PLASMA CONCENTRATIONS OF AMINO ACIDS IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) IN RESPONSE TO NUTRITIONAL AND FEEDING MANAGEMENT

by

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ABSTRACT

This study considered a number of dietary factors that may alter the balance and constancy of amino acid concentrations in the plasma of rainbow trout. Fish given five equal feedings per day had relatively constant plasma amino acid concentrations and deposited more protein and less lipid than did fish fed the same ration at one feeding per day. The rate at which amino acids from the single meal feeding entered the circulation was evidently in excess of the rate at which those amino acids could be utilized for immediate protein synthesis with the result that a higher proportion of them was catabolized and the carbon skeletons employed in lipid synthesis. Supplementation of dietary protein with lysine and methionine in the free form resulted in more rapid appearance of these amino acids in the plasma than occurred with the intact dietary protein alone. A surge of plasma arginine, alanine, histidine, and lysine concentrations observed at 36 h after the fish were fed diets containing a mixture of protein sources suggested delayed digestion of particular proteins. Isonitrogenous substitution of free glycine for that supplied by gelatin delayed the time at which plasma glycine peaked postprandially compared with the response to the gelatin-containing diet. When a diet was supplemented with a mixture of free essential amino acids, elevated concentrations remained in the plasma and muscle pools as long as 26 h after feeding, indicating that dietary supplements of free amino acids may remain available for protein synthesis even when the fish are fed once daily. Plasma concentrations of free amino acids in fish fed different concentrations of dietary lipid indicated that dietary lipid at 24% of the diet had no effect of the lipid on digestion of protein or absorption of amino acids. Postprandial concentrations of plasma amino acids in fish fed fish meal that had

been subjected to heat treatment showed that the predominant nutritional effect of protein denaturation was a reduction in availability of threonine and histidine. In conclusion, the responses of plasma amino acid concentrations to different dietary conditions observed in this study indicate that they provide a useful tool for investigating the effects of various nutritional factors on protein metabolism in fish.

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CHAPTER 1

INTRODUCTION

Free amino acids in plasma of animals are derived from dietary protein, catabolism of tissue proteins, and synthesis. At the same time free amino acids are removed from the plasma for synthesis of tissue protein, or other nitrogenous compounds, and degradation. Consequently, the size of the plasma pool of each amino acid at any time is the result of the balance between input and removal (Munro, 1970). Numerous studies with animals of various species including fish have demonstrated a marked rise of plasma amino acids following consumption of protein (McLaughlan and Morrison, 1968; Eggum, 1972; Nose, 1972; Yamada *et al.*, 1982; Walton and Wilson, 1986; Lyndon *et al.*, 1993). Furthermore, the concentrations of essential amino acids in plasma are positively correlated with their dietary concentrations during the absorptive period. The above relationship suggests that studies of the amino acid pattern appearing in the plasma during and following protein digestion might give an indication of rates and extent of proteolytic release of the amino acids from dietary proteins.

As with other animals, the rate at which different amino acids are absorbed from the digestive tract and enter the circulation have an important bearing on the rate of protein synthesis in fish. The inferior growth rate of rainbow trout observed by Cowey *et al.*, (1992) when fish were fed diets containing large quantities of supplementary free amino acids was assumed by these investigators to be due to the difference in uptake rates into plasma of free amino acids compared with amino acids derived from digestion of intact protein in the diet. In an experiment in which fish were fed a purified diet containing free amino acids , but no source of intact

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protein, there was more rapid appearance and a higher concentration of free amino acids in the plasma (Murai *et al.*, 1987). It was considered that the rapid uptake of amino acids into the plasma enhanced the activities of amino acid-catabolizing enzymes leading to increased amino acid degradation and decreased protein synthesis (Cowey and Luquet, 1983).

From a practical point of view, fish diets often contain a combination of different protein sources. Sometimes supplementary amino acids are added to improve the amino acid profile of the diet (Hardy, 1989). It has been well documented that different protein sources vary in their digestibility and consequently in the efficiency of absorption of amino acids from the gastrointestinal tract. Under this circumstance, the rate at which free amino acids are absorbed into the circulation from the hydrolysis of different dietary proteins in the gastrointestinal tract and from supplementary amino acids may be different. Furthermore, although a formulated diet may have an amino acid profile that meets the requirements of the fish, the availability of amino acids to the fish may be reduced by factors that affect digestibility of the protein.

The present study evolved from the above observations. It was devoted to investigating the responses of plasma amino acid concentrations in rainbow trout under a variety of dietary treatments. The plasma concentrations of individual amino acids were monitored following the feeding of 1) diets containing different protein sources, 2) diets containing different protein sources supplemented with free amino acids, 3) experimental diets at two feeding frequencies, 4) diets containing different lipid concentrations, and 5) diets containing experimental fish meals subjected to over-heating.

CHAPTER 2

LITERATURE REVIEW

2.1 PLASMA FREE AMINO ACIDS

The release and absorption of free amino acids from ingested proteins in fish follows similar processes to those in other animals. Protein consumed in the diet is hydrolyzed enzymatically in the gastrointestinal tract, and the final products of the digestion, i.e. free amino acids, are absorbed into the circulation via the portal blood stream (Nissen, 1992). A proportion of amino acids liberated in the intestinal lumen is also incorporated into proteins of mucosal cells. Moreover, some amino acids may be metabolized within the epithelium of small intestine, for example the transamination of glutamic acid and aspartic acid with pyruvate results in low recoveries of these amino acids from the mucosal cells (Munro and Crim, 1980). After entering the circulation, free amino acids are subjected to a series of metabolic reactions which can be grouped into three categories (Munro and Crim, 1980). First, part of the free amino acids is incorporated into tissue proteins. Because of tissue protein catabolism, these amino acids eventually return to the free pool after a variable length of time and become available for reutilization. Second, part of the free amino acids undergoes catabolic reactions. This leads to loss of the carbon skeleton as CO_2 or its conversion to fat and/or glucose, while the nitrogen is excreted as ammonia in fish. Third, some amino acids are used for synthesis of N-containing compounds, such as purine bases, hormones, nucleic acids, and neurotransmitters. In addition, some nonessential amino acids are synthesized in the body using amino groups derived from other amino acids, and carbon skeletons

from intermediary metabolism (Munro and Crim, 1980; Cowey and Walton, 1989). Consequently, the size of the pool of each free amino acid varies depending upon the rate of entry and removal of respective amino acids into and from the pool (Munro, 1970). The pattern of plasma amino acids has been shown to relate to that of the ingested protein in many animals (Longenecker and Hause, 1959; Hill and Olsen, 1963; McLaughlan and Illman, 1967; Munro,1970; Young and Scrimshaw, 1972). On this basis, amino acids present in greatest abundance in the diet should cause the largest increases in plasma concentrations. This relationship has been confirmed by several researchers (Eggum, 1972; Hagemeister *et al.*, 1990). Some investigators, on the other hand, have failed to establish a relationship between the relative abundance of dietary amino acids and their increase in plasma during the absorptive period. For example, Nasset *et al.*, (1963) could not find any relationship between amino acid composition of the diets and plasma free amino acids in dogs. Despite this contradiction, Johnson and Anderson (1982) were able to qualitatively and quantitatively relate plasma amino acids to dietary amino acids. They used retrospective analysis of plasma amino acid data from a number of studies in which protein quantity and quality of the diet were varied. The level of each plasma amino acid in male weanling rats was examined in relation to their intake from gluten, casein, synthetic amino acid, or zein diets. They found that levels of most amino acids in the plasma were predictable if both the concentration of the amino acid in the diet and chronic level of protein intake were known.

Based on the above relationship, the alterations of plasma amino acid concentrations have been employed as an indicator for various purposes in protein nutrition. Plasma amino acid levels have been investigated in relation to the

limiting amino acid in the diet (McLaughlan and Morrison, 1968; Sarwar *et al.*, 1983), amino acid requirement (Harper, 1977), amino acid imbalance (Peng and Harper, 1970; Peng *et al.*, 1972), and absorption and availability of amino acids (Young and Scrimshaw, 1972; Ostrowski, 1977).

Investigations have also been conducted on the relationship between the state of body protein metabolism and plasma amino acid concentrations (Young and Scrimshaw, 1972). For example, Galibois *et al.*, (1987) have attempted to determine the relationship between protein synthesis rate and concomitant plasma amino acid levels in rats. The rats were fed with four types of proteins, i.e. beef, rapeseed flour, casein, and soybean. Plasma amino acid concentrations in the portal vein and the aorta, and hepatic ribosome aggregation were investigated at different times during the active eating period. Each protein source was found to generate different variations in portal and aortic plasma amino acid levels. Furthermore, proportions of essential amino acids in portal and aortic plasma were characteristic of the protein fed and tended to remain constant. Although no direct relationship was detected between the concentrations and pattern of plasma amino acids, and ribosome aggregation, these two parameters were both affected by dietary protein.

Most of the studies in fish on plasma amino acid levels have been limited to a few species. The investigations have been conducted mostly in rainbow trout, carp, and channel catfish (commercially important species). Some studies have also been carried out in Atlantic salmon, coho salmon, sea bass, tilapia, and goldfish (Cowey *et al.*, 1962; Carrillo *et al.*, 1980; Yamada *et al.*, 1982; Thebault, 1985; Ogata and Arai, 1985). Studies with fish have generally shown similar results to those with mammals in that the plasma amino acid concentrations, particularly essential amino acids,

were positively correlated with their dietary concentrations (Nose, 1972; Plakas *et al.*, 1980; Dabrowski, 1982; Wilson *et al.*, 1985; Walton and Wilson, 1986; Blasco *et al.*, 1991; Lyndon *et al.*, 1993). Concentrations of non-essential amino acids in plasma, however, do not show such correlation, presumably because these amino acids undergo extensive interconversion and metabolism within the body (Cowey and Walton, 1989).

Interesting interspecies differences have been noted in the chronology of the appearance of essential amino acids in the plasma of fish following the provision of a protein containing diet. In rainbow trout, several studies have indicated that after consumption of diets containing intact proteins, such as casein or a commercial pelleted diet, plasma amino acids rose soon after feeding and attained peaks at 6 h or between 12-24 h post-feeding depending on experimental conditions such as water temperature, salinity, fish size, duration of starvation, and types of diets (Nose, 1972; Kaushik and Luquet, 1977b; Walton and Wilson, 1986). Yamada *et al.*, (1981a), on the other hand, reported different results. They force-fed rainbow trout a casein diet, and observed a lag period until plasma amino acids started to rise at 12 h postprandial. Peaks of amino acid concentrations occurred between 24-36 h after feeding. An explanation for the 12 h lag period observed by Yamada *et al.*, (1981a) might conceivably reside in the stress effect on the digestive physiology induced by the forced-feeding protocol (Ash, 1985). While this might be true, Murai *et al.*, (1987) also force-fed rainbow trout a diet containing the same type of protein, but did not observe any lag period. Walton and Wilson (1986) suggested that the period of starvation before the time of feeding was possibly the cause of the delay of stomach emptying resulting in the delay of digestive and absorptive processes.

The times when most amino acids reached peaks after meal consumption in other species of fish have differed from those of rainbow trout. In carp, tilapia and sea bass, most amino acids have been reported to rise within 2 h and reached peaks between 4-6 h depending upon the species (Plakas *et al.*, 1980; Yamada *et al.*, 1982; Thebault, 1985). Differences in the appearance of amino acids in rainbow trout (cold water species) and the species mentioned above (warm water species) were due to temperature and morphology of the digestive tract particularly in the case of carp which is an agastric species. Regarding the latter reason, when compared with the rainbow trout, digestion of casein in carp is very rapid. Ash (1985) postulated that in agastric species the ingested protein will become immediately subjected to the action of proteolytic enzymes and transport systems present within the anterior small intestine.

When different species of fish were fed diets containing synthetic amino acids in comparison with those containing intact protein, a similar phenomenon on the appearance of plasma free amino acids was observed. The plasma amino acids in fish fed free amino acid diets rose and reached their peaks earlier than those in fish fed intact protein diets (Plaskas *et al.*, 1980; Murai *et al.*, 1987; Yamada *et al.*, 1982). Moreover, the examination of stomach contents in rainbow trout by Yamada *et al.*, (1981b) revealed that fish fed an amino acid mixture emptied the stomach more rapidly than fish fed a casein diet. Plakas *et al.*, (1980) also observed that not only were free amino acids in carp that were fed amino acids absorbed at a faster rate, but there were also variations in the response of the plasma amino acids compared with when they were fed intact protein.

The above finding has provided useful information for better understanding of amino acid utilization in fish. For example, it has been reported that fish of several species fail to utilize an amino acid diet or show inferior growth compared to fish fed a diet containing intact protein. In the light of this finding several researchers concluded that the inability of fish to utilize a diet containing free amino acid effectively was due to faster absorption and catabolism of dietary free amino acids before amino acids from intact dietary protein would be available for protein synthesis (Ash, 1985).

Concentrations of plasma amino acids have been used as a parameter to determine amino acid requirements in several species of fish such as channel catfish, rainbow trout, and sea-bass. The success of this technique has varied. For example, Harding *et al.*, (1977) and Wilson *et al.*, (1977) successfully used plasma free amino acid levels to determine the requirements for lysine and methionine in channel catfish. They found that plasma concentrations of these free amino acids increased significantly once the requirement of the amino acid was exceeded on the basis of maximum growth. Kaushik *et al.*, (1988) and Cho *et al.*, (1992), also attempted to relate the plasma concentrations of arginine to growth when estimating the arginine requirement of rainbow trout. The results obtained from both studies, however, indicated that the patterns of change in plasma arginine levels did not provide the means for estimation of the arginine requirement.

Assessment of protein quality has been attempted on the basis of the plasma amino acid profile following ingestion of a test protein. For instance, Ogata *et al.*, (1986) fed carp diets containing various ratios of casein to gelatin and compared free amino acid concentrations in the plasma with dietary amino acid composition

as predictors of growth response to the diets. They concluded that performance of carp can be estimated more precisely from plasma free amino acid level than from the dietary amino acid levels. A similar finding was found in the same species of fish by Murai *et al.*, (1989a) when they fed diets containing soy flour supplemented with methionine.

Lyndon *et al.*, (1993) measured the changes in the free amino acid pools of various tissues, including plasma, of the cod after a single meal, and endeavored to interpret the results in relation to protein synthesis. They found that the patterns of plasma amino acids were similar to those in rainbow trout. They suggested from the results on tissue pools of free amino acids together with the results found in other studies on plasma amino acids that the changes in the concentration of some essential amino acids, particularly tryptophan, have a role in stimulation of protein synthesis. This is because tryptophan, which was consistently found in the lowest level in all the pools, showed a significant increase between 12-24 h post-feeding, this range in time coincided with the time when the rate of protein synthesis was maximum. Their conclusion was based on the assumption that protein synthesis can only proceed if all amino acids are simultaneously present in the tissue. The rate of protein synthesis will be limited by the least abundant essential amino acid present in the precursor pool.

However, the interpretation of plasma amino acids in relation to protein status is a difficult task. This is because qualitative and quantitative changes in the plasma free amino acid profile are determined by a number of factors. These include 1) amino acid composition, digestibility and quantity of protein consumed 2) composition of remaining food constituents 3) rate of stomach emptying 4) rate of

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absorption of amino acids 5) extent to which the gastrointestinal mucosal cells metabolizes various amino acids 6) rate of amino acid removal from the blood which is influenced by numerous metabolic processes associated with amino acid metabolism 7) time of sampling after giving the test meal 8) endogenous protein in the gastrointestinal tract (to a small and temporary extent) and 9) age and strain of animals (Young and Scrimshaw, 1972; Simon, 1989; Munro and Portugal, 1972).

2.2 PROTEIN SOURCES IN SALMONID DIETS

2.2.1 Present Status of the Use of Protein Sources in Salmonid Diets

Fish meal is the major dietary protein source in salmonid diets, ranging from 25-60% of commercial diets (Murai, 1992), and there have been many attempts to partly or completely replace fish meal protein with alternative protein sources. Protein sources that have been studied in salmonids are from animals and their by-products, plants and their by-products, and unconventional protein sources. The animal and animal by-product protein sources include locally available fish meal, meat and bone meal, blood meal, poultry-by-product meal, hydrolyzed feather meal, and, dried milk by-product (Tacon and Jackson, 1985). The plant protein sources include soybean meal and concentrate, rapeseed meal and concentrate, canola meal, cotton seed meal, corn gluten meal, field bean meal, wheat germ meal, broad bean meal, sunflower meal, and sweet lupin meal (Higgs, *et al.*, 1979 and 1991; Tacon and Jackson, 1985; Fowler and Burrows, 1971). The unconventional protein sources include single cell protein (yeast, bacteria, blue green algae), fry larvae, krill meal, leaf protein concentrate, and dried domestic sewage (Tacon and Jackson,

1985; Cowey *et al.*, 1971; Rosenlund, 1986).

Among the proteins from animals and their by-products, poultry-by-products have been reported to have the most potential, even though the results from different laboratories are still contradictory. Gropp *et al.*, (1979) reported that fish meal in practical trout diets could be replaced entirely by a mixture of poultry by-product meal and hydrolyzed feather meal on an isonitrogenous basis if the essential amino acid deficiencies were corrected. The results from studies of Tiews *et al.*, (1979) corresponded well with the work of Gropp *et al.*, (1979) in rainbow trout. Higgs *et al.*, (1979) were unsuccessful in their attempt to completely replace herring meal protein with poultry by-product meal in diets for coho salmon. A mixture of poultry by-product meal with hydrolyzed feather meal, however, was found to successfully replace up to 75% of herring meal in the diet for coho salmon without any reduction in growth rate. Fowler (1991) attempted to partially replace fish meal with poultry by-product meal in diets for chinook salmon. He found that the growth rates and feed efficiencies of chinook fed a diet containing 20% of poultry by-product meal (replaced 50% of fish meal protein) were not different from those fed the diet without poultry by-product meal. Feather meal, usually considered to be an inferior source of protein for fish can be included in salmonid diets by replacing up to 50% of fish meal (Tiews *et al.*, 1979; Higgs *et al.*, 1979; Fowler, 1990). The dietary combination of meat and bone meal and blood meal was mentioned by Tacon and Jackson (1985) to be successfully employed to replace up to 50% of fish meal protein.

Soybean meal would appear to have the most potential of the plant protein sources available as a partial substitute for fish meal in salmonid diets although

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results from many studies have been variable. Reinitz (1980) found that fish fed a diet with 65% soybean meal and no herring meal grew at an acceptable rate and remained in good health. He, therefore, concluded that soybean meal, although not nutritionally equal to herring meal, could be used as the primary source of protein in trout diets. Partial replacement of soybean meal in salmonid diets was reported by several researchers to substitute for 75% of fish meal without any adverse results (Higgs *et al.*, 1979; Alexis *et al.*, 1985; Smith *et al.*, 1988). In other studies, however, the inclusion of soybean meal in salmonid diets at various concentrations has been shown to reduce growth rate and feed efficiency (Fowler, 1980; Dabrowski *et al.*, 1989; Murai *et al.*, 1989a). Murai (1992) pointed out that there are differences among species in the utilization of soybean meal and that rainbow trout are able to utilize this protein source more efficiently than chum salmon. The results of Fowler (1980) support this finding because he found that the growth of chinook and coho salmon fed the same diet as that fed to rainbow trout was depressed and there was concomitant high mortality. Krogdahl (1991) has reported that feeding rainbow trout with soybean products induces histological and chemical changes in the absorptive cells of the intestinal mucosa which may, in turn, alter the digestive process.

Other plant protein sources having a potential for partial replacement of fish meal are canola meal, cotton seed meal, and corn gluten meal (Gropp *et al.*, 1979; Higgs *et al.*, 1979, 1983; Fowler, 1980; March, 1991). The substitution levels are mentioned to be between 5-15% of diets (Tacon and Jackson, 1985). Higgs *et al.*, (1991), however, suggested that undephytinized rapeseed protein concentrate can comprise about 38% of dietary protein (fish meal only 11% of diet) without

adversely affecting growth rate, appetite, feed efficiency and protein utilization of trout.

2.2.2 Restrictions on the Uses of Other Protein Sources as Fish Meal Replacers

Two of the main factors responsible for the differences in the results obtained from many experiments, in which fish meal has been either partially or totally replaced in practical fish diets, are related to amino acid balances and/or protein digestibility. Most of protein sources used as replacement for fish meal have imbalanced amino acid patterns. Many protein sources are deficient in one or more amino acids according to the review of Tacon and Jackson (1985). For example, the concentrations of the sulfur-containing amino acids and lysine are low in all non animal-proteins when compared with fish muscle protein (Rosenlund, 1986). The exception is rapeseed protein concentrate which has an amino acid profile similar to that of fish meal.

Digestibility among various protein sources varies in terms of the rate of digestion and proportions of amino acids released. The digestibility coefficients vary from 37-97% depending upon the source of protein (NRC, 1981, 1983). This wide range of digestibility coefficients of different protein sources is due to various factors: the structure of constituent proteins in products such as animal protein meals, processing conditions, high fiber contents in plant products, and the presence of antinutritional factors (Tacon and Jackson, 1985). In some protein sources, the amino acid profile may be as good as that of fish meal protein, but the availability of some amino acids may be poor. Rosenlund (1986), for instance, found a difference in results obtained from feeding rainbow trout two types of fish meal. Rainbow

trout fed a diet based on low temperature-dried fish meal showed a higher capacity for protein synthesis than did fish fed a diet based on regular processed fish meal. He concluded that low temperature-dried fish meal had better bio-availability of amino acids and that heat treatment during processing may have led to chemical changes with resultant reductions in protein digestibility.

2.3 SUPPLEMENTATION AND UTILIZATION OF AMINO ACIDS

Supplementation of proteins with amino acids is often suggested as a means of improving the utilization of diets formulated with proteins that are deficient in one or more essential amino acids (NRC, 1981, 1983; Cowey, 1979). The efficacy of dietary supplementation with free amino acids for fish, including salmonids, is still inconclusive. Many researchers have shown promising results with single or multiple supplementation of amino acids to the diets for rainbow trout, Atlantic salmon, chinook salmon, channel catfish, and carp (Rumsey and Ketola, 1975; Dabrowska and Wojno, 1977; Gropp *et al.*, 1979; Higgs *et al.*, 1983; Tacon *et al.*, 1983; Abel *et al.*, 1984). Fowler (1980) on the other hand found no benefit from supplementing soybean-fish meal based diets with methionine. The growth rates of chinook and coho salmon were decreased by methionine supplementation of the diet. Aoe *et al.*, (1970) and Dean and Robinette (1983) found similar results with carp and catfish to those obtained by Fowler (1980) with salmon when methionine was used as a dietary supplement.

According to Lovell (1989), fish do not utilize dietary crystalline amino acids as well as chickens or swine. Generally, fish fed diets containing large amounts of free amino acids will show lower growth rates than those obtained when all the

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protein in the diet is supplied as high quality intact protein (Cowey, 1992).

Furthermore, the ability to utilize free amino acids varies within and between

species. Carp used in various experiments, for instance, showed variable results

when diets supplemented with free amino acids were tested (Murai, 1989a; Aoe *et al.*, 1970). Rainbow trout appear to use synthetic amino acids more efficiently than

carp and catfish (Cowey, 1979; Wilson, 1989). It is suggested that if a diet

containing free amino acids is neutralized or adjusted to pH 6.5-6.7 for carp and pH

6.0-8.0 for catfish, the utilization of the diet will be improved (Nose *et al.*, 1974;

Wilson *et al.*, 1977). Dean and Robinette (1983), however, found no effect when

catfish were fed a diet supplemented with amino acids and pH was about 5.9.

Supplementation of free amino acids at excess levels might adversely affect fish

growth. Murai (1989a), for instance, found depression of growth and feed efficiency

in carp fed a diet supplemented with 0.50% methionine (130% above requirements)

as compared with the addition of 0.25% methionine. He suggested that the higher

level of methionine is toxic to fish and that fish might be much more sensitive to

methionine toxicity than land animals when it is added in the free form. Feeding

three to four times the requirement of methionine has been shown to suppress food

intake and to cause near-cessation of growth in land animals (Benevenga and Steel,

1984).

2.4 FACTORS AFFECTING PROTEIN UTILIZATION

Apart from the amino acid balance and digestibility of proteins there are several other factors that affect protein utilization in fish. These include size of fish, composition of diets, feeding practice, and gastric evacuation time (Steffens, 1981; Pfeffer, 1982).

2.4.1 Size of Fish

The ability of fish to utilize protein varies with the size of fish. Small fish (immature fish) seem to utilize proteins less efficiently than large fish (mature fish). A possible important reason may be lower enzyme activities in small fish (Steffens, 1981). Kitamikado and Tachino (1960) found that the proteolytic activity of an extract from the pyloric caeca and intestine of rainbow trout was low in the early stages (body weight less than 4 grams) and increased with growth of the fish.

2.4.2 Composition of Diets

Dietary fiber has been the subject of studies regarding its influence on protein utilization in fish. Its presence might prevent nutrients from becoming available to the fish (Davies, 1985). It has been found that rainbow trout fed diets containing 10 and 20% of cellulose (alpha-floc) displayed poorer growth than fish fed a control diet without added cellulose (Hilton *et al.*, 1983). In contrast to these findings, Davies (1985) found that inclusion of 15% and 20% of cellulose in diets for rainbow trout gave better protein efficiency ratios and net nitrogen utilization values than the control diet containing no cellulose. This positive effect of dietary fiber may have been due to stimulation of cell turnover in the intestinal mucosa and

induction of enzyme secretions which may, in turn, improve protein utilization (Davies, 1985; Simon, 1989). Digestible carbohydrate in diets may also be important as a protein-sparing component. Ufodike and Matty (1989) showed that 20-30% inclusion of corn and potato meal in rainbow trout diet resulted in better protein digestibility and growth performance than that obtained with the control diet. The authors concluded that the improved growth response was due to utilization of carbohydrate energy in place of energy from protein. Beamish and Thomas (1984), on the other hand, found that the apparent digestibility of protein varied inversely with the concentration of dietary starch (10-30%) in rainbow trout.

Dietary lipid influences protein utilization through its protein-sparing action. Increases in dietary lipid to a certain level in fish diets have been shown to promote growth in several studies (Atherton and Aitken, 1970; Lee and Putnam, 1973; Adron *et al.*, 1976; Watanabe *et al.*, 1979; Gropp *et al.*, 1982; De Silva *et al.*, 1991). Higuera *et al.*, (1977) fed rainbow trout with diets containing 6.7%-20.9% of lipid and found a higher biological value (BV) for the diet containing 18% lipid than for the diet containing 6.7% lipid. He suggested that the higher BV obtained with the elevated concentration of dietary lipid was due to availability of calories from the lipid which spared the catabolism of amino acids for energy purposes. The optimum level of dietary lipid varies according to the dietary concentration of protein. Watanabe *et al.*, (1979), for instance, found maximum nitrogen retention with a ratio of 35% protein to 15-20% lipid in rainbow trout. A similar ratio of 36% protein and 16% lipid was suggested by Cho and Kaushik (1990) using rainbow trout in their study. Davies (1989) found that the optimum dietary levels of crude protein and lipid for growing juvenile trout were 40% and 12-14%, respectively. Reinitz and Hitzel

(1980), however, obtained maximum nitrogen retention in rainbow trout with diets containing 26% protein and 11-17% lipid.

Inclusion of lipid at high levels in the diets of animals other than fish has been found to delay gastric evacuation (Church and Pond, 1988). In the case of fish, Windell *et al.*, (1972) suggested that dietary lipid levels of 15% or higher may inhibit gastric motility in rainbow trout. Jobling (1980) found that low-energy diets were evacuated from the stomach of plaice more rapidly than those with a higher energy content. Lie *et al.*, (1988) fed young cod diets containing different levels of dietary protein and fat. They found that the digestibilities of protein and fat were reduced when the fish were fed an imbalanced diet containing 27% protein energy and 61% fat energy. They concluded that the reduction in protein digestibility was due to an overloading of the digestive system with fat. Higuera *et al.*, (1977), on the other hand, reported that dietary lipid levels did not affect protein digestibility in rainbow trout. Furthermore, Wilson *et al.*, (1985) did not find any differences in the serum patterns of free amino acids of channel catfish fed diets with varying ratios of protein to energy.

2.4.3 Feeding Practice

Feeding techniques that have been used, either in laboratory experiments or on fish farms include feeding at a restricted level per day and, feeding to satiation by hand or automatic feeder once or more times daily, or through the use of demand feeders (Tacon and Cowey, 1985; Rumsey, 1991). Feeding at a restricted level prevents the fish from realizing their maximum growth potential for a given diet. Grayton and Beamish (1977) found lower specific growth rates and gross food

conversion efficiencies when rainbow trout were fed a restricted daily ration of 2% of wet body weight as compared to satiation feeding. Feeding to satiation may, depending upon the nature of the diet, size of fish, and temperature, have an advantage over restricted feeding. For instance, Grayton and Beamish (1977) found that rainbow trout fed to satiation two times/day exhibited maximum growth rate. Andrews and Page (1975) also obtained the maximum growth and feed efficiency when catfish were hand-fed to satiation two times per day. Anderson (1988) reported maximum protein utilization in the luderrick, a marine herbivorous fish, fed ad libitum.

2.4.4 Gastric Evacuation Time

Gastric evacuation time plays an important role in digestion and absorption of nutrients in the digestive tract. The period of time during which digestive enzymes may attack their substrates depends on the rate of passage of digesta and this is in turn influenced by various factors. These factors include water temperature, fish size, the amount of food eaten, feeding frequency, diet compositions, type of food, and stress (Talbot, 1985). The effects of these factors on gastric evacuation time are still open to question. The interaction of these factors on gastric evacuation and digestion further increases the complexity of the system as a whole.

CHAPTER 3

EXPERIMENT 1

PATTERNS AND CONCENTRATIONS OF FREE AMINO ACIDS IN THE PLASMA OF RAINBOW TROUT FED FISH MEAL AS THE PRINCIPAL SOURCE OF DIETARY PROTEIN IN COMPARISON WITH (1) A MIXTURE OF PROTEIN SOURCES AND (2) A MIXTURE OF PROTEIN SOURCES PLUS SELECTED AMINO ACIDS

3.1 INTRODUCTION

Studies in several species of fish have shown that growth rate and feed efficiency of fish fed amino acid supplemented diets were poorer than when they were fed a complete protein meal (Aoe *et al.*, 1970; Wilson *et al.*, 1978; Walton *et al.*, 1986). A possible explanation for these observations was that free amino acids added as supplements to diets, may be rapidly absorbed and metabolized before other amino acids derived from digestion of dietary protein reach the sites of protein synthesis. Studies with rainbow trout have demonstrated that when amino acid-based diets are fed to the fish there is more rapid appearance and higher concentrations of free amino acids in the plasma (Yamada *et al.*, 1981). Similarly, when a mixture of protein is consumed, not all of amino acids from the proteins are necessarily available for absorption at the same time as different proteins vary in the rates at which they are hydrolyzed by the digestive enzymes. In both situations, some essential amino acids may not be available in tissues in time to complement other amino acids so that a balanced mixture is available for protein synthesis. Any amino acids that cannot be utilized for protein synthesis because of some limitation

of one or more amino acids will be rapidly oxidized or converted to glucose and fatty acids.

The importance of similar rates of absorption of amino acids from the digestive tract on the profile of free amino acids in the plasma, which are available for protein synthesis at any one time, is a question that has not been given much consideration in salmonid nutrition.

Experiment 1.1 was, therefore, designed to investigate the changes in concentrations of free amino acids in the plasma and the growth responses of rainbow trout fed (1) diets containing different protein sources, (2) diets with and without supplementation of free amino acids, and (3) experimental diets fed either once or five times daily. Experiment 1.2, was similar to Experiment 1.1 except that the amino acid profiles were monitored over a longer period following feeding.

3.2 EXPERIMENT 1.1: EFFECTS OF DIETS AND FEEDING FREQUENCY ON GROWTH AND CHANGES IN PLASMA AMINO ACID CONCENTRATIONS IN RAINBOW TROUT

3.2.1 MATERIALS AND METHODS

3.2.1.1 Diets

The compositions of the three diets formulated for this experiment are shown in Table 1.1. Diet 1 contained herring meal as the principal source of protein. Diet 2 contained herring meal, soybean protein concentrate, gelatin, and corn gluten meal as the protein sources. Diet 3 contained the same proportions of

protein from the respective sources as were used in diet 2 but was supplemented with lysine, methionine and tryptophan to levels equivalent to those of diet 1. Tabulated concentrations of amino acids in feed ingredients (NRC, 1981) were used to estimate the dietary concentrations of amino acids. The protein concentration of each diet was calculated to be approximately 35% (air-dry), with additional nitrogen in diet 3 from the supplementary amino acids (equivalent to approximately 1.3% of protein).

