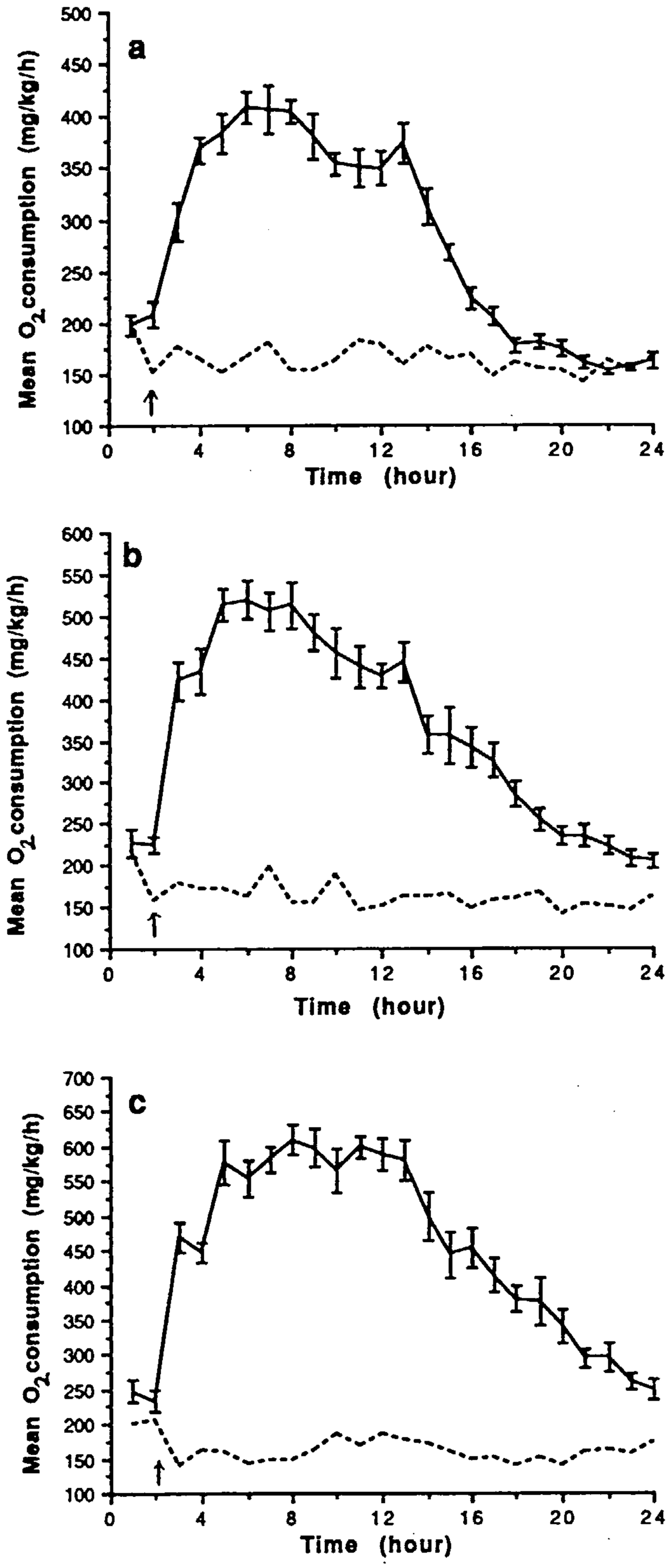
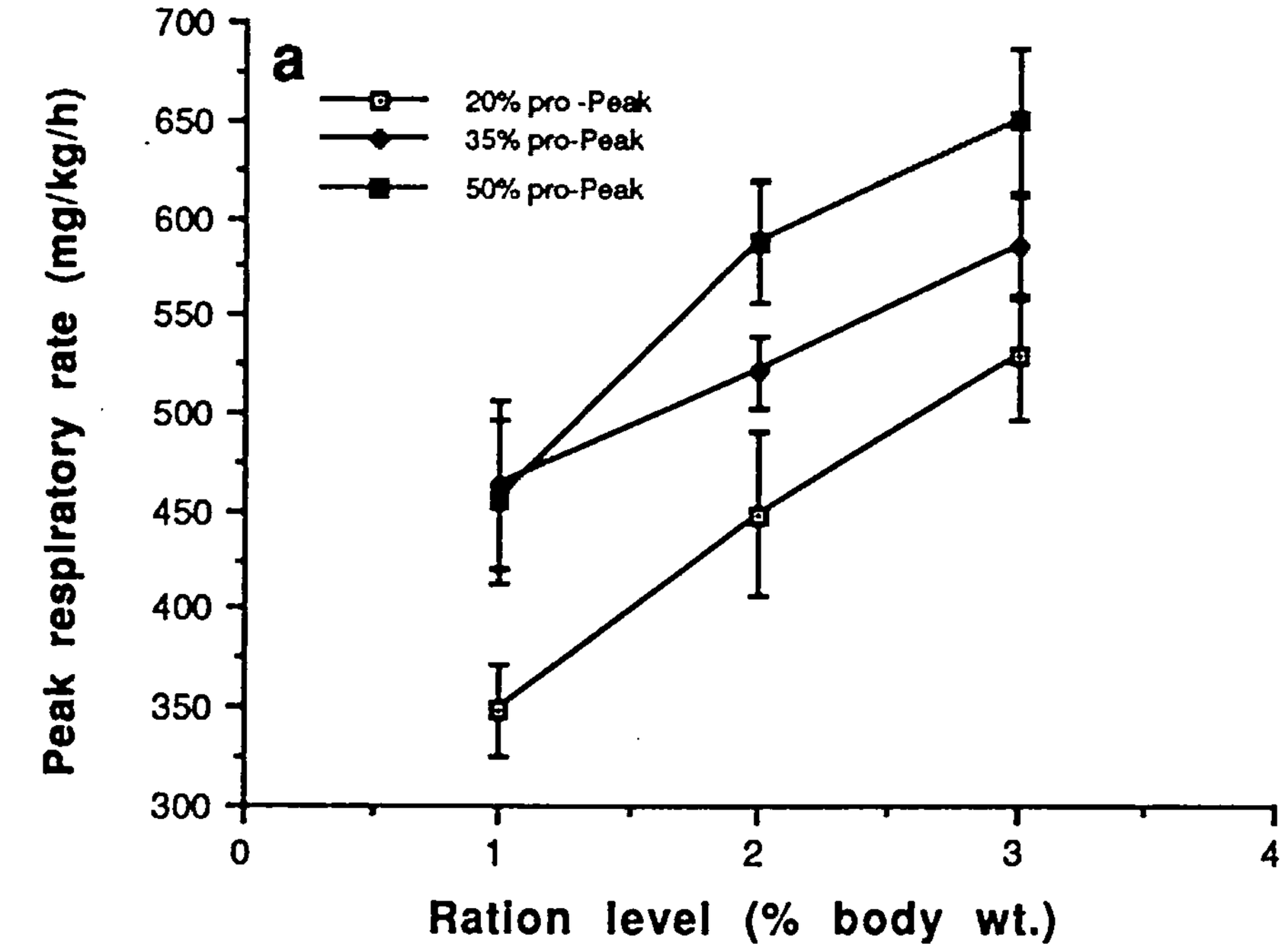


Fig. 5.4 Increase of hourly mean oxygen consumption over routine rate in Cyprinus carpio fed with 50% protein diet (a) fish fed at 1% ration level, (b) fish fed at 2% ration level and (c) fish fed at 3% ration level. Values are hourly arithmetic mean ± SEM, n = 9. Legend : as in Fig. 5.1. Arrow indicates the time of feeding.

Table 5.2 Parameters of the SDA effect of Cyprinus carpio fed 20%, 35% and 50% protein diets at different ration levels. values are arithmetic mean (± SD), n = 9.

Protein level	Ration level	Mean routine rate (unfed) (mg/kg/h)	Mean peak respiratry rate (mg/kg/h)	% increase over routine rate	Time to reach at Peak (h)	Duration above routine rate (h)
20%	1%	171.12 (+_15.95)	348.71 (+_23.46)	104.80 (+_15.37)	4.56 (+_1.16)	11.77 (+_0.79)
	2%	164.06 (+_7.54)	448.26 (+_41.85)	174.56 (+_35.31)	5.67 (+_1.76)	21.33 (+_0.82)
	3%	170.87 (+_13.11)	528.57 (+_31.63)	210.15 (+_22.15)	6.44 (+_0.68)	22 +
35%	1%	153.31 (+_15.48)	463.07 (+_43.56)	213.90 (+_32.31)	3.78 (+_1.03)	14.50 (+_1.22)
	2%	170.48 (+_23.43)	520.71 (+_18.70)	209.71 (+_44.68)	5.89 (+_1.91)	22 +
	3%	163.08 (+_9.75)	585.69 (+_26.89)	260.85 (+-32.23)	5.00 (+-1.88)	22 +
50%	1%	167.18 (+_13.52)	484.80 (+_41.54)	173.07 (+_27.32)	5.55 (+_1.74)	17.88 (+_1.52)
	2%	170.87 (_+19.57)	587.34 (+_30.91)	248.39 (+_44.58)	5.33 (+_1.00)	22+
	3%	158.96 (+_11.72)	648.81 (+_38.34)	310.72 (+_41.29)	6.00 (+_1.94)	22+



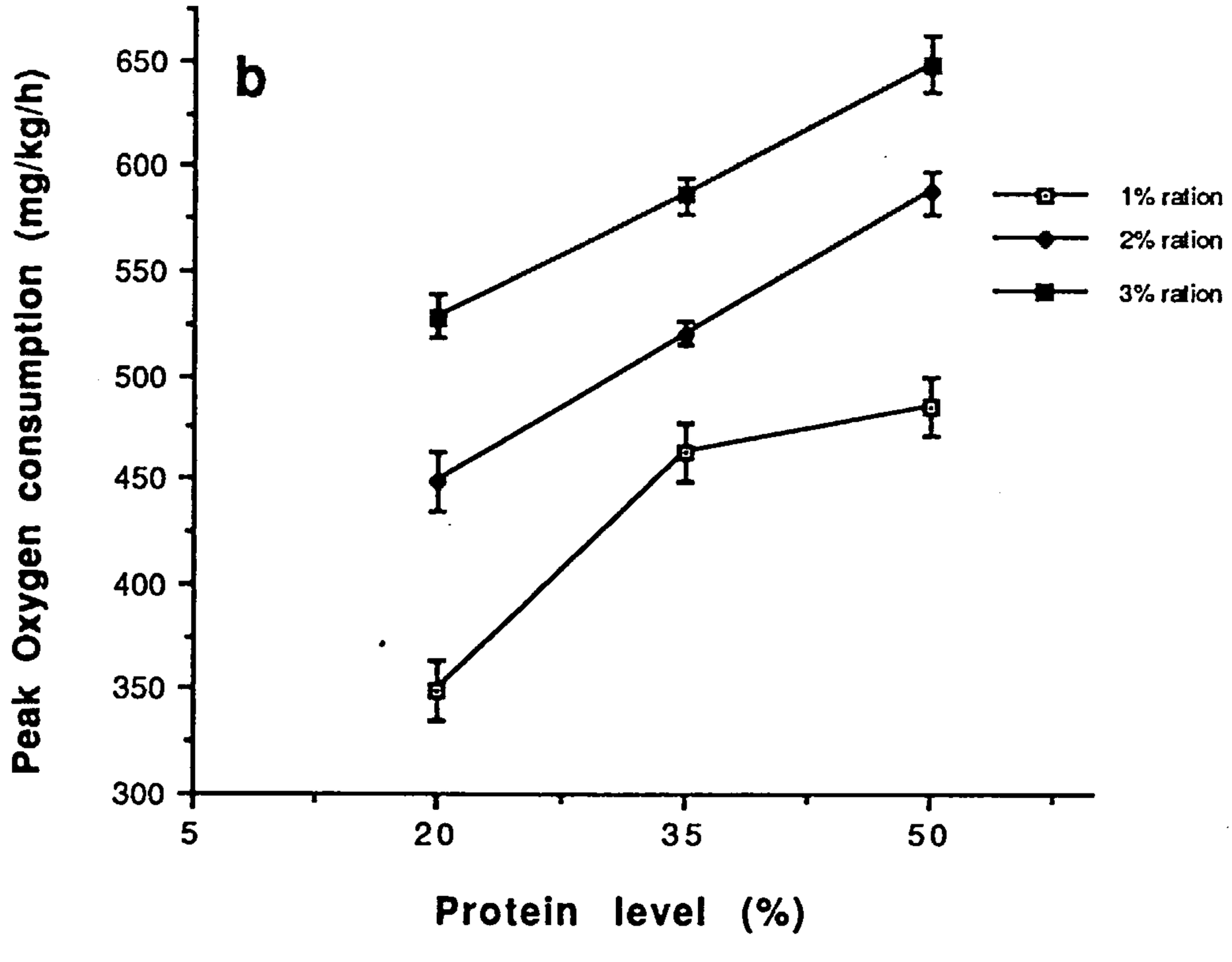


Fig. 5.5 Relationship between changes of (a) ration level and (b) protein level in the diets with mean peak oxygen consumption in Cyprinus carpio.

calculated and were found to range from 104.80 to 310.72%, again increasing with ration level and dietary protein level.

The time to attain these peak values also varied from 4.56 (± 1.16) h for the 1% ration at 20% protein to 6.00 (± 1.99) h for fish fed a 3% ration at 50% protein diet (Table 5.2). But this variation did not appear to follow any quantitative pattern. The duration of increased metabolic rate over routine rate also varied depending on ration level and dietary protein content, although for practical reasons this was not quantified beyond 22 h.

Fig.5.6 a, b, c summarises the daily mean oxygen consumption rates at different ration levels. It can be seen that the rate of increases with dietary protein level and does not markedly vary over the experimental period. The mean oxygen consumption values for fish fed with different diets at each different ration level were significantly different ($p<0.05$) from each other (Table 5.3). Thus, the mean metabolic rate were positively correlated with the protein content of the diet (corr. coeff. = 0.943, 0.887 and 0.998 for 1, 2 and 3% ration level respectively). These data are replotted to show the effect of different ration levels for each dietary protein content (Fig. 5.7). The daily mean metabolic rate at 1, 2 and 3% ration level increased significantly ($p < 0.05$) with each diet and was

Table 5.3 Daily mean metabolic rate during experiments with Cyprinus carpio fed on diet with different protein. Values are Arithmatic mean +_SEM, n = 24.

Ration level	Unfed	1%	2%	3%	Corr. coeff r
	Metabolic rate (mg/kg/h)				
20% protein	166.13 (+_5.60)	216.33 (+_4.92)	284.97 (+_5.26)	337.34 (+_5.70)	0.905
35% protein	164.20 (+_7.78)	257.42 (+_6.95)	338.87 (+_ 7.86)	414.67 (+_ 13.77)	0.996
50% protein	157.74 (+_6.08)	275.58 (+_9.45)	356.90 (+_14.73	445.57 (+_20.15)	0.998
Corr. Coeff. (r)		0.943	0.887	0.998	

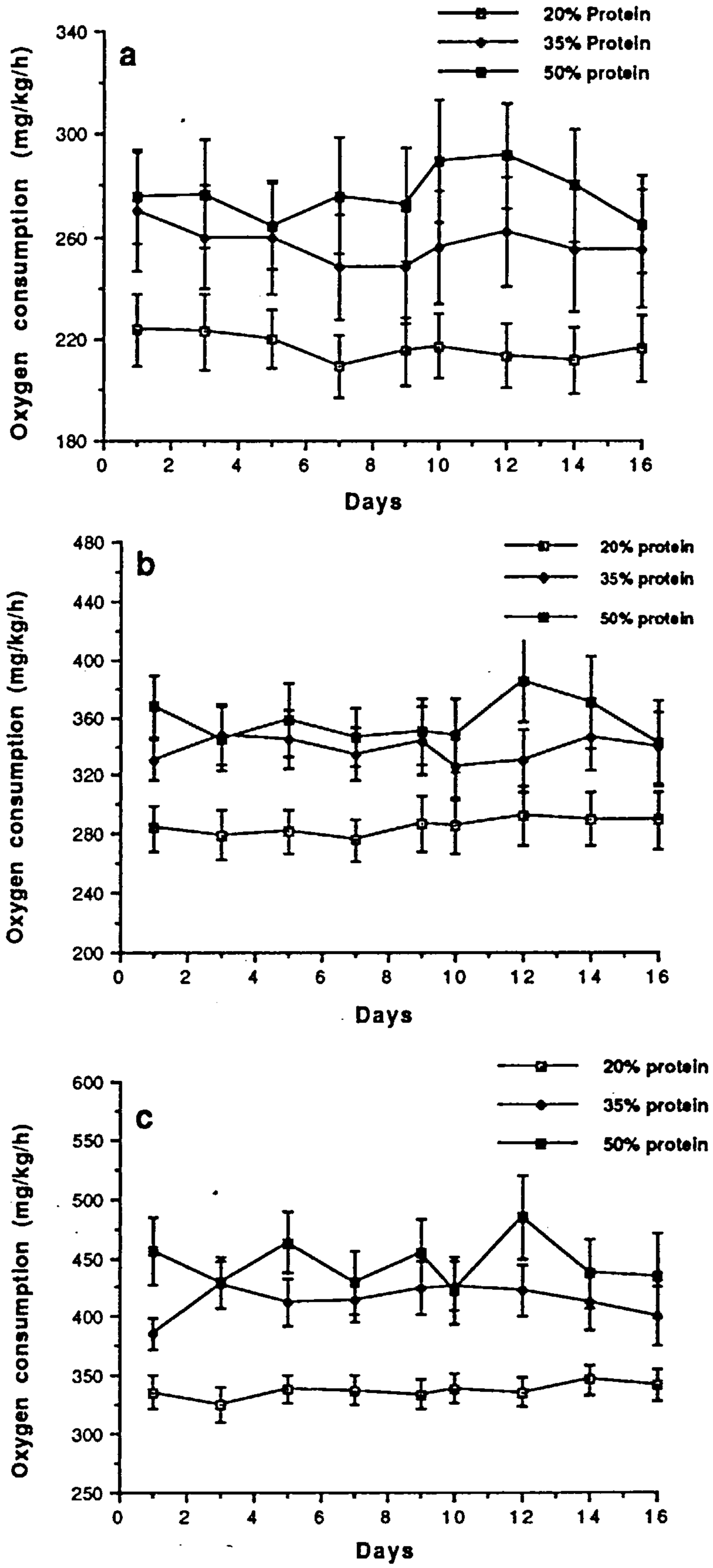


Fig. 5.6 (a - c) Daily mean metabolic rate in Cyprinus carpio fed with different protein diet (a) 1% ration level, (b) 2% ration level, (c) 3% ration level. Values are arithmetic mean +_ SEM, n = 24.

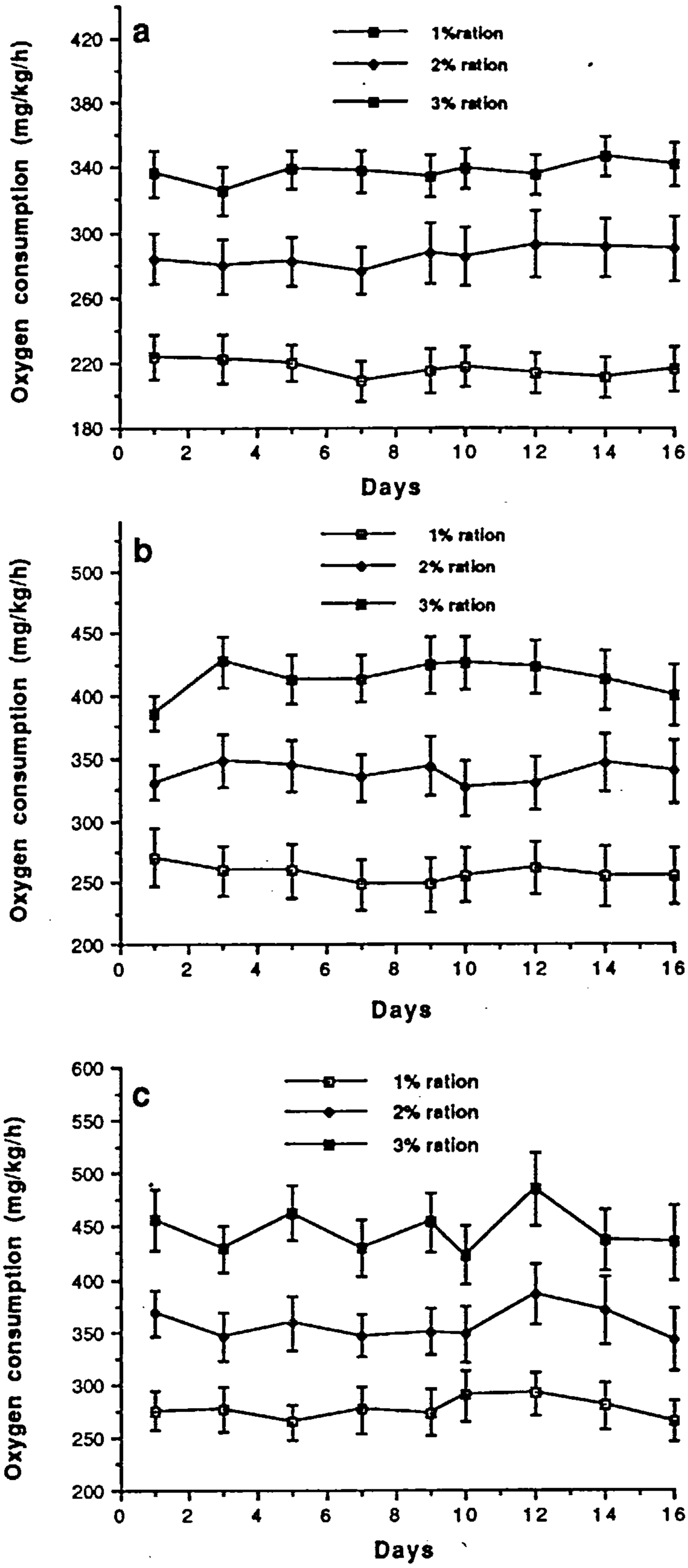


Fig. 5.7 Effect of ration level on oxygen consumption in Cyprinus carpio (a) with 20% protein diet, (b) with 35% protein diet and (c) with 50% protein diet. Values are arithmetic mean +_ SEM, n =

positively correlated with the ration levels (corr. coeff. 0.905, 0.996 and 0.998 for 20, 35 and 50% dietary protein respectively).

Table 5.4 summarises the routine metabolic rate as hourly mean oxygen consumption in the control fish experiments 1, 5, and 9, during the total experimental period. No significant variation ($p > 0.05$) was found in oxygen consumption during this period.

Oxycalorific Coefficient (Q_{ox})

The oxycalorific coefficient (or the oxycaloric or oxycalorific equivalent or the energy equivalent) (Q_{ox}) is the number of joules (J) lost as heat per mg oxygen consumed. This varies depending on the substrate being metabolized. Brafield and Llewellyn (1982) adopted Q_{ox} values for carbohydrate, lipid, and protein metabolism in fish of 14.76, 13.72, and 13.36 J/mg O_2 consumed, and these values were used in this study.

Since the fish feeds used were a mixture of protein, fat and carbohydrate, then a weighted, overall, Q_{ox} was computed for each diet. For example, if in a fish feed protein, fat, and carbohydrates are in the proportion 3 : 1 : 6, then the weighted Q_{ox} is :

Table 5.4 Rate of Routine Respiration in Cyprinus carpio during different experiments.