3.2.1.2 Fish and Rearing Conditions

Rainbow trout used in this experiment ranged in size from 16-31 g. They were divided into three size groups which had average weights of 25.9 ± 2.4, 21.5 ± 1.4, and 18.5 ± 1.5 g (mean $\pm$ SD) and these were referred to as large, medium, and small, respectively. The reason for sorting the fish was to reduce size variation and thus minimize establishment of feeding hierarchies. Fish from each of the size categories were distributed at random into six 150 L tanks with 50 fish/tank (eighteen tanks total). The tanks and facilities were located at the UBC aquarium facilities. The water supply was dechlorinated Vancouver city water. Water temperature was maintained at 13°-14°C by means of a heat exchanger unit. Water flow to each tank was 2 L/min, and dissolved oxygen was 8 ppm. The photoperiod was 24 h. Twelve fish from the same stock (mean weight $\pm$ SD = 16.19 ± 0.83) were killed and frozen for subsequent determination of initial whole body proximate composition. The feeding trial was conducted during November-December, 1989.

Table 1.1. Ingredient (air-dry basis) and proximate composition (dry matter basis) of diets used in Experiment 1.1

Ingredient	Diet 1	Diet 2	Diet 3
	g/kg	g/kg	g/kg
Herring meal (whole steam-dried)	423.5	141.2	141.2
Ground wheat[1]	300.0	300.0	300.0
Gelatin	-	117.4	117.4
Corn gluten meal	-	77.1	77.1
Soybean protein concentrate	-	59.1	59.1
Sardine oil[2]	93.0	118.3	118.3
Bone meal	16.0	38.0	38.0
Dextrin	107.5	88.9	88.9
Premix[3]	40.0	40.0	40.0
Calcium lignosulphonate	20.0	20.0	20.0
L-lysine	-	-	7.5
DL-methionine	-	-	4.0
L-tryptophan	-	-	1.5
Total	1000.0	1000.0	1013.0
Proximate analysis[4]			
Crude protein (%)	36.2	39.0	39.6
Ether-extractable lipid (%)	15.8	15.8	15.8
Ash (%)	8.0	6.4	6.1
Gross energy (kcal/kg)	5288	5364	5461

[1] Autoclaved at 121°C for 1.5 h

[2] Stabilized with 0.05% ethoxyquin

[3] The premix supplied the following per kg of diet as fed (except for diet 3 in which the percentage of each will be proportionally lower): thiamin HCl, 67.3 mg; riboflavin, 104.2 mg; niacin, 400 mg; biotin, 5 mg; folic acid, 25 mg; pyridoxine HCl, 60.8 mg; cyanocobalamine, 0.1 mg; D-calcium pantothenate, 218.3 mg; ascorbic acid, 1500 mg; choline chloride, 4000 mg; inositol, 2000 mg; menadione, 30 mg; vitamin A, 10,000 IU; vitamin D_3, 1000 IU; vitamin E, 1000 IU; Mg (as $MgSO_4$), 380 mg; Mn (as $MnSO_4.H_2O$), 17 mg; Zn (as ZnO), 50 mg; Fe (as $FeSO_4.7H_2O$), 85 mg; Cu (as $CuSO_4.5H_2O$), 2 mg; Co (as $CoCl.6H_2O$), 0.003 mg; K (as K_2SO_4), 895 mg; I (as KIO_3), 5 mg; NaCl (as NaCl), 2836 mg; F (as NaF), 4.5 mg; Se (as $Na_2SeO_3.5H_2O$), 0.10 mg.

[4] The values for crude protein, ether-extractable lipid, and ash were obtained by proximate analyses. The values for gross energy were estimated by ascribing 5.65 kcal/g crude protein, 9.5 kcal/g crude lipid, 4.0 kcal/g carbohydrate (Alexis *et al.*, 1985)

3.2.1.3 Design of Feeding Trial

The experiment consisted of six treatments (i.e. three dietary treatments, and two feeding regimes). Each experimental treatment was assigned to a tank of large, medium, and small size fish. The design of the experiment was, therefore, as follows:

```
                                                       ┌─  Large
                                    ┌─ Fed once daily   ├─  Medium
                                    │                   └─  Small
         Three experimental diets ─┤
                                    │                   ┌─  Large
                                    └─ Fed five times daily ├─ Medium
                                                        └─  Small
```
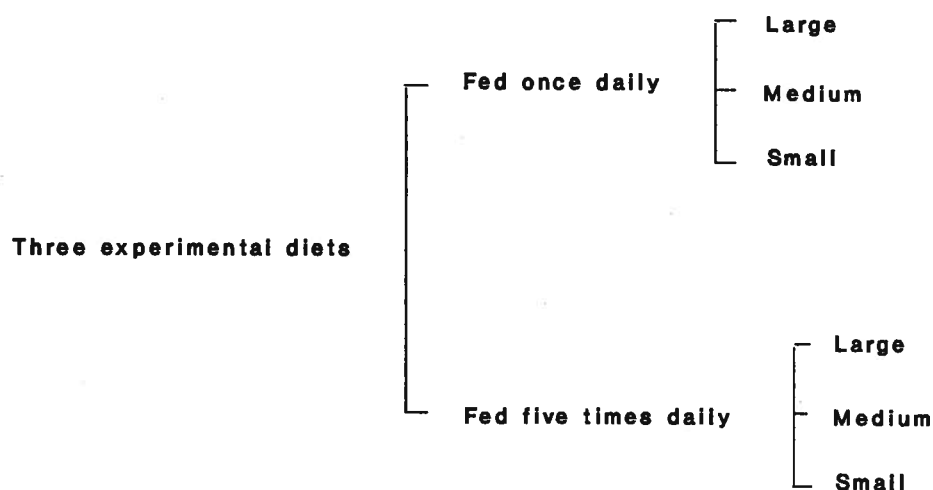
Fish that were fed once daily were fed at 09:30 hours until satiation. The feeding of fish five times daily was done at intervals of 3 h from 06:30 to 18:30 hours. The amount of feed fed to these fish was controlled relative to the average food consumption of the fish in the first group in the previous 2 days. The daily ration was divided equally among the five feedings. The exception was the first 2 days when fish in both feeding regimes were fed to satiation. The total amount of food consumed by both groups was averaged, with respect to the dietary treatment and

size of fish, to determine the amount of feed for day-three feeding of the fish that were fed five times daily. The groups of fish that were fed once daily were located in the inner section of the experimental room in order that they would not be disturbed by the feeding of the groups five times daily.

Fish were gradually accustomed to experimental diets over a four-day period by mixing increasing amounts of the respective diets with the commercial diet (EWOS) that had been fed to the fish during the pre-experimental period. The experimental period was 26 days following acceptance of the experimental diets. The amount of food given to the fish was recorded daily to determine feed efficiency. There was no mortality during the feeding trial. After 26 days, the fish were sampled according to the following protocols for determination of plasma amino acid concentrations, gastrointestinal contents, and carcass composition.

3.2.1.4 Sampling Procedure

Blood was withdrawn from five fish from each tank at each sampling time, i.e. 15 fish were sampled per dietary treatment under each feeding regime. The fish that were fed once daily were bled at 0, 3, 9, and 15 h after feeding, and fish fed five times daily were sampled at 0, 3, and 9 h after the first morning feeding. The feeding for the frequently-fed fish was continued during the sampling period. The reason for limiting the sampling times to three for the latter group of fish was because of the likelihood that disturbance of the fish would affect food intake and digestion. The fish to be sampled were anesthetized with 0.01% tricaine-methanesulfonate (MS-222), and weighed. Blood was collected from the caudal vein artery complex into heparinized tubes by severing the caudal peduncle. The

reason for sampling blood from the caudal vein complex was to obtain samples representative of the systemic circulation.

Blood samples from five fish were pooled per tank and centrifuged at 780x*g* for 15 min. The resultant plasma was collected and kept at -70°C pending analysis for amino acids. After the blood samples were taken, any remaining food was stripped from the digestive tracts of the fish and the carcasses were frozen for subsequent proximate analyses. The contents of the gastrointestinal tracts were weighed.

3.2.1.5 Proximate Analysis

The proximate analyses of formulated diets and fish were conducted according to AOAC methods (AOAC, 1984). The analyses for each pooled sample of five fish for each replicate tank were performed in triplicate. The formulated diets were also subjected to acid hydrolysis for amino acid determination.

3.2.1.6 Amino Acid Analysis

Plasma samples that were kept at -70°C were thawed and deproteinized by the addition of 10% trichloroacetic acid (1:1 v/v), followed by vortexing for 2 s, and centrifugation at 10,000x*g* for 5 min at -4°C. The deproteinized plasma was pipetted into 20 mL test tubes, and 3 mL of diethyl ether were added for each mL of plasma. Lipid was extracted into the ether by vortexing, and the ether layer was pipetted off. The deproteinized, defatted plasma was filtered through a 0.22 μm polycarbonate membrane filter. The filtered plasma samples were kept at -70°C until amino acid analyses could be performed. Plasma amino acid concentrations were determined

using ion exchange chromatography (Beckman Amino Acid Analyzer Model 6300). Identification and quantification of individual amino acids were accomplished with the use of external standards obtained from Sigma (Amino acid standard solution, catalog number A2908). The procedure did not detect tryptophan. In addition, threonine peaks were often eluted with serine. As a result, the concentrations of threonine in some samples were calculated from the area under the co-eluted peak according to Beckman instruction (Beckman, 1982).

3.2.1.7 Statistical Analysis

An analysis of variance with size classes of fish as blocks was performed using a factorial model to examine the effects of feeding frequency and of dietary treatment on fish weight, specific growth rate, feed consumption, feed efficiency, protein gain, productive protein value, lipid gain, energy efficiency, and body proximate composition of fish. In the case of the data for protein gain, lipid gain, dry matter, and proximate composition of the fish, the interaction terms were pooled with the error term because the interactions were not significant. To test differences between treatment means, the Tukey HSD test was employed (Zar, 1984). Correlations were determined on the relationship between the concentrations of plasma and dietary amino acids. The statistical analyses were performed using Systat (Wilkinson, 1990).

3.2.2 RESULTS

3.2.2.1 Comparison Between Amino Acid Composition of Experimental Diets and Requirements

The amino acid compositions of the experimental diets are shown in Table 1.2. Diet 1 (fish meal based diet) contained similar proportions of essential and non-essential amino acids with the ratio of EAA/NEAA of 1.0. Diets 2 and 3 contained lower concentrations of essential amino acids and higher concentrations of non-essential amino acids. The ratios of EAA/NEAA in diet 2 and 3 were 0.7 and 0.8, respectively.

The comparisons between amino acid concentrations in the experimental diets and the requirement values recommended by the NRC (1981) and recently reported values for rainbow trout are shown in Table 1.2. It was found that the concentrations of essential amino acids present in diets 1 and 3 exceeded the requirement levels, expressed as % protein.

3.2.2.2 Responses of Fish to Experimental Diets and Feeding Frequency

The data for initial weight, final weight, weight gain, and feed consumption of fish of the three different sizes subjected to the different treatments are provided in Table 1.3. Carcass composition of fish of different sizes subjected to different treatments is given in Table 1.4. The data in Table 1.3 and 1.4 were used to calculate different indices shown in Table 1.5. Data were subjected to analysis of variance and the significance of the treatment effects is shown in Appendices 1, 2, and 3.

Table 1.2. Amino acid composition of experimental diets and the NRC requirement values

Amino acid	Experimental diet[1]			Requirement	
	Diet 1	Diet 2	Diet 3	Salmon[2]	Trout
	g/16g N				
Arg	6.4	5.7	6.3	6.0	2.0-5.9[a-f]
His	2.2	1.8	2.2	1.8	1.6[f]
Ile	3.8	2.9	2.9	2.2	2.4[f]
Leu	7.1	6.6	6.8	3.9	4.4[f]
Lys	5.9	4.0	5.7	5.0	3.7-6.1[a-c,f]
Met	2.5	1.6	2.5	-	2.2-3.0[f-i]
Met+Cys	3.5	2.5	3.3	4.0	-
Phe	3.7	3.4	3.4	-	3.1[f]
Phe+Tyr	6.4	5.6	5.4	5.1	-
Thr	4.1	3.0	3.0	2.2	3.4[f]
Trp[3]	-	-	-	0.5	0.5-1.4[j-l]
Val	4.6	3.5	3.5	3.2	3.1[f]
Ala	5.5	6.4	6.3		
Asp	8.7	8.1	8.1		
Glu	14.5	14.5	14.6		
Gly	5.9	10.1	10.0		
Pro	4.9	8.0	7.9		
Ser	4.1	3.9	3.9		
Tau	1.0	0.7	0.7		
TAA[4]	87.3	86.5	90.0		
EAA	43.9	35.5	39.2		
NEAA	43.5	51.0	50.8		
EAA/NEAA	1.0	0.7	0.8		

[1] Diet 1 = fish meal based diet, Diet 2 = fish meal, soybean protein concentrate, corn gluten meal, and gelatin, Diet 3 = Diet 2 supplemented with methionine, lysine, and tryptophan.
[2] NRC (1981)
[3] Tryptophan was not detected in the diets by the procedure used.
[4] Excluding asparagine, glutamine, tryptophan, taurine.
[a] The values are cited from Murai (1992); [b] The values are cited from Wilson (1989); [c] Ketola (1983); [d] The values are cited from Steffens (1989); [e] The value is from Cho et al., (1989); [f] The value is from Ogino (1980); [g] Walton et al., (1982); [h] Rumsey et al., (1983); [i] Kim et al.,(1992); [j] Poston and Rumsey (1983); [k] Walton et al., (1984b); [l] Kim et al., (1987)

Mean initial weight, final weight, weight gain, and feed consumption of fish of different sizes, and treatments are shown in Table 1.6. The averaged values of the mentioned parameters were also tabulated for fish fed different diets according to feeding regimes as shown in Table 1.7. As expected, fish in different size categories (large, medium, and small) were significantly ($P < 0.05$) different in weights (initial weight, final weight, and weight gain). With similar mean initial weights, fish fed diet 1 (herring meal-based diet) had greater final weight and weight gain ($P < 0.05$) than those of fish fed either diet 2 (amino acid deficient diet) or diet 3 (diet 2 supplemented with lysine, methionine, and tryptophan), regardless of size and feeding frequency. Moreover, feeding fish either once or five times daily did not affect final weight or weight gain of the fish fed different diets ($P > 0.05$).

In general, larger fish consumed more feed than small fish (Table 1.3). Although it was assumed that the amount of food consumed by the groups of fish fed once daily and five times daily would be similar, the results showed that fish fed five times daily consumed less feed ($P < 0.0002$). There was also a significant interaction between diet and size of fish. Medium and small size fish had lower feed consumption when fed diets 2 and 3 as compared to diet 1, but large fish consumed less of diet 1 compared to diets 2 and 3. The variation in feed consumption among the three size groups was minimum for diet 1 and maximum for diet 3.

Mean specific growth rates, efficiency of feed conversion, productive protein values (PPV = gain in body nitrogen/nitrogen intake), and energy efficiency of fish of different sizes, and on different treatments are shown in Table 1.8. The average values for these parameters were also tabulated for fish fed different diets according to feeding regimes as shown in Table 1.9. No significant ($P > 0.05$) differences in

specific growth rate, feed efficiency, or energy efficiency were found among different sizes of fish. The specific growth rate of fish of different size categories, however, showed the trend that small size fish grew faster than medium size, or large size fish (Table 1.8). Regardless of size of fish and feeding frequency, fish fed diet 1 showed higher ($P < 0.05$) specific growth rate, feed efficiency, and energy efficiency than fish fed either diet 2 or diet 3 (Table 1.9). Fish fed either once daily or five times daily in the present experiment showed similar growth rate, feed conversion efficiency, and energy efficiency ($P > 0.05$)(Table 1.8). The difference in feed consumption between these two groups as mentioned in the previous paragraph was mainly due to very small variation of the data.

In general, protein gain and productive protein values (PPV) for fish fed diet 1 were significantly higher than those for fish fed diets 2 and 3. A significant interaction between diets and sizes of fish, however, was observed ($P < 0.05$) for the PPV values (Table 1.5). The PPV values for fish of different sizes fed either diet 2 or diet 3 were similar. Large fish fed diet 1, however, had significantly higher PPV value than those for medium and small fish fed the same diet. Furthermore, the PPV values for fish that were fed five times daily were slightly but significantly ($P < 0.05$) better than for fish fed once daily (Table 1.9). Data in Table 1.5 indicate that only fish fed diet 3, regardless of the size, had improved PPV when fed five times daily, although the improvement was not statistically significant. Protein gains of fish that were fed experimental diets at two different feeding regimes were similar ($P > 0.05$). Total lipid gain of fish varied with the size, diet, and feeding frequency as shown in Table 1.8.

3.2.2.3 Carcass Composition

Mean whole body proximate composition of fish of different sizes fed different diets according to feeding regimes are provided in Table 1.10. The average values for carcass composition of fish were also tabulated for fish fed different diets according to feeding regimes as shown in Table 1.11. The analyses of fish carcasses showed that the body compositions of fish of the three sizes were similar ($P > 0.05$). Furthermore, no significant differences ($P > 0.05$) were found in the concentrations of body protein, lipid and ash among fish fed diets 1, 2 and 3. The dry matter content of fish fed diet 1 under both feeding regimes, nevertheless, was slightly but significantly higher than those of fish fed diet 2 and diet 3 ($P < 0.05$). Although feeding fish at different frequencies did not have any effect on growth rate or feed efficiency, significant effects were detected in the dry matter, and carcass protein and lipid concentrations. Regardless of diet and size of fish, the dry matter and lipid concentrations in fish that were fed five times daily were significantly lower than in fish that were fed once daily ($P < 0.05$). Moreover, protein concentrations in fish that were fed five times daily were significantly higher than in fish fed once daily ($P < 0.05$). Ash concentrations of fish fed once or five times daily were similar ($P > 0.05$).

Table 1.3. Initial weight, final weight, body weight gain and feed consumption of rainbow trout of different sizes fed different diets either once or five times daily over a 26-day period in Experiment 1.1

Feeding regime	Diet[1]	Size[2]	Initial weight	Final weight	Weight gain	Feed consumption
			(g/fish)	(g/fish)	(g/fish)	(g/fish/day)
Once daily						
	1	L	25.6	50.6	25.0	1.15^{b3}
		M	21.3	47.2	25.9	1.15^{b}
		S	18.6	39.6	21.0	1.01^{d}
	2	L	24.8	48.0	23.3	1.23^{a}
		M	22.1	40.1	17.9	1.04^{cd}
		S	19.8	35.4	16.6	0.97^{de}
	3	L	26.0	48.2	22.2	1.27^{a}
		M	21.0	39.0	18.0	1.07^{c}
		S	18.1	34.6	16.6	0.92^{e}
Five times daily						
	1	L	26.5	52.0	25.6	1.13^{b}
		M	21.8	44.7	22.9	1.12^{b}
		S	18.3	38.6	20.3	0.98^{d}
	2	L	26.5	49.1	22.6	1.21^{a}
		M	24.6	40.0	18.4	1.03^{cd}
		S	18.6	36.3	17.7	0.95^{de}
	3	L	26.0	49.2	23.3	1.25^{a}
		M	21.3	40.4	19.1	1.05^{c}
		S	18.4	34.3	15.9	0.89^{e}

[1] Diet 1 = fish meal based diet; Diet 2 = diet deficient in lysine, methionine, and tryptophan; Diet 3 = diet 2 supplemented with lysine, methionine, and tryptophan.
[2] L = large, M = medium, S = small, each value represents a mean of 50 fish/tank.
[3] Superscript letters in the same column within the same feeding regime refer to Tukey HSD test (Zar, 1984) when the interaction between diets and sizes of fish were detected. Values followed by the same letters are not significantly different (P>0.05). Where there are no superscript letter, no interaction among the means was detected.

Table 1.4. Whole body proximate composition of rainbow trout of different sizes fed different diets either once or five times daily over a 26-day period in Experiment 1.1

Feeding regime	Diet[1]	Size[2]	Dry matter	Protein	Lipid	Ash
			(%)		(% of dry matter)	
Initial			20.12	54.03	31.01	ND[3]
Once daily						
	1	L	30.12	51.27	36.30	7.54
		M	29.86	49.58	37.50	7.41
		S	29.37	50.38	36.45	8.45
	2	L	28.48	50.94	35.30	7.91
		M	29.09	49.68	37.00	7.58
		S	29.40	51.33	34.05	7.56
	3	L	28.94	49.81	37.70	7.48
		M	29.07	50.94	36.10	7.38
		S	28.70	50.84	33.60	7.52
Five times daily						
	1	L	29.92	51.32	37.60	7.39
		M	29.41	50.57	36.00	7.21
		S	29.08	52.14	33.50	7.54
	2	L	29.08	50.56	35.00	7.48
		M	28.38	51.10	34.30	7.72
		S	27.60	51.87	29.51	7.73
	3	L	28.60	51.53	28.55	7.35
		M	28.30	52.91	32.90	8.02
		S	28.71	51.86	34.65	7.38

[1] Diet 1 = fish meal based diet; Diet 2 = diet deficient in lysine, methionine, and tryptophan; Diet 3 = diet 2 supplemented with lysine, methionine, and tryptophan.
[2] L = large, M = medium, S = small, each value represents a pool sample of five fish/tank.
[3] Not determined
No interaction among means in the same column was detected.

Table 1.5. Specific growth rates, feed conversion efficiency, productive protein value and energy efficiency of rainbow trout of different sizes fed different diets either once daily or five times daily over a 26-day period in Experiment 1.1

Feeding regime	Diet[1]	Size[2]	Specific growth rate[3]	Feed efficiency[4]	Protein gain	Productive protein value[5]	Lipid gain	Energy efficiency[6]
					(g/tank)		(g/tank)	
Once daily								
	1	L	2.62^7	0.82	250.9	0.511^{a8}	194.9	0.46
		M	3.06	0.86	233.9	0.471^b	197.3	0.44
		S	2.90	0.80	191.9	0.440^b	153.5	0.40
	2	L	2.54	0.72	212.8	0.375^c	171.7	0.36
		M	2.29	0.68	171.9	0.358^c	147.4	0.37
		S	2.23	0.66	165.3	0.369^c	118.3	0.34
	3	L	2.37	0.67	208.1	0.352^c	180.1	0.36
		M	2.38	0.64	173.9	0.349^c	138.1	0.34
		S	2.49	0.69	151.0	0.351^c	107.9	0.32
Five times daily								
	1	L	2.59	0.87	250.1	0.516^a	205.5	0.48
		M	2.76	0.80	217.0	0.451^b	170.3	0.41
		S	2.87	0.79	186.6	0.444^b	126.2	0.37
	2	L	2.37	0.72	214.7	0.386^c	165.0	0.36
		M	2.37	0.69	173.5	0.367^c	127.3	0.34
		S	2.57	0.72	165.8	0.379^c	93.4	0.30
	3	L	2.45	0.71	219.6	0.377^c	118.2	0.30
		M	2.46	0.70	188.3	0.383^c	122.1	0.33
		S	2.40	0.68	153.7	0.370^c	111.7	0.34

[1] Diet 1 = fish meal based diet; Diet 2 = diet deficient in lysine, methionine, and tryptophan; Diet 3 diet 2 supplemented with lysine, methionine, and tryptophan.

[2] L = large, M = medium, S = small.

[3] = (lnW2-lnW1)*100/(T2-T1).

[4] = Gain in body weight/feed consumption.

[5] = Gain in body nitrogen/nitrogen intake.

[6] = Gain in body energy/GE intake, (body energy was estimated on the basis of carcass gains in protein and lipid using caloric value of 5.7 and 9.5 kcal/g respectively according to March *et al.*, 1985).

[7] Each value represents a mean of 50 fish/tank, except for protein and lipid gain.

[8] Superscript letters in the same column within the same feeding regime refer to Tukey HSD test (Zar, 1984) when the interaction between diets and sizes of fish were detected. Values followed by the same letters are not significantly different (P>0.05). Where there is no superscript letter, no interaction among the means was detected.

Table 1.6. Mean initial weight, final weight, weight gain, and feed consumption of rainbow trout of different sizes fed different diets either once or five times daily over a 26-day period in Experiment 1.1

Main effect	Initial weight	Final weight	Weight gain	Feed consumption
Size[1]	(g)	(g)	(g/fish)	(g/fish/day)
L	25.9 ± 0.4^{a4}	49.6 ± 0.3^{a}	23.7 ± 0.4^{a}	1.207 ± 0.017^{a}
M	21.5 ± 0.4^{b}	41.9 ± 0.3^{b}	20.4 ± 0.4^{b}	1.077 ± 0.017^{b}
S	18.5 ± 0.4^{c}	36.5 ± 0.3^{c}	18.0 ± 0.4^{c}	0.953 ± 0.017^{c}
Diet[2]				
1	22.0 ± 0.4^{a}	45.5 ± 0.3^{a}	23.5 ± 0.4^{a}	1.090 ± 0.017^{a}
2	22.0 ± 0.4^{a}	41.5 ± 0.3^{b}	19.5 ± 0.4^{b}	1.071 ± 0.017^{b}
3	21.8 ± 0.4^{a}	41.0 ± 0.3^{b}	19.2 ± 0.4^{b}	1.075 ± 0.017^{b}
Feeding frequency[3]				
Once daily	21.8 ± 0.3^{a}	42.5 ± 0.3^{a}	20.71 ± 0.36^{a}	1.090 ± 0.017^{a}
Five times daily	22.1 ± 0.3^{a}	42.8 ± 0.3^{a}	20.70 ± 0.36^{a}	1.068 ± 0.017^{b}

[1] L = large, M = medium, S = small, each value is a mean of six values (n=6).

[2] Diet 1 = fish meal based diet; Diet 2 = diet deficient in lysine, methionine, and tryptophan; Diet 3 = diet 2 supplemented with lysine, methionine, and tryptoph, each value is a mean of six values (n=6).

[3] Each value is a mean of nine values (n=9).

[4] Superscript letters in the same column within a main effect refer to Tukey HSD test (Zar, 1984). Values followed by the same letters are not significantly different (P>0.05).

Table 1.7. Mean initial weight, final weight, body weight gain and feed consumption of rainbow trout fed different diets according to feeding regime over a 26-day period in Experiment 1.1

Feeding regime	Diet[1]	Initial weight	Final weight	Weight gain	Feed consumption
		(g)	(g)	(g/fish)	(g/fish/day)
Once daily					
	1	21.8[2]	45.8	24.0	1.103
	2	22.2	41.1	19.3	1.080
	3	21.7	40.6	18.9	1.087
Five times daily					
	1	22.2	45.1	22.9	1.077
	2	22.2	41.8	19.6	1.063
	3	21.9	41.5	19.6	1.063
Pooled SEM		0.5	0.7	0.7	0.002

[1] Diet 1 = fish meal based diet; Diet 2 = diet deficient in lysine, methionine, and tryptophan; Diet 3 = diet 2 supplemented with lysine, methionine, and tryptophan.

[2] Each value is a mean of three values for three sizes of fish, each contains 50 fish, (n=3). No interaction among means in the same column was detected.

Table 1.8. Mean specific growth rate, feed conversion efficiency, productive protein value, and energy efficiency of rainbow trout of different sizes fed different diets either once or five times daily over a 26-day period in Experiment 1.1

Main effect	Specific growth rate[1]	Feed efficiency[2]	Protein gain	Productive protein value[3]	Lipid gain	Energy efficiency[4]
			(g/tank)		(g/tank)	
Size[5]						
L	$2.49 \pm .06^a$	$0.75 \pm .01^a$	226.0 ± 1.9^a	$0.419 \pm .003^a$	172.6 ± 8.4^a	$0.39 \pm .01^a$
M	$2.55 \pm .06^a$	$0.73 \pm .01^a$	193.0 ± 1.9^b	$0.397 \pm .003^b$	150.4 ± 8.4^b	$0.37 \pm .01^a$
S	$2.57 \pm .06^a$	$0.72 \pm .01^a$	169.0 ± 1.9^c	$0.392 \pm .003^b$	118.5 ± 8.4^c	$0.35 \pm .01^a$
Diet[6]						
1	$2.80 \pm .06^{a8}$	$0.82 \pm .01^a$	221.7 ± 1.9^a	$0.472 \pm .003^a$	174.6 ± 8.4^a	$0.43 \pm .01^a$
2	$2.40 \pm .06^b$	$0.70 \pm .01^b$	184.0 ± 1.9^b	$0.372 \pm .003^b$	137.2 ± 8.4^b	$0.35 \pm .01^b$
3	$2.43 \pm .06^b$	$0.68 \pm .01^b$	182.4 ± 1.9^b	$0.364 \pm .003^b$	129.7 ± 8.4^c	$0.33 \pm .01^b$
Feeding regime[7]						
Once daily	$2.54 \pm .05^a$	$0.73 \pm .01^a$	195.5 ± 1.5^a	$0.397 \pm .002^a$	156.6 ± 6.8^a	$0.38 \pm .01^a$
Five times daily	$2.54 \pm .05^a$	$0.74 \pm .01^a$	196.6 ± 1.5^a	$0.408 \pm .002^b$	137.8 ± 6.8^b	$0.36 \pm .01^a$

[1] = (lnW2-lnW1)*100/(T2-T1).

[2] = Gain in body weight/feed consumption.

[3] = Gain in body nitrogen/nitrogen intake.

[4] = Gain in body energy/GE intake, (body energy was estimated on the basis of carcass gains in protein and lipid using caloric value of 5.7 and 9.5 kcal/g respectively according to March *et al.*, 1985).

[5] L = large, M = medium, S = small, each value is a mean of six values (n=6).

[6] Diet 1 = fish meal based diet; Diet 2 = diet deficient in lysine, methionine, and tryptophan; Diet 3 = diet 2 supplemented with lysine, methionine, and tryptophan, each value is a mean of six values (n=6).

[7] Each value is a mean of nine values (n=9).

[8] Superscript letters in the same column within a main effect refer to Tukey HSD test (Zar, 1984). Values followed by the same letters are not significantly different (P>0.05).

Table 1.9. Mean specific growth rates, feed conversion efficiency, productive protein value and energy efficiency of fish fed different diets according to feeding regimes over a 26-day period in Experiment 1.1

Feeding regime	Diet[5]	Specific growth rate[1]	Feed efficiency[2]	Protein gain	Productive protein value[3]	Lipid gain	Energy efficiency[4]
				(g/50 fish)		(g/50 fish)	
Once daily							
	1	2.86[6]	0.83	225.53	0.474	181.90	0.43
	2	2.35	0.69	183.32	0.367	145.82	0.36
	3	2.41	0.67	177.64	0.351	142.02	0.34
Five times daily							
	1	2.74	0.82	217.88	0.470	167.33	0.42
	2	2.44	0.71	184.65	0.377	128.57	0.33
	3	2.44	0.70	187.21	0.377	117.34	0.32
Pooled SEM		0.08	0.01	2.65	0.004	11.82	0.01

[1] = (lnW2-lnW1)*100/(T2-T1).
[2] = Gain in body weight/feed consumption.
[3] = Gain in body nitrogen/nitrogen intake.
[4] = Gain in body energy/GE intake, (body energy was estimated on the basis of carcass gains in protein and lipid using caloric value of 5.7 and 9.5 kcal/g respectively).
[5] Diet 1 = fish meal based diet; Diet 2 = diet deficient in lysine, methionine, and tryptophan; Diet 3 = diet 2 supplemented with lysine, methionine, and tryptophan.
[6] Each value is a mean of three values for three sizes of fish, each contains 50 fish, (n=3). No interaction among means in the same column was detected.

Table 1.10. Mean carcass compositions of rainbow trout of different sizes fed different diets either once or five times daily over a 26-day period in Experiment 1.1

Main effect	Dry matter	Protein	Lipid	Ash
	(%)		(% of dry matter)	
Size[1]				
L	29.19 ± 0.18^{a4}	50.90 ± 0.31^{a}	35.08 ± 0.85^{a}	7.51 ± 0.14^{a}
M	29.02 ± 0.18^{a}	50.79 ± 0.31^{a}	35.61 ± 0.85^{a}	7.36 ± 0.14^{a}
S	28.81 ± 0.18^{a}	51.40 ± 0.31^{a}	33.63 ± 0.85^{a}	7.75 ± 0.13^{a}
Diet[2]				
1	29.63 ± 0.18^{a}	50.88 ± 0.31^{a}	36.20 ± 0.85^{a}	7.59 ± 0.13^{a}
2	28.67 ± 0.18^{b}	50.91 ± 0.31^{a}	34.16 ± 0.85^{a}	7.55 ± 0.13^{a}
3	28.72 ± 0.18^{b}	51.31 ± 0.31^{a}	33.85 ± 0.85^{a}	7.48 ± 0.13^{a}
Feeding frequency[3]				
Once daily	29.22 ± 0.15^{a}	50.53 ± 0.25^{a}	35.98 ± 0.69^{a}	7.65 ± 0.11^{a}
Five times daily	28.79 ± 0.15^{b}	51.54 ± 0.25^{b}	33.56 ± 0.69^{b}	7.43 ± 0.11^{a}

[1] L = large, M = medium, S = small, each value is a mean of six values (n=6).

[2] Diet 1 = fish meal based diet; Diet 2 = diet deficient in lysine, methionine, and tryptophan; Diet 3 = diet 2 supplemented with lysine, methionine, and tryptophan, each value is a mean of six values (n=6).

[3] Each value is a mean of nine values (n=9).

[4] Superscript letters in the same column within a main effect refer to Tukey HSD test (Zar, 1984). Values followed by the same letters are not significantly different (P>0.05).

Table 1.11. Mean carcass composition of rainbow trout fed different diets according to feeding regimes over a 26-day period in Experiment 1.1

Feeding regime	Diet[1]	Dry Matter	Protein	Lipid	Ash
		(%)		(% of dry matter)	
Once daily					
	1	29.78^2	50.41	36.75	7.81
	2	28.99	50.65	35.45	7.69
	3	28.90	50.53	35.80	7.47
Five times daily					
	1	29.47	51.34	35.70	7.38
	2	28.35	51.17	32.94	7.51
	3	28.53	52.10	32.03	7.51
Pooled SEM		0.24	0.43	1.27	0.20

[1] Diet 1 = fish meal based diet; Diet 2 = diet deficient in lysine, methionine, and tryptophan; Diet 3 = diet 2 supplemented with lysine, methionine, and tryptophan.

[2] Each value is a mean of three mean values for three sizes of fish, each was analysed from a pool sample of five fish, (n=3). No interaction among means in the same column was detected.

3.2.2.4 Gastrointestinal Contents

The average wet weights of the gastrointestinal contents from the fish that were fed once daily and five times daily are compared graphically in Figure 1.1. The fish that were fed once daily showed a gradual decline in the amount of gut contents between 3 and 15 h after feeding. The rate of feed passage in fish fed the different experimental diets appeared to be similar. As expected, the fish that were fed five times daily showed less variation in the amounts of gut contents over the experimental period.

3.2.2.5 Plasma Amino Acid Profiles

The concentrations of individual free amino acids in the plasma expressed as μmol/mL and as percentage composition determined at different times after meal consumption are shown in Tables 1.12-1.17. The concentrations of total amino acids (TAA), total essential amino acids (EAA), total non-essential (NEAA), and ratios of EAA/NEAA in the plasma of fish fed the different diets are also tabulated in these tables. The total essential amino acids included arginine, cystine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tyrosine and valine. Since the procedure used in analysing plasma amino acids did not detect tryptophan, it was not reported anywhere. The changes in plasma concentrations of total essential amino acids, total nonessential amino acids, and total amino acids are shown in Figure 1.2. The changes in plasma concentrations of individual free amino acids at different times after meal consumption are depicted in Figure 1.3. It should be mentioned that the fish fed diets once daily had been without feed for 24 h at zero-time i.e. just before feeding. The fish fed five times daily, on the other hand, were without feed for only 12 h at zero-time.
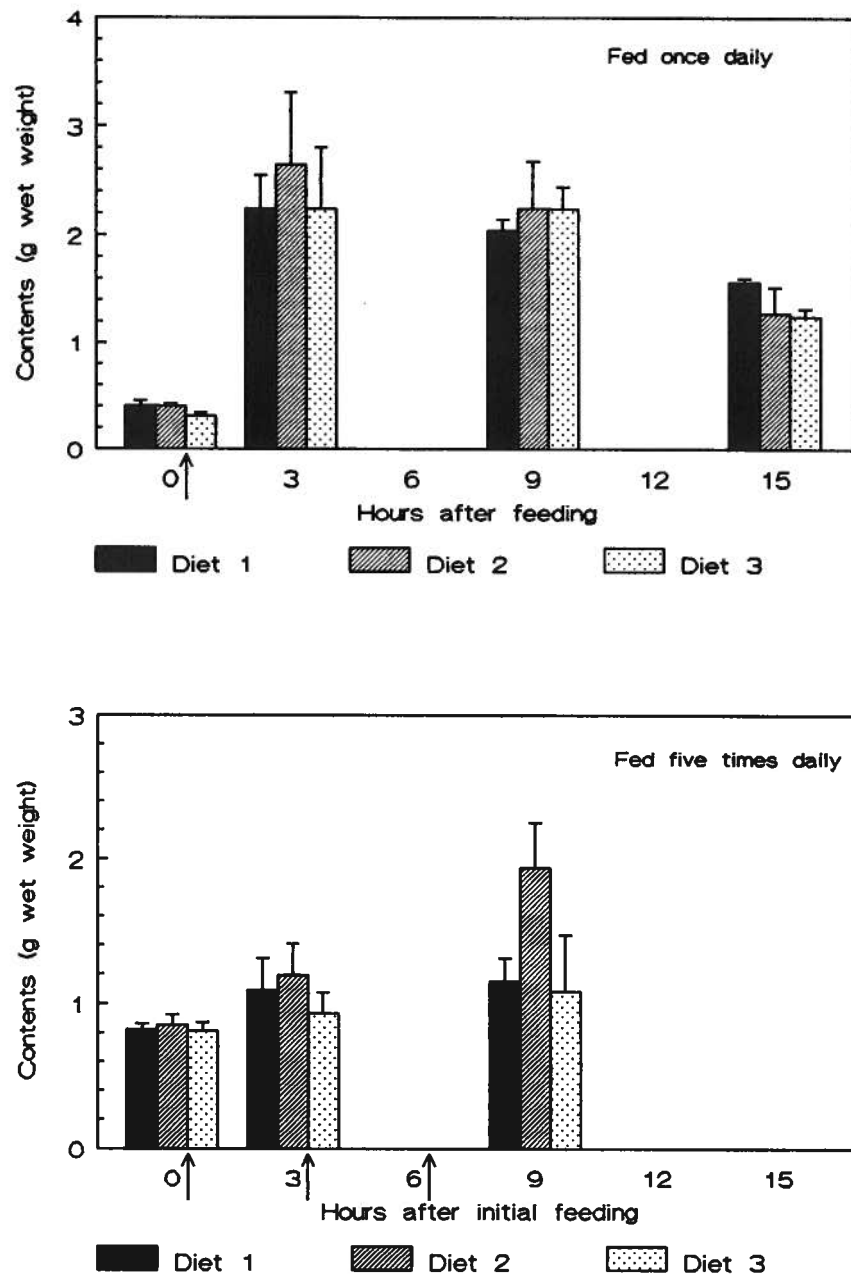
Figure 1.1. Mean wet weight (± SE) of gastrointestinal contents of rainbow trout at different times after feeding in Experiment 1.1; arrows indicate the time of feeding. Each value is a mean of three pooled samples of five fish (n=3).

Plasma Total Amino Acids (TAA), Total Essential Amino Acids (EAA) and Total Non-Essential Amino Acids (NEAA)

As shown in Figure 1.2, feeding regimen affected the patterns of plasma EAA, NEAA, and TAA concentrations in fish fed different diets over the sampling period. In fish that were fed once daily, plasma EAA, NEAA, and TAA concentrations peaked differently depending upon the nature of the diet. Plasma EAA concentrations in fish that were fed diets 1, 2, 3 followed a similar pattern in that they started to rise soon after feeding and reached a peak 9 h postprandial (Figure 1.2a). The patterns of NEAA and TAA concentrations in fish fed diets 2 and 3 were similar. In both treatments, the concentrations of amino acids remained elevated and had not declined before the fish were re-fed at 24 h after the previous feeding. Plasma TAA concentrations in fish that were fed diet 1 resembled the changes of EAA whereas those of NEAA showed only a small increase after feeding. Unlike the fish that were fed once daily, plasma EAA, NEAA, and TAA concentrations of fish fed five times daily, as would be expected, varied comparatively little throughout the sampling period regardless of the diet.

The peak concentration of plasma EAA at 9 h after feeding in fish fed diet 1 once daily was approximately 125% higher than that in fish fed diet 2, and 75% higher than that in fish fed diet 3 (Figure 1.2a). Interestingly, the plasma EAA concentrations in fish fed the different diets five times daily, however, showed only small differences (Figure 1.2a). Distinctively, plasma NEAA concentrations in fish fed diet 2 and 3 were more than two-fold higher than those of fish fed diet 1 under both feeding regimes. As a result, the patterns and levels of TAA concentrations in fish fed diet 2 and 3 resembled the NEAA patterns and concentrations while, the patterns and levels of TAA concentrations in fish fed diet 1 resembled those of

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EAA concentrations (Figure 1.2b and 1.2c).

The ratio of plasma EAA/NEAA concentrations in fish fed the different diets revealed the differences in the concentrations of non-essential amino acids between diet 1 and those of diet 2 and 3 (Tables 1.12-1.17). The ratios of plasma EAA/NEAA in fish fed diet 1 were between 0.78-1.02 and 0.74-0.81 in fish fed once daily and five times daily, respectively (Table 1.12 and 1.15). The ratios of plasma EAA/NEAA of fish fed diet 2 were lower than those of fish fed diet 1 ranging between 0.24-0.36 and 0.25-0.31 in fish fed once daily and five times daily, respectively (Table 1.13 and 1.16). The ratios of plasma EAA/NEAA in fish fed diet 3 were between 0.34-0.45 in fish fed once daily, and 0.35-0.40 in fish fed five times daily (Table 1.14 and 1.17). The ratios were higher than those of fish fed diet 2 regardless of feeding schedule, and lower than those in fish fed diet 1.

Plasma Concentrations of Individual Amino Acids in Fish Fed Different Diets and on Different Feeding Regimes

Essential Amino Acids

In fish fed once daily, patterns of several individual amino acids in fish fed diets 1 and 3 seemed to follow the overall patterns of EAA concentrations. As with plasma EAA patterns, fish fed diet 1 once daily showed fluctuation of most essential amino acids namely histidine, isoleucine, leucine, lysine, methionine + cystine, valine, and threonine over time, i.e. increasing after meal consumption and gradually decreasing subsequently. Plasma concentrations of arginine, phenylalanine, and tyrosine did not, however, show any changes. In fish fed diet 3, plasma concentrations of isoleucine, leucine, lysine, methionine + cystine, and valine

showed similar patterns to that of the overall change in EAA concentrations. The remaining amino acids, however, stayed relatively constant. Plasma concentrations of most essential amino acids in fish fed diet 2 showed only slight increases or remained constant in the period following the meal. There were only two essential amino acids, leucine, and valine, that showed a fluctuation similar to the overall pattern of the plasma EAA (Figure 1.3a-1.3i).

In fish fed five times daily, levels of most essential amino acids in fish fed the three different diets tended to remain fairly stable throughout the sampling period in agreement with the overall pattern of EAA concentrations (Figures 1.3a-1.3e, 1.3i, and 1.3p). Exceptions were plasma concentrations of methionine+cystine, and valine. The levels of plasma valine dropped at 3 h and rose at 9 h after feeding. In contrast, the levels of plasma methionine+cystine started to rise at 3 h after feeding and thereafter remained relatively stable (Figures 1.3f-1.3g).

The most abundant essential amino acid in the plasma was found to differ among the fish that were fed different diets. Valine concentrations were the highest in fish fed diet 1 or 2 under both feeding regimes. The average relative concentrations for this amino acid calculated as a percentage of total amino acids were 11.3% and 9.6% for fish fed diet 1 once daily and five times daily (Tables 1.12 and 1.15); and 4.9% and 4.5% for fish fed diet 2 once daily and five times daily, respectively (Tables 1.13 and 1.16). In contrast, the fish that were fed diet 3 (which was supplemented with lysine) once daily or five times daily had lysine as the most abundant essential amino acid in the plasma constituting 5.4 and 5.0% of TAA respectively (Table 1.14 and 1.17).

Non-Essential Amino Acids

The patterns of individual non-essential amino acids in plasma of fish fed different diets once daily resembled the overall pattern of NEAA (Figures 1.3j-o, 1.2b), except for the levels of glycine in fish fed diets 2 and 3. Plasma glycine in these fish started to rise after feeding and peaked at 15 h. This was different from the NEAA pattern which showed a drop at 3 h after feeding.

The patterns of individual non-essential amino acids in fish fed diets 1 and 3 five times daily were similar to the overall pattern of NEAA. Some non-essential amino acids in fish fed diet 2, however, differed from the overall pattern of NEAA. Plasma alanine, proline, and aspartic acid concentrations dropped at 3 h after feeding (Figures 1.3j-o, 1.2b).

The concentrations of alanine, aspartic acid, glycine, proline and serine in fish fed diets 2 and 3 were considerably higher than those of fish fed the diet 1 under either feeding regime. Differences between fish fed diet 2 and 3 were also found in the levels of the above mentioned amino acids. The levels of these amino acids in fish that were fed diet 2 were higher than those in fish fed diet 3 under both feeding regimes (Tables 1.12-1.17 and Figures 1.3j-p).

Glycine, of the non-essential amino acids, generally represented the highest concentrations, between 27.6-38.8%, of the total plasma amino acids depending upon the diets and feeding regimes. Serine was the second most abundant non-essential amino acid, constituting about 11.7-14.7% of total plasma amino acids depending upon the diet and feeding regime (Tables 1.12-1.17, and Table 1.18).

Table 1.12. Concentrations of plasma amino acids in rainbow trout at different times after feeding diet 1 (fish meal based diet) in Experiment 1.1. Fish fed once daily to satiation.

Amino acid	Hours after feeding							
	0		3		9		15	
	$\bar{X} \pm SEM$[1] (μmol/mL)	%[2]	$\bar{X} \pm SEM$ (μmol/mL)	%	$\bar{X} \pm SEM$ (μmol/mL)	%	$\bar{X} \pm SEM$ (μmol/mL)	%
Essential								
Arg	0.125 ± 0.003	2.5	0.159 ± 0.005	2.5	0.146 ± 0.011	2.1	0.136 ± 0.024	2.5
Cys	0.016 ± 0.002	0.3	0.026 ± 0.002	0.4	0.030 ± 0.007	0.4	0.023 ± 0.001	0.4
His	0.326 ± 0.023	6.5	0.383 ± 0.018	5.9	0.428 ± 0.041	6.3	0.369 ± 0.009	6.7
Ile	0.175 ± 0.017	3.5	0.269 ± 0.020	4.1	0.302 ± 0.055	4.4	0.173 ± 0.033	3.1
Leu	0.307 ± 0.033	6.1	0.476 ± 0.041	7.3	0.568 ± 0.110	8.3	0.297 ± 0.040	5.4
Lys	0.267 ± 0.014	5.3	0.343 ± 0.010	5.3	0.337 ± 0.040	4.9	0.261 ± 0.010	4.7
Met	0.165 ± 0.007	3.3	0.232 ± 0.012	3.6	0.264 ± 0.014	3.9	0.227 ± 0.015	4.1
Phe	0.095 ± 0.004	1.9	0.124 ± 0.009	1.9	0.129 ± 0.002	1.9	0.122 ± 0.004	2.2
Thr	0.218 ± 0.018	4.4	0.244 ± 0.044	3.8	0.292 ± 0.083	4.3	0.205 ± 0.023	3.7
Tyr	0.027 ± 0.010	0.5	0.083 ± 0.005	1.3	0.061 ± 0.020	0.9	0.053 ± 0.014	1.0
Val	0.567 ± 0.048	11.3	0.715 ± 0.056	11.0	0.881 ± 0.148	12.9	0.526 ± 0.042	9.5
Non-essential								
Ala	0.423 ± 0.022	8.5	0.530 ± 0.034	8.2	0.535 ± 0.034	7.8	0.391 ± 0.006	7.1
Asp	0.075 ± 0.004	1.5	0.093 ± 0.005	1.4	0.090 ± 0.016	1.3	0.099 ± 0.008	1.8
Glu	0.105 ± 0.002	2.1	0.175 ± 0.024	2.7	0.181 ± 0.023	2.7	0.167 ± 0.020	3.0
Gly	1.452 ± 0.076	29.0	1.718 ± 0.102	26.5	1.728 ± 0.101	25.3	1.690 ± 0.198	30.6
Pro	0.081 ± 0.010	1.6	0.162 ± 0.006	2.5	0.157 ± 0.098	2.3	0.122 ± 0.022	2.2
Ser	0.582 ± 0.025	11.6	0.758 ± 0.046	11.8	0.780 ± 0.020	14.4	0.657 ± 0.027	12.0
TAA[3]	5.005 ± 0.131		6.434 ± 0.390		6.822 ± 0.623		5.477 ± 0.257	
EAA[4]	2.286 ± 0.119		3.053 ± 0.175		3.443 ± 0.440		2.391 ± 0.065	
NEAA[5]	2.718 ± 0.077		3.380 ± 0.212		3.378 ± 0.185		3.086 ± 0.252	
EAA/NEAA	0.84		0.90		1.02		0.78	

[1] Mean of three pools of plasma (five fish/pool) and standard error of the mean (n=3). [2] % of total amino acids. [3] Total amino acids. [4] Total essential amino acids. [5] Total non-essential amino acids

Table 1.13. Concentrations of plasma amino acids in rainbow trout at different time after feeding diet 2 (amino acid deficient diet) in Experiment 1.1. Fish fed once daily to satiation.

Amino acid	Hours after feeding							
	0		3		9		15	
	$\bar{X} \pm SEM$[1] (μmol/mL)	%[2]	$\bar{X} \pm SEM$ (μmol/mL)	%	$\bar{X} \pm SEM$ (μmol/mL)	%	$\bar{X} \pm SEM$ (μmol/mL)	%
Essential								
Arg	0.118 ± 0.003	1.3	0.155 ± 0.031	1.8	0.118 ± 0.033	1.2	0.134 ± 0.009	1.3
Cys	0.012 ± 0.001	0.1	0.032 ± 0.012	0.4	0.026 ± 0.005	0.3	0.021 ± 0.003	0.2
His	0.346 ± 0.020	3.7	0.348 ± 0.057	4.0	0.346 ± 0.018	3.5	0.261 ± 0.112	2.8
Ile	0.101 ± 0.016	1.1	0.177 ± 0.025	2.1	0.193 ± 0.031	1.9	0.153 ± 0.010	1.5
Leu	0.251 ± 0.045	2.7	0.335 ± 0.046	3.9	0.399 ± 0.058	4.0	0.305 ± 0.006	2.9
Lys	0.223 ± 0.017	2.4	0.255 ± 0.034	3.0	0.237 ± 0.022	2.4	0.236 ± 0.004	2.3
Met	0.070 ± 0.003	0.8	0.126 ± 0.021	1.5	0.130 ± 0.023	1.3	0.118 ± 0.021	1.1
Phe	0.103 ± 0.001	1.1	0.123 ± 0.011	1.4	0.137 ± 0.016	1.4	0.129 ± 0.017	1.2
Thr	0.175 ± 0.013	1.9	0.175 ± 0.046	2.0	0.200 ± 0.034	2.0	0.202 ± 0.027	2.0
Tyr	0.032 ± 0.002	0.4	0.080 ± 0.012	0.9	0.050 ± 0.005	0.5	0.040 ± 0.018	0.4
Val	0.378 ± 0.051	4.1	0.483 ± 0.071	5.6	0.568 ± 0.064	5.7	0.448 ± 0.034	4.3
Non-essential								
Ala	1.014 ± 0.091	10.9	0.717 ± 0.101	8.3	0.935 ± 0.048	9.4	0.953 ± 0.024	9.2
Asp	0.816 ± 0.044	8.8	0.669 ± 0.084	7.8	0.802 ± 0.084	8.1	0.892 ± 0.036	8.6
Glu	0.163 ± 0.017	1.8	0.164 ± 0.016	1.9	0.188 ± 0.014	1.9	0.180 ± 0.016	1.7
Gly	2.780 ± 0.566	29.8	3.215 ± 0.182	37.3	3.579 ± 0.222	36.0	4.100 ± 0.141	36.9
Pro	1.155 ± 0.179	12.4	0.461 ± 0.012	5.4	0.653 ± 0.097	6.6	0.606 ± 0.116	5.9
Ser	1.584 ± 0.156	17.0	1.096 ± 0.114	12.7	1.393 ± 0.237	14.0	1.559 ± 0.087	15.1
TAA[3]	9.318 ± 0.875		8.610 ± 0.803		9.953 ± 0.738		10.340 ± 0.200	
EAA[4]	1.808 ± 0.150		2.289 ± 0.338		2.404 ± 0.206		2.047 ± 0.147	
NEAA[5]	7.510 ± 0.735		6.321 ± 0.484		7.550 ± 0.583		8.289 ± 0.334	
EAA/NEAA	0.24		0.36		0.32		0.25	

[1] Mean of three pools of plasma (five fish/pool) and standard error of the mean (n=3). [2] % of total amino acids. [3] Total amino acids. [4] Total essential amino acids. [5] Total non-essential amino acids

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Table 1.14. Concentrations of plasma amino acids in rainbow trout at different time after feeding diet 3 (supplemented with lysine, methionine, and tryptophan) in Experiment 1.1. Fish fed once daily to satiation.

Amino acid	Hours after feeding							
	0		3		9		15	
	X̄ ± SEM[1] (μmol/mL)	%[2]	X̄ ± SEM (μmol/mL)	%	X̄ ± SEM (μmol/mL)	%	X̄ ± SEM (μmol/mL)	%
Essential								
Arg	0.122 ± 0.015	1.4	0.166 ± 0.014	1.9	0.164 ± 0.017	1.8	0.149 ± 0.013	1.6
Cys	0.027 ± 0.002	0.3	0.024 ± 0.002	0.3	0.030 ± 0.003	0.3	0.033 ± 0.001	0.4
His	0.321 ± 0.023	3.6	0.336 ± 0.040	4.0	0.340 ± 0.024	3.7	0.357 ± 0.012	3.8
Ile	0.107 ± 0.008	1.2	0.169 ± 0.019	2.0	0.166 ± 0.024	1.8	0.142 ± 0.016	1.5
Leu	0.256 ± 0.013	2.8	0.310 ± 0.022	3.7	0.358 ± 0.047	3.9	0.313 ± 0.026	3.3
Lys	0.509 ± 0.023	5.7	0.517 ± 0.045	6.2	0.488 ± 0.038	5.3	0.439 ± 0.028	4.6
Met	0.274 ± 0.014	3.0	0.340 ± 0.018	4.0	0.355 ± 0.029	3.8	0.330 ± 0.018	3.5
Phe	0.098 ± 0.004	1.1	0.130 ± 0.007	1.5	0.114 ± 0.010	1.2	0.120 ± 0.007	1.3
Thr	0.171 ± 0.010	1.9	0.140 ± 0.021	1.7	0.186 ± 0.023	2.0	0.168 ± 0.030	1.8
Tyr	0.024 ± 0.002	0.3	0.058 ± 0.003	0.7	0.039 ± 0.011	0.4	0.044 ± 0.017	0.5
Val	0.394 ± 0.020	4.4	0.431 ± 0.027	5.1	0.515 ± 0.063	5.6	0.432 ± 0.027	4.6
Non-essential								
Ala	0.863 ± 0.045	9.6	0.707 ± 0.060	8.4	0.775 ± 0.026	8.4	0.895 ± 0.055	9.4
Asp	0.730 ± 0.014	8.1	0.601 ± 0.110	7.2	0.627 ± 0.031	6.8	0.689 ± 0.061	7.3
Glu	0.159 ± 0.013	1.8	0.163 ± 0.015	1.9	0.175 ± 0.014	1.9	0.153 ± 0.014	1.6
Gly	2.947 ± 0.140	32.7	2.971 ± 0.335	35.3	3.262 ± 0.127	35.2	3.498 ± 0.160	36.9
Pro	0.847 ± 0.133	9.4	0.353 ± 0.026	4.2	0.568 ± 0.229	6.1	0.615 ± 0.073	6.5
Ser	1.167 ± 0.104	12.9	0.989 ± 0.156	11.8	1.099 ± 0.060	11.9	1.114 ± 0.060	11.7
TAA[3]	9.014 ± 0.510		8.405 ± 0.800		9.260 ± 0.723		9.491 ± 0.381	
EAA[4]	2.303 ± 0.119		2.621 ± 0.162		2.755 ± 0.270		2.526 ± 0.049	
NEAA[5]	6.711 ± 0.395		5.784 ± 0.656		6.506 ± 0.454		6.964 ± 0.361	
EAA/NEAA	0.34		0.45		0.42		0.36	

[1] Mean of three pools of plasma (five fish/pool) and standard error of the mean (n=3). [2] % of total amino acids. [3] Total amino acids. [4] Total essential amino acids. [5] Total non-essential amino acids

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Table 1.15. Concentrations of plasma amino acids in rainbow trout at different times after feeding diet 1 (fish meal based diet) in Experiment 1.1. Fish fed 5 times daily[1].

Amino acid	Hours after feeding					
	0		3		9	
	$\bar{X} \pm SEM^2$ (μmol/mL)	%[3]	$\bar{X} \pm SEM$ (μmol/mL)	%	$\bar{X} \pm SEM$ (μmol/mL)	%
Essential						
Arg	0.111 ± 0.002	2.1	0.154 ± 0.018	2.9	0.130 ± 0.016	2.5
Cys	0.016 ± 0.003	0.3	0.034 ± 0.008	0.6	0.026 ± 0.007	0.5
His	0.333 ± 0.029	6.4	0.334 ± 0.006	6.2	0.310 ± 0.033	5.9
Ile	0.171 ± 0.038	3.3	0.173 ± 0.016	3.2	0.174 ± 0.022	3.3
Leu	0.314 ± 0.077	6.0	0.284 ± 0.014	5.3	0.307 ± 0.030	5.9
Lys	0.268 ± 0.022	5.1	0.280 ± 0.013	5.2	0.235 ± 0.023	4.5
Met	0.171 ± 0.009	3.3	0.225 ± 0.021	4.2	0.206 ± 0.035	3.9
Phe	0.106 ± 0.009	2.0	0.116 ± 0.026	2.2	0.118 ± 0.004	2.3
Thr	0.242 ± 0.034	4.6	0.155 ± 0.007	2.9	0.163 ± 0.014	3.1
Tyr	0.045 ± 0.007	0.9	0.070 ± 0.036	1.3	0.079 ± 0.015	1.5
Val	0.566 ± 0.110	10.8	0.472 ± 0.018	8.7	0.488 ± 0.040	9.3
Non-essential						
Ala	0.412 ± 0.047	7.9	0.403 ± 0.024	7.5	0.398 ± 0.044	7.6
Asp	0.090 ± 0.015	1.7	0.096 ± 0.005	1.8	0.134 ± 0.051	2.6
Glu	0.146 ± 0.026	2.8	0.162 ± 0.012	3.0	0.139 ± 0.008	2.7
Gly	1.565 ± 0.087	30.0	1.712 ± 0.018	31.7	1.645 ± 0.207	31.4
Pro	0.106 ± 0.023	2.0	0.042 ± 0.042	0.8	0.050 ± 0.051	1.0
Ser	0.559 ± 0.050	10.7	0.693 ± 0.026	12.8	0.642 ± 0.051	12.2
TAA[4]	5.222 ± 0.540		5.405 ± 0.172		5.244 ± 0.458	
EAA[5]	2.344 ± 0.333		2.298 ± 0.126		2.234 ± 0.193	
NEAA[6]	2.878 ± 0.233		3.108 ± 0.046		3.008 ± 0.349	
EAA/NEAA	0.81		0.74		0.74	

[1] Total daily intake of feed was equal to the amount consumed by the fish fed once daily, for each respective diet. [2] Mean of three pools of plasma (five fish/pool) and standard error of the mean (n=3). [3] % of total amino acids. [4] Total amino acids. [5] Total essential amino acids. [6] Total non-essential amino acids

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Table 1.16. Concentrations of plasma amino acids in rainbow trout at different times after feeding diet 2 (amino acid deficient diet) in Experiment 1.1. Fish fed 5 times daily[1].

Amino acid	Hours after feeding					
	0		3		9	
	X̄ ± SEM[2] (μmol/mL)	%[3]	X̄ ± SEM (μmol/mL)	%	X̄ ± SEM (μmol/mL)	%
Essential						
Arg	0.124 ± 0.013	1.2	0.165 ± 0.017	1.6	0.143 ± 0.015	1.4
Cys	0.017 ± 0.000	0.2	0.032 ± 0.006	0.3	0.046 ± 0.012	0.5
His	0.342 ± 0.015	3.2	0.361 ± 0.004	3.5	0.361 ± 0.016	3.6
Ile	0.138 ± 0.019	1.3	0.135 ± 0.022	1.3	0.177 ± 0.017	1.8
Leu	0.335 ± 0.046	3.2	0.265 ± 0.021	2.5	0.347 ± 0.037	3.4
Lys	0.226 ± 0.012	2.1	0.236 ± 0.006	2.3	0.225 ± 0.030	2.2
Met	0.083 ± 0.007	0.8	0.115 ± 0.011	1.1	0.148 ± 0.015	1.5
Phe	0.120 ± 0.009	1.1	0.129 ± 0.006	1.2	0.136 ± 0.010	1.4
Thr	0.211 ± 0.036	2.0	0.192 ± 0.041	1.8	0.198 ± 0.020	2.0
Tyr	0.058 ± 0.012	0.6	0.090 ± 0.016	0.9	0.094 ± 0.006	0.9
Val	0.490 ± 0.070	4.6	0.403 ± 0.015	3.9	0.496 ± 0.044	4.9
Non-essential						
Ala	0.965 ± 0.124	9.1	0.892 ± 0.010	8.6	0.828 ± 0.100	8.2
Asp	0.900 ± 0.015	8.5	0.808 ± 0.031	7.8	0.846 ± 0.069	8.4
Glu	0.179 ± 0.011	1.7	0.192 ± 0.012	1.9	0.176 ± 0.020	1.7
Gly	3.847 ± 0.211	36.5	4.400 ± 0.226	42.2	3.806 ± 0.242	37.6
Pro	1.051 ± 0.126	10.0	0.505 ± 0.067	4.9	0.550 ± 0.166	5.4
Ser	1.469 ± 0.053	13.9	1.501 ± 0.051	14.4	1.534 ± 0.245	15.2
TAA[4]	10.533 ± 0.464		10.419 ± 0.282		10.111 ± 1.030	
EAA[5]	2.143 ± 0.161		2.125 ± 0.060		2.370 ± 0.195	
NEAA[6]	8.411 ± 0.343		8.298 ± 0.307		7.740 ± 0.835	
EAA/NEAA	0.25		0.26		0.31	

[1] Total daily intake of feed was equal to the amount consumed by the fish fed once daily, for each respective diet. [2] Mean of three pools of plasma (five fish/pool) and standard error of the mean (n=3). [3] % of total amino acids. [4] Total amino acids. [5] Total essential amino acids. [6] Total non-essential amino acids

Table 1.17. Concentrations of plasma amino acids in rainbow trout at different times after feeding diet 3 (supplemented with lysine, methionine, and tryptophan) in Experiment 1.1. Fish fed 5 times daily[1].