Days of obs	Expt (1) (mg/kg/h)	Energy (kJ/kg/day)	Expt (5) (mg/kg/h)	Energy (kJ/kg/day)	Expt(9) (mg/kg/h)	Energy (kJ/kg/day)
0	166.63 (+_15.58)	54.23	180.61 (+_21.68)	58.78	166.30 (+_16.04)	54.12
1	174.00 (+_20.26)	56.63	173.37 (+_30.78)	56.42	159.21 (+_33.22)	51.81
3	176.42 (+_19.54)	57.41	163.55 (+_16.57)	53.22	162.70 (+_23.56)	52.95
5	162.33 (+_11.61)	52.83	157.80 (+_9.87)	51.35	169.12 (+_27.80)	55.03
7	165.97 (+_17.02)	54.01	163.80 (+_19.68)	53.30	156.82 (+_10.38)	51.03
9	164.17 (+_18.60)	53.42	159.19 (+_10.45)	51.80	155.58 (+_14.21)	50.63
10	168.94 (+_21.72)	54.98	160.22 (+_11.45)	52.14	152.27 (+_14.78)	49.55
12	162.47 (+_22.87)	52.87	156.30 (+_11.83)	50.86	149.43 (+_20.99)	48.63
14	159.92 (+_11.25)	52.04	168.39 (+_19.08)	54.80	153.78 (+_15.49)	50.04
16	160.45 (+_10.24)	52.21	158.78 (+_11.83)	51.67	152.21 (+_17.51)	49.53
Mean	166.13 (+_5.56)	54.06 (+_1.81)	164.20 (+_7.78)	53.43 (+_2.54)	157.74 (+_6.5)0	51.33 (+_2.11)

(13.36 X 3) + (13.72 X 1) + (14.72 X 6)/10 = 14.21 J per each mg oxygen consumed.

An appropriate O_{ox} was calculated by this method for each experimental diet (Appendix I) giving values of 14.34, 14.077, and 13.79 J/mg O_2 for the 20, 35, and 50% protein diets respectively. For starving fish respiring its own tissue, a O_{ox} value of 13.56 J/mgO_2 (Brett and Groves, 1979) has been accepted.

It is assumed that the respiratory substrates were respired in proportions in which they were ingested. This is probably true in case of carbohydrate (glucose) but may not be so in fish fed a protein and fat diet where the amount of dietary fat approaches 30% of the ingested food. The unassimilated fat may alter the O_{ox} used for the diet (Musisi, 1984). However, in the present study the three fish feeds contained only 8% lipid. So it can be assumed that there was no significant effect on O_{ox} at this lipid content. But for the protein substrate the total energy consumed was corrected by reference to the absorption efficiency (AE), which was derived from analysis of the faeces.

Respiratory energy loss, 'R'

Daily mean oxygen consumption in the control, unfed, groups was 166.13 (+_5.56), 164.20 (+_7.78), and 157.74 (+_

6.50) mg/kg/h in experiment 1, 5, and 9 respectively. The energy lost in routine metabolism was calculated using a Q_{ox} value of 13.56 J/mgO_2 and these data were shown in Table 5.4. The energy needed for routine metabolism was 54.06 (+_1.81), 53.43 (+_2.54), and 51.33 (+_2.11) kJ/kg/day in experiments 1, 5, and 9 respectively and these values were not significantly different ($p > 0.05$).

The mean oxygen consumption rates from the different experiments are compiled in Appendix II and the equivalent energy (kJ) expended by the fish was obtained by applying a suitable Oxycalorific Coefficient (Q_{ox}),

As may be expected, the daily ingested energy resulting from the 20, 35 and 50% protein diet had a significantly different ($p < 0.05$) heat of metabolism 'R' which was related directly to protein in the diet (Fig. 5.8a).

The energy lost as heat of metabolism 'R' (kJ) when recalculated as a percentage of 'C' was found to increase significantly ($p < 0.05$) with protein levels in the diet but decreased markedly with increasing ration level (Fig. 5.8b).

The relationship between respiratory energy loss, dietary protein level and ration level in the experiment is given by the following regression model :

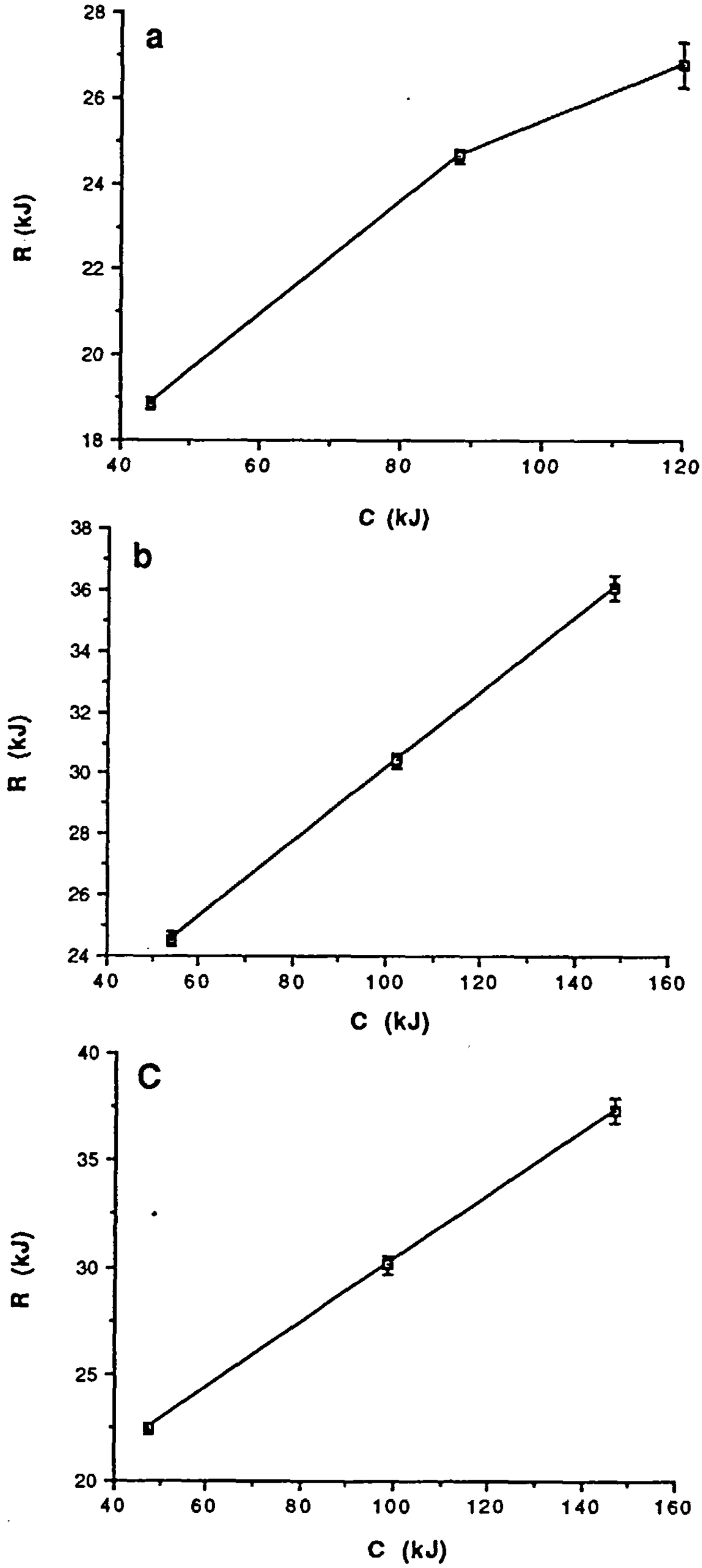


Fig. 5.8a Daily energy ingestion (C) from feed of different ration level and metabolic loss (R) with (a) 20% protein diet, (b) 35% protein diet and (c) 50% protein diet. Values are arithmetic mean +_ SEM, n = 9.

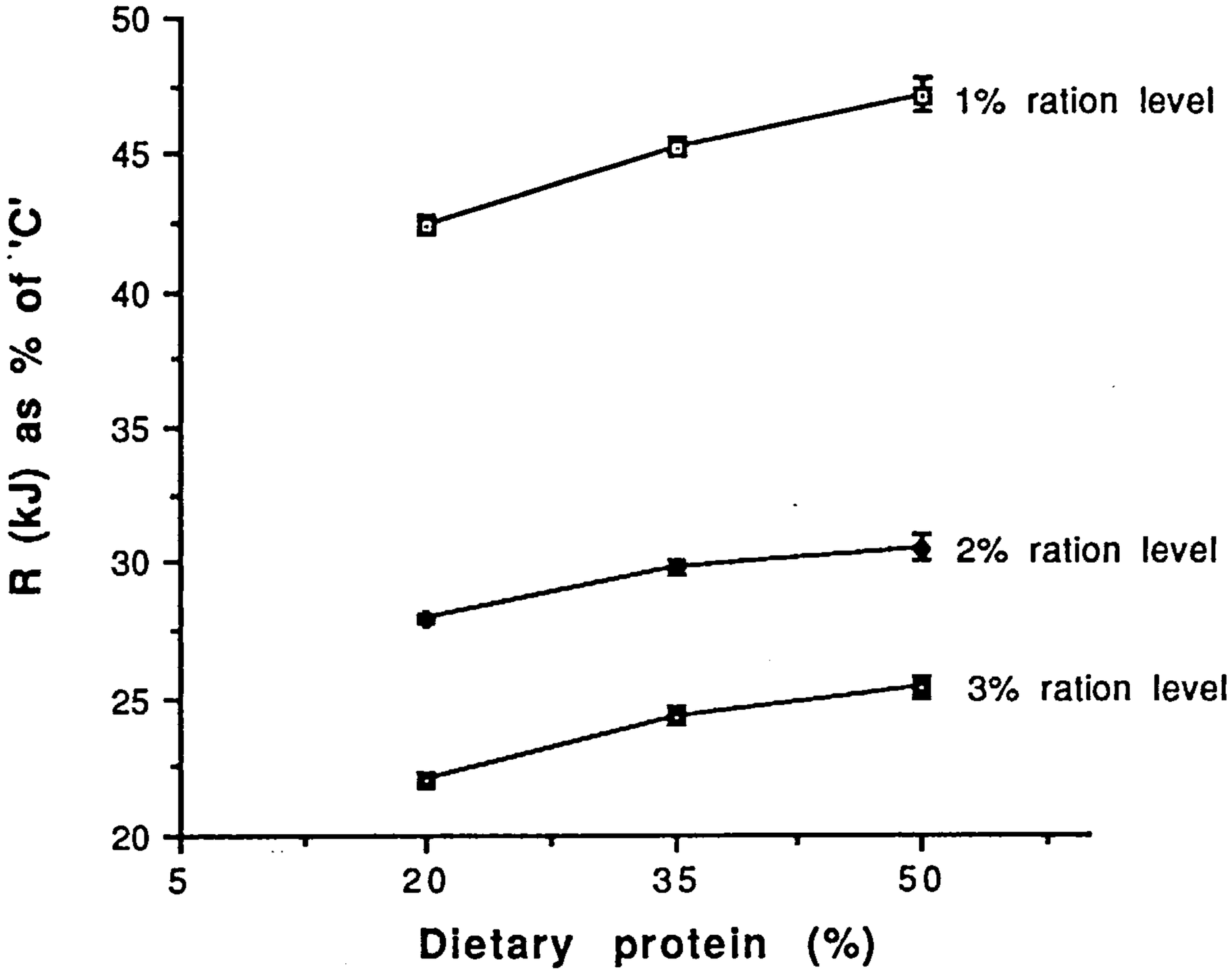


Fig. 5.8b Relationship between energy lost as heat of metabolism 'R' as a percent of consumption 'C' in response to different dietary protein at different ration levels.

$$R = 49.6 + 0.119\ P - 10.5\ r \quad \ldots\ldots\ldots \quad p < 0.05$$

where, R = respiratory energy loss expressed as percentage of 'C'; P = dietary protein level (within the range of 20 - 50%); r = ration level (within 1 - 3% body weight of fish).

5.3.3 Faeces, 'F'

The amount of faeces voided by fish in each experiment was directly related to the amount of feed ingested (Fig. 5.9). Thus, fish fed with 1, 2 and 3% ration level of 20% protein diet (experiments 2, 3 & 4) produced significantly different ($p < 0.05$) amounts of faeces of 0.73, 1.35, and 1.92 g respectively. Similar effects were seen with the other diets. (Values of faecal production are given in Appendix III). The faecal energy (F) varied from 9.55 kJ/day at 1% ration with 20% dietary protein to 29.21 kJ/day at 3% ration with 50% protein diet.

Daily ingestion (C) of 44.45, 54.15 and 47.49 kJ (experiments 2, 6 and 10) produced a faecal energy loss (F) of 9.55, 10.34 and 9.60 kJ, respectively, showing no statistically significant ($p > 0.05$) relationship between faecal energy and dietary protein level (Table 5.5). However, faecal energy increased significantly ($p < 0.05$) with ration levels ($r = 0.984$). For example, with the 20%

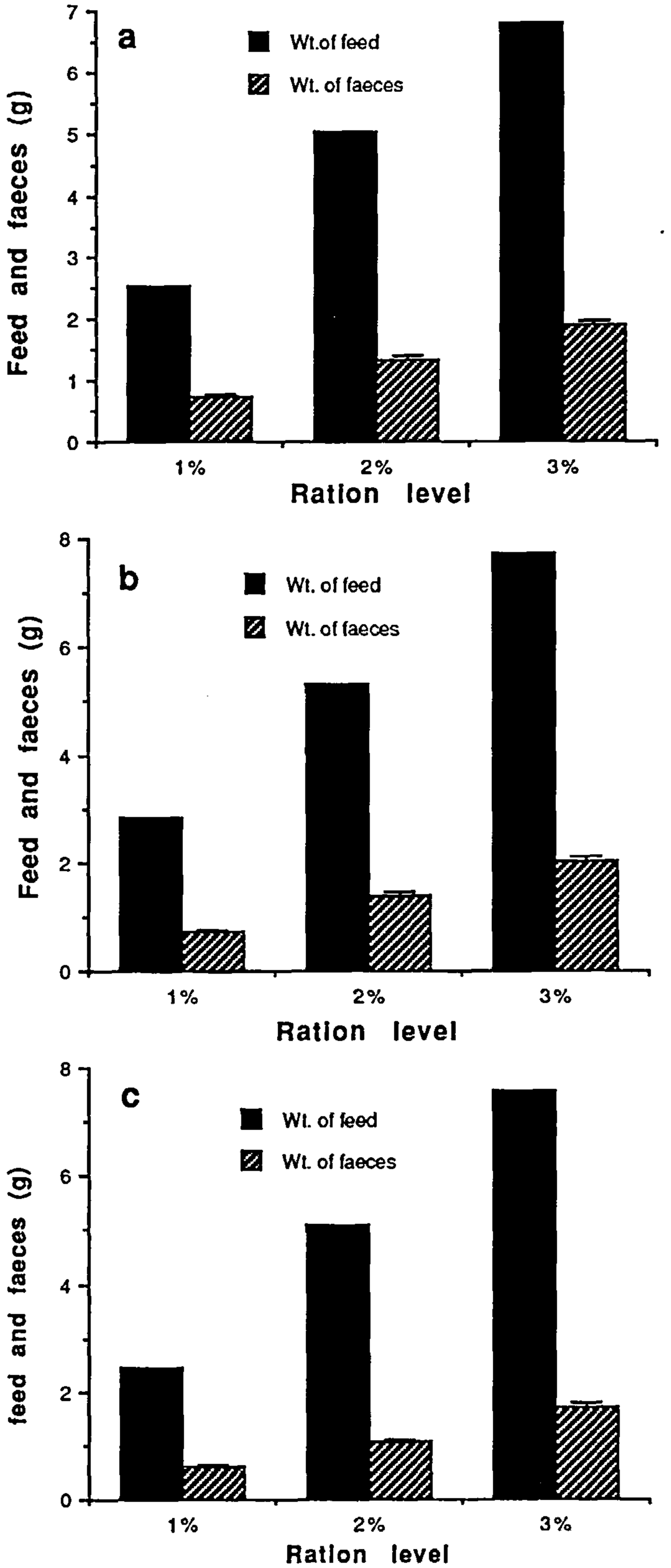


Fig. 5.9 Relationship between the amount of feed ingested with amount of faeces produced at different ration level with diets containing (a) 20% protein, (b) 35% protein and (c) 50% protein. Values are arithmetic mean +_SEM, n = 9.

Table 5.5 Energy of faecal loss (F) and consumption (C). Values are arithmetic mean (+_SD), n = 9.

Expt. No.	protein level (%)	Ration level (%)	C (kJ/day)	Energy in faeces (kJ)	Energy value of faeces (kJ/g)	'F' as % of'C
2	20	1%	44.45	9.55(+_1.14)	13.15(+_0.54)	21.38(+_3.36)
3	20	2%	88.19	20.22(+_2.84)	14.96(+_0.96)	21.78(+_3.28)
4	20	3%	119.87	28.78(+_2.70)	15.00(+_0.48)	24.00(+_2.25)
6	35	1%	54.15	10.34(+_1.86)	14.09(+_0.75)	19.10(+_2.07)
7	35	2%	102.13	19.75(+_1.88)	14.39(+_1.18)	19.34(+_1.84)
8	35	3%	148.03	27.97(+_1.71)	13.94(+_0.63)	18.89(+_2.09)
10	50	1%	47.44	9.60(+_1.83)	15.95(+_0.39)	20.24(+_3.87)
11	50	2%	98.74	19.18(+_2.34)	18.01(+_0.63)	19.42(+_2.36)
12	50	3%	146.96	29.21(+_3.64)	16.95(+_0.77)	19.88(+_2.48)

protein diet, ingested energy (C) of 44.45, 88.19 and 119.87 kJ/day (experiment 2, 3, 4) produced a faecal energy loss (F) of 9.55, 20.22 and 28.78kJ respectively (Table 5.5).

'F' as percent of 'C' was around 19 to 24% (Table 5.5) and did not appear to vary significantly ($P > 0.05$) with either dietary protein or ration level. The correlation coefficient 'r' for 20, 35 and 50% protein diet were 0.347,-0.277 &-0.024 respectively and the correlation coefficient for 1, 2 & 3% ration diet were -0.023, -0.470 & 0.055 respectively.

Apparent Protein Digestibility (%)

Fish fed with higher protein level diets voided more protein in the faeces (fig. 5.10) but this did not appear to be affected by ration level. Apparent protein digestibility (APD) for the different protein diets used in feeding trials at 1, 2 and 3% ration levels have been summarized in Table 5.6 (for details see Appendix IV). The mean APD (%) in fish fed with diets containing 20% protein were significantly lower ($p < 0.05$) than with the 35% and 50% protein diet. Thus the 20% protein diet at 1% ration level had a APD of 71.43 (+_0.72)%, significantly lower ($p < 0.05$) than that with 35 and 50% content protein diet. There was no significant effect of ration level with the 20% protein diet.

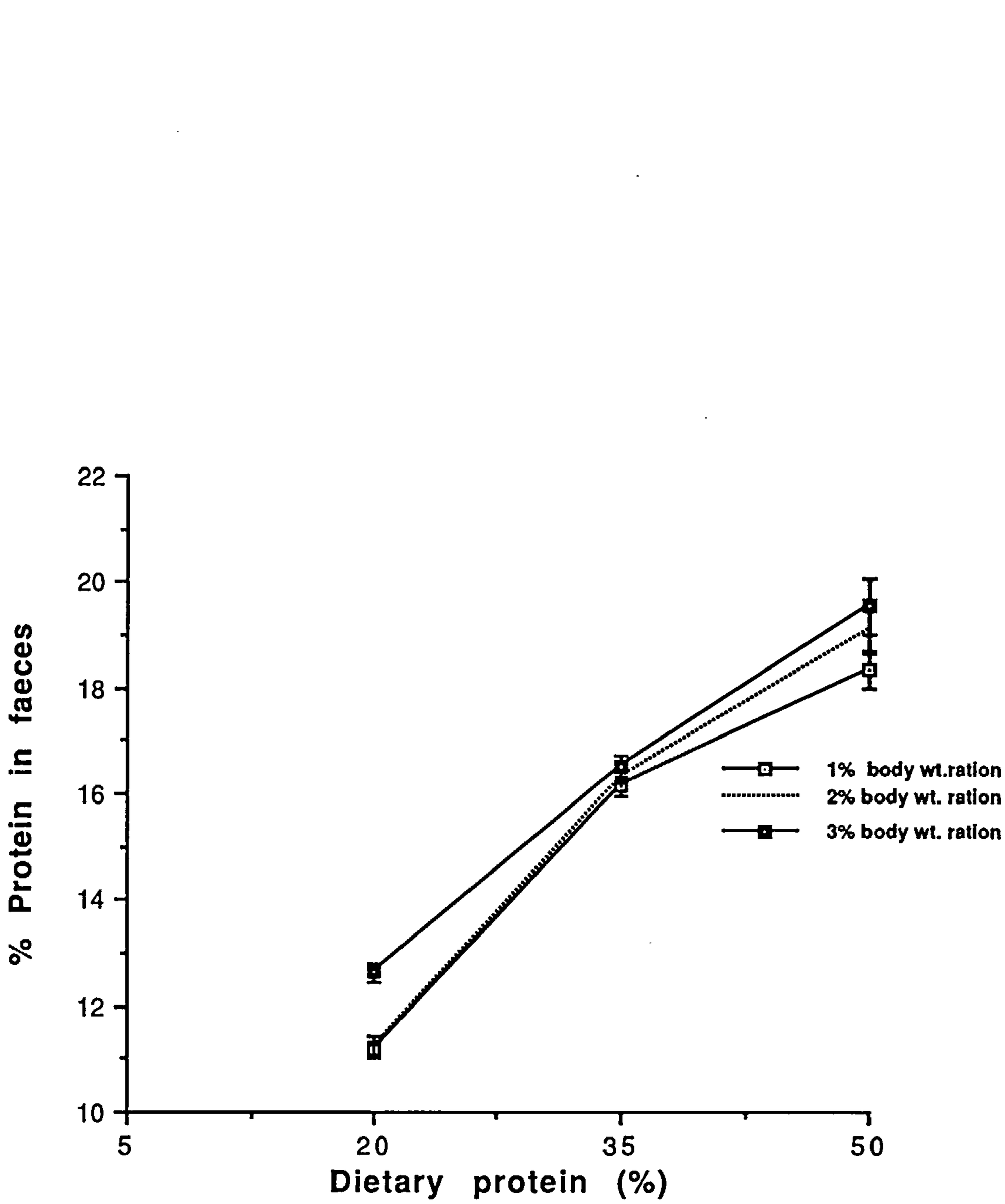


Fig. 5.10 Percent protein voided in faeces after ingestion of diet containing different protein fed by Cyprinus carpio at different ration level. Values are arithmetic mean +_SEM, n = 9.