Amino acid	Hours after feeding					
	0		3		9	
	$\bar{X}$ ± SEM[2] (µmol/mL)	%[3]	$\bar{X}$ ± SEM (µmol/mL)	%	$\bar{X}$ ± SEM (µmol/mL)	%
Essential						
Arg	0.132 ± 0.012	1.4	0.156 ± 0.006	1.8	0.158 ± 0.018	1.8
Cys	0.027 ± 0.002	0.3	0.095 ± 0.047	1.1	0.037 ± 0.007	0.4
His	0.312 ± 0.017	3.3	0.337 ± 0.021	3.9	0.335 ± 0.036	3.8
Ile	0.120 ± 0.007	1.3	0.132 ± 0.009	1.5	0.128 ± 0.017	1.4
Leu	0.297 ± 0.018	3.1	0.254 ± 0.008	3.0	0.262 ± 0.029	2.9
Lys	0.457 ± 0.011	4.8	0.469 ± 0.042	5.4	0.421 ± 0.032	4.7
Met	0.321 ± 0.005	3.4	0.294 ± 0.006	3.4	0.336 ± 0.055	3.8
Phe	0.113 ± 0.008	1.2	0.125 ± 0.008	1.4	0.118 ± 0.013	1.3
Thr	0.199 ± 0.012	2.1	0.157 ± 0.030	1.8	0.170 ± 0.024	1.9
Tyr	0.040 ± 0.008	0.4	0.045 ± 0.012	0.5	0.040 ± 0.005	0.4
Val	0.446 ± 0.030	4.7	0.401 ± 0.013	4.6	0.385 ± 0.033	4.3
Non-essential						
Ala	0.797 ± 0.066	8.4	0.697 ± 0.081	8.0	0.765 ± 0.078	8.6
Asp	0.790 ± 0.017	8.3	0.668 ± 0.019	7.7	0.757 ± 0.046	8.5
Glu	0.200 ± 0.017	2.1	0.183 ± 0.018	2.1	0.170 ± 0.017	1.9
Gly	3.388 ± 0.166	35.6	3.259 ± 0.064	37.4	3.381 ± 0.493	37.9
Pro	0.683 ± 0.086	7.2	0.396 ± 0.066	4.5	0.327 ± 0.047	3.7
Ser	1.189 ± 0.031	12.5	1.057 ± 0.018	12.1	1.139 ± 0.157	12.8
TAA[4]	9.510 ± 0.107		8.725 ± 0.345		8.926 ± 1.000	
EAA[5]	2.464 ± 0.076		2.466 ± 0.038		2.387 ± 0.193	
NEAA[6]	7.046 ± 0.068		6.260 ± 0.309		6.541 ± 0.812	
EAA/NEAA	0.35		0.40		0.36	

[1] Total daily intake of feed was equal to the amount consumed by the fish fed once daily, for each respective diet. [2] Mean of three pools of plasma (five fish/pool) and standard error of the mean (n=3). [3] % of total amino acids. [4] Total amino acids. [5] Total essential amino acids. [6] Total non-essential amino acids

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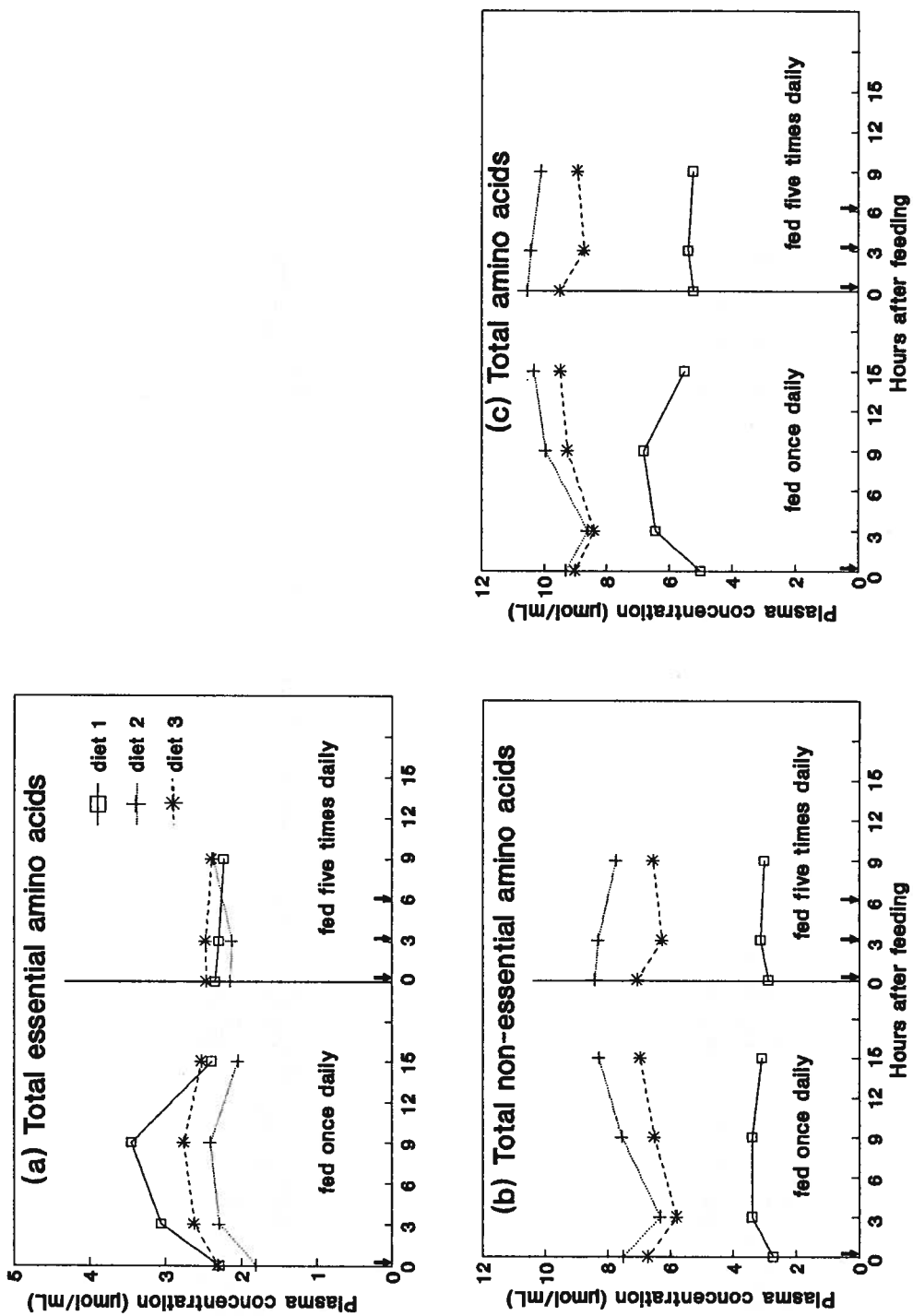
Figure 1.2. Total concentrations of plasma amino acids (essential, non-essential, and total amino acids) determined at different times postprandial for rainbow trout fed once daily and five times daily in Experiment 1.1. Diet 1 = fish meal based diet, diet 2 = diet deficient in methionine, lysine, and tryptophan, diet 3 = diet 2 supplemented with methionine, lysine, and tryptophan. Each point represents a mean of three pools of plasma, five fish/pool, (n=3). Arrows indicate the time of feeding.

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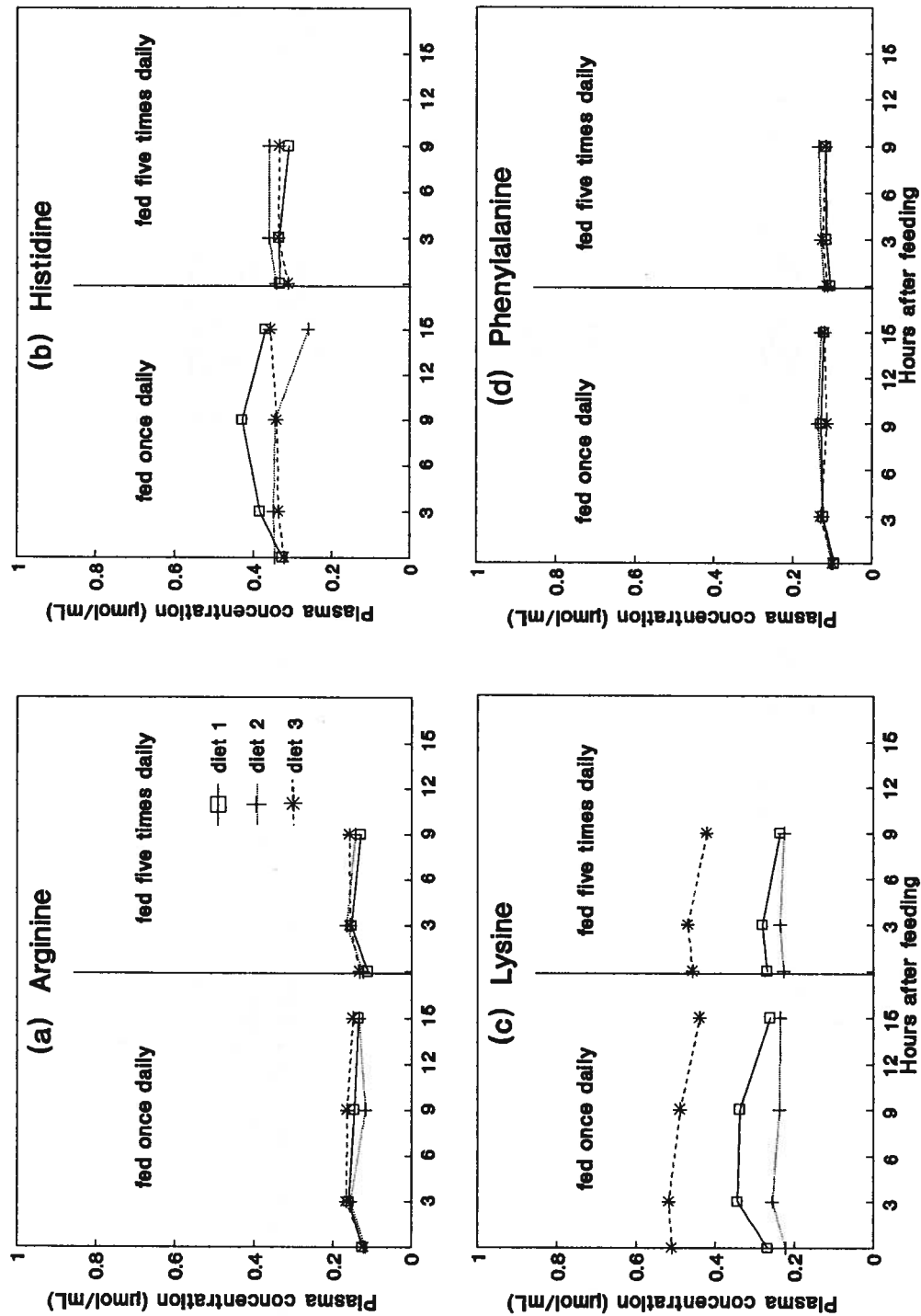
Figure 1.3. Plasma concentrations of amino acids in rainbow trout fed different diets according to different feeding regimes in Experiment 1.1. Diet 1 = fish meal based diet, diet 2 = diet deficient in methionine, lysine, and tryptophan, diet 3 = diet 2 supplemented with methionine, lysine, and tryptophan. Each point represents a mean of three pools of plasma, five fish/pool, (n=3).

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Figure 1.3. (Continued)

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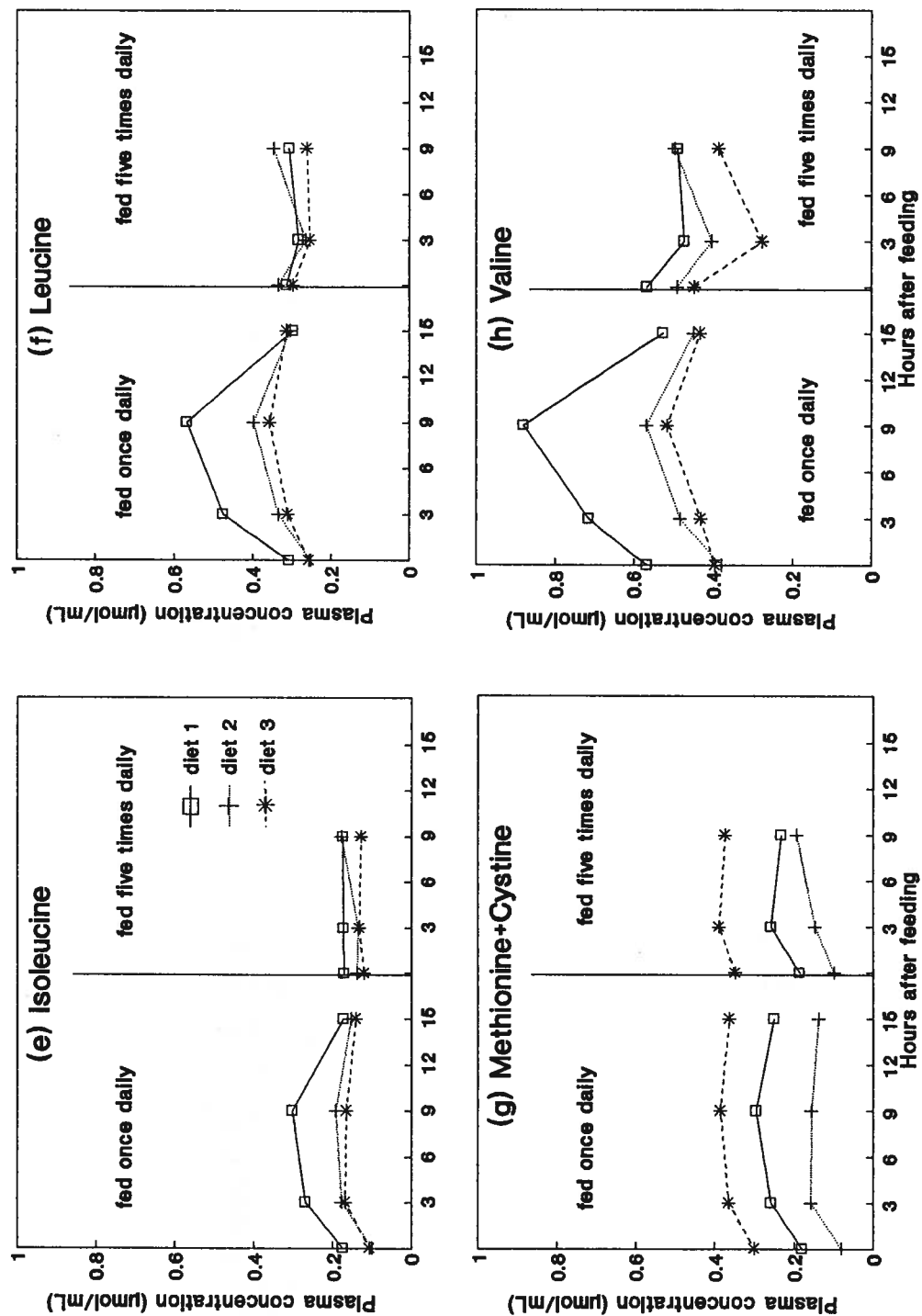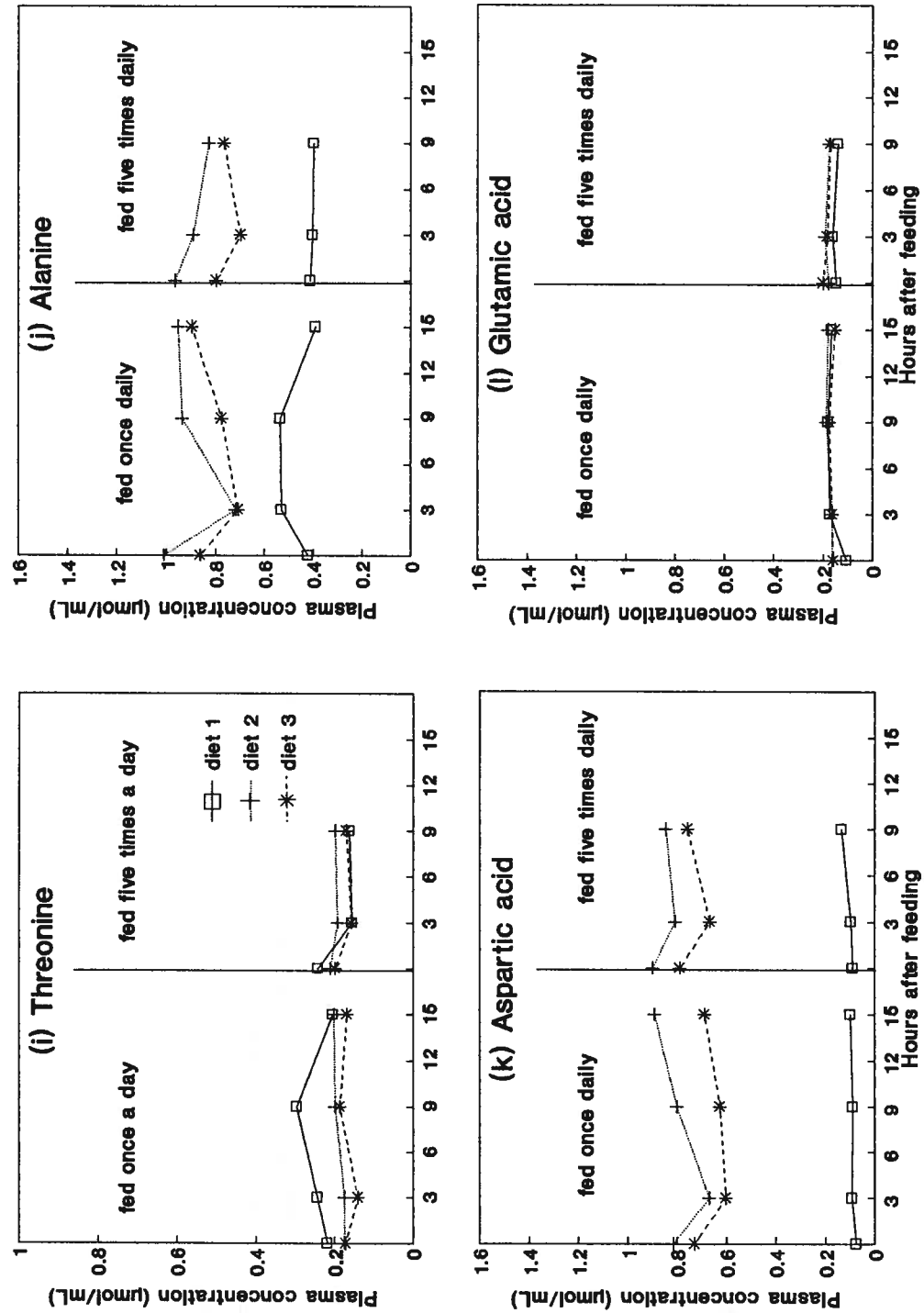
Figure 1.3. (Continued)

Figure 1.3. (Continued)

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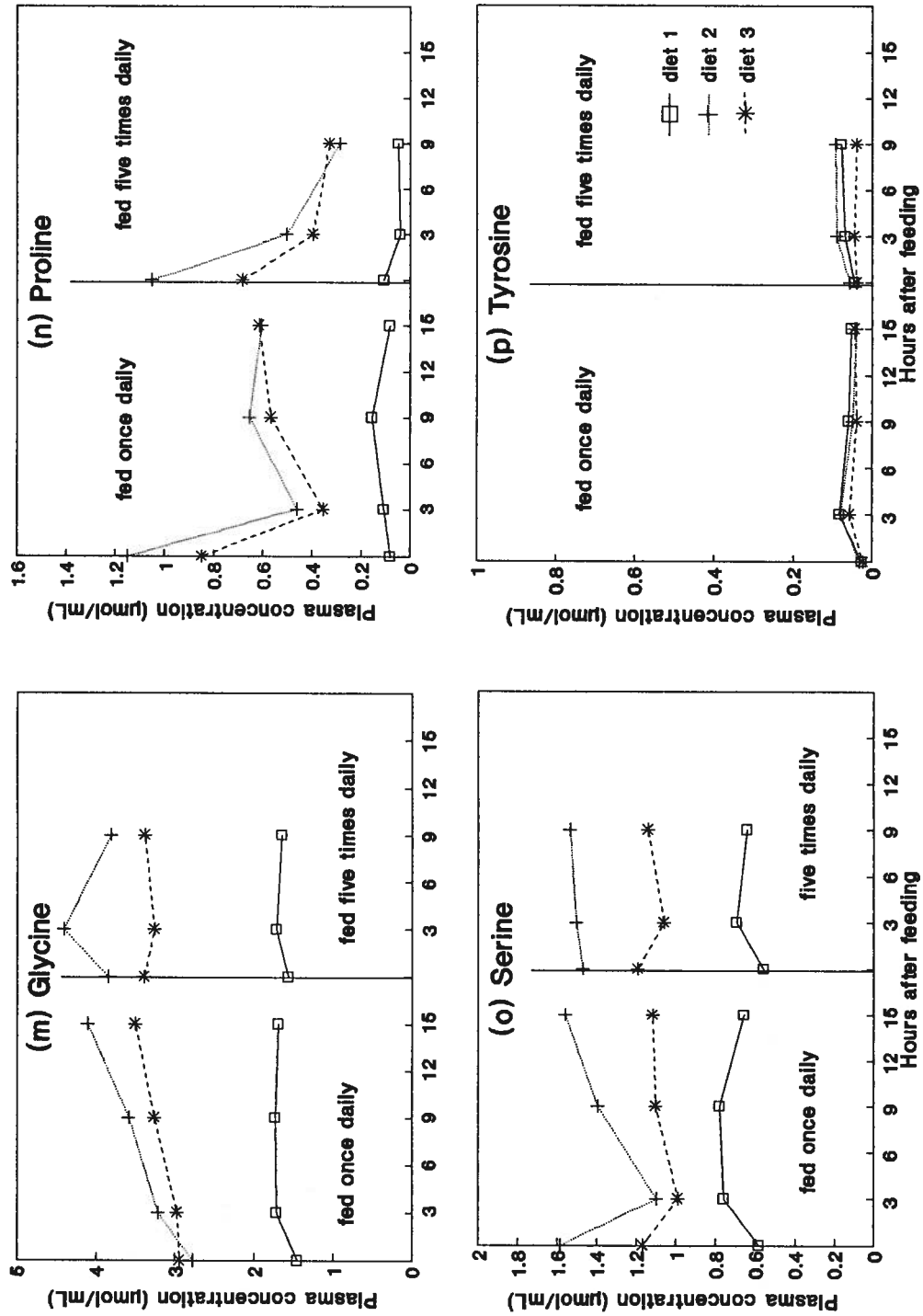
Distribution of Plasma Essential Amino Acids

The data regarding the percentages of individual amino acids in the plasma shown in Tables 1.12-1.17 indicated that the pattern of the distribution of essential amino acids in fish over the sampling times was fairly consistent with respect to the diet fed. The data from Tables 1.12-1.14 on the percentage of essential amino acids relative to the total amino acids at 9 h postprandial in fish fed once daily were rearranged according to their order of abundance, and presented in Table 1.18. The reason why the plasma amino acids at 9 h postprandial were chosen was because plasma concentration of most amino acids attained their peaks at this time and would be expected to reflect the profile of dietary amino acids. Differences between the pattern of distribution of plasma amino acids in fish fed diet 1 and those of fish fed diet 2 and diet 3 were apparent. The order of abundance of plasma valine, leucine, histidine, lysine, tyrosine, and cystine in fish fed diet 2 corresponded well with those of fish fed diet 1. The order of plasma threonine, isoleucine, phenylalanine, methionine, and arginine in fish fed the former diet, however, did not agree with the fish fed the latter diet. With the supplementation of free lysine and methionine in diet 3, fish fed this diet showed a very noticeable deviation of the pattern for lysine, leucine, methionine, histidine, threonine, and isoleucine from that of fish fed diet 1.

Table 1.18. Distribution of plasma amino acids at 9 h postprandial in fish fed different diets once daily in Experiment 1.1

Order[2]	Diet 1[1] AA	%[3]	Diet 2 AA	%	Diet 3 AA	%
Essential						
1	Val	12.9	Val	5.7	Val	5.6
2	Leu	8.3	Leu	4.0	Lys	5.3
3	His	6.3	His	3.5	Leu	3.9
4	Lys	4.9	Lys	2.4	Met	3.8
5	Ile	4.4	Thr	2.0	His	3.7
6	Thr	4.3	Ile	1.9	Thr	2.0
7	Met	3.9	Phe	1.4	Ile	1.8
8	Arg	2.1	Met	1.3	Arg	1.8
9	Phe	1.9	Arg	1.2	Phe	1.2
10	Tyr	0.9	Tyr	0.5	Tyr	0.4
11	Cys	0.4	Cys	0.3	Cys	0.3
Non-essential						
1	Gly	25.3	Gly	36.0	Gly	35.2
2	Ser	14.4	Ser	14.0	Ser	11.9
3	Ala	7.8	Ala	9.4	Ala	8.4
4	Glu	2.7	Asp	8.1	Asp	6.8
5	Pro	2.3	Pro	6.6	Pro	6.1
6	Asp	1.3	Glu	1.9	Glu	1.9

[1] Diet 1 = fish meal based diet; Diet 2 = diet deficient in lysine, methionine, and tryptophan; Diet 3 = diet 2 supplemented with lysine, methionine, and tryptophan.
[2] Amino acids listed in order of concentrations in the plasma.
[3] Percent of total plasma amino acid concentrations.

3.2.2.6 Effects of Supplementary Lysine and Methionine

Supplementary lysine and methionine caused a pronounced increase in their concentrations in plasma of fish fed diet 3 compared with the concentrations in fish fed diet 2. The levels of these two amino acids in fish fed diet 3 under both feeding regimes increased after feeding, and were maintained at a considerably higher level until 24 h after the first feeding. The peak plasma concentration of lysine in fish fed diet 3 once daily was approximately 1.5 and 2.0 times greater than in fish fed diet 1 and diet 2, respectively. Similarly, peak concentrations of plasma methionine + cystine in fish fed diet 3 once daily was approximately 1.3 and 2.5 fold greater than those in fish fed diet 1 and diet 2, respectively. The plasma concentrations of lysine and methionine + cystine in fish fed diet 3 five times daily behaved in a similar manner to those observed in the fish fed once daily (Figure 1.3c and 1.3g).

3.2.2.7 Plasma Taurine Concentrations

Plasma taurine concentrations in fish varied with dietary treatment (Table 1.19, and Figure 1.4). Fish fed diet 1 had higher taurine concentrations than fish fed diet 2 or diet 3 under both feeding regimes. Taurine concentrations in fish fed diet 3 were higher than those in fish fed diet 2 either once daily or five times daily. The fluctuations of plasma taurine concentrations as compared to those of other amino acids were interesting. With once daily feeding, fish fed the different diets showed similar patterns in plasma taurine fluctuation over time. The concentrations were noticeably high at the time of feeding. The concentrations then gradually declined, and subsequently rose again after 9 h. These changes were the reverse of the

changes in methionine concentrations (Figure 1.4). A similar trend up to 9 h post-feeding were also observed in the fish that were fed five times daily.

3.2.2.8 Relationship Between Plasma and Dietary Amino Acids

The comparison between dietary (as μmol/16g N) and plasma amino acids (as μmol/ml of plasma) was made using the concentrations of plasma amino acids measured at 9 h after feeding in fish fed once daily (Table 1.12-1.14). Most amino acids attained peaks at this time. The analysis did not include glutamic acid and aspartic acid because their dietary concentrations also include their amide (glutamine and asparagine), whereas plasma concentrations do not. Significant correlation coefficients ($P < 0.05$) between the levels of amino acids in the plasma and those in the diets were obtained (diet 1, $r = 0.737$; diet 2, $r = 0.915$; diet 3, $r = 0.890$).

Table 1.19. Plasma taurine, methionine, and cystine concentrations in trout fed different diets according to feeding regimes in Experiment 1.1

Hour after feeding	Fed once daily			Fed five time daily		
	Diet 1[1]	Diet 2	Diet 3	Diet 1	Diet 2	Diet 3
	(μmol/mL ± SEM)			(μmol/mL ± SEM)		
Taurine						
0	0.472 ± 0.084[2]	0.241 ± 0.033	0.329 ± 0.014	0.633 ± 0.046	0.252 ± 0.020	0.391 ± 0.033
3	0.450 ± 0.051	0.161 ± 0.044	0.268 ± 0.032	0.447 ± 0.034	0.170 ± 0.017	0.285 ± 0.033
9	0.325 ± 0.157	0.119 ± 0.050	0.201 ± 0.089	0.331 ± 0.053	0.126 ± 0.026	0.258 ± 0.049
15	0.475 ± 0.056	0.165 ± 0.014	0.306 ± 0.030			
Methionine						
0	0.165 ± 0.007	0.070 ± 0.003	0.274 ± 0.014	0.171 ± 0.009	0.083 ± 0.007	0.321 ± 0.005
3	0.232 ± 0.012	0.126 ± 0.021	0.340 ± 0.018	0.225 ± 0.021	0.115 ± 0.011	0.294 ± 0.006
9	0.264 ± 0.014	0.130 ± 0.023	0.355 ± 0.029	0.206 ± 0.035	0.148 ± 0.015	0.336 ± 0.055
15	0.227 ± 0.015	0.118 ± 0.021	0.330 ± 0.018			
Cystine						
0	0.016 ± 0.002	0.012 ± 0.001	0.027 ± 0.002	0.016 ± 0.003	0.017 ± 0.000	0.027 ± 0.002
3	0.026 ± 0.002	0.032 ± 0.012	0.024 ± 0.002	0.034 ± 0.008	0.032 ± 0.006	0.095 ± 0.047
9	0.030 ± 0.007	0.026 ± 0.005	0.030 ± 0.003	0.026 ± 0.007	0.046 ± 0.012	0.037 ± 0.007
15	0.023 ± 0.001	0.021 ± 0.003	0.033 ± 0.001			

[1] Diet 1 = fish meal based diet; Diet 2 = diet deficient in lysine, methionine, and tryptophan; Diet 3 = diet 2 supplemented with lysine, methionine, and tryptophan.

[2] The values shown are means of three pools of plasma, five fish/pool, (n=3).
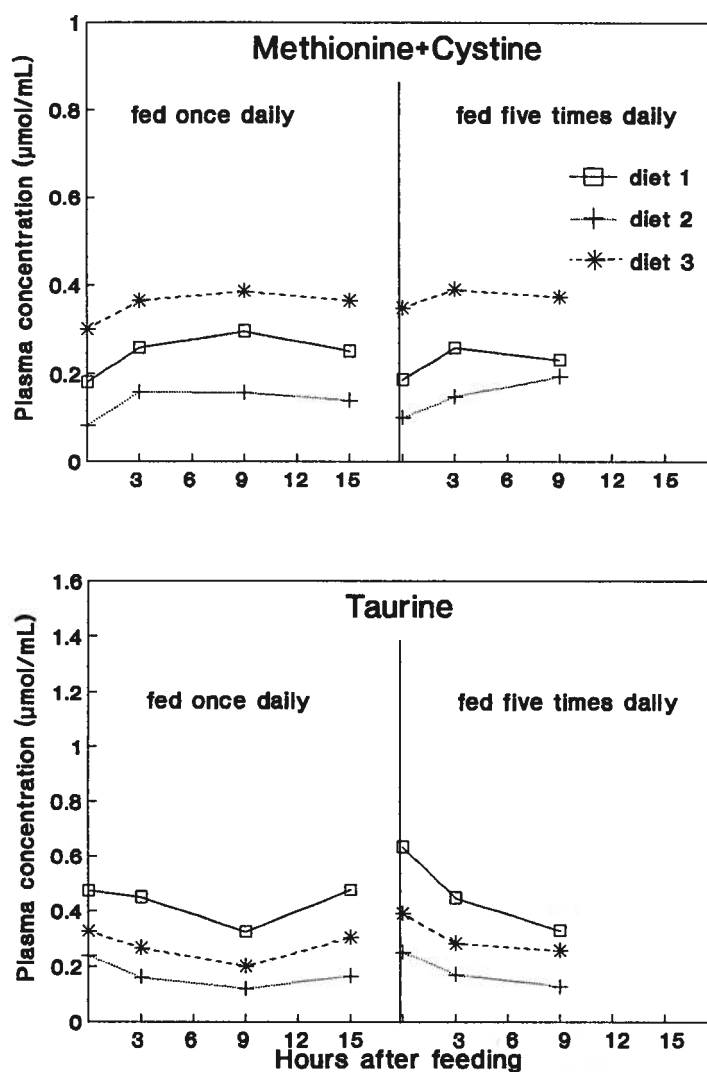
Figure 1.4. Plasma taurine, and methionine + cystine concentrations in rainbow trout fed different diets according to different feeding regimes in Experiment 1.1. Diet 1 = fish meal based diet, diet 2 = diet deficient in methionine, lysine, and tryptophan, diet 3 = diet 2 supplemented with methionine, lysine, and tryptophan. Each data point is a mean of three pools of plasma, five fish/pool, (n=3).

3.3 EXPERIMENT 1.2: DIETARY EFFECTS ON PLASMA CONCENTRATIONS OF AMINO ACIDS MONITORED OVER A 120-HOUR PERIOD FOLLOWING DIET CONSUMPTION

3.3.1 MATERIALS AND METHODS

3.3.1.1 Diets

The ingredient and proximate compositions of the three diets formulated for this experiment are shown in Table 1.20. The ingredients used in the diets were similar to those in experiment 1 except that anchovy meal was used in place of herring meal in all three diets. Diet 3 was supplemented with isoleucine in addition to lysine, methionine, and tryptophan. As with the latter three amino acids, isoleucine was added to mimic the concentration of that calculated to be present in diet 1. The addition of isoleucine was considered advisable because the calculated values of the four supplemental amino acids in diet 3 appeared to be lower than found in the control diet. The formulated diets were analyzed for proximate composition and subjected to acid hydrolysis for later determination of amino acid compositions of the diets according to AOAC methods (AOAC, 1984).

3.3.1.2 Fish

Rainbow trout with a mean weight of 180.4 ± 16.0 g (mean ± SD) were used in this experiment. They were distributed randomly into fifteen 150 L tanks located at the UBC aquarium facilities. Each tank contained ten fish. Each dietary treatment was assigned at random to five tanks of fish.

Table 1.20. Ingredient (air-dry basis) and proximate composition (dry matter basis) of diets used in Experiment 1.2

Ingredient	Diet 1	Diet 2	Diet 3
	g/kg	g/kg	g/kg
Anchovy meal (whole-steam dried)	460.00	153.20	153.20
Ground wheat[1]	300.00	300.00	300.00
Gelatin	-	102.00	102.00
Corn gluten meal	-	79.00	79.00
Soybean protein concentrate	-	60.20	60.20
Sardine oil[2]	93.00	118.00	118.00
Bone meal	9.00	38.00	38.00
Dextrin	88.00	100.00	87.00
Premix[3]	30.00	30.00	30.00
Calcium lignosulphonate	20.00	20.00	20.00
L-lysine	-	-	7.30
DL-methionine	-	-	4.00
L-tryptophan	-	-	1.70
L-isoleucine	-	-	3.50
Total	1000.00	1000.40	1003.70
Proximate analysis[4]			
Crude protein (%)	39.0	39.0	40.3
Ether-extractable lipid (%)	15.5	16.0	16.2
Ash (%)	9.4	7.1	7.1
Gross energy (kcal/kg)	5082	5220	5238

[1] Autoclaved at 121°C for 1.5 h.

[2] Stabilized with 0.05% ethoxyquin.

[3] The premix supplied the following per kg of diet as fed (except for diet 3 in which the percentage of each will be proportionally lower): thiamin HCl, 67.3 mg; riboflavin, 104.2 mg; niacin, 400 mg; biotin, 5 mg; folic acid, 25 mg; pyridoxine HCl, 60.8 mg; cyanocobalamine, 0.1 mg; D-calcium pantothenate, 200 mg; ascorbic acid, 1500 mg; choline chloride, 4000 mg; inositol, 2000 mg; menadione 30 mg; vitamin A, 10,000 IU; vitamin D_3, 300 IU; vitamin E, 1000 IU; Mg (as $MgSO_4$), 380 mg; Mn (as $MnSO_4.H_2O$), 17 mg; Zn (as ZnO), 50 mg; Fe (as $FeSO_4.7H_2O$), 85 mg; Cu (as $CuSO_4.5H_2O$), 2 mg; Co (as $CoCl.6H_2O$), 0.003 mg; K (as K_2SO_4), 895 mg; I (as KIO_3), 5 mg; NaCl (as NaCl), 2836 mg; F (as NaF), 4.5 mg; Se (as $Na_2SeO_3.5H_2O$), 0.10 mg.

[4] The values for crude protein, ether-extractable lipid, and ash were obtained by proximate analyses. The values for gross energy were estimated by ascribing 5.65 kcal/g crude protein, 9.5 kcal/g crude lipid, 4.0 kcal/g carbohydrate (Alexis *et al.*, 1985).

To acclimate the fish to the experimental diets, each experimental diet was gradually mixed with the commercial diet (EWOS) to which the fish were accustomed (from 50:50% to 100:0%), and was given to the fish in the respective tanks once daily. After 5 days of acclimation, i.e. when they fully accepted the experimental diets, fish in each tank were fed the designated diets for ten consecutive days. Fish were fed once daily until satiation in the morning. Satiation was determined when the fish stopped taking pellets. The amounts of food fed to the fish were recorded daily.

During the experiment, the water supply was dechlorinated Vancouver city water. Temperature was maintained between 13.0°C and 13.5°C by control of the heat exchanger unit. Water flow to each tank was 2 L/min, and dissolved oxygen was 8 ppm. Photoperiod was 24 h. There was no mortality.

3.3.1.3 Sampling Procedure

Following the final feeding at 10:00 hour, fish were bled at 3, 12, 24, 36, 48, 72, and 120 h post-feeding. Six fish were taken from one replicate tank for each dietary treatment at 3, 12, 24, 36, and 48 h sampling times. Fish that were sampled in the last two sampling times (72, and 120 h) were taken randomly from all replicated tanks with respect to the dietary treatments. The fish that were sampled were anesthetized with 0.01% tricaine-methanesulfonate (MS-222), and weighed. Blood was withdrawn from the caudal vein/dorsal aorta into 4 mL heparinized vacutainers. Blood samples were immediately centrifuged at 780xg for 15 min. The resultant plasma samples from three fish were pooled (i.e. there were two plasma samples/dietary treatment at each sampling time). The plasma samples were kept at -70°C for subsequent analyses.

3.3.1.4 Amino Acid Analysis

The procedures for the preparation of plasma for analysis and the determination of concentrations of free amino acids were the same as described for Experiment 1.1.

3.3.1.5 Statistical Analysis

The data on growth of fish were subjected to one-way analysis of variance. The analysis was performed using Systat (Wilkinson, 1990). To test the significance of differences between the treatment means, the Tukey HSD test was employed (Zar, 1984).

3.3.2 RESULTS

3.3.2.1 Amino Acid Compositions of Experimental Diets

The amino acid compositions of the experimental diets are shown in Table 1.21. As with the results of analyses conducted on the diets in Experiment 1.1, the essential amino acid levels in diet 1 were higher than those in diet 2 or diet 3. The proportion of EAA to NEAA in diet 1 was close to 1. Diets 2 and 3, on the other hand, contained a higher proportion of non-essential amino acids. The concentration of arginine in diet 1 was markedly lower than the concentration of this amino acid in diet 1 formulated for Experiment 1.1.

Table 1.21. Amino acid composition of experimental diets in Experiment 1.2

Amino acid	Experimental diet[1]		
	Diet 1	Diet 2	Diet 3
		g/16g N	
Arg	4.8	5.3	5.0
His	2.7	3.0	2.6
Ile	3.6	2.9	3.7
Leu	6.6	6.7	6.5
Lys	6.4	4.4	5.9
Met	2.3	1.5	2.3
Cys	1.0	0.9	0.8
Phe	3.9	3.6	3.6
Tyr	2.6	2.3	1.9
Thr	3.8	2.9	2.9
Val	4.3	3.5	3.4
Ala	5.4	6.0	6.0
Asp	8.2	7.8	7.6
Glu	13.3	14.1	14.0
Gly	5.7	9.5	9.1
Pro	4.5	7.3	7.3
Ser	3.7	3.8	3.7
Tau	1.2	0.8	0.7
TAA[2]	82.8	85.3	86.5
EAA	42.1	36.9	38.8
NEAA	40.7	48.4	47.7
EAA/NEAA	1.03	0.76	0.81

[1] Diet 1 = fish meal based diet; Diet 2 = amino acid deficient diet; Diet 3 = diet 2 supplemented with isoleucine, lysine, methionine, and tryptophan.

[2] excluding asparagine, glutamine, and taurine, and tryptophan was not detected by the procedure used.

Table 1.22. Body weight gain and feed consumption of rainbow trout over a 10-day period in Experiment 1.2

Diet[1]	Initial weight	Final weight	Weight gain	Feed consumption
	(g)	(g)	(g/fish)	(g/fish/day)
1	182.0 ± 1.3^2	224.9 ± 3.9	42.9 ± 4.9	4.1 ± 0.20
2	179.9 ± 4.1	218.7 ± 5.9	38.8 ± 7.7	3.8 ± 0.03
3	179.5 ± 1.9	221.9 ± 3.0	42.4 ± 1.2	3.8 ± 0.17

[1] Diet 1 = fish meal based diet; Diet 2 = amino acid deficient diet; Diet 3 = diet 2 supplemented with isoleucine, lysine, methionine, and tryptophan.

[2] The values shown are means ± SEM of 5 replicated tanks, 10 fish/tank, (n=5).

Table 1.23. Specific growth rates and feed conversion efficiencies for rainbow trout fed different diets in Experiment 1.2

Diet[1]	Specific growth rate[2]	Feed efficiency[3]
	%	
1	2.12^{a4}	1.04^a
2	1.95^a	1.04^a
3	2.12^a	1.11^a
Pooled SEM	0.19	0.09

[1] Diet 1 = fish meal based diet; Diet 2 = amino acid deficient diet; Diet 3 = diet 2 supplemented with isoleucine, lysine, methionine, and tryptophan.

[2] = (lnW2-lnW1)*100/(T2-T1)

[3] = Gain in body weight/feed consumption

[4] The values shown are means ± SEM of 5 replicated tanks, 10 fish/tank, (n=5). Values within the same column with the same superscript were not significantly different (Tukey HSD, P>0.05).

3.3.2.2 Responses of Fish to Experimental Diets

The initial weights, final weights, weight gains and feed consumptions of fish fed the different diets are provided in Table 1.22. Values for specific growth rates and feed efficiencies of fish are shown in Table 1.23. The data were subjected to analysis of variance and the results of the analyses are shown in Appendix 5. There were no significant dietary effects on the specific growth rate or feed efficiency.

3.3.2.3 Plasma Amino Acid Profile

The free amino acid concentrations in the plasma of fish expressed as μmol/mL and their percentages as determined at different times after meal consumption are shown in Table 1.24-1.26. The levels of total amino acids (TAA), total essential amino acids (EAA), total non-essential amino acids (NEAA), and ratios of EAA/NEAA in plasma of fish fed the different diets are also tabulated in the tables indicated above. The changes in plasma concentrations of EAA, NEAA, and TAA are shown in Figure 1.5. The changes in plasma concentrations of individual amino acids at different times after meal consumption are depicted in Figure 1.6. It should be noted that the concentrations of plasma amino acids in fish fed different diets at 24 h were used to represent the concentrations of plasma amino acids at 0 h, that is, at the time of feeding.

Plasma Total Essential Amino Acids (EAA), Total Non-Essential Amino Acids (NEAA), and Total Amino Acids (TAA)

The patterns of plasma EAA concentrations in fish that were fed diets 1, 2, and 3 differed from each other during the absorptive period (3-24 h postprandial) as shown in Figure 1.5a. The plasma EAA concentrations in fish fed diet 1 rose from 3

h and peaked between 12-24 h postprandial, after which the concentrations fell sharply at 36 h. Fish fed diet 3 showed an increase in the concentrations of EAA after feeding and peaked at 12 h. The level then dropped at 24 h postprandial. In contrast, the level of EAA in fish fed diet 2 fluctuated very little from 3 h to 36 h after feeding. After 36 h, the concentrations of EAA in fish fed all diets declined from 36 h to reach their lowest level at 48 h. After this time the plasma EAA concentrations increased until 120 h when the experiment was terminated (Figure 1.5a).

The patterns of plasma NEAA and TAA in fish in the different dietary treatment groups displayed a similar trend with respect to the diets fed. Fish that were fed diet 2 and diet 3 showed plasma NEAA and TAA peaks at 12 h after feeding, thereafter the levels gradually declined to the lowest level at 48 h after feeding. After this time concentrations of TAA slightly increased, whereas the concentrations of NEAA slightly decreased. The fluctuations in the concentrations of NEAA and TAA in fish fed diet 1 were similar in that they peaked at 24 h, and declined to the lowest concentrations at 48 h after which the level of both groups increased (Figure 1.5b and, 1.5c).

During the absorptive period, the magnitude of the increase in plasma concentrations of EAA in fish fed the different diets differed in the following order, diet 1 > diet 3 > diet 2 (Figure 1.5a). The concentrations of EAA in fish fed diet 1 at peak (12 h) was approximately 10% and 70% greater than those of fish fed diet 3 and diet 2 respectively. The concentrations of EAA in fish on the different dietary treatments during the post-absorptive period were similar. The concentrations of NEAA in fish fed diet 2 and 3 were similar, and were maintained at concentrations

higher than those in fish fed diet 1 throughout the sampling period, except at 120 h post-feeding (Figure 1.5b). The highest concentrations (at 12 h) of NEAA in fish fed diets 2 and 3 were about double the highest level of NEAA in fish fed diet 1. The differences in the concentrations of TAA between fish fed diet 2 and 3 and those in fish fed diet 1 corresponded with those for NEAA (Figure 1.5c).

The ratio of EAA/NEAA in fish fed the different diets changed over time, showing the influence of diets on the plasma pattern of amino acids. The ratios in fish fed diet 1 ranged between 0.55 and 1.08 depending upon the sampling time, being highest at 12 h and lowest at 36 h postprandial. A ratio of 1 was obtained at 120 h postprandial (Table 1.24). In contrast to the response of fish fed diet 1, the ratios in fish fed diet 2 were lower. The ratios of EAA/NEAA in fish of this group were between 0.28 and 0.72, being highest at 120 h and lowest at 12 h postprandial (Table 1.25). Fish fed diet 3 displayed a similar trend to that seen for fish fed diet 2, with ratios between 0.36 and 0.86 with the highest ratio at 120 h and lowest at 24 h postprandial (Table 1.26).

Plasma Concentrations of Individual Amino Acids in Fish Fed Different Diets

Essential Amino Acids

Fish fed diet 1 displayed some variations among the individual plasma essential amino acids from the overall pattern of EAA, and could be grouped into three groups. The first group included histidine, lysine, methionine + cystine, phenylalanine, and threonine. These amino acids had their peaks at 24 h after feeding, after which time concentrations gradually subsided and reached minimum levels at either 36 or 48 h depending upon the particular amino acids (Figures 1.6b-

d, 1.6g, and 1.6i). The second group were the branched-chain amino acids, isoleucine, leucine, and valine, which attained their peak concentrations at 12 h followed by moderate declines to the fasting level at 36 h postprandial (Figures 1.6e-f, and 1.6h). The last group contained tyrosine and arginine. The concentrations of tyrosine were constant throughout the sampling period (Figures 1.6a). The concentrations of arginine were constant during the absorptive period and dropped at 48 h postprandial (Figures 1.6a).

Most essential amino acids in fish fed diets 2 and 3 followed similar patterns to the overall pattern of EAA for the respective diet. Exceptions were phenylalanine and tyrosine which stayed constant over the test period. Very striking patterns of changes in the plasma concentrations of arginine, histidine, and lysine in fish fed diets 2 and 3 were observed during 24-48 h postprandial. The levels of these amino acids, after being constant or attaining moderate peaks followed by decreases at 24 h, showed increases at 36 h and dropped at 48 h postprandial (Figure 1.6a-1.6c).

Those essential amino acids that were present in greatest amounts varied with the time of sampling, and with diets. Fish that were fed diets 1 and 2 had valine as the most abundant essential amino acid during the absorptive period (3-24 h), histidine at 36 h, and histidine and valine at 48 h post-feeding. At 72 h, histidine, lysine, and valine were present in great amounts in fish fed diet 1, and only lysine and valine in fish fed diet 2. Lysine and valine concentrations dominated in plasma of fish fed diet 1 and 2 at 120 h postprandial (Table 1.24 and 1.25). Fish fed diet 3, however, showed a variation in the amino acids present in the greatest concentrations throughout the sampling period. Leucine and valine, present in

similar amounts, represented the largest proportion of plasma amino acids at 3 h postprandial, methionine during 12 -24 h, lysine at 36 h postprandial. Histidine, lysine, and valine concentrations were predominant in the plasma at 48-72 h postprandial. Lysine, followed by valine, were the most abundant amino acids at 120 h postprandial (Table 1.26).

Non-Essential Amino Acids

It should be recognized that the plasma concentrations of proline throughout the sampling period in fish fed diet 1 were too low to be detected by the amino acid analyzer. As a result, they do not appear in Figure 1.6n.

The plasma patterns of most non-essential amino acids in fish fed diet 1 were similar to the overall pattern of their NEAA. Glycine, proline, and serine in fish diets 2 and 3 resembled the overall pattern of their NEAA (Figures 1.6m-o, and Figure 1.5b). The concentrations of glutamic acid in these fish remained stable. The changes in the concentrations of alanine and aspartic acid in fish fed these diets, however, differed from the overall pattern of NEAA. Plasma alanine in these fish had two peaks, one at 12 h, and the other at 36 h postprandial (Figure 1.6j). The peak at 36 h was similar to those observed for the fluctuations of arginine, histidine, and lysine in fish fed the same diets. While plasma aspartic acid in fish fed diet 3 resembled that of NEAA, the concentration of this amino acid in fish fed diet 2 peaked differently from the overall pattern.

Of the non-essential amino acids, glycine was found as the most abundant, and serine the second most abundant in the plasma of the fish fed each of the diets.

Table 1.24. Concentrations of plasma amino acids in rainbow trout at different times after feeding diet 1 (fish meal based diet) in Experiment 1.2

Amino acid	Hours after feeding													
	3		12		24		36		48		72		120	
	$\bar{X}$ ± SEM[1] (μmol/mL)	%[2]	$\bar{X}$ ± SEM (μmol/mL)	%	$\bar{X}$ ± SEM (μmol/mL)	%	$\bar{X}$ ± SEM (μmol/mL)	%	$\bar{X}$ ± SEM (μmol/mL)	%	$\bar{X}$ ± SEM (μmol/mL)	%	$\bar{X}$ ± SEM (μmol/mL)	%
Essential														
Arg	0.234±.018	4.4	0.220±.014	3.6	0.189±.039	2.8	0.157±.056	3.4	0.057±.019	1.7	0.092±.004	2.2	0.151±.028	3.2
Cys	0.038±.001	0.7	0.055±.006	0.9	0.055±.002	0.8	0.016±.016	0.4	0.072±.071	2.2	0.075±.000	1.8	0.073±.008	1.6
His	0.326±.006	6.2	0.334±.008	5.5	0.381±.032	5.7	0.307±.019	6.6	0.214±.039	6.5	0.301±.067	7.3	0.339±.004	7.2
Ile	0.208±.000	3.9	0.320±.021	5.3	0.247±.025	3.7	0.097±.021	2.1	0.098±.053	3.0	0.120±.028	2.9	0.191±.019	4.1
Leu	0.350±.003	6.6	0.543±.048	9.0	0.419±.030	6.3	0.156±.002	3.4	0.150±.077	4.5	0.199±.042	4.8	0.294±.024	6.3
Lys	0.285±.035	5.4	0.297±.022	4.9	0.353±.005	5.3	0.279±.007	6.0	0.144±.049	4.4	0.289±.048	7.0	0.401±.036	8.6
Met	0.170±.005	3.2	0.222±.007	3.7	0.268±.040	4.0	0.111±.000	2.4	0.103±.035	3.1	0.092±.000	2.2	0.104±.002	2.2
Phe	0.189±.027	3.6	0.183±.031	3.0	0.226±.019	3.4	0.128±.041	2.8	0.162±.044	4.9	0.109±.018	2.7	0.131±.000	2.8
Thr	0.193±.014	3.7	0.200±.018	3.3	0.277±.038	4.2	0.116±.031	2.5	0.068±.013	2.1	0.119±.032	2.9	0.148±.016	3.2
Tyr	0.072±.019	1.4	0.087±.000	1.4	0.096±.021	1.4	0.053±.006	1.1	0.086±.044	2.6	0.024±.012	0.6	0.064±.006	1.4
Val	0.466±.000	8.8	0.684±.034	11.3	0.582±.053	8.7	0.223±.032	4.8	0.212±.105	6.4	0.299±.049	7.3	0.451±.040	9.6
Non-essential														
Ala	0.593±.071	11.2	0.568±.048	9.4	0.605±.052	9.1	0.596±.096	12.8	0.366±.063	11.1	0.433±.106	10.5	0.554±.077	11.8
Asp	tr[3]	0.0	0.120±.043	2.0	0.072±.009	1.1	0.077±.004	1.7	0.086±.026	2.6	0.097±.007	2.4	0.059±.009	1.3
Glu	0.318±.048	6.0	0.356±.003	5.9	0.413±.046	6.2	0.335±.022	7.2	0.225±.069	6.8	0.249±.018	6.0	0.166±.013	3.6
Gly	1.154±.074	21.8	1.139±.002	18.8	1.624±.134	24.4	1.269±.092	27.3	0.755±.213	22.8	0.894±.202	21.7	0.735±.121	15.7
Pro	tr		tr		tr		tr		tr		tr		tr	
Ser	0.691±.015	13.1	0.730±.023	12.1	0.860±.089	12.9	0.737±.061	15.8	0.509±.188	15.4	0.725±.147	17.6	0.829±.023	17.7
TAA[4]	5.286±.150		6.058±.114		6.668±.891		4.657±.009		3.307±1.392		4.118±.761		4.687±.161	
EAA[5]	2.530±.028		3.144±.130		3.093±.293		1.643±.021		1.367±.510		1.720±.264		2.344±.159	
NEAA[6]	2.756±.178		2.914±.116		3.575±.313		3.014±.013		1.940±.564		2.398±.480		2.343±.002	
EAA/NEAA	0.92		1.08		0.87		0.55		0.70		0.71		1.00	

[1] Mean of two pools of plasma (three fish/pool) and standard error of the mean (n=2). [2] % of total amino acids. [3] present in a very small amount. [4] Total amino acids. [5] Total essential amino acids. [6] Total non-essential amino acids

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Table 1.25. Concentrations of plasma amino acids in rainbow trout at different times after feeding diet 2 (amino acid deficient diet) in Experiment 1.2

Amino acid	Hours after feeding													
	3		12		24		36		48		72		120	
	X̄ ± SEM[1] (μmol/mL)	%[2]	X̄ ± SEM (μmol/mL)	%	X̄ ± SEM (μmol/mL)	%	X̄ ± SEM (μmol/mL)	%	X̄ ± SEM (μmol/mL)	%	X̄ ± SEM (μmol/mL)	%	X̄ ± SEM (μmol/mL)	%
Essential														
Arg	0.204±.027	3.2	0.209±.015	2.4	0.214±.042	3.0	0.255±.028	4.3	0.138±0.62	3.0	0.199±.008	3.9	0.175±.052	3.7
Cys	tr[3]		0.041±.010	0.5	0.035±.013	0.5	0.005±.005	0.1	tr		0.053±.006	1.0	0.046±.001	1.0
His	0.251±.039	3.9	0.254±.000	3.0	0.243±.040	3.4	0.313±.019	5.3	0.239±.026	5.2	0.280±.009	5.5	0.260±.005	5.5
Ile	0.118±.003	1.8	0.127±.015	1.5	0.099±.000	1.4	0.098±.005	1.7	0.090±.016	2.0	0.153±.024	3.0	0.147±.009	3.1
Leu	0.230±.015	3.6	0.315±.035	3.7	0.261±.002	3.6	0.197±.003	3.4	0.170±.018	3.7	0.251±.035	4.9	0.245±.031	5.2
Lys	0.204±.027	3.2	0.147±.002	1.7	0.134±.033	1.9	0.238±.026	4.1	0.136±.022	3.0	0.327±.005	6.4	0.346±.058	7.3
Met	0.110±.031	1.7	0.096±.006	1.1	0.078±.009	1.1	0.073±.005	1.2	0.070±.016	1.5	0.092±.001	1.8	0.072±.001	1.5
Phe	0.187±.007	2.9	0.190±.021	2.2	0.197±.024	2.7	0.154±.005	2.6	0.138±.019	3.0	0.157±.006	3.1	0.140±.019	3.0
Thr	0.081±.003	1.3	0.117±.012	1.4	0.088±.025	1.2	0.076±.016	1.3	0.062±.023	1.3	0.070±.013	4.3	0.109±.016	2.3
Tyr	0.061±.003	1.0	0.042±.042	0.5	0.050±.011	0.7	0.047±.010	0.8	0.043±.016	0.9	0.065±.004	1.3	0.064±.023	1.4
Val	0.270±.032	4.2	0.347±.027	4.0	0.268±.006	3.7	0.243±.008	4.1	0.229±.032	5.0	0.350±.037	6.9	0.367±.015	7.8
Non-essential														
Ala	0.785±.124	12.3	0.864±.215	10.0	0.678±.165	9.4	0.881±.026	15.0	0.645±.098	14.0	0.598±.004	11.7	0.586±.050	12.4
Asp	0.271±.048	4.2	0.337±.029	3.9	0.335±.083	4.7	0.076±.048	1.3	0.163±.084	3.5	0.224±.024	4.4	0.053±.031	1.1
Glu	0.196±.005	3.1	0.322±.025	3.7	0.266±.005	3.7	0.314±.037	5.4	0.222±.040	4.8	0.250±.004	4.9	0.186±.018	3.9
Gly	2.142±.403	33.4	3.189±.119	37.1	2.531±.934	35.2	1.680±.323	28.6	1.392±.209	30.1	1.227±.028	24.1	1.163±.064	24.6
Pro	0.429±.000	6.7	0.864±.519	10.0	0.634±.351	8.8	0.289±.000	4.9	0.197±.000	4.3	tr		tr	
Ser	0.868±.140	13.1	1.143±.105	13.3	1.089±.242	15.1	0.928±.047	15.8	0.688±.062	14.9	0.796±.067	15.6	0.764±.027	16.2
TAA[4]	6.406±1.038		8.604±1.158		7.196±1.738		5.866±.593		4.621±.387		5.091±.199		4.721±.340	
EAA[5]	1.715±.113		1.884±.013		1.664±.116		1.699±.040		1.314±.250		1.996±.140		1.969±.150	
NEAA[6]	4.691±.925		6.719±.963		5.532±1.782		4.168±.553		3.307±.025		3.094±.062		2.752±.191	
EAA/NEAA	0.37		0.28		0.30		0.41		0.40		0.66		0.72	

[1] Mean and standard error of the mean (n=2). [2] % of total amino acids. [3] present in a very small amount [4] Total amino acids. [5] Total essential amino acids. [6] Total non-essential amino acids

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Table 1.26. Concentrations of plasma amino acids in rainbow trout at different times after feeding diet 3 (diet 2 supplemented with isoleucine, methionine, lysine, and tryptophan) in Experiment 1.2

Amino acid	3 X̄ ± SEM[1] (μmol/mL)	3 %[2]	12 X̄ ± SEM (μmol/mL)	12 %	24 X̄ ± SEM (μmol/mL)	24 %	36 X̄ ± SEM (μmol/mL)	36 %	48 X̄ ± SEM (μmol/mL)	48 %	72 X̄ ± SEM (μmol/mL)	72 %	120 X̄ ± SEM (μmol/mL)	120 %
Essential														
Arg	0.223±.016	3.3	0.196±.059	2.1	0.155±.002	2.3	0.178±.001	2.7	0.127±.041	2.8	0.201±.009	4.2	0.302±.024	6.3
Cys	0.043±.001	0.6	0.068±.000	0.7	0.040±.000	0.6	0.049±.001	0.8	0.043±.006	1.0	0.058±.001	1.2	0.062±.007	1.3
His	0.206±.020	3.0	0.256±.001	2.7	0.211±.010	3.1	0.301±.011	4.6	0.227±.011	5.0	0.271±.003	5.7	0.248±.058	5.2
Ile	0.231±.024	3.4	0.266±.019	2.8	0.111±.003	1.6	0.109±.002	1.7	0.084±.001	1.9	0.115±.011	2.4	0.156±.015	3.2
Leu	0.385±.027	5.6	0.401±.041	4.2	0.217±.005	3.2	0.196±.016	3.0	0.151±.013	3.3	0.191±.023	4.0	0.260±.023	5.4
Lys	0.319±.090	4.7	0.324±.022	3.4	0.187±.004	2.7	0.329±.040	5.0	0.216±.039	4.8	0.290±.046	6.1	0.455±.033	9.4
Met	0.266±.040	3.9	0.508±.023	5.4	0.367±.047	5.4	0.228±.017	3.5	0.156±.033	3.4	0.100±.081	2.1	0.079±.006	1.6
Phe	0.174±.006	2.5	0.208±.001	2.2	0.180±.003	2.6	0.142±.025	2.1	0.107±.036	2.4	0.136±.006	2.9	0.094±.017	1.9
Thr	0.081±.001	1.2	0.149±.001	1.6	0.099±.020	1.4	0.093±.007	1.4	0.052±.006	1.1	0.064±.002	1.3	0.138±.013	2.9
Tyr	0.059±.006	0.9	0.047±.007	0.5	0.045±.004	0.7	0.031±.030	0.5	0.037±.000	0.8	0.064±.005	1.3	0.025±.016	0.5
Val	0.386±.019	5.7	0.407±.028	4.3	0.206±.002	3.0	0.236±.002	3.6	0.210±.003	4.6	0.278±.029	5.9	0.409±.029	8.5
Non-essential														
Ala	0.712±.073	10.4	0.775±.062	8.2	0.690±.139	10.1	0.856±.177	12.9	0.652±.009	14.4	0.520±.081	11.0	0.518±.155	10.7
Asp	0.237±.023	3.5	0.414±.059	4.4	0.362±.008	5.3	0.324±.013	4.9	0.123±.018	2.7	0.218±.017	4.6	0.121±.005	2.5
Glu	0.212±.023	3.1	0.290±.005	3.1	0.305±.009	4.5	0.323±.016	4.9	0.295±.026	6.5	0.250±.004	5.3	0.195±.027	4.0
Gly	2.013±.269	29.5	3.118±.001	33.0	2.286±.043	33.4	1.985±.281	30.0	1.386±.390	30.7	1.238±.208	26.1	0.960±.308	19.9
Pro	0.496±.016	7.3	0.801±.066	8.5	0.381±.055	5.6	0.314±.006	4.8	tr[3]		tr		tr	
Ser	0.793±.065	11.6	1.230±.010	13.0	0.994±.054	14.5	0.920±.013	13.9	0.673±.095	14.8	0.752±.046	15.9	0.804±.136	16.7
TAA[4]	6.835±.704		9.458±.212		6.837±.175		6.612±.125		4.538±.521		4.744±.135		4.826±.820	
EAA[5]	2.370±.236		2.831±.024		1.818±.080		1.890±.027		1.392±.070		1.767±.101		2.230±.189	
NEAA[6]	4.464±.469		6.627±.053		5.018±.094		4.722±.097		3.146±.484		2.977±.035		2.597±.631	
EAA/NEAA	0.53		0.43		0.36		0.40		0.44		0.59		0.86	

[1] Mean of two pools of plasma (three fish/pool) and standard error of the mean (n=2). [2] % of total amino acids. [3] present in a very small amount [4] Total amino acids. [5] Total essential amino acids. [6] Total non-essential amino acids

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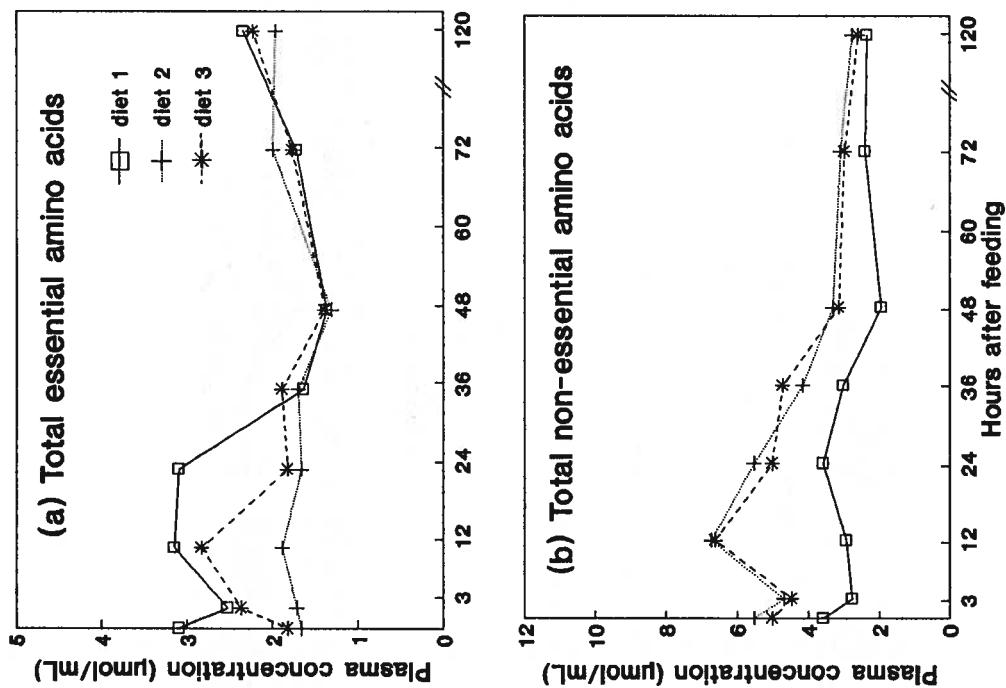
Figure 1.5. Total concentrations of plasma amino acids (essential, non-essential, and total amino acids) in rainbow trout determined at different times after feeding in Experiment 1.2. Diet 1 = fish meal based diet, diet 2 = amino acid deficient diet, diet 3 = diet 2 supplemented with isoleucine, methionine, lysine, and tryptophan. Each point represents a mean of two pools of plasma, three fish/pool, (n=2).

Figure 1.6. Plasma concentrations of amino acids in rainbow trout determined at different times after feeding experimental diets in Experiment 1.2. Diet 1 = fish meal based diet, diet 2 = amino acid deficient diet, diet 3 = diet 2 supplemented with isoleucine, methionine, lysine, and tryptophan. Each point represents a mean of two pools of plasma, three fish/pool, (n=2).