Table 5.6 Apparent protein Digestibility in Cyprinus carpio fed with different protein diets at different ration levels. (values from Appendix IV)

Ration level (% body weight)	Expt. No.	Protein level in diet (%)	Apparent Protein Digestibilty (%)
	2	20	71.43 (+_ 0.72)
1	6	35	80.40 (+_ 1.08)
	10	50	81.83 (+_ 2.44)
	3	20	71.59 (+_ 1.01)
2	7	35	80.75 (+_ 1.10)
	11	50	82.12 (+_ 2.45)
	4	20	71.74 (+_ 1.85)
3	8	35	80.86 (+_ 1.91)
	12	50	82.72 (+_ 2.99)

The comparatively higher APD values in both 35 and 50% protein diet at same ration level did not differ significantly ($p > 0.05$) from each other or between ration level.

Assimilation efficiency (AE)

All the energy ingested was not used up by the fishes because it was not assimilated. The values for AE (expressed as percentage) were determined from the following formula :

$$AE = [(C - F) / C] /100$$

where, C = Energy of food

F = Energy of faeces

Values for assimilation efficiencies has been calculated from Appendix III and are shown in Table 5.7 in which the AE values ranged from 74.09 (+_ 3.65) percent with 20% protein diet at 2% ration level to 81.80 (+_2.17) percent with 35% protein at 3% ration level.

AE for the 20% protein diet was consistently lower than the other two diets. The AE for the three diets did not change significantly ($p > 0.05$) with ration level.

The relationship between assimilation efficiency (AE) with dietary protein is given by the following regression equation:

$$AE = 74.2 + 0.137\ P \quad \text{.....} \quad p < 0.05$$

Where, AE = assimilation efficiency expressed as percentage; P = dietary protein level (20 - 50% range)

5.3.4 **Nitrogenous excretion (U) and energy calculation.**

Only ammonia was measured as nitrogenous excretion from water flowing from the metabolism chambers. The 24 hr-mean ammonia excretion in unfed fish (control) in experiment 1, 5 and 9 were very similar and showed no significant variation with time (Fig. 5.11). The mean daily excretory rates were 5.04 (+_0.63), 5.20 (+_0.50) and 4.94 (+_0.33) mg/kg/h in experiment 1, 5 and 9 respectively (Table 5.8).

The hourly ammonia excretion for fed fish over the experimental period was converted into mg/kg/h and is represented in a series of figures which resemble the response in oxygen consumption after feeding. Generally, fish fed with different protein diets showed an increase of nitrogenous excretion over the unfed condition (endogenous nitrogen excretion). Feeding the 20% protein

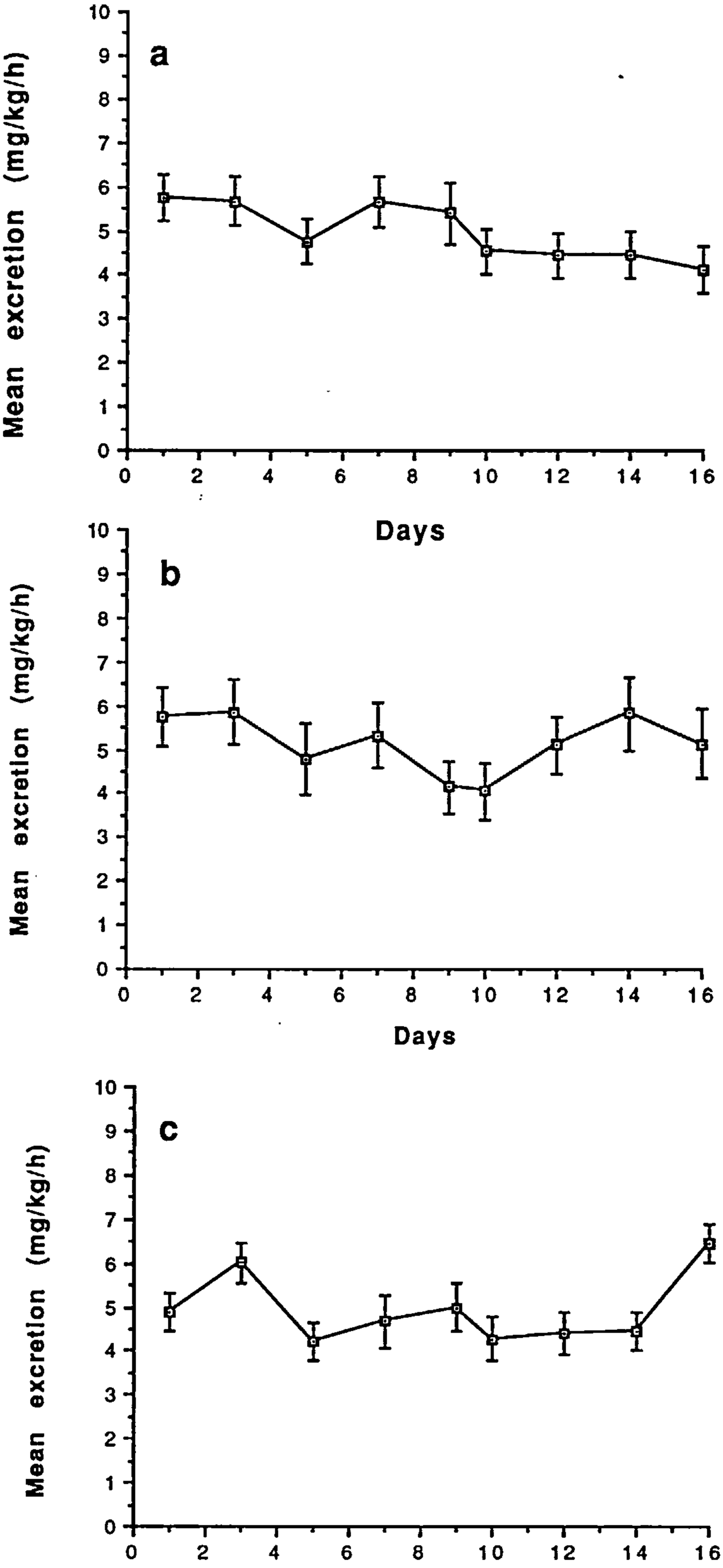


Fig. 5.11 Mean daily variation of excretion (NH_3 - nitrogen) of Cyprinus carpio in unfed condition in (a) experiment 1 (b) experiment 5 and (c) experiment 9. Values are arithmetic means +_ SEM, n = 24.

Table 5.8 Ammonia Excretion (U) for unfed Cyprinus carpio during experiments.

Days of obs.	Experiment 1 (mg/kg/h)	Equiv. energy (kJ/tank/d)	Experiment 5 (mg/kg/h)	Equiv. energy (kJ/tank/d)	Experiment 9 (mg/kg/h)	Equiv. energy (kJ/tank/d
0	5.61 (+_1.36)	0.84	6.13 (+_1.81)	0.86	5.03 (+_1.40)	0.74
1	5.75 (+-1.60)	0.86	5.73 (+_2.04)	0.80	4.90 (+_1.39)	0.72
3	5.66 (+_1.63)	0.85	5.86 (+_2.30)	0.82	6.02 (+_1.44)	0.89
5	4.76 (+_1.54)	0.71	4.77 (+_2.44)	0.67	4.20 (+_1.30)	0.62
7	5.64 (+_1.71)	0.84	5.33 (+_2.19)	0.75	4.67 (+_1.77)	0.69
9	5.39 (+_2.09)	0.81	4.15 (+_1.84)	0.58	5.00 (+_1.68)	0.74
10	4.54 (+_1.50)	0.68	4.05 (+_1.98)	0.42	4.28 (+_1.52)	0.63
12	4.44 (+_1.54)	0.66	5.10 (+_1.94)	0.86	4.40 (+_1.41)	0.65
14	4.47 (+_1.59)	0.67	5.82q (+_2.48)	0.82	4.46 (+_1.32)	0.66
16	4.10 (+_1.59)	0.61	5.13 (+_2.30)	0.73	6.46 (+_1.39)	0.95
Mean, $\bar{X}$=	5.04 (+_0.63)	0.75 (+_0.10)	5.20 (+_0.71)	0.73 (+_0.14)	4.94 (+_0.75)	0.73 (+_0.11)

content diet at 1% ration level in experiment 2 (Fig. 5.12a - i) resulted in an increase of excretory rate over the following 24 hr period. The rate of excretion started to increase soon after feeding the fish and continued for several hours eventually falling to the unfed level.

The increase in mean hourly excretory rate over the unfed rate when fed 1, 2 and 3% rations (experiment 2, 3 and 4) is shown in Fig. 5.13 and it can be seen that both of the peak rate of excretion and duration of this increased rate depends on the ration level in the diet. A similar increase in excretory rate and the duration of the response with ration level in fish was seen with the 35% protein diet (experiments 10, 11 and 12); the response was significantly higher than with the 20% protein diet and increased with increase of ration level (Fig. 5.14). With 50% protein diet a much higher excretion than those at 20 and 35% protein diet was observed, again, increasing with increasing ration size (Fig. 5.15).

There was no significant difference ($p > 0.05$) between mean daily variation in excretion (U) in fish fed with same ration diet (Fig 5.16). However, the mean ammonia excretion rates were significantly different ($p < 0.05$) from each other with different ration levels and with dietary protein levels (Figs. 5.16a - c, 5.17a - c) both factors causing an increase (for details see Appendix V).

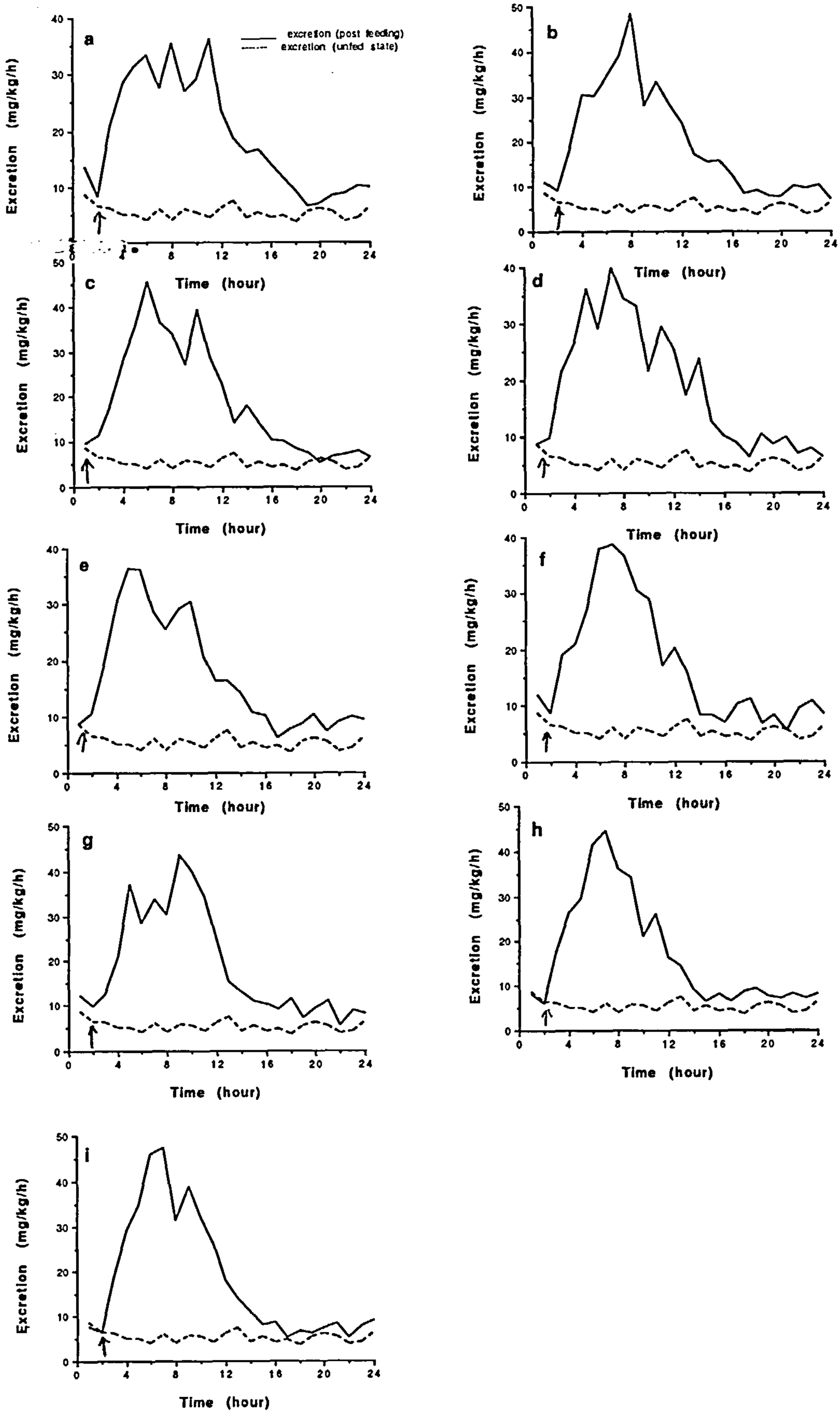


Fig. 5.12 (a - i) Hourly change of excretion (NH_3 - nitrogen) of Cyprinus carpio fed with 20% protein diet at 1% ration level. (a - i) shows changes of nitrogen excretion during the days - 1, 3, 5, 7, 9. 10, 12, 14, and 16 respectively. Feeding started at 10'0 clock (10.00 hr) morning. Arrow indicates the time of feeding.

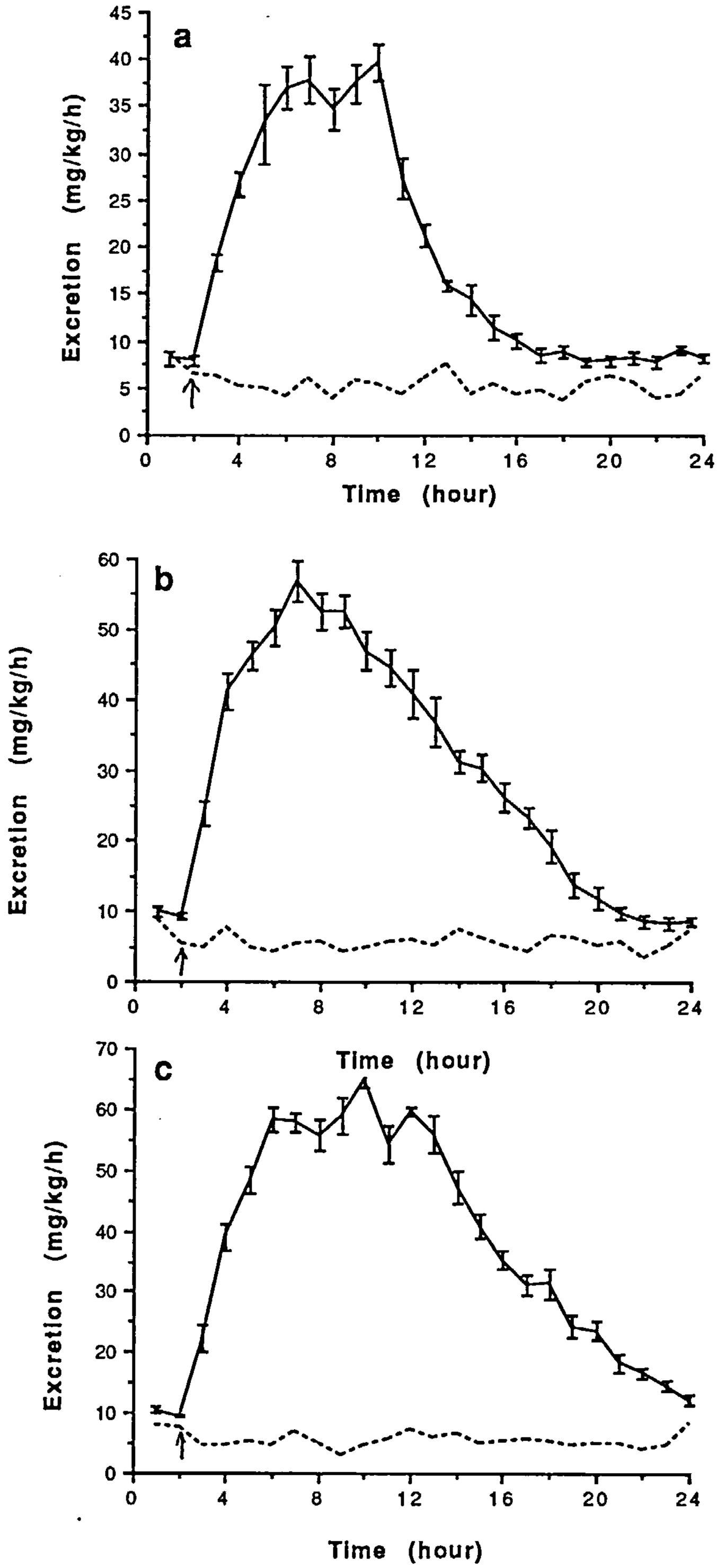


Fig. 5.13 Changes in hourly mean ammonia excretion of Cyprinus carpio fed 20% protein at (a) 1 percent ration level, (b) 2 percent ration level and (c) 3 percent ration level. Values are arithmetic means +_SEM, n = 9. Arrows indicate the time of feeding.

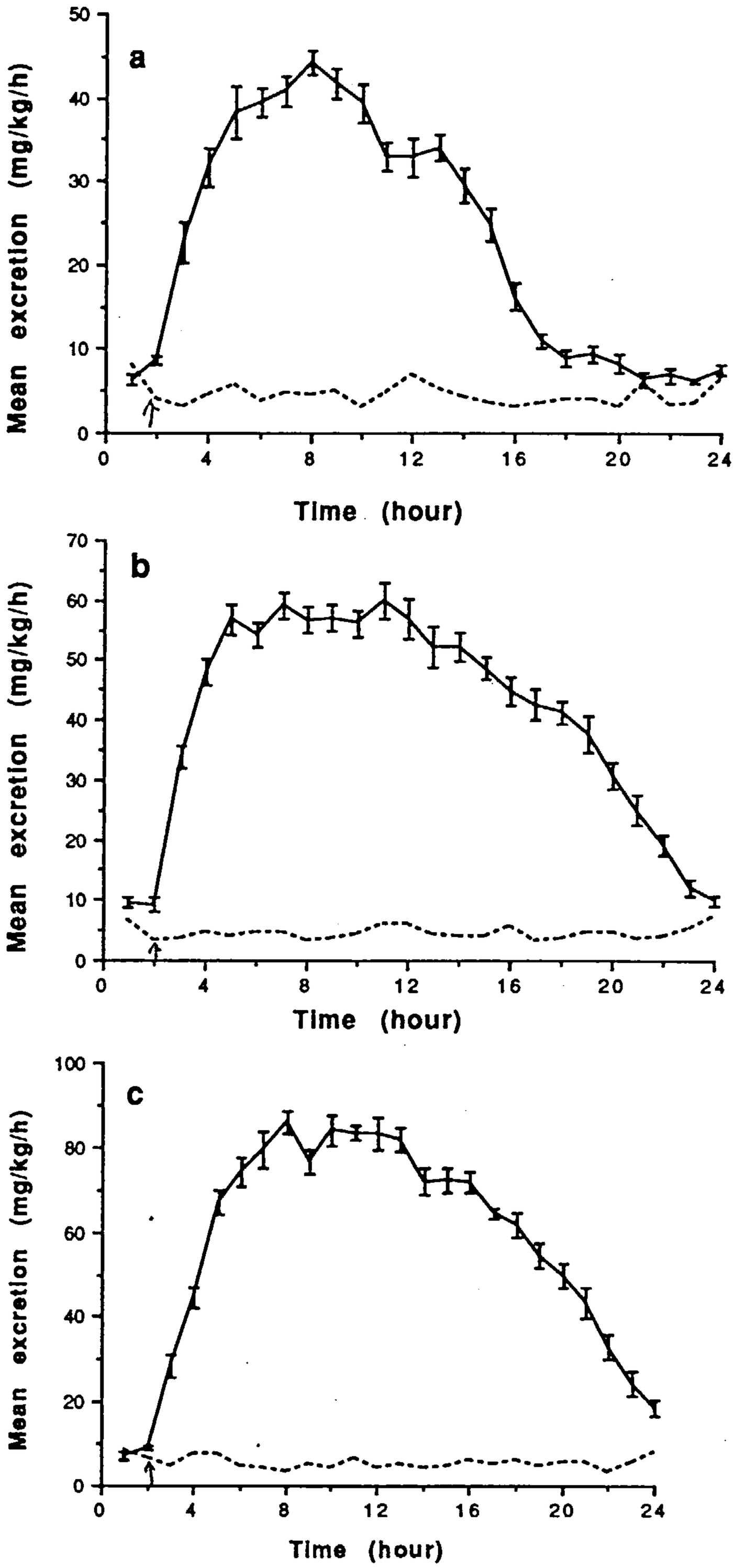


Fig. 5.14 Hourly mean variation (24 hr observation) of excretion of Cyprinus carpio fed with 35% protein diet at (a) 1% ration level, (b) 2% ration level and (c) at 3% ration level. Values are hourly arithmetic mean ± SEM, n = 9. Legend : as in Fig. 5.1. Arrow indicates the time of feeding.

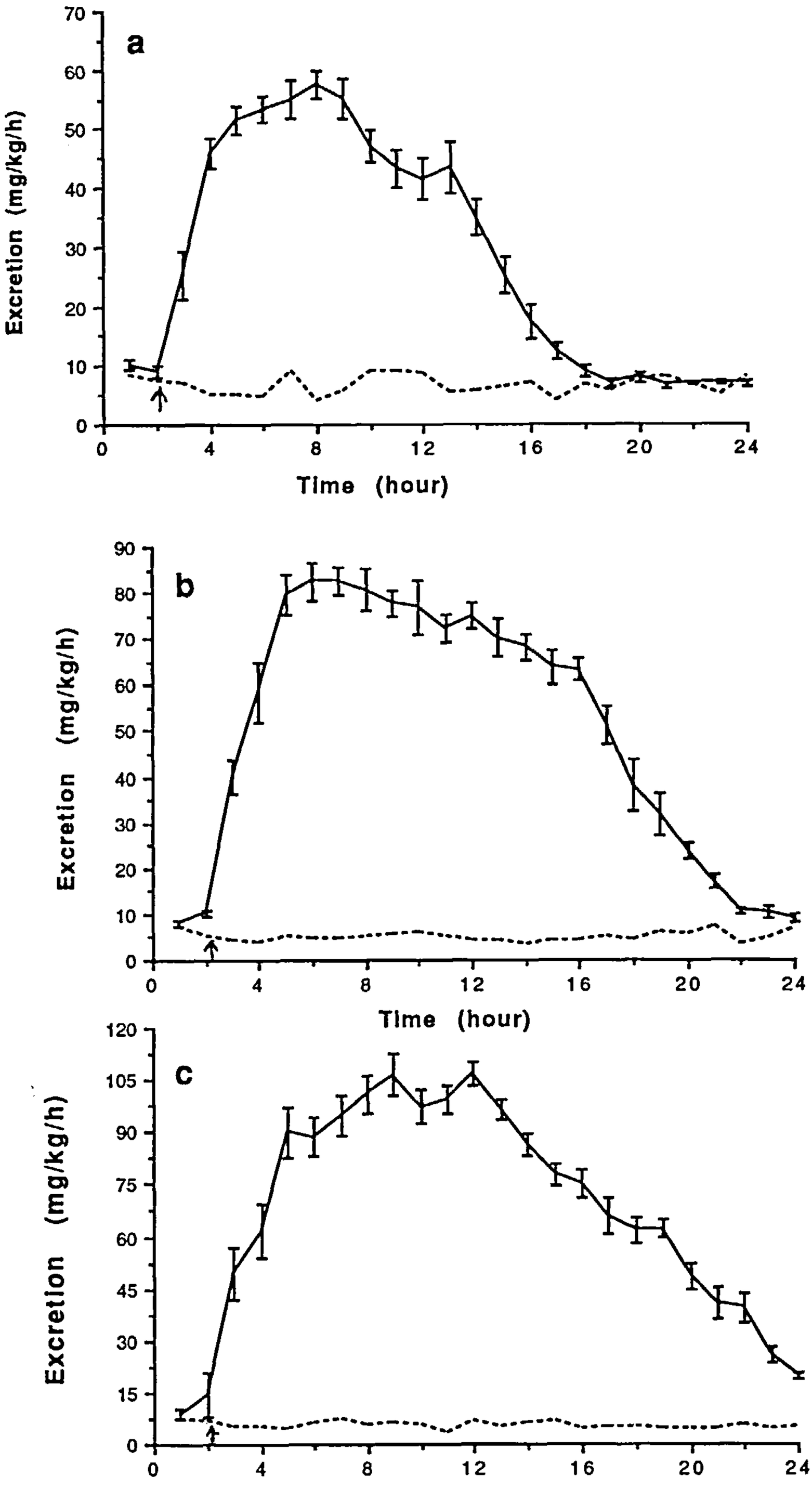


Fig. 5.15 Hourly mean variation in ammonia excretion during 24 hr day in Cyprinus carpio fed with 35% protein diet at (a) 1% ration level, (b) 2% level and (c) 3% ration level. Values are arithmetic means +_SEM, n = 9. Arrow indicates the time of feeding.