Figure 1.6. (Continued)

(i) Threonine

(j) Alanine

(k) Aspartic acid

(l) Glutamic acid

diet 1
diet 2
diet 3

Plasma concentration (μmol/mL)

Hours after feeding

Figure 1.6. (Continued)

82

(m) Glycine

(n) Proline

(o) Serine

(p) Tyrosine

diet 1
diet 2
diet 3

Figure 1.6. (Continued)

83
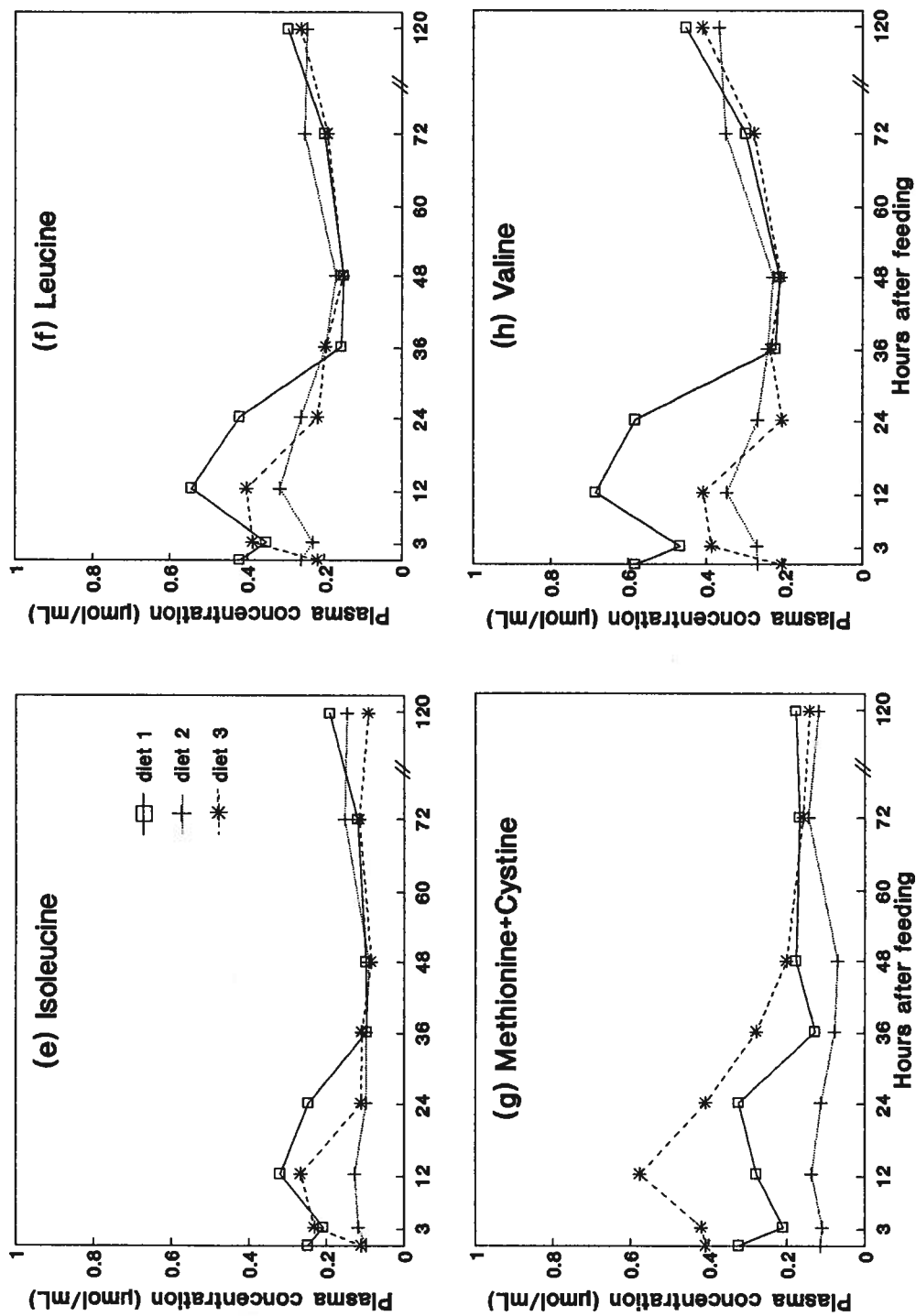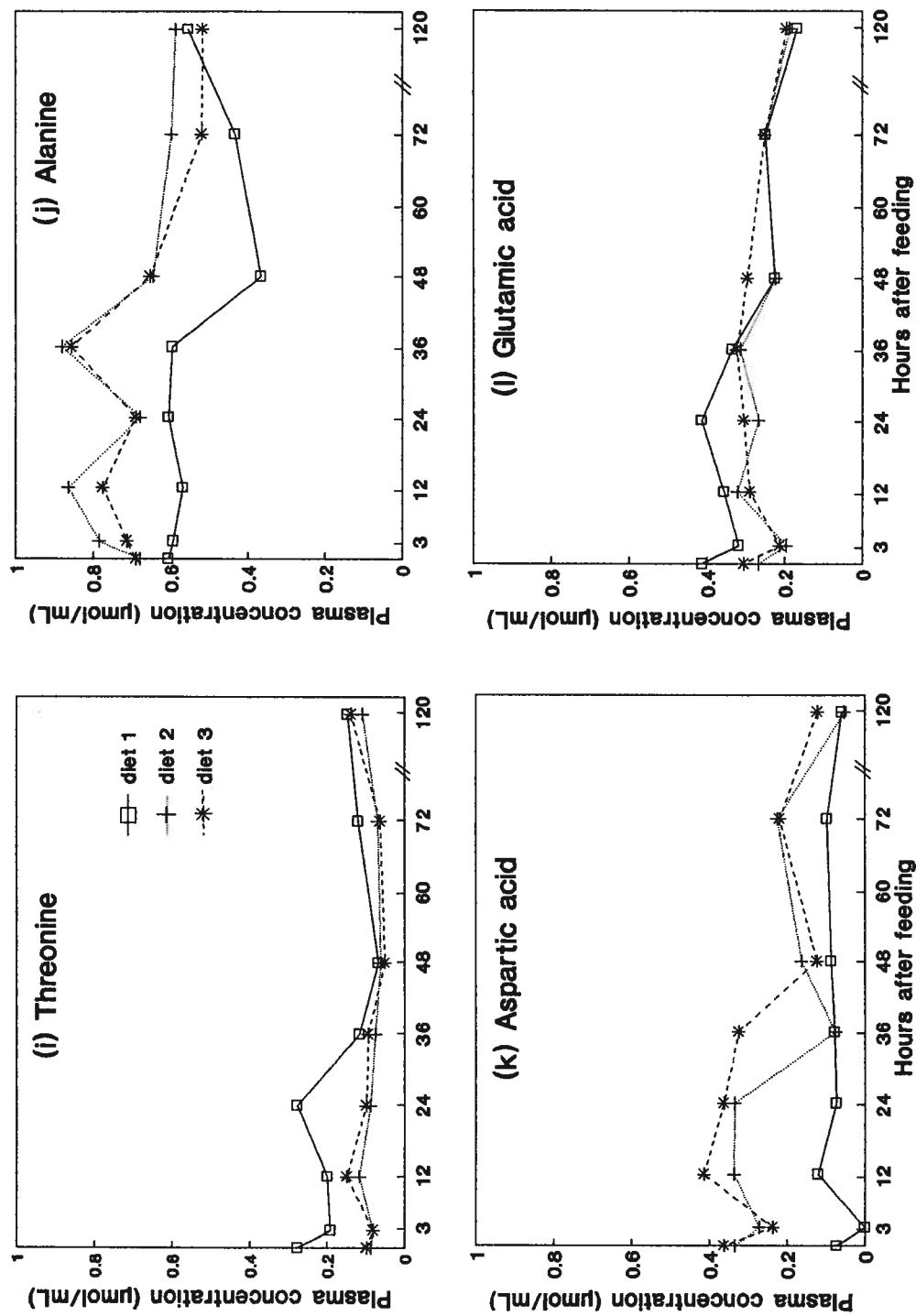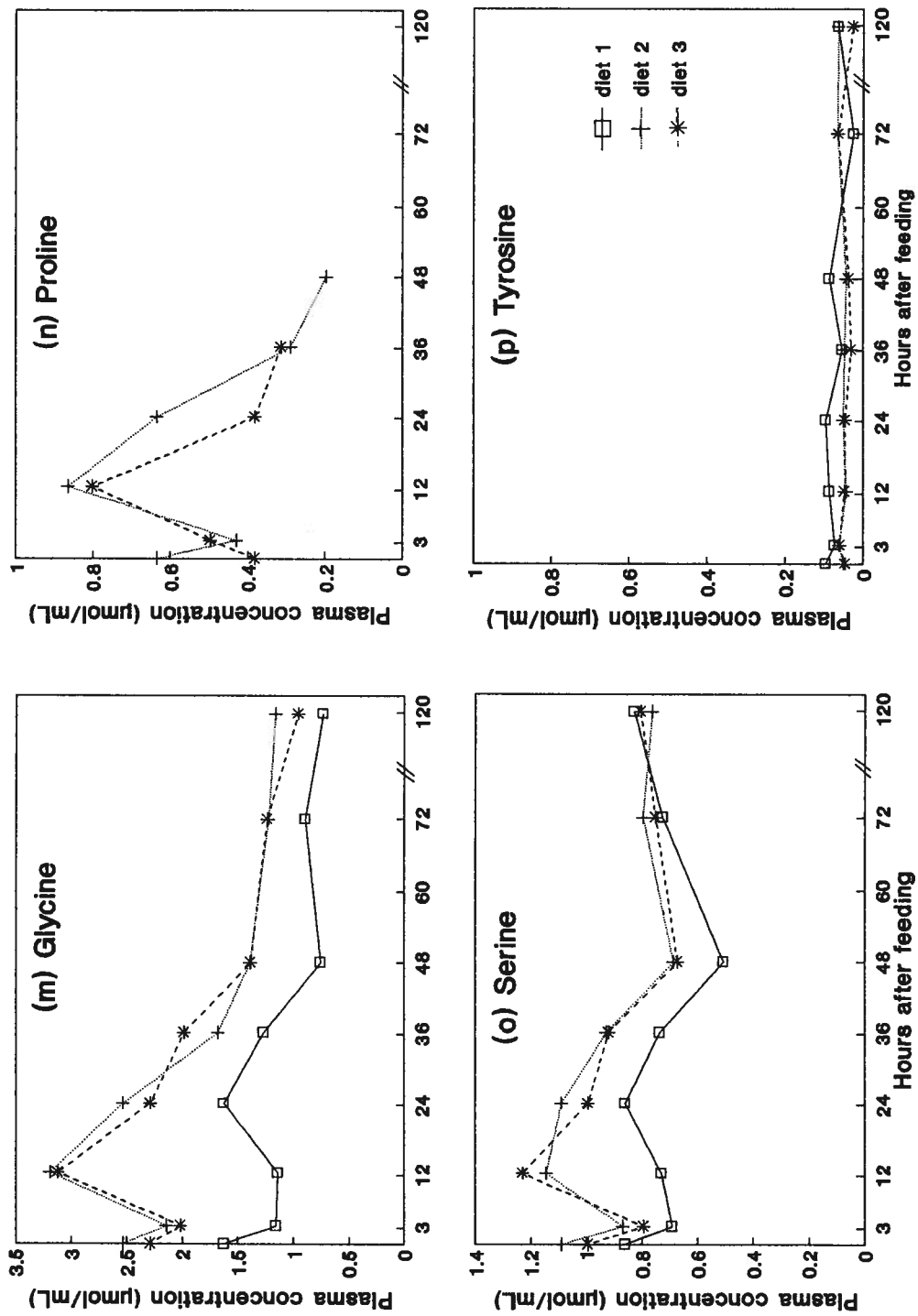
3.3.2.4 The Effects of Dietary Amino Acid Supplementation on Plasma Amino Acids

Dietary supplements of lysine and methionine resulted in pronounced increases in their plasma concentrations in fish fed diet 3 compared with those fed diet 2 (Figures 1.6c, and 1.6g). The plasma concentrations of lysine in fish fed diet 3 at 3 and 12 h were very much higher than those in fish fed diet 2. The concentration dropped at 24 h postprandial. The level was, however, maintained above that of fish fed diet 2 at the same sampling time and beyond. The elevation of methionine + cystine concentrations in fish fed diet 3 was remarkable. The concentration at the peak (12 h) was about double and five-fold higher than the concentrations in fish fed diet 1 and 2, respectively (Figure 1.6g). Furthermore, the addition of isoleucine in diet 3 caused an elevation in its plasma concentrations in fish fed this diet. Interestingly, leucine and valine also showed increases in their concentrations in a similar manner to isoleucine in fish fed this diet. In comparison with plasma concentrations of branched-chain amino acids in fish fed diet 2, the concentrations of these amino acids in fish fed diet 3 were very much higher between 3 and 12 h postprandial. The concentrations subsequently declined to the same levels as those in fish fed diet 2 at 24 h postprandial (Figures 1.6e-f, and 1.6h).

3.3.2.5 Plasma Taurine Concentrations

Plasma taurine concentrations in fish fed the different diets are shown together with methionine and cystine concentrations in Table 1.27. The changes in plasma taurine, and methionine + cystine concentrations are illustrated graphically in Figure 1.7. Plasma taurine concentrations were the highest in fish fed diet 1. Furthermore, the concentrations in fish fed diet 3 were generally higher than those

in fish fed 2. As shown in Figure 1.7, the concentrations of taurine in fish fed diets 1 and 3 rose from 3 h postprandial, and peaked at 36 h postprandial after which the concentrations declined at 48 h. Plasma taurine concentrations in fish fed diet 2, however, showed a different pattern of fluctuation in that they peaked twice, once at 12 h postprandial and the other at 36 h. The concentrations dropped at 48 h, and rose again at 72-120 h after the last feeding. The pattern of the fluctuation of taurine was similar to the results observed in Experiment 1.1 in that the concentrations of taurine tended to rise when methionine + cystine concentrations started to decline.

3.3.2.6 Relationship Between Plasma and Dietary Amino Acids

A correlation analysis between plasma (as μmol/ml of plasma) and dietary amino acids (as μmol/16g N)was performed. The values for plasma amino acids used in the analysis were the concentrations at 12 h postprandial when most amino acids attained their peaks (Table 1.24-1.26). Significant correlation coefficients ($P < 0.05$) between the levels of amino acids in the plasma and those in the diets (except for glutamic acid and aspartic acid) were obtained (diet 1, $r = 0.845$; diet 2, $r = 0.929$; diet 3, $r = 0.893$).

Table 1.27. Concentrations of plasma taurine, methionine, and cystine in rainbow trout at different times after feeding experimental diets in Experiment 1.2

Amino acid	Hours after feeding							Mean
	3	12	24	36	48	72	120	
	X̄ ± SEM[2] (μmol/mL)	X̄ ± SEM (μmol/mL)	X̄ ± SEM (μmol/mL)	X̄ ± SEM (μmol/mL)	X̄ ± SEM (μmol/mL)	X̄ ± SEM (μmol/mL)	X̄ ± SEM (μmol/mL)	X̄ ± SEM (μmol/mL)
Taurine								
Diet 1	1.193 ± .111	1.397 ± .137	1.612 ± .264	1.672 ± .129	1.178 ± .263	1.152 ± .031	1.106 ± .056	1.367 ± .120
Diet 2	0.718 ± .107	1.249 ± .065	1.055 ± .132	1.266 ± .150	0.909 ± .154	1.092 ± .074	1.209 ± .113	1.071 ± .100
Diet 3	0.773 ± .002	1.176 ± .142	1.291 ± .168	1.538 ± .003	1.258 ± .091	1.202 ± .068	1.466 ± .138	1.243 ± .130
Methionine								
Diet 1	0.170 ± .005	0.222 ± .007	0.268 ± .040	0.111 ± .000	0.103 ± .035	0.092 ± .000	0.104 ± .002	0.153 ± .036
Diet 2	0.110 ± .031	0.096 ± .006	0.078 ± .009	0.073 ± .005	0.070 ± .016	0.092 ± .001	0.072 ± .001	0.084 ± .008
Diet 3	0.266 ± .040	0.508 ± .023	0.367 ± .047	0.228 ± .017	0.156 ± .033	0.100 ± .081	0.079 ± .006	0.243 ± .080
Cystine								
Diet 1	0.038 ± .001	0.055 ± .006	0.055 ± .002	0.016 ± .016	0.072 ± .071	0.075 ± .000	0.073 ± .008	0.055 ± .011
Diet 2	tr[3]	0.041 ± .010	0.035 ± .013	0.005 ± .005	tr	0.053 ± .006	0.046 ± .001	0.036 ± .021
Diet 3	0.043 ± .001	0.068 ± .000	0.040 ± .000	0.049 ± .001	0.043 ± .006	0.058 ± .001	0.062 ± .007	0.052 ± .006

[1] Diet 1 = fish meal based diet; Diet 2 = amino acid deficient diet; Diet 3 = diet 2 supplemented with isoleucine, lysine, methionine, and tryptophan.

[2] Each value represents a mean of two pools of plasma, three fish/pool, and standard error of the mean (n=2).

[3] Present in a very small amount

86
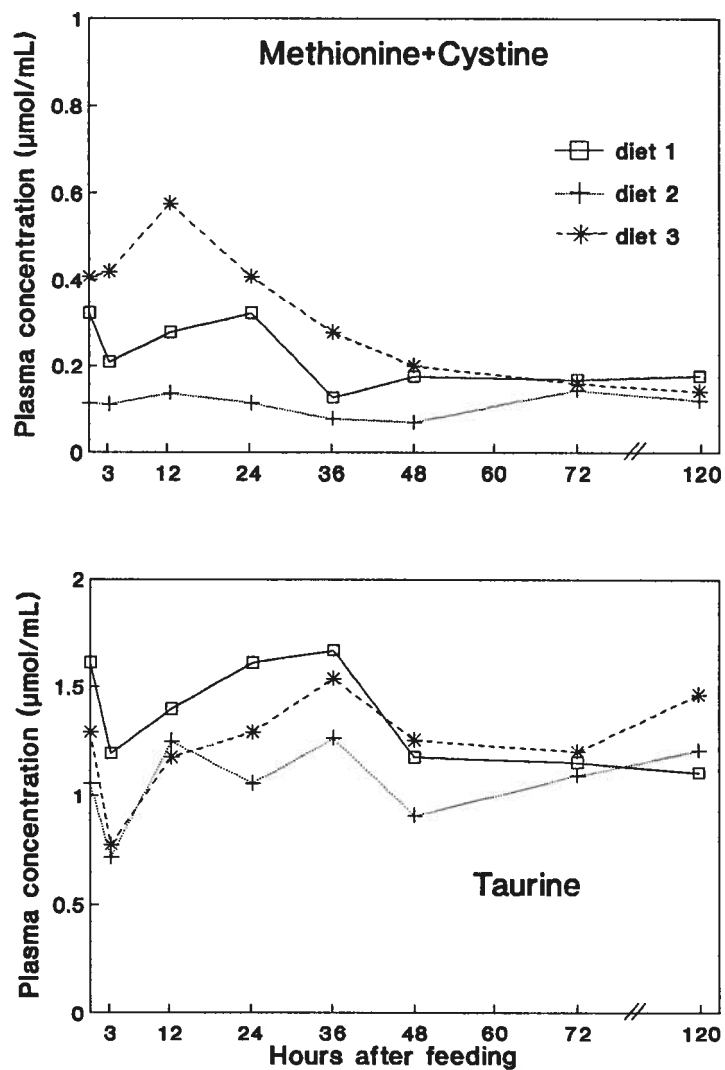
Figure 1.7. Plasma taurine, and methionine+cystine concentrations in rainbow trout fed different diets in Experiment 1.2. Diet 1 = fish meal based diet, diet 2 = amino acid deficient diet, diet 3 = diet 2 supplemented with isoleucine, methionine, lysine, and tryptophan. Each points represents a mean of two pools of plasma, three fish/pool, (n=2).

3.4 DISCUSSION

In Experiment 1.1, the fish were sorted initially into three groups based on body weight to reduce the error term in analysis of variance of the data. Another advantage of segregating the fish on the basis of body weight was the reduction in the variation in feed intake of individual fish due to the feeding hierarchy that normally occurs when fish of a large size range are in the same tank. The results for final weight, weight gain, and feed consumption showed good consistency within the same size category of fish. Although large and medium-sized fish fed diet 1 (fish meal based diet) consumed similar amounts of food, this did not affect the comparison between the results obtained from fish that were fed the control diet and test diets. Furthermore, growth rate, feed efficiency, and energy efficiency showed that fish of different sizes responded to experimental diets and feeding frequency in a similar manner.

The results for growth rate, feed efficiency, protein gain, productive protein value, and energy efficiency showed that fish fed diet 1 (fish meal based diet) had better growth, and protein and energy utilization than noted for fish fed either diet 2 (amino acid defic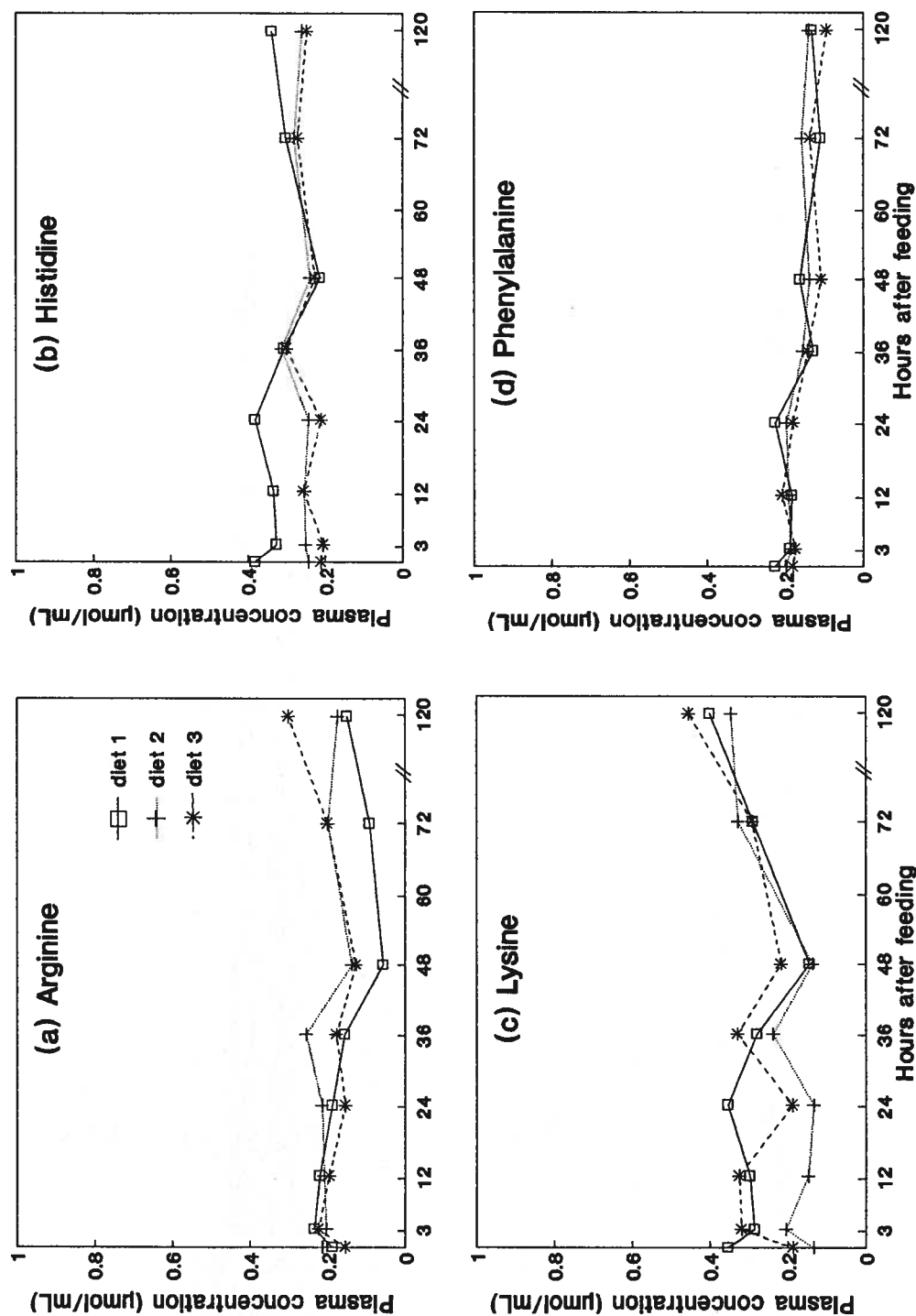ient diet) or diet 3 (diet 2 supplemented with methionine, lysine, and tryptophan). Furthermore, fish that were fed diet 3 showed comparable growth to that of fish that were fed diet 2. Dietary supplementation with free amino acids in diet 3 was, therefore, ineffective in improving growth and nutrient utilization of rainbow trout in comparison with fish fed diet 2 which was similar but not supplemented with amino acids in Experiment 1.1.

It has been theorized that fish do not efficiently use supplemental amino acids when fed only once a day because crystalline amino acids may be absorbed

from the intestine and oxidized before amino acids derived from protein digestion are available for absorption (Robinson, 1992). The feeding of fish five times daily in this experiment showed apparent constancy in the supply of amino acids derived from both intact dietary protein and supplementary free amino acids to the plasma, and ultimately to the tissue sites of protein synthesis. Growth of fish fed diet 3 (diet supplemented with lysine, methionine, and tryptophan) five times per day in Experiment 1.1, however, was not improved significantly. The results from this study were different from those found in carp by Yamada *et al.*, (1981b) who found that the growth rate of carp fed an amino acid diet dramatically improved when the number of feedings increased. This might be explained by a species difference, and the nature of diets used in the experiments. Carp is a stomachless fish, and the retention time of food in the digestive tract, particularly of purified diets such as an amino acid mixture is, therefore, shorter than that in salmonids, e.g. rainbow trout. Continuous provision of feed to carp when fed a free amino acid mixture is, consequently, necessary.

The body composition of fish fed five times daily in this experiment, nevertheless, was significantly higher in protein concentration and lower in dry matter and lipid concentrations than those in fish fed once daily. Although, protein gain by the two groups of fish was similar, the lipid content in fish fed the different diets was reduced when they were fed five times daily. These differences were obvious, particularly in fish that were fed diet 3, which showed a 3% increase in protein concentration and a 10% reduction in lipid concentration. A higher productive protein value (PPV) for fish fed five times daily was also observed. This observation was similar to that reported in rats. Cohn (1963) summarized the

results of several studies carried out on the effects of feeding frequency on body composition of rats. He observed that the body of meal-fed rats contained more fat but less protein and water than that of rats fed ad libitum, and vice versa. Feeding fish five times per days in the present experiment appeared to be a factor in the regulation of the intermediary metabolism of dietary protein. In fish fed five times daily, the load of amino acids derived from ingested protein was used for protein synthesis more efficiently. By contrast, when the same amount of food was fed once daily, the load of amino acids derived from protein digestion seemed to exceed the capacity of the fish for protein synthesis. Excess amino acids in this group of fish were evidently catabolized and the reduced carbon skeletons of the amino acids were converted to fat. The results corresponded with those reported for rats by Cohn *et al.*, (1963).

Steffens (1981) stated that a reliable indication of protein utilization in fish by means of growth rate will be reached only if the experiment lasts for at least 2-3 months with a two-week adaptation period. Kaushik *et al.*, (1988) found in their experiment that the response of growth rate and feed conversion ratio to the supplementation of arginine in the diet was only demonstrated in rainbow trout during the last 3 weeks of their experiment which lasted 9 weeks. Rumsey and Ketola (1975), on the other hand, concluded from studies with rainbow trout and other fish that experiments of 2-6 weeks duration appeared to be adequate in the studies of protein and amino acids metabolism. March *et al.*, (1985) also demonstrated in their study that a short term bioassay of 21 days was sufficient to evaluate dietary protein quality in rainbow trout previously selected for uniformity of size. The experimental period of 27 days in Experiment 1.1, therefore, was likely

adequate.

A possible explanation for the lack of response to either the amino acid supplementation or feeding frequency treatment was an imbalance in the amino acid profile of diet 3. Rumsey and Ketola (1975) and Ketola (1982) did not find any improvement in growth of rainbow trout when a diet containing soybean meal was supplemented with a single amino acid or several combinations of amino acids, for example, histidine + methionine + lysine. They obtained better results when the mixtures of several amino acids were added to the diet to simulate the concentration of essential amino acids in trout eggs or isolated fish protein. They concluded that diets containing soybean meal had disproportionate levels of amino acids, thus causing a reduction in growth rate. The same situation of amino acid imbalance may have occurred in the present experiment.

Diet 3 in this experiment was supplemented with methionine, lysine, and tryptophan to the levels present in the control diet (fish meal based diet). Comparisons between amino acid concentrations in this diet, the requirement values for juvenile salmon as recommended by the NRC (1981), and more recently reported values for rainbow trout revealed that the concentrations of all essential amino acids in the diets were above the requirements (Table 1.2). There was no assurance, however, that all the amino acids were totally available to the fish. Moreover, the balance of dietary essential amino acids may not have been optimal. This was revealed by the results on the distribution of essential amino acids in the plasma of fish fed the different diets (Table 1.18). The pattern of plasma essential amino acids in fish fed either diet 2 or 3 deviated from the pattern in fish fed the fish meal based diet.

The results of Experiment 1.2 showed that growth of fish tended to be improved when they were fed diet 3 which contained supplementary isoleucine, lysine, methionine, and tryptophan. The inclusion of isoleucine in this diet in Experiment 1.2 may have adjusted the balance of amino acids. A decisive conclusion could not be drawn from this because the improvement in growth was not statistically significant. Furthermore, the sizes of fish in Experiment 1.2 differed from those in Experiment 1.1. The average weight of rainbow trout in Experiment 1.1 was 18-21 g whereas that in Experiment 1.2 was 180 g. Larger fish may utilize amino acids supplemented in the free form in diets more efficiently than smaller fish. Variations in growth response results due to fish size have been observed in other laboratories (Dabrowski, 1986; Murai *et al.*, 1989b).

The ratio of essential to non-essential amino acids either in the diets or the plasma is important in relation to protein quality and utilization in mammals (Young and Zamora, 1968; Hartog and Pol, 1972; Fujita *et al.*, 1981; Mercer *et al.*, 1989). Noteworthy results on the ratio between plasma total essential to total non-essential amino acids were also found in Experiments 1.1 and 1.2. During the absorptive period, fish fed diet 1 (control diet) had a ratio of close to 1 in fish fed five times daily, and a ratio of 1 in fish fed once daily to satiation. Plasma ratios of essential to non-essential amino acids in fish fed diet 2 or 3, on the other hand, were considerably lower. This reflected the amino acid composition of the diets as the ratio of EAA to NEAA in the different diets also agreed with the ratios in the plasma of fish fed respective diets. The high levels of plasma non-essential amino acids in fish fed diets 2 and 3 resulted in higher levels of total plasma amino acids than in fish fed diet 1 in both Experiment 1.1 and 1.2 (Figures 1.2 and 1.5).

The differences between the plasma concentrations of essential, and non-essential amino acids in fish fed the different diets had disappeared 48-72 h postprandial. The increase in the concentrations of most essential amino acids in the plasma by 120 h after feeding indicated the input of free amino acids from the catabolism of tissue protein. The increased ratios of plasma EAA/NEAA in the fish on the different dietary treatment groups at this sampling time, particularly those of fish fed diet 2 and 3 (ratio of approximately 1), suggested the contribution of amino acids from the catabolism of the body protein.

The time at which plasma amino acids reached their peaks in fish fed diet 1 in Experiment 1.2 differed from the results in Experiment 1.1. Amino acid concentrations in fish fed this diet in Experiment 1.2 peaked between 12 and 24 h postprandial, whereas those for fish fed diet 1 in Experiment 1.1 peaked at 9 h and had declined at 15 h postprandial. The difference between the two experiments could possibly have been due to the differences in size of the fish in the respective experiments. A greater amount of food consumed by the larger fish in this experiment was probably evacuated at a slower rate than the food ingested by the smaller fish in Experiment 1.1 resulting in the delay of the appearance of free amino acids in the plasma. Nose (1972) found similar results on the time course of plasma amino acid concentrations when rainbow trout weighing 150 g were fed a commercial diet. In his study, most free amino acids began to increase immediately after feeding and attained maximum concentrations between 12 and 24 h after feeding. Another factor which might affect gastric evacuation and digestion in fish was the sampling method. Fish were sampled from the same tank at every sampling time in Experiment 1.1, whereas fish were taken from different tanks in Experiment

1.2. The disturbance associated with catching the fish in Experiment 1.1 may have caused stress to the remaining fish that were left in the tanks and sampled at the next sampling time.

In Experiment 1.1, the elevations of the plasma amino acids lysine and methionine in fish fed diet 3 were appreciably higher (Figures 1.3c and 1.3g) than in fish fed the other two diets. Although, the amounts of both amino acids were increased in diet 3 to equal the concentrations in diet 1, the plasma concentrations in fish fed diet 3 (fed once and five times daily) were approximately double those of fish fed diet 1. The same phenomenon was observed in Experiment 1.2 and indicated that synthetic lysine and methionine were effectively absorbed from the digestive tract of fish fed diet 3. A rise of plasma concentrations in response to dietary supplementation with free amino acids has been reported in other studies in fish (Barash, 1984; Murai *et al.*, 1989a). In the present study, plasma methionine in fish fed diet 3 was maintained at a level higher than in fish fed diets 1 and 2 until 36 h after feeding. Although the concentrations of plasma lysine in fish fed diet 3 dropped abruptly at 24 h after feeding in Experiment 1.2, lysine concentration was maintained at a higher level than in fish fed diet 2 until 48 h after feeding.