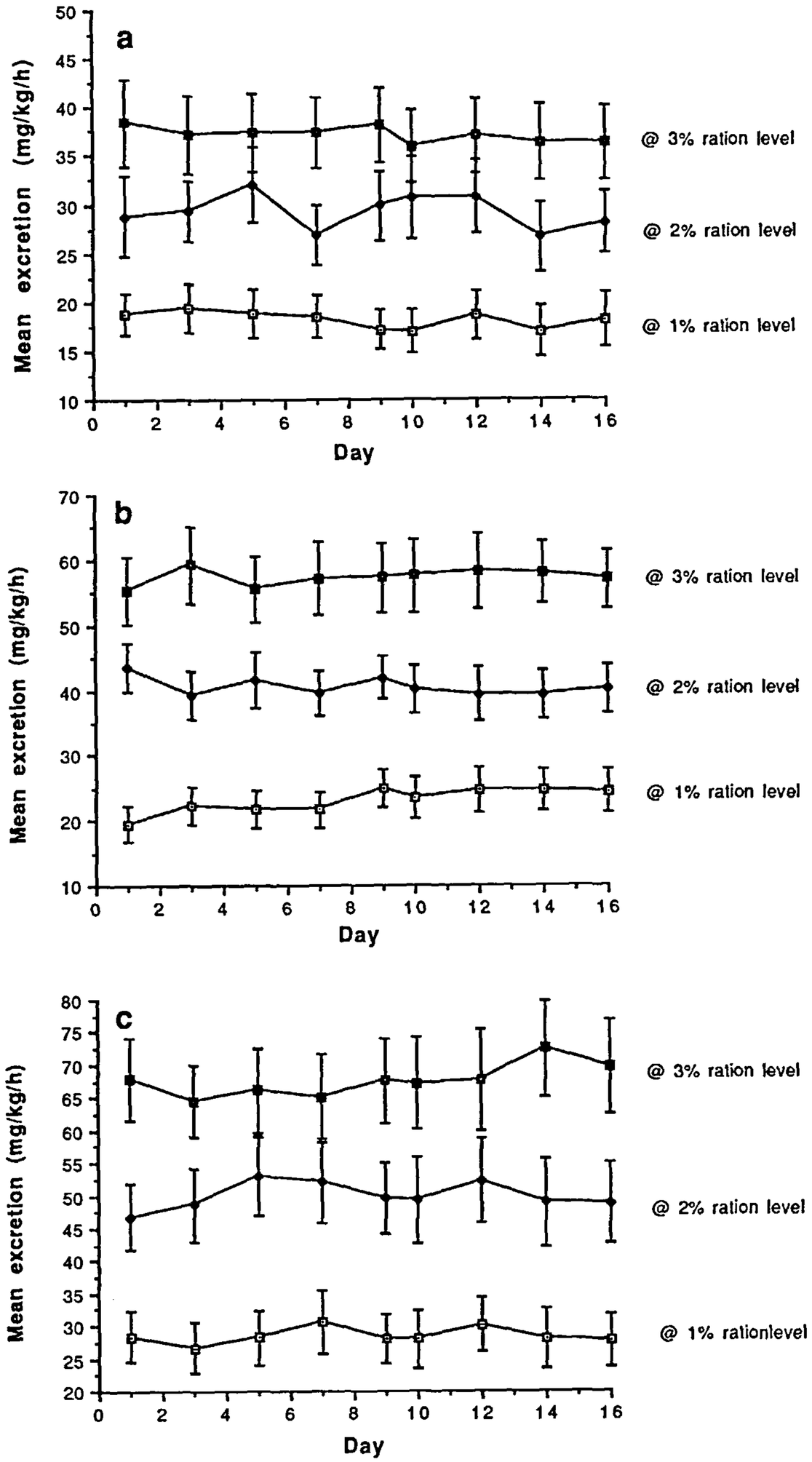


Fig. 5.16 Mean daily variation in ammonia excretion in Cyprinus carpio when fed with (a) 20% protein diet, (b) 35% protein diet and (c) 50% protein diet at different ration levels. Values are arithmetic mean +_ SEM, n = 24.

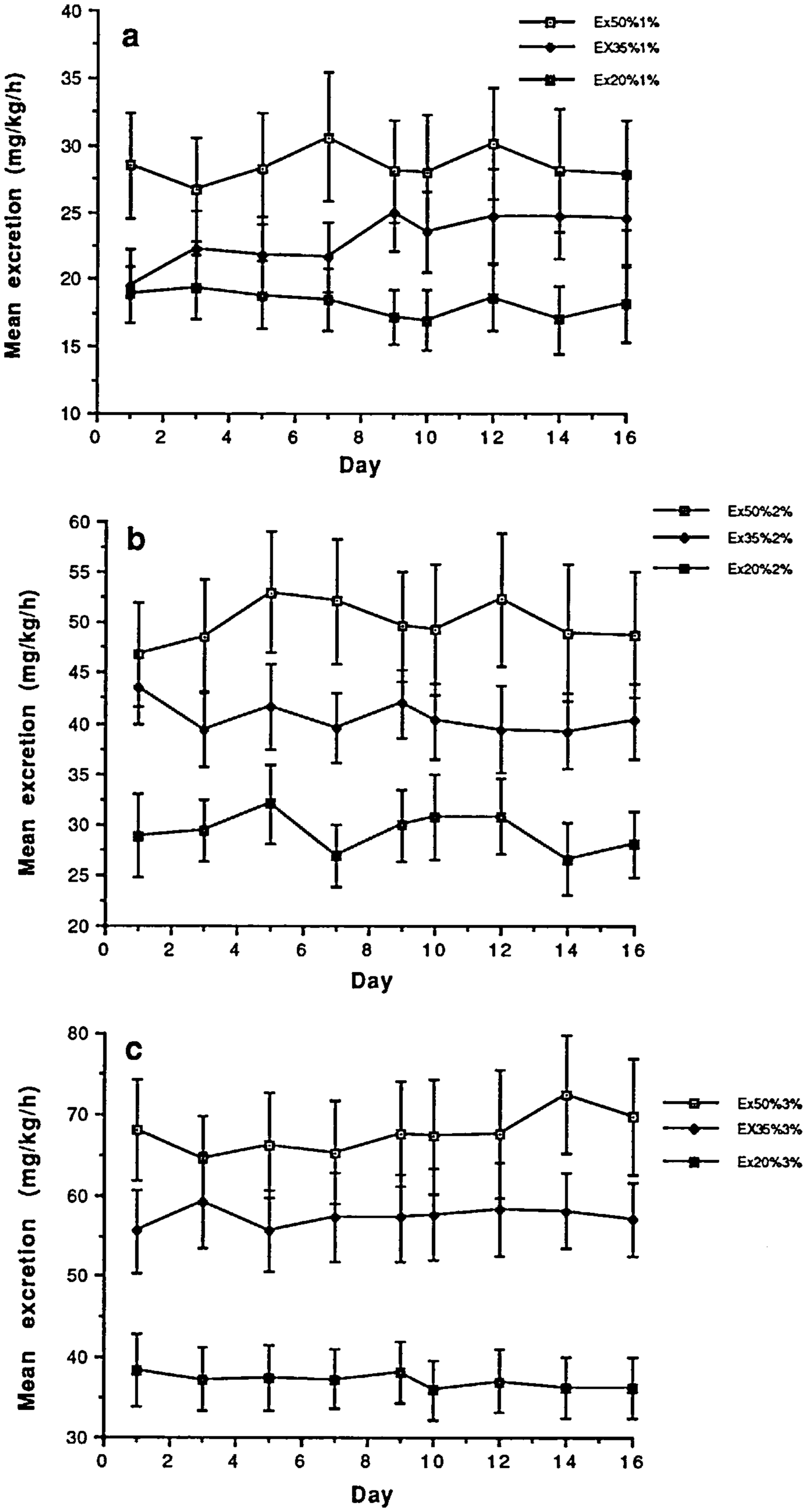


Fig. 5.17 Mean daily variation in ammonia excretion in Cyprinus carpio when fed with different protein diets with ration at (a) 1 percent body weight, (b) 2 percent body weight and (c) 3 percent body weight. Values are arithmetic mean +_ SEM, n = 24.

Table 5.9 summarises the ammonia excretion of Cyprinus carpio fed on the different diets and energy. The energy lost as kJ/day in 'U' varied from 2.77 with 1% ration of 20% protein diet to 10.18 at 3% ration level with 50% protein diet showing an increase of energy loss with increase of ration level. When expressed as a percentage of 'C', U can be seen to vary from 4.19 to 8.74 (Table 5.9). There is an increase in 'U' as a % of 'C' with dietary protein content, but a clear decrease with ration level in all three diets.

The regression model expressing the relationship between energy lost in excretion with different dietary protein level used at different ration level is given by:

$$U = 4.97 + 0.092\ P - 0.846\ r \qquad .... \ p < 0.05$$

where, U = energy loss in excretion expressed as percentage, P = dietary protein within 20 - 50% range and r = ration level used (range 1 - 3% body weight).

5.3.5 Growth (P)

The growth performances of fish fed with the different protein level diets are summarized in Table 5.10. Any changes of wet weight or dry weight were considered as

Table 5.9 Rates of NH_3 nitrogen excretion by Cyprinus carpio in relation to protein and ration level.

Protein in diet	Expt. No.	Ration (%body wt)	C (kJ)	N	U (mg/kg/h) Mean (+_SD)	Energy (kJ/day) Mean(+_SD)	'U' as % of 'C' (+SD)
20%	1	0		9			
	2	1%	44.45	9	18.32 (+_0.89)	2.77 (+_0.12)	6.22 (+_0.27)
	3	2%	88.19	9	29.28 (+_1.85)	4.38 (+_0.27)	4.97 (+_0.31)
	4	3%	119.87	9	37.16 (+_0.81)	5.01 (+_0.11)	4.19 (+_0.13)
35%	5	0		9			
	6	1%	54.14	9	23.05 (+_1.87)	3.88 (+_0.32)	7.17 (+_0.59)
	7	2%	102.13	9	40.57 (+_1.47)	6.44 (+_0.25)	6.30 (+_0.23)
	8	3%	148.03	9	57.19 (+_1.21)	8.79 (+_1.86)	5.93 (+_0.12)
50%	9	0		9			
	10	1%	47.44	9	28.42 (+_1.21)	4.14 (+_0.17)	8.74 (+_0.37)
	11	2%	98.74	9	49.94 (+_2.05)	7.59 (+_0.31)	8.00 (+_0.33)
	12	3%	146.96	9	67.58 (+_2.41)	10.18 (+_0.36	6.93 (+_0.25)

Table 5.10 Growth summary of experiments in Cyprinus carpio during 16 days feeding trial. Values were calculated at the start and end of the experiments.

Expt. No.	Ingest-ion'C' (kJ/d)	Wet wt. at start (g)	wet wt at end (g)	Wet wt. change (g)	Final dry wt. of fish (g)	(Dry wt./ wet wt.) %	Dry wt. change (g)	Energy value of carcass (kJ/g)	Growth 'P'(kJ)	'P' as % 'C'
1	Unfed	251.64	236.60	-15.04	52.93	22.37	-3.36	20.14	-67.76	--
2	44.45	253.43	260.40	+6.97	59.31	22.78	1.58	20.75	32.79	4.60
3	88.19	251.12	291.22	40.10	68.71	23.58	9.46	20.36	192.60	13.65
4	119.87	226.47	286.68	60.21	71.31	24.87	14.97	20.63	308.83	16.10
5	Unfed	236.25	222.13	-14.12	49.47	22.27	-3.14	20.20	-63.42	---
6	54.14	282.58	292.63	10.05	63.54	21.74	2.18	20.78	45.40	5.24
7	102.13	266.42	336.43	70.01	70.83	21.05	14.74	20.63	304.03	18.60
8	148.03	257.42	373.50	116.14	81.72	21.88	25.41	21.28	540.75	22.83
9	Unfed	248.00	232.00	-16.0	49.78	21.46	-3.43	20.33	-69.73	---
10	47.44	244.80	253.60	8.80	55.98	22.07	1.94	21.03	40.84	5.38
11	98.74	255.10	335.22	80.12	77.39	23.09	18.50	20.79	384.60	24.34
12	146.96	253.00	377.12	124.12	83.80	22.17	27.52	21.71	597.40	25.41

"growth" in the experiments.

In experiments 1, 5 and 9 the unfed, control fish consistently lost some of their body weight (Table 5.10) due to combustion of their own tissues during starvation. Growth increased as the dietary protein level increased and over the 16 day trial period, better growth was attained with the higher protein diets and higher ration levels (Fig. 5.18a, b and 5.19a, b). Maximum growth (49.05% wet weight change and 32.84% dry weight change) was observed with 50% protein diet at 3% ration level (Table 5.10).

From Figs. 5.18a, b, the maintenance ration for Cyprinus carpio can be obtained by noting the ration level corresponding zero growth. Feed conversion ratio (FCR) was inversely related to protein content and ration levels (Figs. 5.19b and 5.18c). The FCR fell dramatically from 5.8 at 1% ration with 20% protein diet to value ranged from 0.98 at 3% ration of 50% protein diet to 1.3 at 35% protein diet. This phenomenon was also clear with the other diets. There was also an improvement in FCR as dietary protein increased. Protein efficiency ratio (PER) was directly related to ration in the diet (Fig. 5.18d) and inversely related to the protein content of the diet (Fig. 5.19c).

The growth 'P' as a percent of ingested energy was 4.60, 5.24 and 5.38% with diets having 20, 35 and 50% protein at the 1% ration level (Table 5.10).

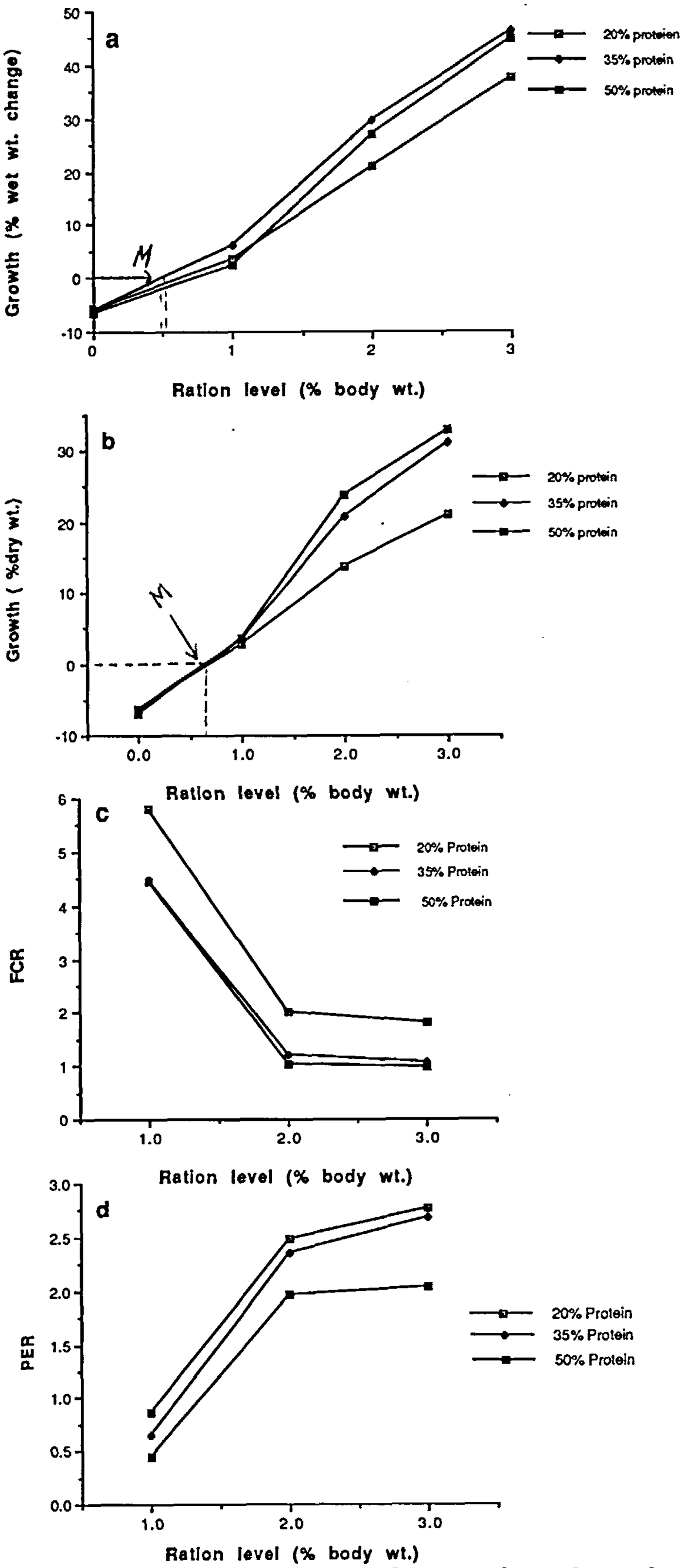


Fig. 5.18 Relationship between the level of ration size of different protein diets and (a) growth (wet Wt.), (b) growth (dry wt.), (c) feed conversion ratio. (FCR) and (d) protein efficiency ratio (PER) in Cyprinus carpio. M = maintenance ration.

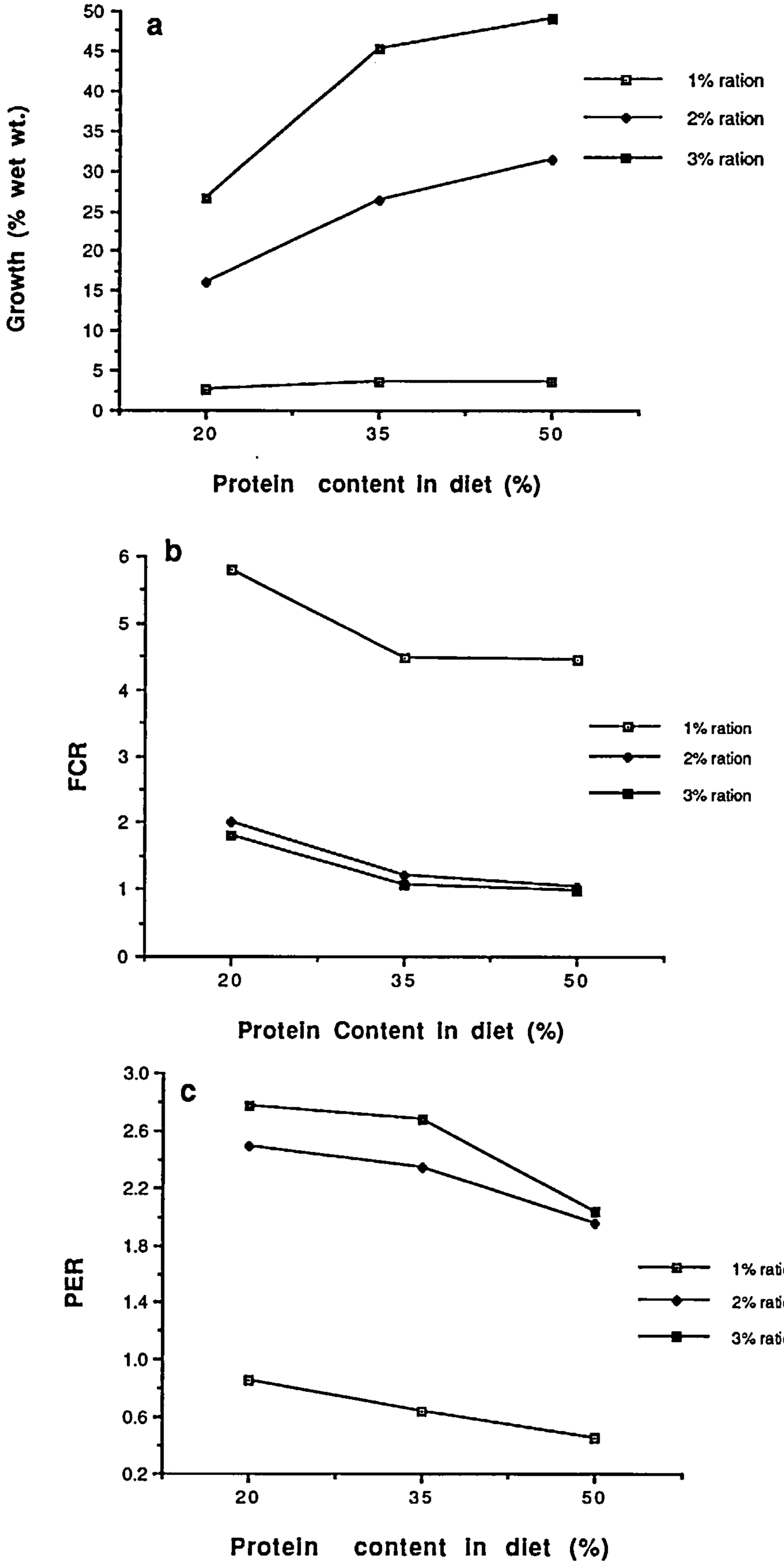


Fig. 5.19 Relationship between the dietary protein level at different ration diet and (a) growth (b) feed conversion ratio (FCR) and (c) protein efficiency ratio (PER) in Cyprinus carpio.

However, at the 2% ration level growth was 13.65. 18.60 and 24.34 percent of 'C' from 20, 35 and 50% protein diets respectively and similarly, was 16.10, 22.83 and 25.41 percent of 'C' at the 3% ration level.

The carcass analysis of the fish at the end of the experiments is shown in Table 5.11 and the results of the Analysis of Variance for each component. are given in Table 5.12. Significant differences were seen after 16 days of the experiments except for moisture in unfed fish (experiments 1, 5 and 9), dry matter in fish fed 35% protein diet (experiments 3, 7 and 11), crude protein in unfed fish and in fish fed with 20% protein diet (experiments 2, 6 and 10), crude lipid in fish fed 35% protein diet, ash in unfed fish were found (Table 5.12) from analysis.