There have been studies, both in fish and in other animals, showing that amino acid oxidation is influenced by the level of amino acid in that the rate of oxidation increases with increments of amino acids, and a surplus of amino acids will be oxidized rapidly if they are not used for protein synthesis (Brett and Zala, 1975; Walton *et al.*, 1982; Harper, 1983; Young *et al.*, 1985; Kim *et al.*, 1992). Thebault (1985), for example, found in sea-bass that the level of plasma methionine in response to dietary supplementation reached a peak and then returned to the

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pre-feeding level sooner than was the case with other essential amino acids. He concluded, therefore, that supplementary free methionine was absorbed and degraded faster than methionine originating from dietary protein. The continued elevation of plasma methionine and lysine until 36 h after feeding in the present study is contradictory to the above observation. It is possible that the enzymes responsible for oxidation of these amino acids were overloaded (Harper *et al.*, 1970) so that oxidation of lysine and methionine was not rapid and high plasma concentrations were maintained for a relatively long period.

The patterns of change in the concentrations of plasma branched-chain amino acids in Experiment 1.2 were very interesting. Diet 3 in this experiment was supplemented with isoleucine to raise the concentration to that present in diet 1. Not only was the level of plasma isoleucine in fish fed diet 3 elevated over that occurring with diet 2, but the concentrations of leucine and valine were also increased. A similar behaviour of branched-chain amino acids was reported in a study of channel catfish fed diets containing increasing amounts of isoleucine (Wilson *et al.*, 1980). Although an antagonistic interaction between branched-chain amino acids has been extensively documented for other animals (Harper, 1964; Harper, *et al.*, 1970; Harper *et al.*, 1984; Block, 1989), little information is available for fish. The evidence for the antagonistic interaction among these amino acids based on growth data was observed by Chance *et al.*, (1964) in chinook salmon, and Wilson *et al.*, (1980) in channel catfish. Choo *et al.*, (1991) did not find such an interaction in rainbow trout when the fish were fed diets containing excess leucine. Plasma concentrations of isoleucine and valine were not affected by the changes of dietary leucine concentrations. The fish fed a high leucine diet (13.4% of diet),

however, exhibited gross lesions (scoliosis, and deformed opercula). Choo *et al.*, (1991) attributed these signs to "toxicity" of excess dietary leucine. The parallel alteration of leucine and valine to that of isoleucine found in Experiment 1.2 suggested an antagonism among these amino acids in rainbow trout.

A significant phenomenon regarding the changes of the plasma concentrations of arginine, histidine, lysine and alanine in fish fed diets 2 and 3 was observed in Experiment 1.2. These plasma amino acids in fish fed the respective diets exhibited similar patterns of change in that they either remained constant or peaked during 3-12 h postprandial and then rose at 36 h. Diets 2 and 3 in this experiment were both formulated with the same proportions of protein sources. The surges in the concentrations of arginine, histidine, lysine, and alanine at 36 h could be interpreted as the result of delayed digestion of particular proteins. Although, the data on the composition of amino acids in the dietary protein was informative as to the level of each amino acid which was supplied in the diet, it could not be ascertained when the constituent amino acids would be released, absorbed and available for protein synthesis following diet consumption.

As with other studies on rainbow trout, glycine was found in the highest concentration among plasma non-essential amino acids in Experiments 1.1 and 1.2 (Nose, 1972; Gras *et al.*, 1982; Walton and Wilson, 1986). The magnitude of the concentrations, however, depends on the nature of the food eaten. For example, fish fed diets 2 or 3 in both experiments had more than double the concentrations of plasma glycine relative to fish fed diet 1. This was a result of gelatin in diet 2 and 3 which is rich in glycine. Likewise, Hill *et al.*, (1961) observed a rise in plasma glycine concentration in chickens fed a diet containing 10% glycine. Alanine,

aspartic acid, proline, and serine concentrations in fish fed diets 2 or 3 were also very high. The higher levels of alanine and proline in fish fed these two diets were undoubtedly a result of gelatin in the diet (Tables 1.2, and 1.20).

The higher concentrations of plasma serine in fish fed diets 2 or 3 than in fish fed diet 1 are assumed to be a cosequence of metabolism of plasma glycine in the former fish. Glycine and serine are readily interconvertible, and glycine can be condensed to yield serine mostly for gluconeogenesis (Bender, 1985). As with mammals under normal conditions, the major pathway of glycine catabolism is probably via the glycine cleavage system, whereby the carboxyl carbon is released as CO_2 and methylene carbon as $N^{5.10}$-methylenetetrahydrofolate, and the NH_4 is released (Walton and Cowey, 1981). In the present experiment, the plasma concentrations of glycine were unusually high. The conversion of glycine to serine is a control mechanism for maintaining of the balance of the amino acids in the plasma of the fish (Schepartz, 1973). Since the formation of one molecule of serine from glycine requires two molecules of glycine, this pathway is probably an effective way to reduce the excess glycine in the circulation of fish. Serine formed from this reaction is the substrate for gluconeogenesis (Bender, 1985; Walton and Cowey, 1982).

Aspartic acid is usually low in fish plasma (Dabrowski, 1982). The elevated concentrations of plasma aspartic acid in this study were possibly the result of an imbalance of amino acids in diets 2 and 3 as well. The mechanism involved in the rise of this amino acid is not clear.

The high level of plasma taurine observed in the present study has also been reported in various fish (Nose, 1972; Wilson and Poe; 1974 Plaskas *et al.*, 1980; Walton and Wilson, 1986). The origin and metabolism of taurine in fish has not been studied intensively. In mammals and some other animals, taurine is synthesized mainly in the liver from sulfur amino acids (cysteine). Evidence for the biosynthesis of taurine in fish is limited. The source of taurine has been, therefore, believed to be from diets (Sakaguchi *et al.*, 1988; van Waarde, 1988). The results on the concentrations of plasma taurine in the present study were in accord with this conclusion in that fish fed diet 1 (fish meal based diet, see Table 1.2) had a significantly higher plasma taurine concentration than those of fish fed diets 2 or 3. The results from several studies, however, have shown that fish may synthesize taurine since the level of plasma taurine is significantly increased when the fish are fed a diet containing excess methionine (Murai *et al.*, 1989a; Cowey *et al.*, 1992). Yokoyama and Nakazoe (1989) also found an induction of cysteine dioxygenese activity, the enzyme which catabolizes the first step reaction in taurine formation, in the liver of rainbow trout when the fish were fed a diet containing excess sulfur amino acids. The concentrations of plasma taurine in fish fed diet 3 (methionine supplemented diet) were higher than those in fish fed diet 2 which was not supplemented with methionine. Furthermore, plasma taurine concentrations were low when the plasma concentrations of methionine were increasing, but rose when plasma methionine declined (Table 1.19). Degradation of methionine to taurine is thereby suggested.

In conclusion, the results on plasma amino acids indicated that they were positively correlated with the dietary amino acids concentrations. Dietary supplements of free amino acids were absorbed more rapidly, and they were maintained in the plasma at levels higher than those in the diet containing similar ingredients but without amino acid supplementation until 36 h after feeding. This finding indicated that free amino acids added to the diets were still available to tissues for protein synthesis at least at the time of routine feeding. Diets supplemented with amino acids in this study did not improve the growth of rainbow trout even when the fish were fed more frequently. This could be explained by an unidentified imbalance of amino acids present in the diets. Feeding fish five times daily when the fish were pair-fed to the control group fed once daily did not have any beneficial effects on growth or feed conversion efficiency. The results on the body composition of the fish, however, showed that feeding fish more frequently affected the intermediary metabolism in the fish. The carcasses of fish fed five times daily contained higher protein concentrations and lower lipid concentrations.

The high concentrations of serine observed in the present study indicated that the excess of glycine absorbed from the diet was converted to serine. Also, the results found in Experiment 1.2 suggested that different proteins from various protein sources in diets were digested at different rates judging from the time of appearance of amino acids in the plasma. This could be useful information for consideration when diets containing different kinds of proteins are formulated.

CHAPTER 4

EXPERIMENT 2

PATTERNS AND CONCENTRATIONS OF FREE AMINO ACIDS IN THE PLASMA OF RAINBOW TROUT FED DIETS CONTAINING PROTEIN FROM A MIXTURE OF DIFFERENT PROTEIN SOURCES WITH AND WITHOUT DIETARY SUPPLEMENTS OF SELECTED AMINO ACIDS

4.1 INTRODUCTION

The results from Experiment 1 showed that plasma concentrations of glycine in fish fed diets 2 and 3 (containing gelatin) were strikingly high. Plasma amino acid profiles in response to a diet formulated to be the same as diet 2 and the diet containing synthetic glycine were, therefore, investigated in the present experiment. Assuming that the crystalline amino acids were in a readily available form, the rate at which they enter the circulation should be faster than the rate at which the intact proteins were digested and the amino acids absorbed into the circulation.

The amino acid pattern of fish carcass protein has been claimed to be a good quick reference for formulation of fish diets (Arai, 1981; Ketola, 1982; Ogata *et al.*, 1983; Wilson and Poe, 1985). Fish fed diets supplemented with amino acids to simulate the pattern of fish whole body protein have shown better growth and feed efficiency than those fed diets with suboptimal amino acid balance. Plasma amino acid profiles in response to a diet supplemented with several essential amino acids to simulate the amino acid pattern of fish carcass protein were, therefore, investigated in the present experiment.

4.2 MATERIALS AND METHODS

4.2.1 Diets

The compositions of the four diets formulated for this experiment are shown in Table 2.1. Diet 1 (control diet) contained herring meal, corn gluten meal, and soybean protein concentrate as the main protein sources. The concentrations of essential amino acids in the control diet, which were calculated from tabulated values (NRC 1981), were equivalent to or higher than the stated requirements of rainbow trout (Table 1.2, Experiment 1). Diet 2 in this experiment was formulated to be similar to diet 2 in Experiment 1, i.e. the diet containing herring meal, corn gluten meal, soybean protein concentrate, and gelatin as principal protein sources. Diet 3 was formulated to have herring meal, corn gluten meal, and soybean protein concentrate as predominant protein sources. The amounts of protein provided by herring meal, corn gluten meal, and soybean protein concentrate were in the same proportion as in diet 2, i.e. 2 herring meal: 1 corn gluten meal: 1 soybean protein concentrate. Glycine was added to diet 3 in an amount comparable to that supplied by gelatin in diet 2. This enables comparison of the speed by which glycine was absorbed following digestion of gelatin by fish fed diet 2. Diet 4 was formulated with herring meal, corn gluten meal, and soybean protein concentrate as major sources of protein with the proportions of each identical to those in diet 3. This diet was supplemented with arginine, histidine, lysine, methionine, and threonine to simulate the amino acid profile of trout muscle which was reported by Wilson and Cowey (1985). The formulated diets were analyzed for protein and amino acid concentrations as described previously.

Table 2.1. Ingredient and proximate composition of diets employed in Experiment 2.

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4
	g/kg	g/kg	g/kg	g/kg
Herring meal (whole steam-dried)	231.89	155.00	215.38	215.38
Corn gluten meal	124.16	83.00	115.32	115.32
Soya protein concentrate	90.04	60.00	83.36	83.63
Ground wheat[1]	300.00	300.00	300.00	300.00
Gelatin	-	102.00	-	-
Herring oil[2]	105.52	114.74	107.50	107.50
Dextrin	64.89	100.26	72.94	72.91
Bone meal	13.50	15.00	13.50	13.50
Calcium lignosulfonate	30.00	30.00	30.00	30.00
Premix[3]	40.00	40.00	40.00	40.00
L-Arginine	-	-	-	2.63
L-Histidine ($HCl.H_2O$)	-	-	-	3.78
L-lysine HCl	-	-	-	14.28
DL-Methionine	-	-	-	0.98
L-Threonine	-	-	-	4.16
L-Glycine	-	-	22.00	-
Total	1000.00	1000.00	1000.00	1004.07
Proximate analysis[4]				
Protein (%)	38.2	38.5	38.7	38.7
Ether-extractable lipid (%)	14.0	14.0	14.0	14.0
Ash (%)	5.4	4.4	5.1	5.1
Gross energy (kcal/kg)	4833	4880	4788	4812

[1] Autoclaved at 121°C for 1.5 h

[2] Stabilized with 0.05% ethoxyquin

[3] The premix supplied the following per kg of the diet as fed (except for diet 4 in which the percentage of each will be proportionally lower): thiamine HCl, 60 mg; riboflavin, 100 mg; niacin, 400 mg; biotin, 5 mg; folic acid, 25 mg; pyridoxine HCl, 50 mg; cyanocobalamine, 0.1 mg; D-calcium panthothenate, 200 mg; ascorbic acid, 1500 mg; choline chloride, 4000 mg; inositol, 2000 mg; vitamin K, 30 mg; vitamin A, 10,000 IU; vitamin D_3, 1000 IU; vitamin E, 1000 IU; Mg (as $MgSO_4$), 380 mg; Mn (as $MnSO_4.5H_2O$), 30 mg; Zn (as ZnO), 70 mg; Fe (as $FeSO_4.7H_2O$), 85 mg; Cu (as $CuSO_4.5H_2O$), 2 mg; Co (as $CoCl.6H_2O$), 0.003 mg; K (as KH_2PO_4), 895 mg; P (as KH_2PO_4), 2070 mg; I (as KIO_3), 5 mg; F (as NaF), 4.5 mg; Se (as $Na_2SeO_3.5H_2O$), 0.10 mg.

[4] The values for dietary protein were from determination (on dry matter basis), and for ether-extractable, ash, and gross energy were from the calculation (air-dry basis) using analysis values of each ingredient. The values for gross energy were estimated by ascribing 5.65 kcal/g crude protein, 9.5 kcal/g crude lipid, 4.0 kcal/g carbohydrate (Alexis et al., 1985).

4.2.2 Facilities

Twelve 200 L tanks designated as A-L were set up at the UBC aquarium facility at the West Vancouver Laboratory of Fisheries and Oceans, Canada. They were indoor tanks, and supplied with seawater (32 ppt). The water temperature ranged from 9.0-11.0°C, and dissolved oxygen was 8.5 ppm during the experiment. Photoperiod was 14 h. Mortality of the fish was 8% over the entire experiment.

4.2.3 Fish

Rainbow trout that were already acclimated to seawater, were used in this experiment. The fish were distributed randomly so that each of the 12 tanks had 15-16 fish. The average initial weights of each group were in the range of 237.9 ± 9.3 g to 250.1 ± 20.1 g (mean $\pm$ SE). The four experimental diets were then assigned randomly to the tanks with three replicate tanks of fish per diet.

Before the experiment began, the fish were accustomed to the diets by gradually substituting the test diets for the commercial diet (EWOS) which had been fed previously. The fish accepted the test diets after 3 days. Thereafter, fish were daily fed to satiation their prescribed diets for 17 days. Records of daily feed consumption were maintained.

4.2.4 Sampling Procedure

Fish sampling was done at two different times.

1. After the fish had been on their respective diets for 7 days, blood was withdrawn at different times after feeding for amino acid analyses. On the sampling day, the fish were fed to satiation. Then at 3, 9, 15, and 24 h after feeding, four fish

from each dietary treatment were caught and anesthetized with 0.01 % tricaine-methanesulfonate (MS-222). To avoid disturbance of fish in other tanks, the fish were taken from one of the replicate groups for each dietary treatment at each sampling time. The exception was the last sampling time when they were randomly taken from all replicated tanks with respect to the dietary treatment. The fish were weighed and blood was taken with heparinized vacutainers from the caudal vessels. Blood was centrifuged at 750xg for 10 min and plasma samples from two fish from the same treatment were pooled i.e. two pooled samples/dietary treatment. The plasma was frozen immediately in liquid nitrogen, and stored at -70°C for subsequent analyses.

2. Ten days after the first sampling, the fish were sampled a second time. After feeding fish to satiation on day 17 at 08:00 hour, blood was withdrawn from four fish per dietary treatment 26 and 36 h later by the procedure described for the previous sampling. After bleeding, a piece of white muscle from the area below the dorsal fin of each fish was excised immediately. The time required to take the muscle sample was less than 10 s. Pieces of muscle were frozen immediately in liquid nitrogen, and held at -70°C until analyzed for free amino acids.

4.2.5 Amino Acid Analysis of Plasma and Muscle

Plasma samples were treated as described previously for Experiment 1.1. Muscle samples were extracted for free amino acid analysis after homogenization with trichloroacetic acid to deproteinize tissue protein. Five grams of the muscle from each fish were homogenized for 10 min with 10 % (v/v) trichloroacetic acid in the ratio of 1/3 (w/v) using a Virtis No. 45 homogenizer. The homogenization was

done at low temperature by immersing the homogenizing cup in an ice bath during the homogenization. The homogenate was centrifuged at 20,000xg (at 4°C) for 20 min. The supernatant was decanted into a 50 mL test tube and further treated in the same way as described for plasma samples.

4.2.6 Statistical Analysis

Correlation analyses were applied to test the relationships between plasma and muscle free amino acids using Systat (Wilkinson, 1990).

4.3 RESULTS

4.3.1 Amino Acid Composition of Experimental Diets

The amino acid composition of the experimental diets as determined on acid hydrolysates is shown in Table 2.2. The concentrations of respective essential amino acids in diets 1, 3, and 4 were higher than those in diet 2. Diets 1 and 4 contained higher proportions of essential than non-essential amino acids. The ratios of EAA/NEAA were 1.1 and 1.2 in diets 1 and 4, respectively. The ratio of EAA/NEAA in diet 3 was 1.0. Diet 2 had the lowest concentration of essential amino acids and the highest concentration of nonessential amino acids with the ratio of EAA/NEAA of 0.8.

Table 2.2. Amino acid composition of experimental diets employed in Experiment 2

	Experimental diet[1]			
	Diet 1	Diet 2	Diet 3	Diet 4
	g/16g N			
Arg	9.3	8.3	8.3	8.7
His	2.9	2.7	3.4	3.1
Ile	4.0	3.2	3.4	3.8
Leu	8.5	7.5	8.5	8.7
Lys	5.3	4.7	5.3	7.9
Met	2.2	1.7	2.0	2.3
Cys	1.4	1.1	1.4	1.4
Phe	4.9	3.9	4.8	4.8
Tyr	3.4	2.4	3.3	3.3
Thr	3.6	3.2	3.4	4.5
Val	4.7	3.8	4.4	4.5
Ala	5.8	6.6	5.2	5.4
Asp	8.3	8.3	7.6	7.7
Glu	17.5	16.1	15.9	16.6
Gly	5.1	10.4	10.6	4.7
Pro	6.1	8.4	5.4	4.3
Ser	4.6	4.3	4.3	5.7
TAA[2]	97.4	96.5	97.1	97.1
EAA[3]	50.2	42.4	48.1	52.8
NEAA[4]	47.2	54.1	49.0	44.4
EAA/NEAA	1.1	0.8	1.0	1.2

[1] The main protein sources were as follows : diet 1 = fish meal, corn gluten meal, and soybean protein concentrate; diet 2 = fish meal, corn gluten meal, soybean protein concentrate, and gelatin; diet 3 = fish meal, corn gluten meal, soybean protein concentrate, and glycine; diet 4 = fish meal, corn gluten meal, and soybean protein concentrate, and supplemented with arginine, histidine, lysine, methionine, and threonine.
[2] Total amino acids.
[3] Total essential amino acids, except tryptophan which was not detected by the procedure used.
[4] Total non-essential amino acids.

4.3.2 Initial Weight and Feeding Behavior

The mean initial weights of the fish used in the experiment are shown in Table 2.3. Comparisons of the growth of the fish fed the different diets were not justifiable because fish were sampled at different times from the groups.

Feed consumption per fish varied with dietary treatment and feeding period. In general, food consumption of fish decreased between period 1 and 3. It was observed that fish fed diet 3 (glycine supplemented diet) had a poor appetite.

Table 2.3. Mean initial weights of fish in different experimental treatments employed in Experiment 2

Diet	Initial weight
	(g)
1	243.3 ± 3.0[1]
2	250.1 ± 20.1
3	237.9 ± 9.3
4	250.0 ± 5.5

[1] Mean of three replicate tanks (15-16 fish/tank) and standard error of the mean, (n=3).

4.3.3. Plasma Amino Acid Profile in Fish Fed Experimental Diets for Seven Days

Data on plasma amino acid concentrations in fish at 3, 9, 15, and 24 h after feeding different experimental diets for 7 days (first sampling) are shown in Tables 2.4-2.7. The levels of total amino acids (TAA), total essential amino acids (EAA), total non-essential amino acids (NEAA), and ratios of EAA/NEAA of fish fed the different diets are also tabulated in these tables. The changes in plasma concentrations of total essential amino acids, total non-essential amino acids, and total amino acids are shown in Figure 2.1. The changes in plasma concentrations of individual amino acids at different times after meal consumption are depicted in Figure 2.2. It should be noted that concentrations of amino acids at 24 h postprandial were applied to 0 h values in all figures presented here. Furthermore, values for amino acids at 36 h were from the second sampling at which time the fish had been fed for 17 days.

Plasma Total Amino Acids (TAA), Total Essential Amino Acids (EAA), Total Non-Essential Amino Acids (NEAA)

Plasma EAA, NEAA, TAA concentrations in fish fed the different diets could be placed into two groups according to their patterns of change over the sampling time. One group included the fish whose EAA, NEAA, and TAA in the plasma reached their highest concentrations at 9 h postprandial. The other group contained those fish whose amino acid concentrations rose more slowly and peaked later at 15 h postprandial. The former group included fish that were fed diets 2 and 4, and the latter group included fish that were fed diets 1 and 3. There was a further difference between fish that were fed diets 2 and 4 in that the levels of EAA,

NEAA, and TAA in fish fed diet 4 abruptly decreased after reaching the peak, whereas the levels of amino acids tended to plateau between 9 and 15 h in fish fed diet 2 (Figure 2.1).

As shown in Figure 2.1a, concentrations of plasma EAA in fish fed diets 1, 3, and 4 were generally similar and were higher than those in fish fed diet 2. Differences between the concentrations of NEAA in fish fed diets 2 and 3 as compared to fish fed diets 1 and 4 were prominent. The NEAA concentrations in the fish fed the former diets were more than double those of the fish fed the latter diets (Figure 2.1b and Tables 2.4-2.7). As expected, the patterns and magnitude of changes in TAA in fish fed diets 2 and 3 (Figure 2.1c) resembled the changes of NEAA.

Plasma ratios of EAA/NEAA in fish fed diets 1 and 4 were noticeably higher than those in fish fed diets 2 and 3, and higher than in fish fed any diet in the previous experiments (Tables 2.4-2.7).

Plasma Concentrations of Individual Amino Acids in Fish Fed Different Diets

Essential Amino Acids

The changes in the concentrations of most plasma essential amino acids, particularly branched-chain amino acids, in fish fed the different diets were similar to the overall pattern of EAA with respect to the diets. In fish fed diets 2 and 4, most essential amino acids started to rise and reached their highest concentrations at 9 h postprandial, whereas those in fish fed diets 1 and 3 started to rise and peaked later at 15 h after feeding. Some deviation from this pattern was observed in the

changes in the levels of lysine, arginine, and methionine in fish fed diet 4. These plasma amino acids increased as soon as 3 h after feeding.

Non-Essential Amino Acids

Postprandial changes in the concentrations of individual non-essential amino acids in the plasma of fish fed diets 1 and 4 resembled the overall pattern of NEAA (Figures 2.2j-o and 2.1b). Interesting results were observed in the changes in glycine, alanine, and serine concentrations in fish fed diets 2 and 3. The plasma concentrations of glycine in fish fed diet 2, which contained gelatin, a protein rich in glycine, increased more rapidly than in fish fed diet 3, which contained free glycine. Furthermore, elevated concentrations of this amino acid were maintained until 24 h after feeding in fish fed diet 2 (contained gelatin) whereas the levels abruptly dropped after 15 h in fish fed diet 3 (contained free glycine but no gelatin). While increases in plasma alanine, and proline concentrations in fish fed diet 2 reflected the levels in the diet, increases in plasma serine concentrations generally did not but rather corresponded to the changes in glycine concentrations. Moreover, plasma serine concentrations in fish fed diet 3 increased to similar levels to those in fish fed diet 2 (Figure 2.2o). A similar trend was observed for plasma alanine in fish fed diet 3 (Figure 2.2j).

Irrespective of whether the diets were supplemented with gelatin or glycine, glycine and serine were the non-essential amino acids present in largest amounts in the plasma of the fish (Tables 2.4-2.7).

Table 2.4. Concentrations of plasma amino acids in rainbow trout at different times after feeding diet 1 (fish meal, corn gluten meal, soy protein) on day 7 in Experiment 2.

Amino acid	Hours after feeding							
	3		9		15		24	
	$\bar{X}\pm SEM$[1] (µmol/mL)	%[2]	$\bar{X}\pm SEM$ (µmol/mL)	%	$\bar{X}\pm SEM$ (µmol/mL)	%	$\bar{X}\pm SEM$ (µmol/mL)	%
Essential								
Arg	0.339 ± 0.002	5.7	0.256 ± 0.004	3.8	0.353 ± 0.037	4.2	0.252 ± 0.041	4.1
Cys	0.028 ± 0.002	0.5	0.048 ± 0.002	0.7	0.051 ± 0.006	0.6	0.035 ± 0.005	0.6
His	0.360 ± 0.016	6.0	0.352 ± 0.002	5.3	0.368 ± 0.021	4.4	0.340 ± 0.045	5.5
Ile	0.381 ± 0.092	6.4	0.420 ± 0.058	6.3	0.638 ± 0.035	7.6	0.460 ± 0.003	7.4
Leu	0.800 ± 0.202	13.4	0.916 ± 0.109	13.8	1.445 ± 0.063	17.2	1.175 ± 0.045	18.9
Lys	0.341 ± 0.063	5.7	0.297 ± 0.018	4.5	0.321 ± 0.049	3.8	0.249 ± 0.062	4.0
Met	0.092 ± 0.040	1.5	0.198 ± 0.005	3.0	0.154 ± 0.022	1.8	0.113 ± 0.044	1.8
Phe	0.243 ± 0.010	4.1	0.288 ± 0.005	4.3	0.280 ± 0.010	3.3	0.243 ± 0.032	3.9
Thr	0.155 ± 0.018	2.6	0.197 ± 0.027	3.0	0.320 ± 0.028	3.8	0.222 ± 0.012	3.6
Tyr	0.166 ± 0.021	2.8	0.180 ± 0.007	2.7	0.185 ± 0.013	2.2	0.170 ± 0.033	2.7
Val	0.773 ± 0.159	13.0	0.816 ± 0.108	12.3	1.284 ± 0.052	15.3	0.950 ± 0.005	15.3
Non-essential								
Ala	0.484 ± 0.023	8.1	0.585 ± 0.071	8.8	0.484 ± 0.054	5.8	0.358 ± 0.035	5.8
Asp	0.032 ± 0.007	0.5	0.054 ± 0.001	0.8	0.036 ± 0.000	0.4	0.045 ± 0.024	0.7
Glu	0.178 ± 0.024	3.0	0.185 ± 0.002	2.8	0.306 ± 0.018	3.7	0.196 ± 0.048	3.1
Gly	0.675 ± 0.137	11.3	0.770 ± 0.121	11.6	0.957 ± 0.113	11.4	0.628 ± 0.100	10.1
Pro	0.213 ± 0.023	3.6	0.277 ± 0.012	4.2	0.374 ± 0.028	4.5	0.216 ± 0.034	3.5
Ser	0.698 ± 0.011	11.7	0.816 ± 0.002	12.3	0.832 ± 0.007	9.9	0.574 ± 0.087	9.2
TAA[3]	5.960 ± 0.447		6.654 ± 0.096		8.390 ± 0.343		6.225 ± 0.560	
EAA[4]	3.680 ± 0.605		3.969 ± 0.278		5.399 ± 0.232		4.209 ± 0.232	
NEAA[5]	2.280 ± 0.158		2.685 ± 0.182		2.991 ± 0.113		2.016 ± 0.328	
EAA/NEAA	1.61		1.48		1.80		2.09	

[1] Mean of two pools of plasma (two fish/pool) and standard error of the mean (n=2). [2] % of total amino acids. [3] Total amino acids. [4] Total essential amino acids. [5] Total non-essential amino acids.

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Table 2.5. Concentrations of plasma amino acids in rainbow trout at different times after feeding diet 2 (fish meal, corn gluten meal, soy protein, gelatin) on day 7 in Experiment 2.

Amino acid	Hours after feeding						
	3		9		15		24
	$\bar{X}\pm SEM$[1] (µmol/mL)	%[2]	$\bar{X}\pm SEM$ (µmol/mL)	%	$\bar{X}$ (µmol/mL)	%	$\bar{X}\pm SEM$ (µmol/mL)	%

Essential								
Arg	0.247 ± 0.070	3.4	0.261 ± 0.065	2.3	0.338	3.0	0.271 ± 0.029	3.0
Cys	0.037 ± 0.008	0.5	0.051 ± 0.010	0.5	0.071	0.6	0.041 ± 0.005	0.5
His	0.352 ± 0.036	4.8	0.413 ± 0.033	3.6	0.435	3.9	0.370 ± 0.019	4.0
Ile	0.242 ± 0.002	3.3	0.406 ± 0.075	3.6	0.382	3.4	0.313 ± 0.073	3.4
Leu	0.534 ± 0.034	7.3	0.897 ± 0.141	7.9	0.881	7.9	0.837 ± 0.146	9.1
Lys	0.298 ± 0.040	4.1	0.368 ± 0.047	3.2	0.298	2.7	0.195 ± 0.051	2.1
Met	0.107 ± 0.003	1.5	0.176 ± 0.023	1.6	0.148	1.3	0.104 ± 0.016	1.1
Phe	0.220 ± 0.001	3.0	0.293 ± 0.035	2.6	0.248	2.2	0.234 ± 0.001	2.6
Thr	0.153 ± 0.048	2.1	0.357 ± 0.073	3.1	0.415	3.7	0.279 ± 0.002	3.0
Tyr	0.110 ± 0.010	1.5	0.161 ± 0.022	1.4	0.117	1.1	0.115 ± 0.007	1.3
Val	0.561 ± 0.014	7.7	0.870 ± 0.116	7.6	0.829	7.4	0.767 ± 0.105	8.4
Non-essential								
Ala	0.663 ± 0.064	9.0	0.990 ± 0.269	8.7	0.945	8.4	0.635 ± 0.012	7.0
Asp	0.151 ± 0.020	2.1	0.239 ± 0.034	2.1	0.260	2.3	0.190 ± 0.003	2.1
Glu	0.230 ± 0.028	2.8	0.229 ± 0.008	2.0	0.301	2.7	0.198 ± 0.005	2.2
Gly	1.779 ± 0.226	24.3	3.401 ± 0.178	29.8	3.116	27.8	2.797 ± 0.128	30.6
Pro	0.942 ± 0.282	12.8	0.896 ± 0.225	7.9	1.169	10.4	0.841 ± 0.192	9.2
Ser	0.737 ± 0.048	10.0	1.391 ± 0.221	12.2	1.246	11.1	0.962 ± 0.012	10.5
TAA[3]	7.337 ± 0.590		11.400 ± 1.444		11.197		9.148 ± 0.531	
EAA[4]	2.861 ± 0.105		4.253 ± 0.510		4.162		3.525 ± 0.465	
NEAA[5]	4.476 ± 0.485		7.147 ± 0.935		7.04		5.623 ± 0.066	
EAA/NEAA	0.51		0.60		0.60		0.63	

[1] Mean of two pools of plasma (two fish/pool) and standard error of the mean (n=2). [2] % of total amino acids. [3] Total amino acids. [4] Total essential amino acids. [5] Total non-essential amino acids.

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Table 2.6. Concentrations of plasma amino acids in rainbow trout at different times after feeding diet 3 (fish meal, corn gluten meal, soy protein, supplemented with glycine) on day 7 in Experiment 2.