The energy content (kJ/g) of carcass in unfed fish was slightly, but significantly, lower ($p < 0.05$) than in others. There was also a small but significant effect of input energy from different protein level diets on the resultant carcass energy. So a significantly higher ($p < 0.05$) carcass energy was observed with 3% ration of 50% protein diet than that in other experiments. Although there was a small increase in energy content in carcass with an increase of protein in the diet, no clear significant correlation was found between the effect of ration level in

Table 5.11 Carcass analysis : Dry matter and proximate composition and energy of fishes used in different experiments. Values are arithmetic means of triplicate sample +_SD.

Expt*. No.	Ration level (%)	Moisture (%)	Dry matter (%)	Crude protein (%)	Crude lipid (%)	Ash (%)	Carcass energy (kJ/g)
20%(1)	unfed	77.63 (+_0.30)	22.37 (+_0.15)	70.68 (+_0.17)	10.73 (+_0.16)	18.59 (+_0.16)	20.09 (+_0.05)
20%(2)	1%	77.22 (+_0.22)	22.78 (+_0.58)	69.00 (+_0.25)	15.76 (+_0.27)	15.24 (+_0.14)	20.75 (±0.070)
20%(3)	2%	76.41 (+_0.25)	23.58 (+_0.53)	68.93 (+_0.23)	17.04 (+_0.31)	14.03 (+_0.15)	20.36 (±0.074)
20%(4)	3%	75.13 (+_0.43)	24.87 (+_0.30)	69.31 (+_0.19)	16.96 (+_0.39)	13.73 (+_0.28)	20.63 (±0.074)
35%(5)	unfed	77.73 (+_0.30)	22.27 (+_0.25)	71.06 (+_0.41)	10.07 (+_0.18)	18.87 (+_0.24)	20.40 (±0.095)
35%(6)	1%	78.25 (+_0.40)	21.74 (+_0.36)	68.65 (+_0.21)	16.83 (+_0.24)	14.52 (+_0.17)	20.78 (±0.14)
35%(7)	2%	78.94 (+_0.35)	21.05 (+_0.58)	69.17 (+0.14)	16.65 (+_0.49)	14.18 (+_0.07)	20.63 (±0.10)
35%(8)	3%	78.11 (+_0.24)	21.88 (+_0.47)	69.31 (+_0.27)	15.98 (+_0.47)	14.71 (+_0.19)	21.28 (±0.08)
50%(9)	unfed	78.54 (+_0.20)	21.46 (+_0.18)	70.25 (+_0.22)	11.31 (+_0.12)	18.44 (+_0.23)	20.33 (±0.08)
50%(10)	1%	77.90 (+_0.31)	22.07 (+_0.31)	69.09 (+_0.30)	17.59 (+_0.15)	13.32 (+_0.11)	21.03 (±0.10)
50%(11)	2%	76.90 (+_0.50)	23.09 (+_0.33)	69.66 (+_0.17)	17.28 (+_0.33)	13.06 (+_0.15)	20.79 (±0.11)
50%(12)	3%	77.82 (+_0.34)	22.17 (+_0.47)	69.01 (+_0.30)	16.89 (+_0.12)	14.10 (+_0.19)	21.71 (±0.07)

* Expt. No. denotes % protein diet in experiment number in the parenthesis. e.g. 20%(2) denotes 20% protein diet used in experiment 2.

Table 5.12 Summary of Analysis of Variance of the effect of ration on the chemical composition and energy content of Cyprinus carpio.

Parameter	protein content in diet (%)	F- value	P
Moisture	Unfed	1.87	0.234
	20%	34.30*	0.001
	35%	5.28*	0.047
	50%	5.81*	0.040
Dry matter	Unfed	3.47	0.090
	20%	14.08*	0.005
	35%	2.58	0.155
	50%	6.67*	0.030
Crude protein	Unfed	3.30	0.108
	20%	2.55	0.150
	35%	147.30*	<0.001
	50%	5.83*	0.039
Crude lipid	Unfed	65.00*	<0.0001
	20%	14.25*	0.005
	35%	3.38	0.104
	50%	7.60*	0.023
Ash	Unfed	3.13	0.117
	20%	45.67*	<0.001
	35%	9.30*	0.014
	50%	50.50*	<0.001
Energy	Unfed	2.41	0.176
	20%	20.36*	0.002
	35%	48.35*	<0.001
	50%	69.87*	<0.001

*Significantly different.

the diet and the resultant carcass energy content (Table 5.11)

5.3.6 Compilation of energy budget

From these experimental data for C, R, F, U and P, a simple energy budget equation can be constructed, based on the following formula :

$$C = R + F + U + P$$

'P' in this energy budget compilation was considered in two ways,'predicted growth' (P_1) and 'observed growth' (P_2) respectively. Predicted growth was calculated by the method of difference from the energy budget equation. The 'percent balance of energy' of the budget was given by

(Energy measured as outgoing ÷ energy as ingoing) X 100

i.e. $\frac{(P_2 + R + F + U) \text{ X } 100}{C}$

Using the experimental data, simple regression equations for the various energy components R, F, U, P_1 and P_2 with ingestion 'C' are given in Table 5.13. The correlation coefficients for these relationships are also cited in the table.

Table 5.13 Regression analysis of the changes of various energy components with ingestion at different protein diets using the simple model Y = a + bX, where, Y represents R, F, U, P_1 or P_2 ; C represents ingestion; r = correlation coefficient.

Parameters	Protein content in diet(%)	Regression equation	Corr. Coeff. r
R vs C	20%	R = 14.4 + 0.107C	0.987
F vs C	20%	F = -1.87 + 0.254C	0.989
U vs C	20%	U = 1.52 + 0.030C	0.988
P_1 vs C	20%	P_1 =-14.1 + 0.609C	0.978
P_2 vs C	20%	P_2 =-8.13 + 0.229C	0.968
R vs C	35%	R = 17.9 + 0.123C	0.977
F vs C	35%	F = 0.301+ 0.188C	0.988
U vs C	35%	U = 1.06 + 0.052C	0.993
P_1 vs C	35%	P_1 = -19.3 + 0.637C	0.997
P_2 vs C	35%	P_2 = 14.9 + 0.330C	0.982
R vs C	50%	R = 15.4 + 0.149C	0.990
F vs C	50%	F = 0.087 + 0.197C	0.998
U vs C	50%	U = 1.37 + 0.0608C	0.984
P_1 vs C	50%	P_1= -16.9 + 0.593C	0.981
P_2 vs C	50%	P_2 = -12.9 + 0.350C	0.993

Table 5.14 summarizes the daily energy budget calculated from the experimental data (see sections 5.3.1 to 5.3.4).

A significant difference was found ($P < 0.05$) between the values for calculated or predicted growth (P_1) from the energy budget equation and observed growth (P_2). Although P_1 and P_2 were different , P2 increased significantly ($p < 0.05$) with P_1 (Fig. 5.20) at different rations used in different protein content diet. The percent balance of energy ranged from 66.64 in experiment 4 to 81.96 in experiment 11 (Table 5.15).

Daily energy budgets expressed as a percentage of consumption 'C' for Cyprinus carpio from different experiments (Table 5.15 and Fig. 5.21) showed at respiration energy, 'R' increases with increased protein content in the diet and decreases with increased ration of same diet. There was no clear variation in the relationship between 'F' and 'C'. Like 'R', 'U' increased linearly with increase of protein in diet and decreased with increased ration.

From the various data for C, R, F, U and P the combined regression equation with different dietary protein at different ration levels for energy budget was calculated as follows:

Table 5.14 Daily energy budgets of Cyprinus carpio in relation to protein and ration level.(Values are calculated from observation over 16 days of feeding).

Diet	Ration (% body wt)	Consumption (kJ) 'C'	Respiration (kJ) 'R'	Faecal loss (kJ) 'F'	Excretion (kJ) 'U'	Growth (kJ) Predicted 'P_1'	Growth (kJ) observed 'P_2'	Balance (%) [(P2+R+F+U) X 100] / C
20% Protein	1%	44.45	18.86 (±0.42)	9.55 (±1.14)	2.77 (±0.12)	13.27	2.04	74.74
	2%	88.19	24.63 (±0.45)	20.22 (±2.84)	4.38 (±0.27)	38.96	12.04	69.47
	3%	119.87	26.80 (±0.47)	28.78 (±2.70)	5.01 (±0.11)	59.28	19.30	66.64
35% Protein	1%	54.14	24.55 (±0.65)	10.34 (±1.86)	3.88 (±0.32)	15.37	2.83	76.84
	2%	102.13	30.42 (±0.68)	19.75 (±1.88)	6.44 (±0.25)	45.52	19.00	74.03
	3%	148.03	36.07 (±1.16)	27.97 (±1.70)	8.79 (±0.18)	75.20	33.80	72.03
50% Protein	1%	47.44	22.45 (±0.74)	9.60 (±1.83)	4.14 (±0.17)	11.25	2.55	81.66
	2%	98.74	30.13 (±1.24)	19.18 (±2.34)	7.59 (±0.31)	41.84	24.03	81.96
	3%	146.96	37.31 (±1.68)	29.21 (±3.61)	10.18 (±0.36)	70.26	37.34	77.60
Unfed	0	0	13.59	0	0.75		-3.76	381.38
	0	0	12.62	0	0.73		-3.52	379.26
	0	0	12.73	0	0.74		-3.87	348.06

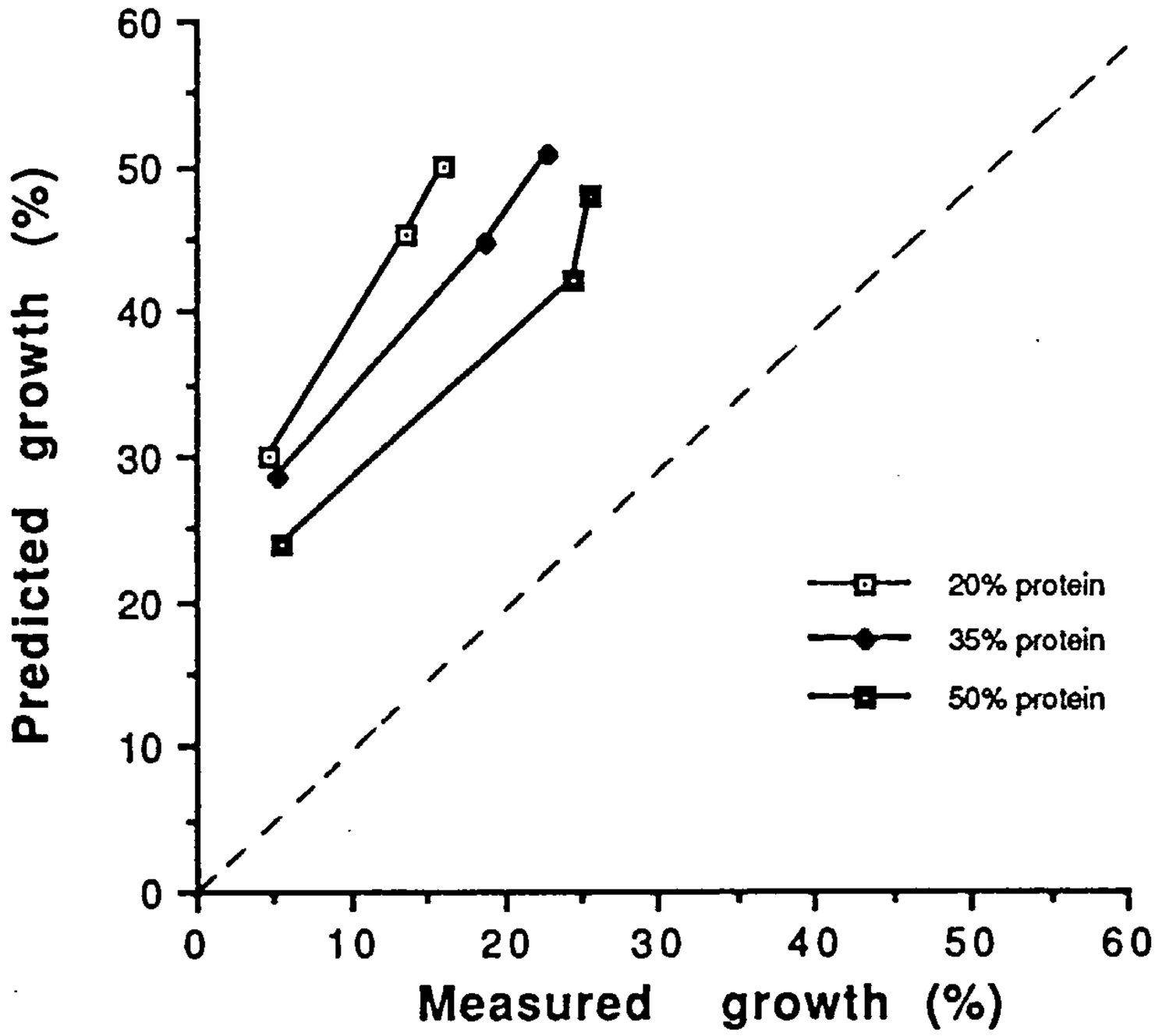


Fig. 5.20 Relatioship between measured growth and predicted growth in Cyprinus carpio with 20, 35 and 50% protein diet used at different ration levels.

Table 5.15 Daily percentage Energy Budget in Cyprinus carpio over experimental periods.

Protein in diet	Ration (% body wt.)	Consumption 'C'	Respiration 'R'	Faecal loss 'F'	Excretion 'U'	Growth (P)		Balance (%)
						Predicted P_1	observed P_2	
	1% ration	100	42.38	21.38	6.22	30.02	4.60	74.74
20%	2% ration	100	27.92	21.78	4.97	45.33	13.65	69.47
Protein	3% ration	100	21.97	24.00	4.19	49.84	16.10	66.64
	1% ration	100	45.23	19.10	7.17	28.50	5.24	76.84
35%	2% ration	100	29.78	19.34	6.30	44.58	18.60	74.03
Protein	3% ration	100	24.33	18.89	5.93	50.85	22.83	72.03
	1% ration	100	47.12	20.24	8.74	23.90	5.38	81.66
50%	2% ration	100	30.51	19.42	8.00	42.07	24.34	81.96
Protein	3% ration	100	25.38	19.88	6.93	47.81	25.41	77.60

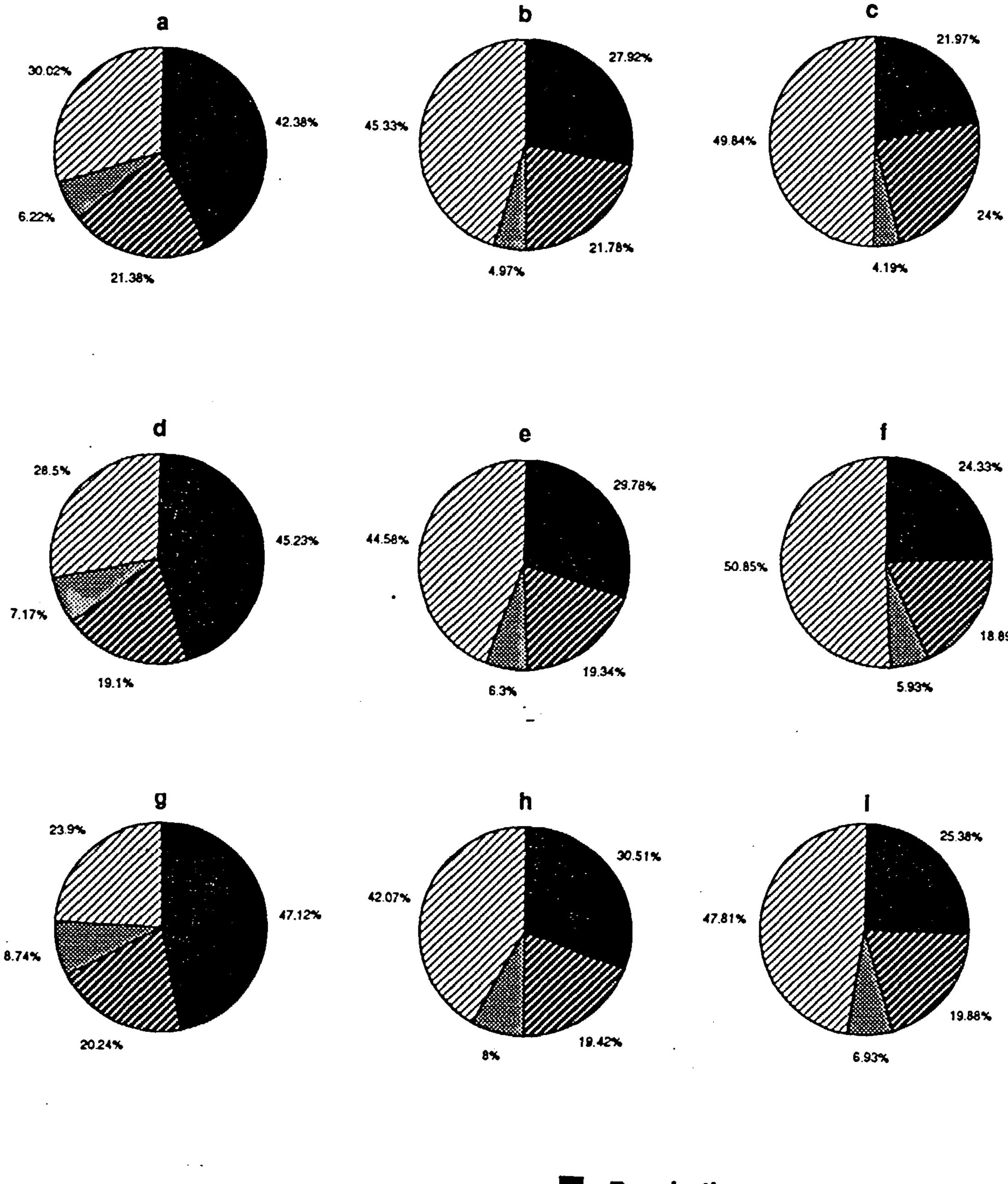


Fig. 5.21 Percentage daily energy budget showing energy distribution:

a. 20% protein diet at 1% ration
b. 20% protein diet at 2% ration
c. 20% protein diet at 3% ration
d. 35% protein diet at 1% ration
e. 35% protein diet at 2% ration
f. 35% protein diet at 3% ration
g. 50% protein diet at 1% ration
h. 50% protein diet at 2% ration
i. 50% protein diet at 3% ration .

$$P_1 = 70.5 - 0.035\ p + 1.58\ r - 0.899\ R + 0.036\ U - 0.139\ F$$

where, P_1 = predicted growth: p = dietary protein level (ranged from 20 - 50%); r = ration level (1 - 3% body wt.), R = energy loss in respiration; F = energy loss in faeces; U = energy loss in excretion.

5.4 DISCUSSION

The experimental system

A number of studies has been made of respiratory performance of different fish using closed respirometers (Ahmed and Mazid, 1968; Kutty, 1972; and Caulton 1978). These authors noted that respiratory rates measured in such experiments were generally higher than those derived from open-circuit respirometers. The main cause of this phenomenon is the lack of opportunity to fully acclimate fish to the experimental chambers prior to making measurements. It might also be true that a fish inside a small respirometer chamber is not completely free from stress. This was to some extent observed in the previous study (Chapter 3) with common carp, Cyprinus carpio, which exhibited occasional jerky movements inside the respirometer chambers. However, in this study the larger metabolism

chambers allowed fish inside to move freely and also sufficient time for acclimation to the new system.

Many experiments have shown that standard metabolic rates (mg/kg/h) are much lower than routine rates measured using closed respirometers (Mann, 1968; Elliot, 1969; Muir and Niimi,(1972); Huisman, 1974; Brett, 1976a). This study showed only a small difference between mean standard metabolic rates (152 mgO_2/kg/h) measured by closed respirometer (see Chapter 3) and mean routine metabolic rates (163 mgO_2/kg/h) measured by open circuit-metabolism chambers (experiments 1, 5 and 9). This observation is in agreement with the comments of Caulton (1978).

The air water-interface had an insignificant influence on the O_2 diffusion in this type of system (Anna, personal communication) and was ignored in measuring O_2 consumption. There was little disturbance caused by the fish inside the chambers so that insignificant disturbances in air-water interface took place and this was also ignored in calculating oxygen consumption and nitrogenous excretion.

A constant water flow-rate of 40l/h in the system showed no significant fluctuation of oxygen content in the air saturated water throughout the experiment. The system was thus sufficient to provide the oxygen demand of higher than 3.0

mg/l (Chiba, 1965) during feeding metabolism and no sign of stress for oxygen was observed at any time in the system.

The oxygen probe for measuring oxygen consumption in the system was recalibrated twice a week by standard Winkler's titration method (Stirling, 1985) and showed no significant variation (+_0.01mg/l only). Water samples for ammonia measurements were taken from the same site of oxygen measurement in the cuvette. The design of the metabolism chambers for collection of faecal matter was based on a system devised by Cho et al. (1985). The sloped perspex sheet inside the metabolism chambers facilitated the easy accumulation of all faecal matter leading to the bottom of the faecal column. Thus, overall, the experimental system showed its suitability for measuring all the components (R, F, U and eventually, P) of the energy budget.

Oxygen consumption & Respiration (R)

A knowledge of metabolism (i.e. the energy lost as heat) is basic to any energy budget because it reflects the total energy expended by the fish in resting or routine metabolism and in activities like feeding and swimming and can comprise a large fraction of the total energy intake (Soofiani and Hawkins, 1985).

This study showed a large range of increase of respiratory peak values over routine rate from about 104.80% with 20% protein content diet at 1% ration level to up to 310.72% with 50% protein content diet (Table 5.2) at 3% ration level. This large variation in peak values was dependent on both the protein content of the diet and the ration levels as shown by Cho (1982). This post-feeding oxygen consumption is the specific dynamic action (SDA).

Since there was no scope for separate measurement of SDA in this study, (except for the first two days of measuring routine rate) the oxygen consumption was considered as total energy of metabolism comprising routine, feeding and active metabolism or in short, metabolism, designated by 'R'. In all trials the increase in oxygen consumption caused by R_s, R_f and R_a showed a similar pattern of increase shortly after feeding reaching a peak and falling again more or less rapidly to the pre-feeding levels (Fig. 5.1 - Fig. 5.4)

Although the total increase of oxygen consumption is also affected by the activity of fish, the cause of this increase over routine rate is mainly due to SDA and is determined chiefly by the quantity and quality of feed, apart from environmental factors such as temperature (LeGrow and Beamish, 1986). This study showed a significant ($p < 0.05$) increase in metabolic rate with the increase of dietary protein (Fig. 5.5).

Certain differences between species exist in respect of duration of the heat increment and the peak value of oxygen consumption, which can be roughly double the routine value (Steffens, 1989). In this study, the mean increase in peak value over routine rate (Table 5.2) ranged from 2.03 times with 20% protein content diet at 1% ration level to 4.08 times with 50% protein content diet at 3% ration levels. These higher peak values are clearly related to higher feeding levels with different protein diet.

Routine metabolism represents 'normal activity' in which the fish can swim at will without outside disturbance. The term 'active rate' was originally used to describe the maximum aerobic activity of which a fish is capable but now is often used to describe any level of forced activity (Brafield, 1985). In this study the design of the metabolism chamber allowed the fish to be free of stress, and, when compared to a natural situation, leading rather a quiet life which was evident by their resting at the bottom corner of the chamber. Thus, a lower level of activity was maintained than would have been the case when searching food or escaping from predators. Therefore, the post-feeding increase of metabolic rate in this study may be roughly considered as being due to feeding metabolism.

For a range of species, Brett and Groves (1979) quoted an average increase in feeding metabolic rate over standard and

routine rates of 3.7 and 1.7 respectively. A similar increase in mean feeding metabolic rates (better, total metabolic rate 'R') over routine rates was observed in this study with common carp Cyprinus carpio, values ranging from 1.3 times in experiment 2 with 20% protein content diet to 2.82 times in experiment 12 (Table 5.3) having 50% protein content diet. Jobling (1981) showed that the maximum rates of post - feeding oxygen consumption appear to be approximately double 'low routine' rates for most fish species. But, in Anguilla anguilla, feeding metabolic rates R_f, of only 1.2 - 1.3 times the pre-fed routine rates were noted (Knights, unpublished results).

In fish the post-feeding energy loss through SDA is considered by many authors to be related to the food consumed without distinguishing between the different functions of maintenance and growth and SDA is taken as a certain ration food energy (Beamish, 1974). Kerr (1971a) and Kitchell et al. (1974) remarked that there is a linear relationship between SDA and food consumption when the food consumption exceeds the maintenance metabolism. Many workers have found that the magnitude of the post-prandial effect increases in relation to meal size (Hamada & Ida, 1973 ; Schalles & Wissing, 1976; Caulton, 1978; Vahl and Davenport, 1979; Jobling and Davies, 1980), although the opposite view was also reported by Averett (1969) and Tandler and Beamish (1979). Although SDA was not measured separately from total

metabolism in this study, it was seen that there was significant ($P < 0.05$) increase of 'R' with increase of ration from the same diet (Fig. 5.7). Similarly, an increase of dietary protein from the same ration levels resulted in a significant increase of 'R' (Fig. 5.6).

Costs of 'R_f' ranging from 9 - 20% of ingested energy from natural foods have been reported by Brett and Groves (1979) and Jobling (1981). Similar results were revealed in this study where the values (calculated from Appendix 1) ranged from 10.51% of 'C' with 20% protein content diet increasing to 16.11% of 'C' with the 50% protein content diet. This confirms the increasing metabolic cost with increase of protein content in the diet seen in other SDA experiments (for details see chapter 4).

Faeces and Faecal energy (F)

Of the food energy ingested (C) only a part is absorbed and used for metabolic processes and the non-absorbed portion is voided as faeces. Together with the nondigestible fraction of the diet, faeces contain sloughed intestinal epithelial cells, mucus, catabolized digestive enzymes and bacteria (Brett and Groves, 1979).In many cases the accuracy of faecal measurement is debateable because suspended and

soluble fractions may be lost in the system and 100% collection may also not be possible resulting in an underestimate of faecal loss. As far as possible the experimental system in this study assured good collection of faecal solids although the leaching fraction remains unknown.

The ratio of faeces to feed (dry wt.) was 1 : 3.48 - 4.78 (23 - 29% of dry feed weight). This was similar to that recorded by Musisi (1984) in her energy budget studies for tilapia. The values for faeces as % of 'C' from different studies shows a big range from 17% (Brocksen et al.,1968) up to 84% (Bryan, 1975).

Brett and Grove (1979) quoted values of 20% and 41% of ingested energy 'C' for faecal energy loss 'F' in well-fed young carnivores and herbivores respectively. From this study the faecal energy loss in Cyprinus carpio ranged from 18.89 to 24.00% of ingested energy with different diets and protein content of the diet had a significant ($p < 0.05$) influence on faecal energy. Winberg (1956) in his theoretical energy budget for a typical carnivorous fish described the faecal loss as 16% of ingested energy. Similarly, a faecal energy loss of 11.87% to 27.30% in Sarotherodon mossambicus with different ration diets was reported by Musisi (1984).

But Cui (1987) found small values for faecal loss 'F' ranging from 2.72% to 13.01% for Phoxinus phoxinus fed on enchytraeid worms. He did not clarify the cause of such small amount of faecal loss although a major factor in all studies is the fibre content of the diet.

A better understanding of protein digestibility coefficients is a prerequisite to evaluate the quality of a diet for fish and potential synthesis of new tissue. Dietary protein utilization was measured as 'Apparent Protein Digestibility' (Table 5.6) which is the fraction excreted in the faeces expressed as percentage of intake. Although determination of digestibility coefficient by the balance method is commonly used for many farm animals (Morgulis, 1918; Tunison, 1942; Hanaoka, et al., 1948; Furukawa et al., 1953; Bondi, et al., 1957, Hepher, 1988), the inert indicator method is mostly used for fish (Peters and Hoss, 1974). In spite of some constraints and drawbacks, most researchers use this method as it seems to reduce the difficulties associated with the balance method (Hepher, 1988) and measurement of all ingestion and faecal production is not necessary. It has been shown that there are many factors responsible for affecting digestibility such as fish species (Refstie & Austreng, 1981), fish age (Windell et al., 1978), physiological conditions, such as stress, (Job, 1977), water temperature (Brett et al.,1969; Shcherbina and Kazlauskene, 1971), water salinity (MacLeod, 1977), food composition

(Sandbank and Hepher, 1978), form of food, method of preparation (Lovell, 1984) and so on.

Apparent Protein Digestibility in this study was found to be less with lower protein content of the diet. Thus, the 20% protein content diet was less digestible than the 35% and 50% protein (Table 5.6). In comparison, digestibility of protein from sunflower oil meal by 2 year old common carp at 16 - 17, 22 and 26 - 27°C was 81.7, 80.0 and 75.1%, respectively (Shcherbina and Kazlauskene, 1971). With rice meal common carp showed a digestibility coefficient for protein of 86.0, 81.0 and 88.3% at 2, 4 and 6% feeding level respectively (Bondi et al., 1957).

The lower digestibility coefficient with 20% protein diets in this study may be due to the higher inclusion of carbohydrate (starch and dextrin) (Tunison et al., 1942, 1943, 1944) which could have inhibited proteolytic enzyme activity (Falge et al., 1978).

Although Windell et al. (1978) could not demonstrate any relationship between ration size and digestibilty for rainbow trout, the studies of Vens-Cappell (1978) showed that the possibility of such an effect should be considered. For a 1% daily increase in the daily feed supply, the digestibility of crude protein fell by 3%. An increase in

the daily ration was also found to impair digestibility in young carp (2.5 - 50g) (Schada, 1982). Bigger carp (1500g) however did not show significant diffences in protein digestibility at feeding rates of 5% - 2.5% (Schwarz and Kirchgessner, 1982b). No significant change of protein digestibility with increasing ration size was shown in the present study (Table 5.6).

If C and F in an energy budget experiment are measured accurately in long-term respirometry, it may be assumed that the difference between the two is the energy absorbed and assimilated by the fish. Thus, assimilation efficiency, perhaps better called absorption efficiency indicates the proportion of food energy actually taken into the fish (Brafield, 1985).

From bioenergetics studies, assimilation efficiency or absorption efficiency [(C-F)/C X 100] is a very useful measure since it is the only indication of the energy potentially available from energy consumed (i.e. gross or total energy, C) for metabolism (R) and observed growth (P). Brett and Groves (1979) and Elliott(1979) mentioned in their reviews that more information is required on the effects of temperature and body size but conclude that Winberg's (1956) assumption that absorption efficiencies (AE) in fish range from 83 - 85% is too high for herbivores and often too low for carnivores. Instead, they and Fischer (1979) quote

typical values of about 40 - 50% for herbivores and 70 - 90% for carnivores with higher values for smaller fishes, tending to increase with temperature and falling with ration size. In this study with common carp, these values ranged from 74 - 82% and were not significantly affected ($p > 0.05$) by smaller ration levels (1% of body wt.) (Table 5.7) but increased slightly with ration size in the 50% protein content diet. Similar values (84.02%) for AE in Sarotherodon mossambicus were reported for a 50% protein diet by Musisi (1984) although the 83.69% for trout fingerling II feed in her experiment may have been due to differences of pellet stability and leaching out more rapidly by trout pellet II which was prepared commercially.

In the present study a mean AE of 81.80% for fish feeding with 35% protein content diet ad libitum was very close to the 81.60% for perch (Perca fluviatilis) found by Spillet (1978) and 78.12% found for grass carp (Ctenopharyngodon idella) by Ang (1980) fed on Gammarus (Table 5.16). A comparison for assimilation efficiencies in some fishes are given in Table 5.16. The differences in values may be due to several factors such as temperature, species used in the study, quality of the food and so on. Thus, Sarotherodon mossambicus when fed on a high protein diet gave an AE mean value of 84.02% (Musisi, 1984) whereas gold fish (Carassius auratus) (Spillett, 1978) fed on the same diet had a mean AE value of 79.2%, and grass carp (Ctenopharyngodon idella)

Table 5.16 Comparison of Assimilation Efficiency (AE) of perch, gold fish, grass carp, and tilapia fed on different diets.

Diet	Name of fish	Experimental temperature	AE (%)	Source of References
Protein	Gold fish	10°C	79.20	Spillett(1978)
	grass carp	10°C	82.65	Ang (1980)
	Tilapia	27°C	84.02	Musisi (1984)
Protein and fat	Gold fish	10°C	47.10	Spillett(1978)
	grass carp	10°C	57.33	Ang (1980)
	Tilapia	27°C	75.98	Musisi (1984)
Protein and carbohydrate	Gold fish	10°C	86.90	Spillett(1978)
	grass carp	10°C	80.69	Ang (1980)
	Tilapia	27°C	79.47	Musisi (1984)
Gammarus	Perch	10°C	81.60	Spillett(1978)
Gammarus	grass carp	10°C	78.12	Ang (1980)
Tubifex	Tilapia	27°C	87.19	Musisi (1984)
Balanced diet (20, 35 and 50% protein)	Common carp	28°C	74.09 to 81.80	This study

(Ang, 1980) gave a mean AE value of 82.65%. The variation of mean AE in our study may be due to the better quality of the food and ration differences in the diets.

Nitrogenous excretion (U)

In addition to faecal losses (F) nitrogenous excretion (U) represents an end product of protein metabolism within the animal (Cowey, 1980). The main nitrogenous product in fishes is ammonia, largely formed in the liver and excreted passively at the gills (Wood, 1958; Forster and Goldstein ,1969; Payan and Matty, 1975; Steffens, 1989). Between 80 to 98% of the nitrogenous excretion in freshwater fish is reported to occur as ammonia (Brett, 1962; Forster and Goldstein, 1969; Solomon and Brafield, 1972; Mclean and Fraser, 1974; Brett and Zala, 1975) and between 75 to 85 % in marine fish (Jobling, 1981).

Urea, and other nitrogen compounds are excreted in much smaller amounts (Smith, 1929; Forster and Goldstein, 1969) where the kidneys by contrast play a minor role as excretory organs. The percentage of ammonia nitrogen from total nitrogen excreted by common carp Cyprinus carpio was reported as 66 - 94% at 20^{o}C (Infante, 1974).

In determining the calorific value of ammonia some authors (e.g. Cowey and Sargent, 1972) consider that, since ammonia has no carbon fragment, it is devoid of residual energy. Therefore, if the end product of protein metabolism is ammonia alone, the metabolizable energy of protein just equals the digestible energy and in that case it is 23.62 kJ/g (5.65 kcal/g) digested protein. This approach has been criticized by several physiologists. A completely opposite view was taken by Elliott and Davison (1975). They calculated the energy value of ammonia, for 'specific enthalpy of combustion', from its heat of formation. From this calculation they have derived a value of 347.78 kJ/mol (83.2 kcal/mol) or (5.94 kcal/g) ammonia N equivalent to 24.83 J/mg NH_3 - N. In this study the later value was accepted in calculating energy value for ammonia.

After feeding, the rate of excretion may be many times greater than that beforehand, as was shown with rainbow trout (Salmo gairdneri) by Paulson (1980). A similar, immediate, increase in excretion was recorded by Kaushik (1980) with rainbow trout and carp. Brett and Zala (1975) also working with trout showed a peak value of 4 times the normal rates with the ammonia excretion reaching its peak 4 - 4½ h after feeding. Similar results were noted by Guerin-Ancey (1976b) in sea bass (Dicentrarchus labrux) and Rychly and Marina (1977) in rainbow trout.

In the present study post-feeding fish had an increased ammonia excretion in a pattern similar to the SDA response (Fig. 5.13 - 5.15). Ammonia excretion increased with the protein content and hence, nitrogen content of the diet (evident from Fig. 5.17). A similar effect was reported by Caulton (1978) where the amount of nitrogen excreted as ammonia by Tilapia rendalli depends largely on the quantity of protein assimilated during feeding. He assumed that ammonia is the main, if not the only, end product of protein catabolism. The same phenomenon was recorded by Zorm (1984) with common carp, Cyprinus carpio. The greater the intake of nitrogen, the higher was the peak and the longer the period of elevated excretion (Fig. 5.13 - 5.15). Kaushik (1980) also noted a peak in ammonia excretion 4 to 6 hours after food intake by carp. Marina (1977) showed a post-prandial increase in ammonia excretion, the peak values being observed after 4 hrs in sockeye salmon (Oncorhynchus nerka) 6 hrs in the trout and 11 hrs in the coho salmon (Oncorhynchus kisutch).

The rates of ammonia nitrogen excretion in this study were about 121 mg NH_3/kg/day. Comparatively smaller values for endogenous nitrogen excretion of 87 mg NH_3/kg/day at 20°C and 104 mg NH_3/kg/day at 27°C were noted for carp of about 200g by Ogino et al. (1973). Dabrowski (1976, quoted from Dabrowski, 1977) also reported values of 176 mg NH_3/kg/day. Larger value of 346.8 mg NH_3/kg/day for endogenous nitrogen

excretion by bluegill sunfish (Lepomis macrochirus) at 29.4 - 32.2°C was recorded by Savitz (1969). But Nose (1961) and Jauncey (1982) reported 169 and 128 mg NH_3/kg/day for endogenous nitrogen excretion in Salmo gairdneri and Oreochromis mossambicus of 5.3g and 1.6 - 1.8g weight fish respectively. Table 5.17 lists some of the reported values for ammonia excretion in different fishes and the values that found in this study clearly fit within this range.

In general, nitrogenous excretion (U) is the smallest component of the energy budget, accounting only for about 7% of the consumed energy in carnivores (Brett and Groves, 1979). This study of the omnivore, Cyprinus carpio, showed an excretory energy loss of 4.19% to 8.74% of consumed energy 'C' which was positively dependent on the amount of protein in the diets. In herbivores this excretory energy comprises only 2% of ingested energy (Brett and groves, 1979). Winberg (1955) in his theoretical energy budget mentioned 'U' as 4% of 'C', while Nielsen and Jurgensen (1982) listed this excretory loss as 5% of 'C'. Musisi (1984) measured 'U' in Sarotherodon mossambicus as 6.72% of 'C'.

Growth (P)

The process of multiplication in cell numbers and increase of cell volume of any organism is "growth" (Needham, 1964).

Table 5.17 Published values for ammonia excretion in perch, gold fish, grass carp, tilapia and common carp.

Name of fish	Experimental temperature	Feeding regime	Ammonia excretion mg NH_3/g/day	Source of Reference
Perch	10°C	unfed	0.40	Solomon & Brafield
Perca fluviatilis	10°C	starving	0.17	(1972)
Gold fish	10°C	unfed	0.377	Spillet (1978)
C. auratus	10oC	starving	0.223	same author
	20°C	starving	0.105	Iowata, 1970
Grass carp	14°C	unfed	0.212	Ang (1980)
C. idella	14°C	starving	0.195	Ang (1980)
	22°C	unfed	0.932	Ang (1980)
	22°C	starving	0.665	Ang (1980)
Tilapia	27°C	unfed	1.329	Musisi (1984)
S. mossambicus	27°C	starving	0.859	Musisi (1984)
	27°C	well fed	1.652	Musisi (1984)
Common carp	16-18°C	starved	0.052	kaushik (1980)
C. carpio	20°C	starved	0.072	Ogino *et al.* (1973)
C. carpio	27°C	starved	0.086	Ogino *et al.* (1973)
	16-18°C	starved	0.052	kaushik (1980)
C. carpio	28°C	unfed	0.121	This study
	28°C	well fed	1.622	This study

If the quantity of food ingested exceeds that required for the maintenance of the body, growth takes place in the form of positive changes in length, volume or mass of the fish which is the principal aim of fish production in aquaculture. The many factors influencing growth were summarized by Brett and Groves (1979), and Hepher (1988). The growth per unit time is the growth rate, while the specific growth rate (SGR) denotes the average daily growth as a percentage of the initial weight. Both of these indices are useful in providing an instantanious estimate for growth.

Initial and final wet weights usually taken over a period in excess of two weeks are sufficient to estimate the gain in wet weight and dry weight during the course of a trial. Initial dry weight then can be estimated from the knowledge of final wet and dry weights (Table 5.10). The wet wt. to dry wt. ratio can be influenced by several factors. The energy value of dried total fish carcass was used to determine the value for growth 'P'. Although it is probably better to express the energy content in ash free terms (Brafield, 1985) this study described the values as total sample weight and the ash values are given where necessary.

The scope for growth is the range of dietary energy ingested between the maintenance ration (when no growth occurs) and maximum ration (when maximum growth occurs). Within the

scope for growth, higher growth is observed with a higher ration. This relationship was demonstrated by Ivlev (1945) and termed by him the 'first order growth coefficient (K_1)'. When the assimilated part of the food was considered this became the 'second order growth coefficient (K_2), given by :

$$K_1 = W/R\ T \quad \text{(First order)}$$

$$K_2 = W / p\ R\ t \quad \text{(Second order)}$$

where, R t = total ration and p R t = assimilated ration. Paloheimo and Dickie (1965, 1966b) reviewed the results of various feeding experiment and modified Ivlev's equation so that for a given type of food, the logarithm of growth efficiency K_1 is linearly related to the ration size, which they called the K line. Many studies have confirmed this form of relationship between growth and ration (e.g. Huisman, 1976). Only carp, Cyprinus carpio has been shown to experience a reduced growth rate at maximum ration (Huisman, 1974, 1976) whereby the efficiency of food utilization is decidedly less at some submaximal point thus defining an optimum ration.

The unfed fish in the work reported here lost weight after 18 days in the metabolism chamber, suffering a net loss of tissue energy (Table 5.10). Compared to other vertebrates, fish require a high protein intake for growth.

It must of course be remembered that the minimum dietary protein depends on a variety of factors.

In the present study, there was a straight line increase of growth with a slight decrease at the highest ration. Other workers have suggested a straight line for this relationship. Brett (1979) described this relationship as the tangent from origin to the growth rate (% wt./day) and the ration line touches it at a point where the ratio between the two is maximal. Brett (1979) considered the food ration at this point as optimal.

The interpretation of the effect of dietary protein on growth is not easy because the results are affected by many interrelated factors (Hepher, 1988). For maximum growth most fishes require 35 to 50% protein in their diets with a few exceptions, for example channel catfish (Hastings, 1973, Tiemeier and Deyoe, 1980). In the present trials the growth with 20% protein diets was significantly lower than with 35% and 50% protein content diets. Many studies cite high protein for highest growth (Hepher, 1988, pp 183). In this study 50% protein diet showed highest growth at 3% ration levels (Table 5.10, Figs. 18a, 19a). From other studies, carp showed highest growth rates at 35% protein (Jauncey, 1981), 38.4% protein (Sin, 1973a), 38.5% (Ogino and Saito, 1970) and 40.6% (Ogino et al., 1976). Ogino and Saito (1970) found that protein gain in young carp reached its peak at a

protein level of 38%; further increase in dietary protein level only resulted in fat deposition. In some cases, a peak in growth rate was observed at a certain level beyond which there was no additional growth, and sometimes even decrease in growth rate (Dupree and Sneed, 1966; Ogino et al., 1976). Similar observations were made by Nose (1971) and Watanabe et al. (1979) for growth of rainbow trout. Fish fed on diets low in protein and rich in carbohydrate are usually fatter than those fed a comparatively protein rich diet (Lee and Putnam, 1973; Ogino et al., 1976; Reinitz and Hitzel, 1980; Hepher et al., 1983). In this study no such clear relationship was found in fish fed low protein, high carbohydrate diets (Table 5.10, Fig. 5.18a) and this was confirmed by the data in carcass composition.

Food conversion ratio (FCR) in this study improved as ration size increased and was notably poor at 1% ration. FCR also improved with increase of dietary protein (Table 5.10, Figs. 5.18c, 5.19b). Normally, the better the food quality for growth, the lower the FCR. In similar experiments with carp, Huisman (1976) showed a decrease of FCR value from 1.41 to 1.14 when the ration was increased from 1 to 3% of the body weight although further increase to 5, 7 and 9% again increased the FCR to 2.76. He commented that there should be a feeding level up to which the FCR is better for growth. Another experiment with young rainbow trout showed FCR of 1.66, 1.61 and 1.94 at a daily ration of 1, 2 and 3%

of the body weight (Huisman, 1976). The larger FCR value for the fish in the present study with 1% ration diet was inevitable because the energy intake and scope for growth was very limited.

The effectiveness of protein on growth can also be evaluated from the protein efficiency ratio (PER) which changes with the level of protein in the diet. PER responds differently in different fishes. Thus, Nose (1971) working with rainbow trout found that PER increases with dietary protein up to an optimum amount consumed and above this PER goes down. These changes in PER are associated with both the utilization of a portion of protein for maintenance and to the overall energy level of the ration. A similar pattern of increase was observed by Cowey et al.(1972) in plaice (Pleuronectes platessa) where the PER was 1.0 at 20% protein, increased to 1.7 at 40% protein and then fell to 1.0 at 70% protein.

By contrast, Ogino et al. (1976) and Takeuchi et al. (1978b) found that PER decreases with increase of protein in diets. Similarly, in this study, PER decreased from 2.49 at 20% to 2.35 at 35% and then to 1.96 at 50% protein diet when a 2% ration fed. Similar trends were clear at other ration levels (Fig. 5.19c). Many authors have noted the same effect (Ogino and Saito, 1970, for common carp; Dabrowski, 1977, for grass carp; Takeuchi et al., 1978b, and Watanabe et al., 1979, for rainbow trout; Mazid et al., 1979, for Tilapia zilli;

Jauncey, 1982, for Sarotherodon mossambicus ; Papaparaskeva-Papotsoglou and Alexis, 1986, for Mugil capito). But Cowey et al. (1972) for plaice (Pleuronectes platessa) point out that the maximum PER in fish occurs at much higher level of protein than in rats. This type of difference in the curves may be due to differences in the energy content in the diets (Ogino et al., 1976). With semi-synthetic feeds (e.g. using casein as a protein source) PER may achieve over 3.0. Thus a 35% protein and 18% lipid diet gave a PER of 3.3 in rainbow trout (Takeuchi et al., 1978b). In rearing rainbow trout fingerlings, PER values ranged from 2.2 to 2.7 (Yu et al.,1977; Gamygin and Kanid'ev, 1977; Steffens and Albrecht, 1979b) and market size trout showed a PER of 2.04 (Steffens and Albrecht, 1975). However, in Huisman's (1976) study on rainbow trout at 15°C PER's of only 1.22, 1.26 and 1.04 at 1, 2 and 3% ration level were recorded. But Hepher (1988) concluded that PER increases with protein up to an optimum amount of consumed protein when its utilization is optimal, decreasing thereafter.

Water in the body of fish is known to vary considerably. A water content of 86.9% was recorded by Brett et al. (1969) in starved sockeye salmon (Oncorhynchus nerka) fingerlings at 20°C and 71.3% water at 15°C in fish fed to excess. In the present study the range of moisture content varied from 75.13% to 78.94% and are not significantly different between treatments. Whereas, Musisi (1984) found a range of 68.9% to

84.9% in Sarotherodon mossambicus. Similarly, in brown trout (Salmo trutta) an increase of pelleted ration size resulted in a wet weight gain and water content decreased (Elliott 1976a). In the present study significantly ($p < 0.05$) higher crude protein content and lower lipid were observed with control, unfed fish and these results are similar to those reported by Jauncey (1982). Other workers have found that when fish are starved there is a gradual decrease in protein content with an increase of moisture content (Idler and Clemens, 1959; Phillips et al., 1966; Brett et al., 1969; Edwards et al., 1969; Niimi, 1972; Elliot, 1976a). Whereas, Brett et al. (1969) in sockeye salmon (Oncorhynchus nerka) and Staple and Nomura (1976) in rainbow trout detected a significant negative correlation between lipid and moisture.

Ash levels were significantly higher in unfed fish reflecting the general reduction in protein and lipid contents (Table 5.11). In Musisi's (1984) study with tilapia (Sarotherodon mossambicus) this value ranged from 10.8 to 21.29% and Jauncey (1982) working with carp reported values of 11.2 to 22.89%. In both cases the lowest values were from well-fed fish and the highest values were for starved fish as was noted in this study (Table 5.11).

Elliott (1976a) noted for brown trout (Salmo trutta) that protein, fat and energy value of the fed fish increased with increasing ration. This was seen in the present work only

for crude lipid and energy value (Table 5.11). Crude protein levels in the carcass of different treatments did not vary significantly which might be explained due to individual variation in the fish. In experiments with rainbow trout (Salmo gairdneri) Grayton and Beamish (1977) suggested that the levels of water, fat and protein were not affected signifcantly by differences in ration levels although body fat increased significantly at the highest ration level. Garling et al. (1976) observed that % moisture content, protein and ash content of channel catfish (Ictalurus punctatus) was decreased when lipid concentration was increased in the body components. In the present study only ash content decreased in the fed fish during the trial period. As may be expected, unfed fish had the lowest carcass energy content (20.27 +_0.16 kJ/g) and this was significantly different from the fed fish (Table 5.11). It was not possible to measure the energy value of the fish tissue at the beginning of the experiment, and so any changes during the period of experiments could not be measured.

Energy budget

The energy budget equation used in this study is given by

$$C = R + F + U + P$$

where it is assumed that work done by the fish on its surroundings and vice versa is small enough to be ignored (Weigert, 1968; Brafield and Llewellyn, 1982). According to the laws of thermodynamics all the energy ingested in a biological systems of fish must turn up in one form or another through metabolism (R), faecal loss (F), nitrogenous excretion (U) and growth (P).

The empirical regression equations derived from the experimental data fitted the original data well but tended to overestimate growth slightly. Predicted growth (%) was always greater than observed growth (%) (Fig. 5.20) made it a sense of understanding that there might be some clue in these experiments for less growth (P_2) in energy budget.

For determining 'P', two terms 'P_1' for calculated growth considering 'C' as 100% and 'P_2' for actual growth are introduced in this study. The balance of the energy budget is considered by comparing the actual growth 'P_2' seen in the experiment. P_1 is calculated as a part of the energy allocation in the energy budget (Table 5.14).

The relationships of the components (C, R, F, U & P) of the daily energy budget to variation of dietary protein level and ration level have already been discussed. The pattern of energy allocation in the daily budget in this study with

balance of energy less than 100% in all fed fishes and from 348 to 381% for unfed fishes. To survive, fish must meet at least their resting metabolic demands from food energy. At low ration (experiments with 1% ration levels) a large proportion of food energy was allocated to resting metabolism. This proportion decreases as the ration energy and dietary protein increases (Table 5.14). The daily energy budgets of carp are shown in Table 5.15 and Fig. 5.21 and it is clear that with increasing ration level a larger amount of energy was available for predicted growth (P_1). A similar observation was recorded for energy allocation in Phoxinus phoxinus (Cui, 1987) with increase of ration size. Overall energy balance in these experiments varied from 66.64% to 81.96%. Energy balance was calculated from the equation:

$$\text{Energy balance} = [(R + F + U + P_2) / C] \times 100$$

Since the energy entering the system must always be equal to energy leaving it, the energy balance should be 100%. In this study of energy budgets the energy balance is never more than 100% (Table 5.14) and energy balance varied from 66.64% to 81.96%.

Musisi (1984) working with Sarotherodon mossambicus found an energy balance for starved fishes was 621.53%, whereas in the present study a mean value of 369.56%, with a range from 348.06 to 381.38% was obtained. This may be due to the differences of the duration of the experiments and the size

of the fish used. With a supermaintenance ration, Musisi obtained an energy balance of 102.24% and 66.01 - 107.12% with tubifex and protein diets respectively. But with a 'protein and carbohydrate' diet the balance was found to be rather less (73.85 - 85.58%) than that found with a 'protein and fat' diet (106.58 - 111.57% at supermaintenance level. This indicates the effect of diet composition on the energy balance. Energy balance with a supermaintenance ration was less than 100% for perch (Perca fluviatilis) (Solomon and Brafield, 1972), in perch and goldfish (Spillet, 1978), in grass carp (Ang, 1980) and in Sarotherodon mossambicus (Musisi, 1984). For a maintenance ration with S. mossambicus Musisi found a balance ranging from 93.59 - 111.70% with a mean of 103.17% and the percentage balance in submaintenance experiments was found higher than 100%. Like these studies, Carter and Brafield (1991) reported similar energy balance of less than 100% with Ctenopharyngodon idella and the values were 81.1 to 93.4% with high protein (70%) diet and 61 to 74% with Lemna but a higher value of 103% was obtained with the high lipid diet containing much higher energy content (24.05 kJ dry g^{-1}).

Clearly, the energy balance in the present experiments is below 100%, implying that some of the energy was lost or not measured. One source of error is in the consumed energy C, where it is assumed that all of the diet entered the fish. There may infact have been some losses due to particles escaping from the opercula or to unobserved, uneaten

pellets. Some of the energy expended for respiration (R) was not accounted for during the later part of each data-collection day (which may be 3 - 6% roughly, of total 'R') when SDA effects extended beyond 24h. A small amount of faecal energy escaped due to unavoidable leaching (as 4% of total 'F' determined by Elliot, 1976, in brown trout) into the water could not be counted in the energy budget. Some part of 'F' may also have been decomposed by bacteria in the faecal column. The unmeasured 'U' such as urea (as was found between 2.77 and 19.90% of the total nitrogen in Ctenopharyngodon idella by Carter and Brafield, (1991); 6 to 7% in Cyprinus carpio (Smith, 1929) and 25% in starved common carp (Vellas, et al,, 1970, quoted from Cui, 1987) and other nitrogenous wastes may also be an energy source which was not counted in these experiments.

The efficiency of utilization of dietary energy for growth is affected by different factors. Among them are the degree of utilization of carbohydrate (Ivlev, 1939a), weight of fish (Mironova, 1976), dietary composition (Hepher et al.1983), amount of food consumed, hormonal control (De Silva and Balbontin, 1974), feeding level (Huisman, 1976; Huisman et al. 1979) and so on. Wagner and McKeown (1985) found that the growth of juvenile rainbow trout, Salmo gairdneri, cycled with accelerated and reduced growth every 3 to 4 weeks. These findings suggest that there may be a cyclic growth efficiency. Short-term studies on the effects

of nutritional history on the feeding and growth of fish would provide an insight into the fish growth regulatory mechanism (Cui, 1987). From the fore-going discussion it is clear that without an understanding of the regulatory nature of fish growth, applications of the relationship between energy budget components in short term trials to the field situations requires caution.

Although the models resulting from this study failed to fully predict the actual growth of Cyprinus carpio, nevertheless such energetics models provide a useful tool for identifying problems that exist in the study of fish growth and future research. The more digestible the diet, the better will be the assimilation efficiency (AE) and hence more energy can be utilized for growth. This manipulation is the ultimate aim of the feed formulation for aquaculture, since the more intensive the aquaculture system the greater is the importance of supplementary feeding and their costs. The most efficient feeding level for good growth can only be predicted from an energy budget when the correct supply of energy and essential food nutrients under optimum growth conditions are available and the factors on which growth depends are fully understood. Hopefully, future studies in these fields will naturally help to improve the knowledge of fish bioenergetics and growth but may also enhance the performance of energetics models of fish growth to a point where they can act as a predictive tool. Moreover, the study of the behaviour of these models can be expected to identify new problems for research.

CHAPTER VI

General Discussion

Studies of bioenergetic have implications for both basic science and applied fields such as animal production and aquaculture. An understanding of production processes in a biological system requires a knowledge of the integration of three main components - energy supply, metabolism and growth. Metabolism is basic to all energetic studies since it reflects the total expenditure of energy for maintenance, activity of feeding and other activities which replenish the energy supply (Beamish and Dickie, 1966). A large number of factors affect most of the aspects of the physiology of animals, and the production of comprehensive energy budget based models depends on being able to measure the effects of these factors on each budget variable. These data can be derived from laboratory studies on respiration and nutrition (Ross et al., 1988) whilst some of the factors must be evaluated in the field.

In many countries malnutrition and undernutrition is a chronic problem, and the increasing and pressing demand for low-cost, protein-rich food has clearly shown the importance of rapid establishment of aquaculture. Thus

apart from the intrinsic scientific interest in the study of energy utilization by growing animals, bioenergetic approaches and the use of predictive energetic models are of potentially great benefit in aquaculture and their development is dependent on better liaison between experimental scientists and practical fish farmers (Knights, 1985). The main components of the energy budget, the energy taken as food (C), the energy dissipated in metabolism (R), the energy lost in faeces (F) and nitrogenous excretion (U) and the surplus energy available for growth of somatic tissue and reproductive tissue (P) are all relevant to an understanding of the survival, growth and overall production of fish (Soofiani and Hawkins, 1985).

In general, animals restrict their activities to being day-active, diurnal, or night-active, nocturnal. An alternative and well known daily rhythm is where animals are dawn and dusk active, and this is known as crepuscular activity. The study of these rhythms has obvious significance in the study of energetics (Hill, 1976) and the present study has clearly shown that changing photoperiod influences the resting metabolic rate of common carp (chapter 3). The total daily resting metabolic rate showed no significant change during day (light) and night (dark) time but did show a significant reduction during total illumination or total dark period.

The resting metabolic rate in constant conditions is profoundly reduced from that in the normal photoperiod (12L : 12D). Brett (1979) suggested that light acts as a "directive factor" stimulating the brain-pituitary responses and radiating through the endocrine and sympathetic systems. He also noted that natural periodicity of light acts as an inducer of growth hormone and anabolic steroids and can influence the locomotor activity of fish in association with the stimulation of the thyroid.

The estimates of energy expenditure of carp in normal and altered photoperiods, calculated from long-term oxygen consumption observations, were consistent over the experimental period. The daily energy expenditure calculated from the study of resting metabolism (chapter 3) is in broad agreement with the values obtained in the SDA study (chapter 4) and also the routine metabolism of starved fishes held in the metabolism chambers (chapter V). In all cases, the crepuscular rhythmicity was noticeable, with only a slight increase in routine rate due to spontaneous activity in the larger metabolism chambers. This consistency suggests that the data can be applied to a long-term natural energy budget where the environmental and physiological condition can be specified. By contrast, Korowin - Kossakowski et al., 1981 (quoted from Steffens, 1989) noted that the oxygen consumption for routine metabolism (studies conducted using an annular chamber) of

common carp kept in groups, was 25% lower than that in isolated fishes.

It sometimes becomes difficult to measure resting metabolism because of the difficulty in eliminating the spontaneous activity of fish (Beamish and Mookherjii, 1964). For this reason some authors prefer indirect measurements of resting rate from the respiration curve at different swimming velocities and then extrapolating back to zero activity (Fry, 1971, Priede, 1985).

Resting metabolism as measured in the laboratory interests the fish farmer only as a source of information leading to a better understanding of the relationship between metabolism and the various factors affecting it. Resting rate alone cannot be directly utilized for design work or for compiling feeding charts until more information is available on maintenance metabolism under natural conditions (generally maintenance metabolism is higher than resting rate in natural condition) (Hepher, 1988). Data are also needed on how much food is necessary to supply this metabolizable energy for maintenance requirements.

In the study of SDA (chapter IV) different aspects of post prandial metabolic rate in common carp fed with different protein content diet was investigated. In these trials, it

was shown that the oxygen uptake, SDA magnitude and SDA coefficient was affected by the dietary protein content and that the oxygen uptake and SDA magnitude was proportional to ration level. The protein requirement of carnivorous, herbivorous and omnivorous fishes is uniformly high and is in the range of 35 - 70% dry weight of food (Pandian, 1987). The primary sources of metabolic energy in fish are lipid and protein, rather than lipid and carbohydrate as in mammals. Thus, protein acts as both a structural component and as an energy source (Brett and Groves, 1979) and this accounts for the very high demand for proteins in fishes. These two opposing metabolic trends have led not only to the decrease in food energy intake but also to the increase in the protein energy to total energy ratio. Thus, optimum total energy to protein energy is around 2 : 1 in the fish, whereas it is 10 : 1 for ruminants (Williamson and Payne, 1980).

Laboratory estimates of SDA can be applied to fish in the natural environment or in culture, provided that information on the food is available. An important additional matter is the physical representation of the diet which should be similar in the laboratory and in the wild. Thus, a partly macerated, more highly digestible diet given in the laboratory may lead to an underestimate of feeding metabolism for fish in the field, especially where the fish feed on large entire prey organisms (Soofiani and

Hawkins, 1985). Estimates of SDA depend upon the actual food intake, and so the estimates of 'C' must be precise.

The distinction between feeding excitation and activity and the metabolic processing (SDA) components of feeding metabolism are difficult to make (Soofiani and Hawkins, 1985). However, Smith et al. (1978a, b) working with Salmo iredeus and Jobling and Davies (1980) working with Pleuronectus platessa found that neither sham feeding nor kaolin-feeding evoked the post-prandial elevation of oxygen uptake. In studies with Salmo iredeus, Cho and Slinger (1979) and Cho et al. (1976) found that sham feeding did elevate post-prandial oxygen consumption, but only by about 1 - 2% of the increases when fish were fed normally.

In the present study, the activity of the fish was controlled and minimised. The values obtained for SDA thus be of great utility in intensive fish culture because of the possible relationship with growth and post-prandial oxygen demands. Staples and Namura (1976) suggested that because of the ease of finding, consuming and digesting high-energy commercial diets, less energy has to be expended in feeding and metabolic processing than is the case with natural foods. Furthermore, they contend that the energy costs of processing food relative to energy intake are probably not affected by body size, a view

supported by Brett and Groves (1979). According to Jobling (1981, 1983a) SDA is an inescapable cost of growth and he suggests that SDA and growth are interactive, with high rates of growth reflecting high rates of metabolism and high SDA, rather than being competitive where a diet inducing high SDA will reduce the amount of food energy available for growth. Jobling's interpretation is almost certainly correct and can be explained on the basis of levels of nitrogen turnover and excretion. Thus, if much protein has to be catabolized to produce energy, an increased level of transamination and deamination will occur resulting in an increase in metabolic rate.

Jobling (1981) noted that little energy is required for fat storage whereas the conversion of glucose to glycogen or fat is relatively expensive in terms of high energy phosphate yield. Temporary storage of amino acids requires no energy whereas the energetic costs of protein synthesis are substantial. In view of the complexities of these transformations it is not unusual that researchers have reported conflicting data regarding SDA.

The increasing information regarding post-prandial oxygen consumption and growth rates suggests the possibility that metabolic rate measurement could be used as a research tool in the rapid estimation of growth potential of fish or the nutritional status of different dietary formula-

tions. A number of studies carried out under laboratory conditions have shown that there is a relationship between feeding rates and metabolic expenditure leading to a close relationship between metabolism and growth (Brett, 1976; Staples and Namura, 1976; Vivekanandan and Pandian, 1977).

In considering the total energy budgets of carp (chapter V) it is important to remember that the metabolic rate in fish is affected by a very large number of factors, both biotic and abiotic (Brett and Groves 1979). The many different conditions and different methods adopted by previous workers, mean that comparisons of their results with those in the present study must be interpreted very carefully. However, in general, it was considered that, in this study, 'C' was not underestimated as the feed as taken was closely observed. In addition, although some leaching from faeces may have occurred with a net underestimation of 'F' it should be noted that dissolved organic materials from faeces may contribute only 4% or less to the total faeces as in case of brown trout (Salmo trutta) (Elliott, 1976a).

Ammonia can be extremely toxic to fish if allowed to accumulate in the body. Small increase in ambient ammonia levels can have many deleterious effects on fish resulting in a reduction of excretion and a net increase of ammonia in the tissue of fish (Guerin-Ancey, 1976b; Randall and

Wright, 1987). For a better understanding of different aspects of potential endogenous nitrogen and excretory nitrogen from different proteinaceous diets in energy metabolism it is essential to make continuous quantitative appraisal of nitrogen excretion (Kaushik et al., 1982) in energy budget experiments. The relatively higher levels of excreted ammonia recorded in this study compared to other studies with common carp may indicate the greater dependence on protein in diets and the higher experimental temperatures. Nitrogen budgeting is often worth further consideration because the protein component of commercial diets is a major cost factor (Steffens, 1981).

Laboratory studies of fish feeding may be carried out for a number of purposes including understanding the physiology of the feeding and digestive process, investigations of fish husbandry, diet formulation and finally, extrapolation to the natural population. The choice of experimental method is of critical importance because of its effect on food consumption, evacuation and digestion. Moreover, such feeding experiments for energetics studies provide important data both for the development of the theories on feeding strategies and for assessing the agreement between prediction and actual observed performances (Talbot, 1985). In the present study of energy budgets (chapter V), common carp in the weight range 50 to 80g, were fed a maximum ration of 3% body

weight/day, which was well below the 12% (three meals each 4%) and 4% (ad libitum) per day used in studies on Sarotnerodon mossambicus (Musisi, 1984) and Phoxinus phoxinus (Cui, 1987), respectively. In both cases the initial weight of the fisnes was small and hence, good for growth response. Observed growth in the present study was found to be lower than predicted growth.

Many laboratory studies on the growth of fish have been based on short periods (few weeks) (Cui, 1987), although long-term studies are also common. Brown (1946b) working on brown trout, Salmo trutta, and De Silva and Balbontin (1974) working on herring, Clupea harengus, found that growth rates fluctuated with time and short-period cycles of slow and rapid growth were under hormonal control. Stirling (1977) also noted marked short term fluctuations of growth, growth efficiency and food intake of Dicentrarchus labrax. Similarly, Wagner and McKeown, (1985) found a cyclic phase of accelerated and reduced growth every 3 to 4 weeks during their 4 months experiment on juvenile rainbow trout, Salmo gairdneri. Such cycles may be a manifestation of growth regulation according to Hubbell (1971), who stated that living organisms are capable of regulating their growth rate to maintain physiological homeostasis. The confirmation of such a hypothesis requires long-term growth experiments on fish given maximum food under constant conditions and with

frequent monitoring of growth rates. Care should also be taken to account for seasonality, maturation and reproduction.

The method of determining the components R, F, and U for the energy budget experiment is time consuming and needs essentially continuous monitoring over at least a 24 hour period. Therefore, control of a long term experiment by a single person is very difficult. It was partly for this reason that the energy budget experiment was scheduled for the minimum acceptable period. This type of long-term respirometry for measuring oxygen consumption, ammonia production and faeces collection and analysis would be best if highly automated or operated by a group of workers so that the trials could run for a longer period.

The basic component of animal tissue is protein and this is an essential nutrient for maintenance and growth. Therefore, the dietary protein and its ratio to the metabolizable energy becomes of prime importance. Generally a range of 25 to 60% crude dietary protein in the total diet have been described as the requirement for optimum growth of fish and Cyprinus carpio require about 30% to 50% (Hepher, 1988). One aim of researchers has been concerned with reducing the protein content of diets without affecting the food conversion efficiency (FCR). The view is that the dietary protein must be used only

for growth and spared as an energy source rather than being wasted. This is because excess protein is not only wasteful to the fish culturist but can also actually bring about decrease in growth due to excess ammonia causing stress and sometimes gill disease.

In this study on energy budgets the maximum growth was observed with 50% dietary protein. But from an economic point of view, the optimum growth rate could be considered to occur with 35% dietary protein as it was not very much less than that with 50% dietary protein in comparison to the cost difference.

Perhaps surprisingly, there are no existing reports on the complete energy budgets for common carp, (Cyprinus carpio) although some components of the budgets (resting metabolic rate, SDA) have been investigated. This study, therefore, represents the first compilation of the energy budget components both separately and simultaneously for Cyprinus carpio. The results presented here may be used to define the metabolic energy required to maintain common carp in its resting state even under different photoperiod regimes and taking account of different dietary proteins at varying ration levels. The regression models obtained in the different experiments are of particular value in forming a preliminary model of energetic pathways in this species.

The application of the principles of thermodynamics to fish energetics is a relatively recent endeavour, but one much entertained by physiologists, nutritionists, and ecologists. An understanding of the efficient transformation of biological energy has become an important issue in world affairs. It can be expected that studies of energetics and growth will develop greatly in the future contributing to our understanding of metabolic processes and tissue growth thereby benefiting fish production and ultimately the human population.

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APPENDIX 1

Oxycalorific coefficients for different fish diets

Q_{ox} for protein, lipid and carbohydrate is 13.36, 13.72 and 14.76 respectively.

(a) 20% protein diet

Proximate composition:	Protein (%)	Lipid (%)	Carbohydrate (%)
	19.74	7.64	57.06

$$Q_{ox} = \frac{(19.74 \times 13.36 + 7.64 \times 13.72 + 57.06 \times 14.76)}{(19.74 + 7.64 + 57.06)}$$

$$= 14.34$$

(b) 35% protein diet

Proximate composition:	Protein (%)	Lipid (%)	Carbohydrate (%)
	35.14	7.43	40.19

$$Q_{ox} = \frac{(35.14 \times 13.36 + 7.43 \times 13.72 + 40.19 \times 14.76)}{(35.14 + 7.43 + 40.19)}$$

$$= 14.07$$

(c) 50% protein diet

Proximate composition:	Protein (%)	Lipid (%)	Carbohydrate (%)
	49.88	7.34	22.18

$$Q_{ox} = \frac{(49.88 \times 13.36 + 7.43 \times 13.72 + 22.18 \times 14.76)}{(49.88 + 7.34 + 22.18)}$$

$$=13.79$$

APPENDIX II

Respiration (R) by Cyprinus carpio fed with 20% Protein diet (Energy value of feed @ 17.57 kJ/g diet and energy eq. 14.34 J/mg O_2)

Fish: 253.43g; Ration @ 1% body wt.; Energy in feed (C)= 44.45kJ

Day of obs.	R (mg/kg/hr)	Equivalent energy (kJ/tank/day)	'R' as % of 'C'
1	223.39	19.48	43.83
3	222.70	19.42	43.68
5	219.70	19.10	42.97
7	209.03	18.23	41.00
9	214.99	18.75	42.18
10	216.90	18.91	42.54
12	213.20	18.59	41.42
14	211.21	18.42	41.44
16	215.90	18.83	42.36
Mean, X	216.33 (+-4.92)	18.86 (+_0.42)	42.38 (+_0.99)

Fish wt. 251.12g; Ration @ 2% body wt.; Energy in feed (C)= 88.19kJ

1	283.76	24.52	27.81
3	279.32	24.14	27.37
5	281.85	24.36	27.62
7	276.31	23.88	27.07
9	286.45	24.76	28.07
10	285.08	24.64	27.93
12	292.27	25.26	28.64
14	290.05	25.07	28.42
16	289.42	25.01	28.36
Mean, X =	284.94 (+_5.23)	24.63 (+_0.45)	27.92 (+_0.51)

fish wt. 226.47g; Ration @3% body wt.; Energy in feed (C)= 119.87kJ

1	330.16	25.20	21.97
3	325.61	25.37	21.16
5	338.80	22.13	22.13
7	357.40	26.30	21.94
9	334.30	25.07	21.75
10	339.17	25.44	22.16
12	335.80	25.17	21.94
14	346.30	26.97	22.50
16	341.70	26.63	22.21
Mean, X =	337.34 (+_5.70	26.30 (+_0.47)	21.97 (+_0.37)

Respiration (R) by Cyprinus carpio fed with 35% Protein diet (Energy value of feed @ 19.17 kJ/g feed and Energy eq. 14.077 J/mg O_2

Fish wt. 282.58g; Ration @ 1% body wt.: Energy in feed (C)= 54.14kJ

Day of obs.	R (mg/kg/hr)	Equivalent energy (kJ/tank/day)	'R' as % of 'C'
1	270.40	25.31	46.67
3	260.00	24.82	45.84
5	259.54	24.77	45.75
7	248.84	23.76	43.87
9	247.99	23.68	43.72
10	255.80	24.42	45.10
12	262.60	25.01	46.19
14	254.89	24.34	44.94
16	255.39	24.38	45.03
Mean, X	257.20 (+-6.95)	24.55 (+_0.65)	45.23 (+_0.99)

Fish wt. 266.42g; Ration @ 2% body wt.; Energy in feed (C)= 102.13 kJ

1	330.36	29.74	29.11
3	347.90	31.31	30.66
5	344.07	30.97	30.32
7	334.43	30.10	29.47
9	343.90	30.95	30.30
10	328.80	29.36	28.75
12	330.08	29.71	29.09
14	345.04	31.15	30.49
16	339.08	30.51	29.88
Mean, X =	333.07 (+_5.18)	30.42 (+_0.68)	29.78 (+_0.66)

Fish wt. 257.42g; Ration @ 3% body wt.; Energy in feed (C) = 148.03 kJ

1	356.00	33.57	22.67
3	427.50	37.18	25.11
5	413.00	35.92	24.27
7	413.80	35.99	24.31
9	424.80	36.94	24.95
10	420.40	37.08	25.05
12	422.70	36.76	24.83
14	412.50	35.87	24.23
16	400.33	34.81	23.52
Mean, X =	414.74 (+_13.77)	36.07 (+_1.16)	24.33 (+_0.81

contd.

Rate of Respiration (R) by Cyprinus carpio fed with 50% Protein diet (Energy value of feed @ 19.36 kJ/g and Q_{ox} = 13.79 J/mgO_2

Fish wt. 244.60g; Ration @ 1% body wt.; energy in feed (C)= 47.44 kJ

Day of obs.	R (mg/kg/hr)	Equivalent energy (kJ/tank/day)	'R' as % of 'C'
1	266.29	21.57	45.46
3	276.76	22.42	47.30
5	264.21	21.40	45.10
7	275.94	22.36	47.16
9	272.76	22.09	46.56
10	289.40	22.45	49.43
12	291.43	23.61	49.81
14	280.53	22.73	47.95
16	264.96	21.47	45.29
Mean, X	275.58 (+-9.45)	22.45 (+_0.74)	47.12 (+_1.62)

Fish wt. 255.10g; Ration @ 2% body wt.; Energy in feed (C)= 98.74 kJ

1	367.53	31.03	31.43
3	347.30	29.15	29.52
5	358.10	30.23	30.61
7	346.10	29.22	29.59
9	349.90	29.54	29.91
10	347.35	29.33	29.69
12	385.45	32.54	32.95
14	370.58	31.29	31.68
16	341.30	28.85	29.22
Mean, X =	356.90 (+_14.73)	30.13 (+_1.24)	30.51 (+_1.26)

Fish wt. 253.00g; Ration @ 3% body wt.; Energy in feed (C) = 146.96 kJ

1	456.10	38.56	25.23
3	433.18	36.94	24.45
5	462.90	39.26	26.71
7	429.10	36.07	24.54
9	454.04	38.01	25.86
10	422.71	35.39	24.08
12	484.36	40.56	27.59
14	437.14	36.60	24.90
16	434.71	36.39	24.76
Mean, X =	445.57 (+_20.15)	37.31 (+_1.68)	25.38 (+_1.14

Appendix III

FAECAL ENERGY (F) **With 20% dietary protein**

Fish wt. 253.0g; Ration @ 1% body wt.; Energy value of 'C'= 44.45KJ

Dry wt.of faeces (g)	Energy value of faeces (KJ/g)	Total energy in faeces 'F' (KJ)	'F'as % of 'C' (F/CX100)	Assimilation efficiency (C-F)/CX100
0.8340	12.69	10.58	23.80	76.19
0.609	12.86	7.83	17.61	77.89
0.7413	13.26	9.83	22.11	77.88
0.8140	13.33	10.85	24.40	75.59
0.7213	13.69	9.87	22.21	77.79
0.865	13.42	11.60	26.09	73.90
0.645	12.12	7.81	17.57	82.46
0.700	13.90	9.73	21.88	78.11
0.603	13.06	7.88	17.73	82.27
X= 0.7258 (+ .0968)	X= 13.15 (+ 0.54)	X= 9.55 (+ 1.141)	X= 21.38 (+ 3.36)	X= 78.00 (+ 2.83)

Fish wt. 251.12g; Ration @ 2% body wt.; Energy value of 'C'= 88.19 KJ

1.5102	16.23	24.51	27.79	69.49
1.4016	15.03	21.07	23.89	73.72
1.3506	14.93	20.16	22.86	74.08
1.0020	15.61	15.64	17.73	79.89
1.5020	15.69	23.56	26.72	69.70
1.3290	14.89	19.79	21.26	74.56
1.3160	13.76	18.10	20.53	76.72
1.5300	14.12	21.60	24.49	72.23
1.2180	14.39	17.53	19.88	77.46
X = 1.348 (+ 0.183)	X= 14.96 (+ 0.79)	X= 20.22 (+ 2.84)	X= 21.78 (+ 3.28)	X=74.09 (+ 3.65)

Fish wt.226.47g; Ration @ 3% body wt.; Energy value of 'C'= 119.87 K

1.902	15.92	30.28	25.26	74.73
2.012	14.79	29.76	24.83	75.17
1.941	15.07	29.25	24.40	75.59
2.136	14.78	31.57	26.33	73.66
2.041	15.33	31.28	26.09	76.42
1.839	14.43	26.53	22.13	77.86
2.009	15.21	30.56	25.49	74.51
1.569	15.16	23.78	19.84	77.63
1.807	14.38	25.98	21.67	78.32
X = 1.917 (+ 0.166)	X= 15.00 (+ 0.48)	X = 28.78 (+2.70)	X = 24.00 (+ 2.25)	= 75.98 (+ 2.25)

Contd.---

FAECAL ENERGY (F) With 35% dietary protein

Fish wt. 282.58g ; Ration: @ 1% body wt.; Energy value of 'C'=54.15kJ

Dry wt. of faeces (g)	Energy value of faeces (KJ/g)	Total energy in faeces 'F'(KJ)	'F' as % of 'C' (F/CX100)	Assimilation efficiency (C-F)/CX100
0.8626	14.12	12.14	22.49	77.60
0.8106	13.99	11.34	20.94	79.06
0.7041	14.03	9.87	18.24	81.77
0.6912	13.70	9.47	17.49	82.51
0.6810	15.08	10.27	18.96	81.36
0.8340	15.02	12.53	23.13	76.86
0.4534	13.63	6.18	21.41	88.59
0.7082	14.57	10.32	19.06	80.94
0.8688	12.64	10.98	20.28	79.72
X=0.7349 (+_0.130)	X=14.09 (+_0.75)	X=10.344 (+_1.86)	X=19.10 (+_2.07)	X=80.93 (+_3.44)

Fish wt. 266.41g; Ration @ 2% body wt.; Energy value of 'C'= 102.13kJ

1.3040	15.63	20.38	19.95	80.04
1.4216	14.41	20.48	20.05	79.94
1.675	12.98	21.74	21.28	81.25
1.385	14.33	19.84	19.42	81.58
1.561	13.06	20.39	19.96	80.03
1.400	15.54	21.76	21.30	78.69
1.236	15.67	19.37	18.96	81.03
1.455	12.86	18.71	18.32	81.68
1.003	15.10	15.14	14.82	81.63
X=1.382 (+ 0.192)	X= 14.39 (+ 1.18)	X=19.75 (+ 1.88)	X=19.34 (+ 1.84)	X= 80.65 (+ 1.95)

Fish wt. 257.42g; Ration @ 3% body wt.; Energy value of 'C'= 148.03 kJ

1.961	13.12	25.73	17.38	82.61
2.016	14.08	28.38	19.17	80.82
2.380	13.34	27.74	21.45	81.26
1.524	14.00	25.54	14.40	82.71
2.368	13.21	31.28	21.13	78.86
1.976	14.33	28.32	19.12	80.86
1.835	15.09	27.69	18.70	81.29
1.970	14.26	28.09	18.97	81.03
2.077	14.05	28.98	19.71	86.77
X= 2.011 (+ 0.26)	X= 13.94 (+ 0.63)	X= 27.97 (+ 1.71)	X= 18.89 (+ 2.09)	X=81.80 (+ 2.17)

FAECAL ENERGY(F) With 50% dietary protein

Fish wt. 244.80g; Ration @ 1% body wt.; Energy value of 'C' = 47.44kJ

Dry wt.of faeces (g)	Energy value of faeces (KJ/g)	Total energy in faeces 'F' (KJ)	'F' as % of 'C' (F/CX100)	Assimilation efficiency (C-F)/CX100
0.6210	16.30	10.12	21.33	78.67
0.5821	15.29	8.90	18.76	81.23
0.5881	15.96	9.38	19.79	80.22
0.6034	16.06	9.69	20.43	79.57
0.3649	16.20	5.91	22.45	87.53
0.6234	16.41	10.23	21.56	78.43
0.8003	15.88	12.70	26.77	73.22
0.5612	15.36	8.62	18.17	81.83
0.6757	16.07	10.85	22.87	77.13
X=0.6022 (+ 0.114)	X= 15.95 (+ 0.39)	X= 9.60 (+ 1.83)	X=20.24 (+ 3.87)	X=79.76 (+ 3.87)

Fish wt. 255.10g ; Ration @ 2% body wt.;Energy value of 'C' = 98.74kJ

1.112	18.21	20.25	20.50	79.49
1.026	17.34	17.79	18.01	81.98
0.9653	18.48	15.89	16.09	83.90
1.0970	18.62	20.42	20.69	79.32
0.9735	18.34	17.85	18.08	81.92
1.231	16.63	20.47	20.73	79.27
1.2210	18.08	23.88	24.18	75.81
0.9860	17.96	17.70	17.93	82.07
0.9850	18.50	18.36	18.59	81.41
X =1.066 (+0.141)	X=18.01 (+ 0.63)	X=19.18 (+ 2.34)	X=19.42 (+ 2.36)	X=80.57 (+ 2.36)

Fish wt. 253.0g; Ration @ 3% body wt. Energy value of 'C' = 146.96 KJ

1.783	17.49	31.18	21.27	78.78
1.8014	15.41	29.30	19.94	80.06
1.6310	16.33	26.63	18.12	81.88
1.8406	17.41	34.55	23.50	76.49
1.8960	17.56	33.29	22.65	77.34
1.623	17.49	28.39	19.32	80.68
1.710	17.60	30.10	20.48	79.52
1.537	16.93	22.64	15.40	84.59
1.686	16.31	26.81	18.24	81.76
X=1.7233 (+ 0.196)	X=16.95 (+ 0.77)	X= 29.21 (+ 3.64)	X= 19.88 (+ 2.48)	X= 80.12 (+ 2.47)

APPENDIX IV

Apparent protein digestibility(%) with different protein diets.

@ 1% ration level

Dry wt.of faeces (g)	Protein in feed(%)	Protein in faeces (%)	Apparent Protein Digestibility (%)
0.834	20	11.37	71.21
0.609	20	10.97	72.46
0.741	20	11.41	71.40
0.814	20	10.90	71.89
0.721	20	11.32	70.77
0.865	20	11.39	70.23
0.645	20	11.71	71.88
0.700	20	10.08	72.08
X = 0.7258 (+_0.968)	X= 20	X = 11.18 (+_ 0.46)	X= 71.43 (+_0.72)

@ 2% ration level

Dry wt. of of faeces(G)	Protein in feed (%)	Protein in faeces (%)	Apparent Protein Digestibility(%)
1.5102	20	10.64	72.49
1.4016	20	10.39	73.47
1.3506	20	10.71	71.67
1.002	20	11.38	70.46
1.502	20	11.66	71.12
1.320	20	11.73	71.01
1.3160	20	10.91	72.49
1.5300	20	11.79	71.53
1.2180	20	11.92	70.09
X=1.348 (+_0.183)	X=20	X=11.23 (+_0.54)	71.59 (+_ 1.01)

@ 3% ration level.

1.902	20	12.13	72.12
2.012	20	11.79	74.33
1.941	20	12.03	73.36
2.136	20	13.18	73.56
2.041	20	12.30	71.00
1.839	20	13.66	68.11
2.009	20	12.79	70.66
1.569	20	13.02	72.39
1.807	20	12.76	70.11
X=1.917 (+-0.116)	X= 20	X= 12.63 (+_0.48)	X = 71.74 (+_ 1.85)

@ 1% ration level (contd..)

Dry wt. of faeces (g)	Protein in feed(%)	Protein in Faeces(%)	Apparent Protein Digestibility (%)
0.862	35	16.02	80.67
0.810	35	15.31	81.32
0.704	35	15.74	81.02
0.691	35	16.36	79.78
0.681	35	15.79	80.44
0.834	35	16.64	79.30
0.453	35	19.91	79.57
0.708	35	15.88	82.77
0.869	35	16.67	79.41
X=0.735 (+_0.13)	X=35	X=16.14 (+_0.53)	80.40 (+_1.08)

@ 2% ration level.

Dry wt. of faeces (g)	Protein in feed (%)	Protein in faeces (%)	Apparent Protein Digestibility (%)
1.304	35	16.32	80.44
1.422	35	17.11	81.19
1.675	35	16.98	79.91
1.383	35	16.18	80.55
1.561	35	16.45	81.57
1.400	35	15.63	81.68
1.236	35	16.08	79.11
1.455	35	15.89	82.60
1.003	35	16.37	79.72
X=1.382 (+_0.19)	X=35	X=16.33 (+_0.48)	X=80.75 (+_1.10)

@ 3% ration level (contd.)

Dry wt. of faeces (g)	Protein in feed (%)	Protein in Faeces (%)	Apparent Protein Digestibility (%)
1.961	35	16.49	82.17
2.016	35	16.61	81.89
2.380	35	17.30	78.05
1.524	35	15.80	82.16
2.368	35	16.30	82.51
1.976	35	16.43	80.35
1.835	35	15.96	83.09
1.970	35	16.64	78.45
2.077	35	15.79	79.11
X=2.010 (+_0.26)	X=35	X=16.49 (+_0.60)	X=80.86 (+_1.91)

contd...

@ 1 % ration level

Dry wt. of Faeces (g)	Protein in Feed (%)	Protein in Faeces (%)	Apparent Protein Digestibility (%)
0.621	50	19.71	81.71
0.582	50	18.31	82.19
0.588	50	19.47	78.19
0.603	50	17.13	84.17
0.365	50	17.87	83.08
0.623	50	18.07	81.11
0.800	50	19.11	77.67
0.561	50	17.36	84.79
0.676	50	17.84	82.68
X=0.602 (+_0.114)	X=50	X=18.32 (+_0.92)	X=81.73 (+_2.44)

@ 2% ration level (contd..)

Dry wt. of Faeces (G0	Protein in Feed (%)	Protein in faeces (%)	Apparent Protein Digestibility(%)
1.112	50	20.11	80.63
1.026	50	19.37	83.69
0.860	50	20.17	78.97
1.097	50	19.08	85.11
0.974	50	20.11	79.70
1.231	50	19.80	84.36
1.321	50	17.40	84.63
0.986	50	17.67	82.59
0.9924	50	18.31	79.46
X = 1.006 (+_0.141)	X = 50	X = 19.11 (+_1.08)	X = 82.12 (+_2.45)

@ 3% ration level

Dry wt. of Faeces (g)	Protein in Feed (%)	Protein in faeces (%)	Apparent protein Digestibility(%)
1.783	50	20.63	82.08
1.901	50	18.71	84.87
1.631	50	20.39	81.75
1.985	50	20.07	82.34
1.896	50	17.46	86.69
1.623	50	21.28	79.12
1.710	50	19.88	80.62
1.337	50	18.79	84.41
1.644	50	18.65	82.58
X = 1.723 (+_0.196)	X = 50	X = 19.54 (+_1.21)	X = 82.72 (+_2.29)

Appendix V

Nitrogenous Excretion (U) by common carp fed with 20% Protein diet. (@ 17.57 kJ/g and @ 24.83 J/mg NH_3

Fish wt. 253.43g (4 fish with mean weight 63.35 +_5.95g)
Ration @ 1% body wt. Energy in 'C'= 44.45kJ

Day of obs.	U (mg/kg/hr)	Equivalent energy (kJ/tank/day)	'U' as % of 'C'
1	18.84	2.85	6.40
3	19.39	2.92	6.59
5	18.82	2.84	6.39
7	18.48	2.79	6.28
9	17.11	2.59	5.82
10	18.50	2.79	6.28
12	18.65	2.81	6.34
14	16.98	2.56	5.77
16	18.12	2.74	6.16
Mean, X	18.32 (+-0.89)	2.77 (+_0.12)	6.22 (+_0.27)

Fish wt. 251.12g Ration @ 2% body wt.; Energy value of 'C'= 88.19kJ

1	28.85	4.31	4.89
3	29.36	4.39	4.98
5	32.01	4.79	5.43
7	26.89	4.02	4.56
9	29.84	4.47	5.06
10	31.10	4.65	5.28
12	30.72	4.60	5.21
14	26.66	4.00	4.52
16	28.06	4.12	4.76
Mean, X =	29.28 (+_1.85)	4.38 (+_0.27)	4.97 (+_0.31)

Fish wt. 226.47g ; Ration @ 3% body wt.; Energy value of 'C'= 119.87kJ

1	38.46	5.19	4.35
3	37.23	5.02	4.21
5	37.38	5.04	4.22
7	37.29	5.03	4.20
9	38.12	5.14	4.31
10	35.93	4.85	4.06
12	37.04	5.00	4.19
14	36.30	4.90	4.10
16	36.63	4.94	4.14
Mean, X =	37.16 (+_0.61)	5.01 (+_0.11)	4.19 (+_0.13)

Contd....

Nitrogenous Excretion (U) by Cyprinus carpio fed with 35% protein diet.
(energy value of feed @ 19.17kJ/g feed and 24.83 J/mg NH_3)

Fish wt. 282.58g; Ration @ 1% body wt. Energy in feed (C)= 54.14kJ

Day of obs.	U (mg/kg/hr)	Equivalent energy (kJ/tank/day)	'U' as % of 'C'
1	19.51	3.29	6.07
3	22.25	3.75	6.92
5	21.74	3.66	6.76
7	21.64	3.64	6.73
9	24.96	4.20	7.76
10	23.48	3.95	7.30
12	24.68	4.16	7.69
14	24.69	4.16	7.69
16	24.48	4.12	7.63
Mean, X	23.05 (+-1.87)	3.88 (+_0.32)	7.17 (+_0.59)

Fish wt. 266.42g; Ration @ 2% body wt.; Energy in feed (C)= 102.13 kJ

1	43.60	6.92	6.77
3	39.38	6.25	6.12
5	41.62	6.61	6.46
7	39.55	6.28	6.14
9	41.97	6.66	6.52
10	40.20	6.38	6.24
12	39.42	6.26	6.13
14	39.16	6.22	6.08
16	40.23	6.39	6.25
Mean, X =	40.57 (+_1.47)	6.44 (+_0.25)	6.30 (+_0.23)

Fish wt. 257.42g; Ration @ 3% body wt.; Energy in feed (C)= 148.03 kJ

1	55.48	8.51	5.75
3	59.28	9.09	6.14
5	55.62	8.53	5.76
7	57.20	8.77	5.92
9	57.24	8.78	5.93
10	57.61	8.84	5.97
12	58.22	8.93	6.03
14	58.08	8.91	6.01
16	56.94	8.73	5.90
Mean, X =	57.29 (+_1.21)	8.79 (+_0.186)	5.93 (+_0.12)

Nitrogenous Excretion (U) by Cyprinus carpio fed on 50% dietary protein (Energy value @ 19.36 kJ.g feed and 24.83 J/mg NH_3).

Fish wt. 244.80g; Ration @ 1% body wt.; Energy in feed (C): 47.44 kJ

Day of obs.	U (mg/kg/hr)	Equivalent energy (kJ/day)	'U' as % of 'C'
1	28.45	4.15	8.75
3	26.63	3.88	8.19
5	28.19	4.11	8.67
7	30.59	4.46	9.41
9	28.02	4.09	8.62
10	27.92	4.07	8.59
12	30.12	4.39	9.27
14	28.13	4.10	8.65
16	27.76	4.05	8.54
Mean, X	28.42 (+-1.21)	4.14 (+_0.17)	8.74 (+_0.37)

Fish wt. 255.10g ; Ration @ 2% body wt.; Energy in feed (C): 98.74 kJ

1	46.84	7.12	7.51
3	48.58	7.38	7.79
5	52.99	8.06	8.50
7	52.10	7.92	8.35
9	49.60	7.54	7.95
10	49.29	7.49	7.90
12	52.26	7.94	8.38
14	48.98	7.45	7.85
16	48.80	7.42	7.83
Mean, X =	49.94 (+_2.05)	7.59 (+_0.31)	8.00 (+-0.33)

Fish wt. 253.00g; Ration @ 3% body wt.; Energy in feed (C): 146.96 kJ

1	68.04	10.26	6.98
3	64.39	9.71	6.60
5	66.12	9.97	6.79
7	65.19	9.83	6.68
9	67.53	10.18	6.93
10	67.22	10.13	6.90
12	67.59	10.19	6.93
14	72.44	10.92	7.43
16	69.68	10.51	7.14
Mean, X =	67.58 (+_2.41)	10.18 (+_0.36)	6.93 (+_0.25