Amino acid	Hours after feeding							
	3		9		15		24	
	$\bar{X}$±SEM[1] (μmol/mL)	%[2]	$\bar{X}$±SEM (μmol/mL)	%	$\bar{X}$±SEM (μmol/mL)	%	$\bar{X}$±SEM (μmol/mL)	%
Essential								
Arg	0.216 ± 0.084	3.4	0.241 ± 0.057	3.8	0.287 ± 0.006	2.4	0.204 ± 0.126	2.4
Cys	0.026 ± 0.026	0.4	0.046 ± 0.004	0.7	0.072 ± 0.010	0.6	0.051 ± 0.012	0.6
His	0.322 ± 0.051	5.1	0.280 ± 0.024	4.4	0.470 ± 0.001	3.9	0.338 ± 0.052	3.9
Ile	0.303 ± 0.084	4.8	0.240 ± 0.026	3.7	0.568 ± 0.053	4.8	0.564 ± 0.105	6.5
Leu	0.651 ± 0.187	10.2	0.491 ± 0.056	7.7	1.326 ± 0.121	11.1	1.442 ± 0.117	16.6
Lys	0.254 ± 0.050	4.0	0.229 ± 0.013	3.6	0.263 ± 0.006	2.2	0.209 ± 0.075	2.4
Met	0.113 ± 0.021	1.8	0.129 ± 0.003	2.0	0.199 ± 0.016	1.7	0.182 ± 0.046	2.1
Phe	0.213 ± 0.004	3.3	0.250 ± 0.008	3.9	0.287 ± 0.021	2.4	0.242 ± 0.042	2.8
Thr	0.149 ± 0.034	2.3	0.169 ± 0.073	2.6	0.504 ± 0.016	4.2	0.257 ± 0.138	3.0
Tyr	0.114 ± 0.020	1.8	0.144 ± 0.002	2.3	0.193 ± 0.017	1.6	0.175 ± 0.026	2.0
Val	0.641 ± 0.183	10.1	0.515 ± 0.041	8.0	1.150 ± 0.107	9.6	1.145 ± 0.140	13.2
Non-essential								
Ala	0.480 ± 0.087	7.6	0.536 ± 0.030	8.4	0.766 ± 0.006	6.4	0.629 ± 0.276	7.3
Asp	0.058 ± 0.005	0.9	0.053 ± 0.009	0.8	0.065 ± 0.022	0.6	0.028 ± 0.005	0.3
Glu	0.143 ± 0.034	2.3	0.148 ± 0.019	2.3	0.274 ± 0.016	2.3	0.194 ± 0.037	2.2
Gly	1.788 ± 0.396	28.1	1.929 ± 0.025	30.1	3.611 ± 0.259	30.2	1.936 ± 0.999	22.3
Pro	0.156 ± 0.038	2.5	0.191 ± 0.019	3.0	0.451 ± 0.011	3.8	0.216 ± 0.068	2.5
Ser	0.731 ± 0.128	11.5	0.826 ± 0.061	12.9	1.468 ± 0.004	12.3	0.853 ± 0.387	9.8
TAA[3]	6.358 ± 1.433		6.420 ± 0.259		11.955 ± 0.077		8.665 ± 2.580	
EAA[4]	3.002 ± 0.745		2.734 ± 0.134		5.320 ± 0.329		4.809 ± 0.880	
NEAA[5]	3.337 ± 0.689		3.686 ± 0.124		6.635 ± 0.252		3.856 ± 1.702	
EAA/NEAA	0.90		0.74		0.80		1.24	

[1] Mean of two pools of plasma (two fish/pool) and standard error of the mean (n=2). [2] % of total amino acids. [3] Total amino acids. [4] Total essential amino acids. [5] Total non-essential amino acids.

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Table 2.7. Concentrations of plasma amino acids in rainbow trout at different times after feeding diet 4 (fish meal, corn gluten meal, soy protein, supplemented with arginine, histidine, lysine, methionine, and threonine) on day 7 in Experiment 2.

Amino acid	Hours after feeding							
	3		9		15		24	
	$\bar{X}$±SEM[1] (μmol/mL)	%[2]	$\bar{X}$±SEM (μmol/mL)	%	$\bar{X}$±SEM (μmol/mL)	%	$\bar{X}$±SEM (μmol/mL)	%
Essential								
Arg	0.323 ± 0.041	6.4	0.330 ± 0.006	4.2	0.341 ± 0.072	5.2	0.178 ± 0.085	3.0
Cys	0.029 ± 0.000	0.6	0.053 ± 0.006	0.7	0.040 ± 0.006	0.6	0.025 ± 0.002	0.4
His	0.344 ± 0.016	6.8	0.440 ± 0.008	5.6	0.367 ± 0.016	5.6	0.332 ± 0.003	5.6
Ile	0.307 ± 0.041	6.1	0.535 ± 0.049	6.8	0.495 ± 0.049	7.5	0.524 ± 0.008	8.8
Leu	0.665 ± 0.067	13.1	1.120 ± 0.103	14.1	1.165 ± 0.123	17.7	1.216 ± 0.022	20.4
Lys	0.561 ± 0.016	11.1	0.739 ± 0.123	9.3	0.484 ± 0.134	7.4	0.355 ± 0.008	6.0
Met	0.145 ± 0.007	2.9	0.268 ± 0.014	3.4	0.177 ± 0.015	2.7	0.090 ± 0.002	1.5
Phe	0.232 ± 0.003	4.6	0.329 ± 0.029	4.2	0.230 ± 0.005	3.5	0.238 ± 0.003	4.0
Thr	0.205 ± 0.008	4.1	0.409 ± 0.086	5.2	0.286 ± 0.049	4.3	0.271 ± 0.004	4.5
Tyr	0.155 ± 0.015	3.1	0.224 ± 0.031	2.8	0.163 ± 0.021	2.5	0.152 ± 0.002	2.6
Val	0.638 ± 0.106	12.6	0.978 ± 0.103	12.3	0.952 ± 0.095	14.5	1.083 ± 0.020	18.1
Non-essential								
Ala	0.279 ± 0.014	5.5	0.521 ± 0.159	6.6	0.415 ± 0.088	6.3	0.271 ± 0.007	4.5
Asp	0.040 ± 0.018	0.8	0.051 ± 0.003	0.6	0.023 ± 0.002	0.4	0.028 ± 0.001	0.5
Glu	0.166 ± 0.014	3.3	0.200 ± 0.008	2.5	0.234 ± 0.019	3.6	0.178 ± 0.005	3.0
Gly	0.434 ± 0.114	8.6	0.588 ± 0.141	7.4	0.474 ± 0.066	7.2	0.446 ± 0.009	7.5
Pro	0.108 ± 0.003	2.1	0.348 ± 0.077	4.4	0.213 ± 0.029	3.2	0.166 ± 0.007	2.8
Ser	0.435 ± 0.020	8.6	0.792 ± 0.231	10.0	0.522 ± 0.026	7.9	0.414 ± 0.009	6.9
TAA[3]	5.065 ± 0.011		7.925 ± 0.659		6.582 ± 0.783		5.970 ± 0.028	
EAA[4]	3.604 ± 0.138		5.425 ± 0.046		4.700 ± 0.554		4.460 ± 0.011	
NEAA[5]	1.461 ± 0.149		2.500 ± 0.613		1.882 ± 0.229		1.510 ± 0.038	
EAA/NEAA	2.47		2.17		2.50		2.95	

[1] Mean of two pools of plasma (two fish/pool) and standard error of the mean (n=2). [2] % of total amino acids. [3] Total amino acids. [4] Total essential amino acids. [5] Total non-essential amino acids.

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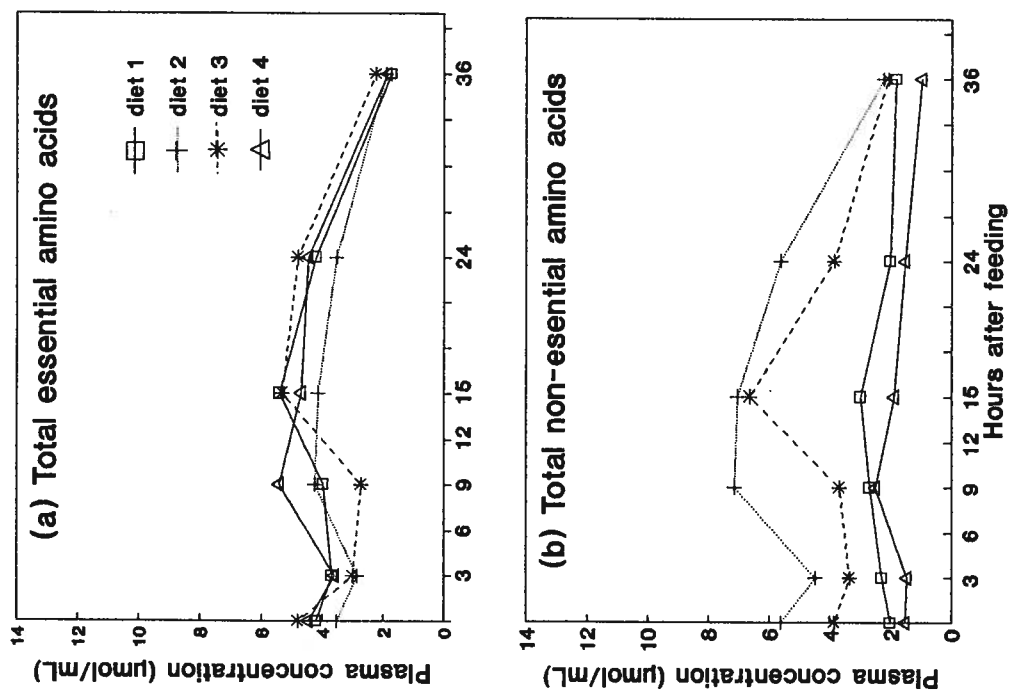
Figure 2.1. Total concentration of plasma amino acids (essential, non-essential, and total amino acids) in rainbow trout determined at different times after feeding experimental diets on day 7 in Experiment 2. Diet 1 = fish meal, corn gluten meal, soy protein; diet 2 = fish meal, corn gluten meal, soy protein, gelatin; diet 3 = fish meal, corn gluten meal, soy protein, and glycine; diet 4 = fish meal, corn gluten meal, soy protein, supplemented with arginine, histidine, lysine, methionine, and threonine. Each point represents a mean of two pools of plasma, two fish/pool, (n=2).

115
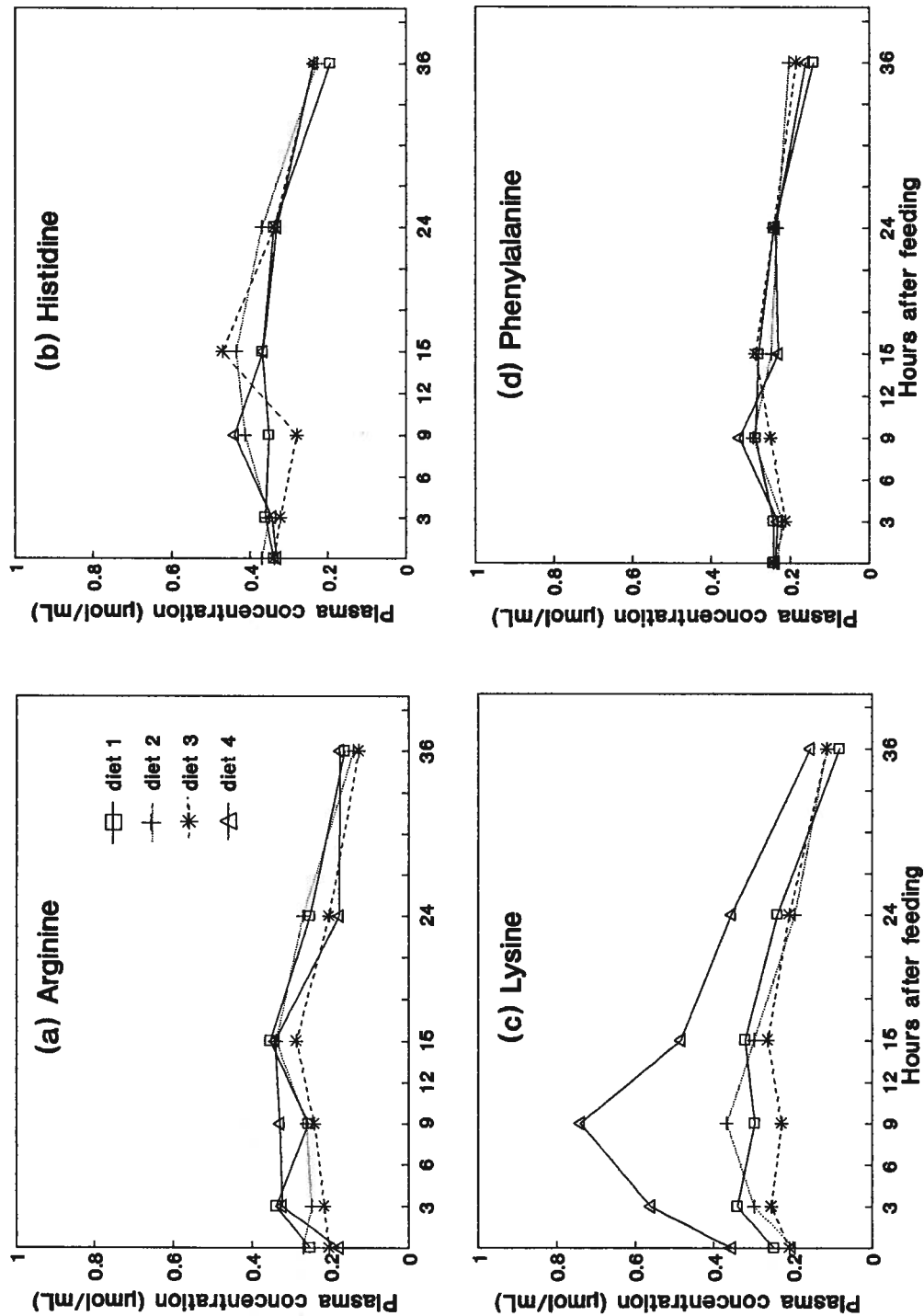
Figure 2.2. Plasma concentrations of amino acids in rainbow trout determined at different times after feeding experimental diets in Experiment 2. Diet 1 = fish meal, corn gluten meal, soy protein; diet 2 = fish meal, corn gluten meal, soy protein, gelatin; diet 3 = fish meal, corn gluten meal, soy protein, and glycine; diet 4 = fish meal, corn gluten meal, soy protein, supplemented with arginine, histidine, lysine, methionine, and threonine. Each points represents a mean of two pools of plasma, two fish/pool, (n =2).

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Figure 2.2. (Continued)

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(i) Threonine

(j) Alanine

(k) Aspartic acid

(l) Glutamic acid

diet 1
diet 2
diet 3
diet 4

Figure 2.2. (Continued)

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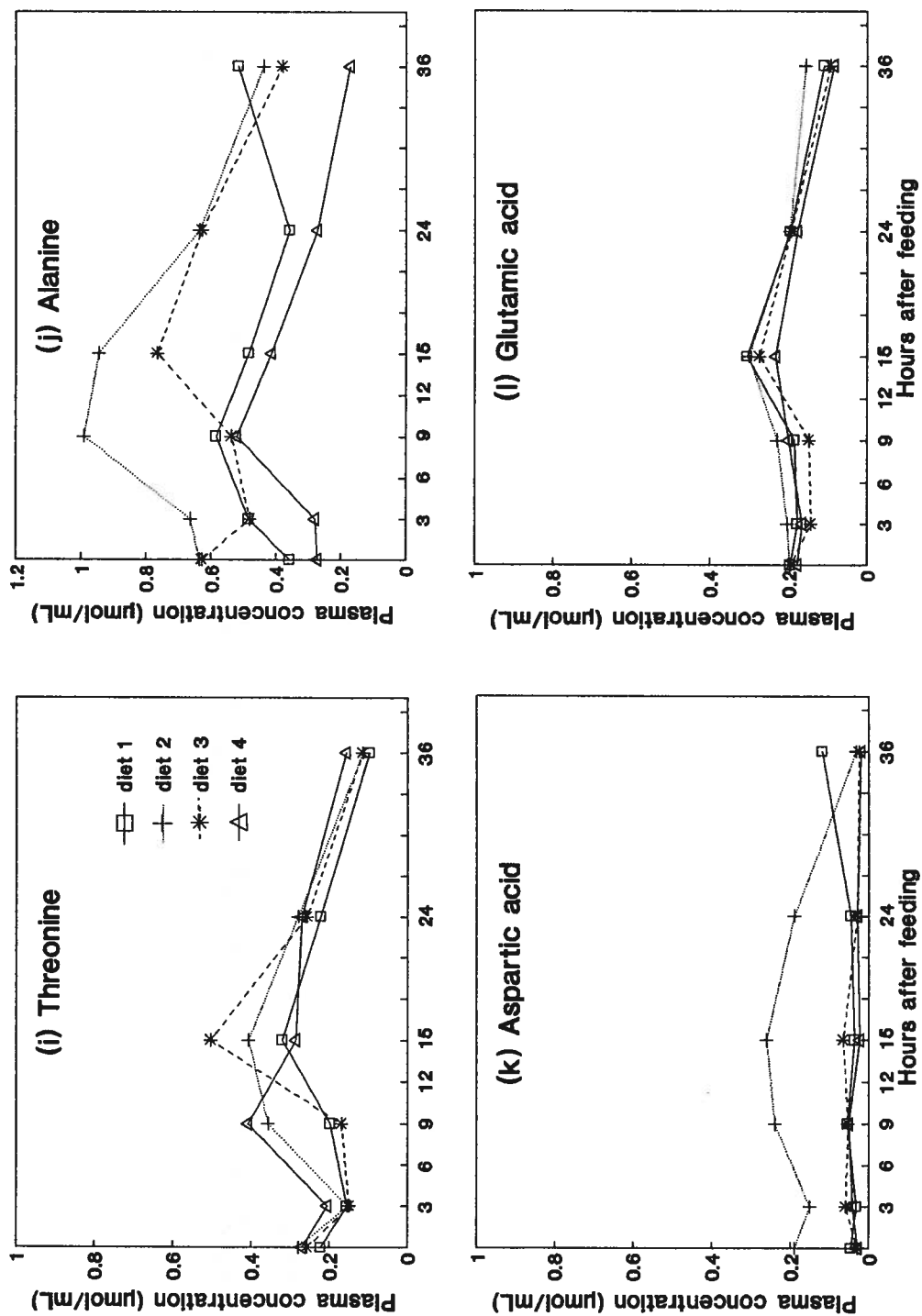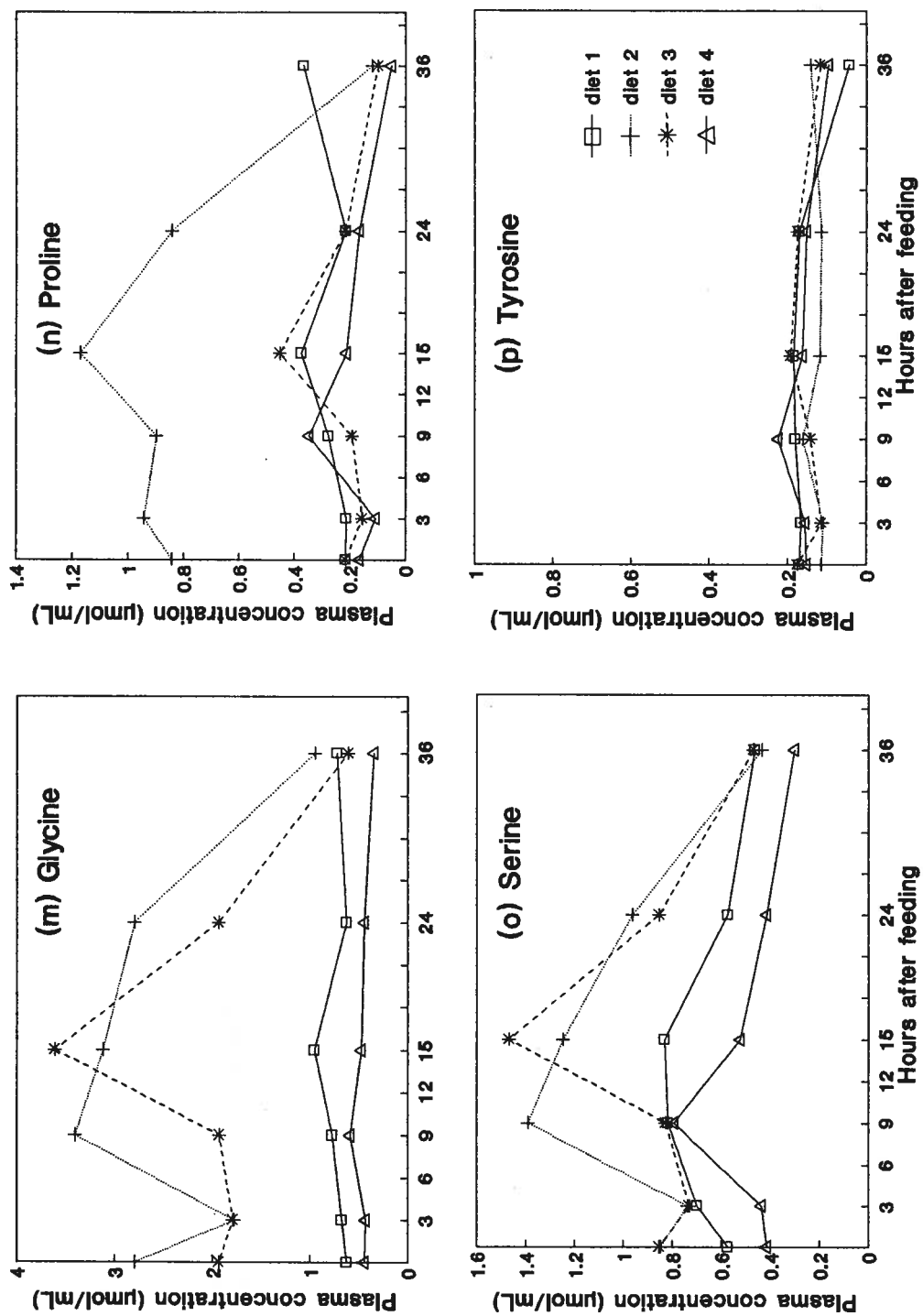
Figure 2.2. (Continued)

4.3.4. Relationship Between the Concentrations of Free Amino Acids in Plasma and Muscle

The concentrations of plasma free amino acids in blood samples collected at 26 and 36 h after feeding the experimental diets for 17 days are shown in Table 2.8. Their relative concentrations, expressed as percentage of total amino acids, are calculated and shown in Table 2.9. The levels of muscle free amino acids and their relative concentrations, as percentage of total amino acids, at the same sampling time are shown in Tables 2.10 and 2.11, respectively.

It was observed that the total concentrations of free amino acids in the muscle were several fold higher than in the plasma at all sampling times (Tables 2.8 and 2.10). Comparison of plasma and muscle TAA concentrations in fish fed different diets revealed that whereas plasma TAA concentrations in fish decreased between 26 and 36 h after feeding, the muscle TAA concentrations were more stable. Muscle EAA concentrations in fish fed diet 4 which contained supplementary amino acids were higher than in fish fed other diets, particularly at 26 h postprandial (approximately 30% higher). Furthermore, muscle NEAA concentrations in fish fed diet 3 containing free glycine were higher than in fish fed other diets, and approximately 35% higher than in fish fed diet 2 which contained similar amounts of dietary glycine regardless of sampling time.

Although the total free amino acid concentrations in muscle were higher than in plasma, concentrations of branched-chain amino acids in plasma were roughly double those in the muscle (Tables 2.8 and 2.10).

The levels of most free essential amino acids in muscle were positively correlated with the plasma levels in fish fed the experimental diets (Table 2.12).

Exceptions were arginine, histidine, and threonine. With respect to non-essential amino acids, significant correlations between the levels in plasma and muscle were detected only in the case of aspartic acid and proline. The results on the levels of glycine in plasma and muscle of fish fed diets 2 and 3 were interesting. While the glycine concentrations in plasma of fish fed diets 2 and 3 were equally high at 26 and 36 h postprandial, the muscle levels of this amino acid in fish fed diet 2 were remarkably lower than in fish fed diet 3 (Tables 2.8 and 2.10). A similar phenomenon was also observed for the levels of serine between the same groups of fish at 26 h postprandial.

Since diet 4 was supplemented with arginine, histidine, lysine, methionine, and threonine, the comparison between the levels of these amino acids in muscle and the plasma free pool of these fish and those of fish fed diets 1, 2 and 3 between 26 and 36 h postprandial were depicted graphically (Figures 2.3 and 2.4). The plasma concentrations of lysine, methionine, and threonine in fish fed diet 4 at 26 h after feeding were higher than those in fish fed diet 3, which contained similar proportions of dietary ingredients. In muscle, the levels of histidine, lysine and threonine in fish fed diet 4 were prominently higher than those in fish fed diet 3 as well as diets 1 and 2 at 26 h after feeding (Figure 2.4). The higher concentrations of these amino acids in muscle of fish fed diet 4 than in fish fed diet 3 were also observed at 36 h after feeding. In plasma, on the other hand, neither free arginine nor histidine, and in muscle neither free arginine nor methionine were appreciably different among fish fed different diets.

Regardless of the differences in the levels of individual amino acids due to dietary differences, the amino acids that characterized the plasma and muscle free amino acids differed. In plasma, the branched-chain amino acids and glycine were the predominant essential and non-essential amino acids, respectively (Table 2.9). In muscle, histidine characterized the essential amino acid pool making up approximately 15-24% of TAA concentrations, and glycine dominated the non-essential amino acid pool, constituting 46.6-59.8% of TAA concentrations (Table 2.11).

Table 2.8. Concentrations of plasma amino acids in rainbow trout at 26 and 36 h after feeding the experimental diets[1] on day 17 in Experiment 2.

Amino acid	26 h after feeding				36 h after feeding			
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 1	Diet 2	Diet 3	Diet 4
	$\bar{X}\pm$SEM (μmol/mL)[2]				$\bar{X}\pm$SEM (μmol/mL)			
Essential								
Arg	0.112±.005	0.133±.040	0.123±.008	0.179±.073	0.128±.007	0.165±.011	0.142±.053	0.176±.002
Cys	0.036±.000	0.047±.007	0.044±.002	0.042±.004	0.028±.008	0.022±.005	0.032±.006	0.015±.015
His	0.332±.026	0.371±.015	0.308±.026	0.337±.002	0.194±.008	0.228±.005	0.236±.020	0.237±.025
Ile	0.300±.060	0.319±.012	0.306±.095	0.400±.013	0.136±.021	0.147±.038	0.190±.024	0.129±.054
Leu	0.787±.181	0.784±.004	0.751±.147	0.999±.051	0.350±.047	0.422±.115	0.503±.091	0.327±.153
Lys	0.185±.015	0.236±.014	0.217±.068	0.379±.051	0.114±.004	0.082±.009	0.115±.001	0.156±.030
Met	0.175±.024	0.149±.005	0.160±.002	0.226±.010	0.069±.007	0.075±.010	0.093±.026	0.108±.013
Phe	0.205±.011	0.212±.008	0.236±.006	0.236±.008	0.185±.014	0.141±.002	0.203±.022	0.161±.006
Thr	0.241±.002	0.315±.036	0.207±.051	0.382±.061	0.115±.025	0.098±.017	0.115±.027	0.157±.037
Tyr	0.142±.005	0.095±.006	0.179±.007	0.166±.017	0.118±.035	0.044±.004	0.142±.020	0.096±.026
Val	0.703±.139	0.817±.044	0.701±.159	0.825±.025	0.288±.041	0.476±.098	0.460±.088	0.300±.124
Non-essential								
Ala	0.319±.056	0.738±.201	0.414±.051	0.370±.045	0.517±.016	0.439±.087	0.380±.122	0.172±.061
Asp	0.035±.010	0.193±.064	0.059±.002	0.021±.000	0.024±.008	0.119±.021	0.031±.010	0.020±.005
Glu	0.142±.030	0.209±.005	0.187±.035	0.144±.004	0.094±.001	0.110±.018	0.156±.040	0.084±.020
Gly	0.624±.053	2.047±.183	2.393±.276	0.641±.082	0.608±.176	0.726±.166	0.948±.203	0.347±.113
Pro	0.194±.004	0.749±.084	0.225±.056	0.193±.023	0.097±.015	0.366±.107	0.119±.033	0.050±.019
Ser	0.542±.010	0.943±.141	0.787±.120	0.539±.014	0.468±.020	0.462±.085	0.433±.049	0.302±.071
TAA[4]	5.075±.552	8.359±.655	7.296±1.108	6.081±.025	3.533±.082	4.123±.759	4.299±.833	2.837±.770
EAA[5]	3.219±.418	3.479±.012	3.232±.567	4.173±.067	1.724±.121	1.902±.275	2.232±.376	1.862±.481
NEAA[6]	1.856±.134	4.880±.668	4.065±.540	1.908±.043	1.809±.203	2.221±.484	2.067±.457	0.975±.289
EAA/NEAA	1.7	0.7	0.8	2.2	0.95	0.86	1.08	1.91

[1] Diet 1 contained fish meal, corn gluten meal, soy protein concentrate, diet 2 contained basal ingredients as in diet 1 and gelatin, diet 3 contained basal ingredients as in diet 1, diet 4 contained the same ingredients as in diet 3 but supplemented with arginine, histidine, lysine, methionine, and threonine.
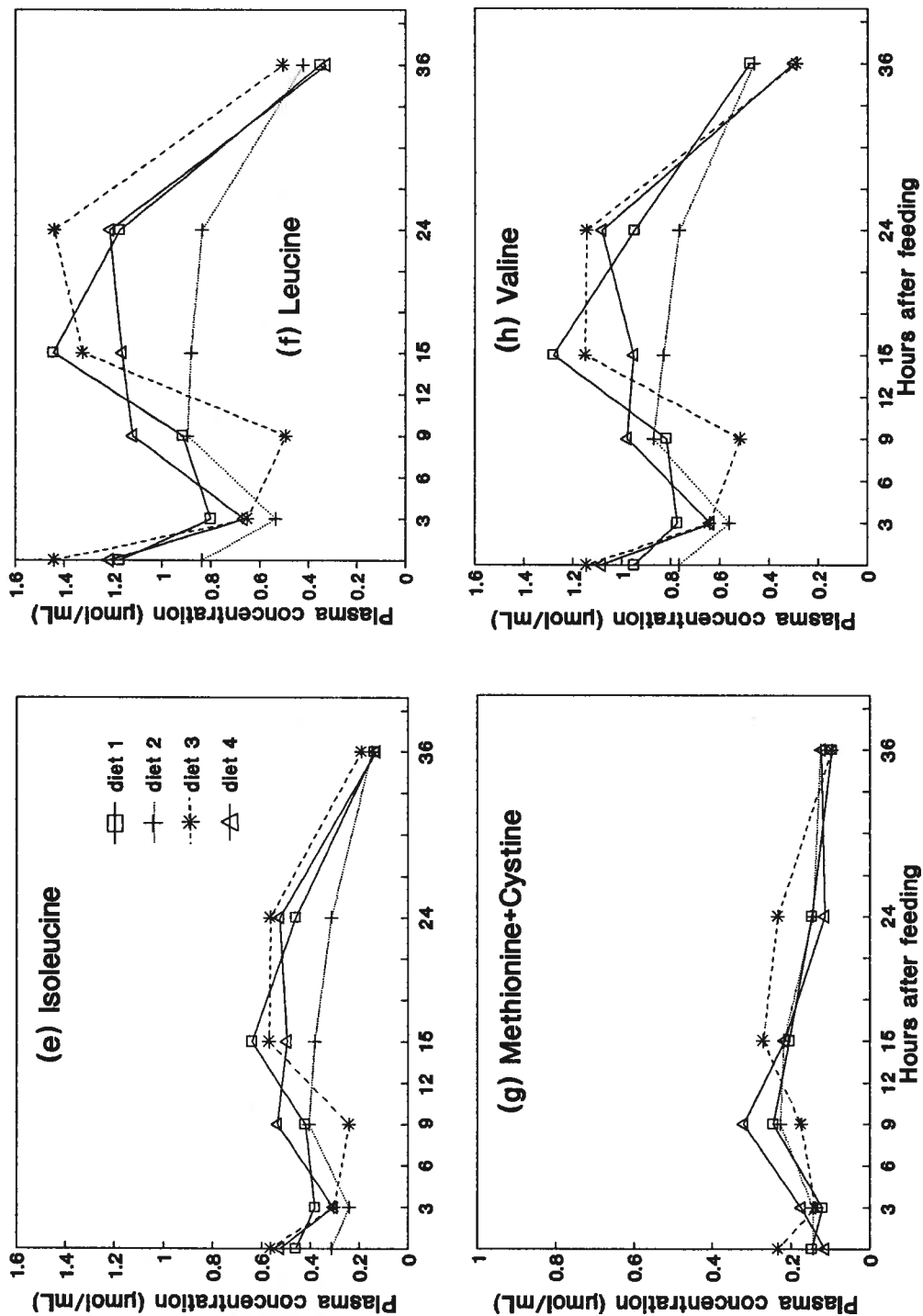[2] Means of two pools of plasma (two fish/pool) and standard error of the means (n=2). [3] present in trace amount. [4] Total amino acids. [5] Total essential amino acids. [6] Total non-essential amino acids.

Table 2.9. Relative concentrations of plasma free amino acids (expressed as a percentage) in rainbow trout at 26 and 36 h after feeding the experimental diets[1] on day 17 in Experiment 2.

Amino acid	26 h after feeding				36 h after feeding			
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 1	Diet 2	Diet 3	Diet 4
Essential								
Arg	2.2	1.6	1.8	3.0	3.6	4.0	3.3	6.2
Cys	0.7	0.6	0.6	0.7	0.8	0.5	0.7	0.5
His	6.5	4.4	4.2	5.5	5.5	5.5	5.5	8.4
Ile	5.9	3.8	4.2	6.6	3.8	3.6	4.4	4.6
Leu	15.5	9.4	10.3	16.4	9.9	10.2	11.7	11.5
Lys	3.6	2.8	3.0	6.2	3.2	2.0	2.7	5.5
Met	3.5	1.8	2.2	3.7	2.0	1.8	2.2	3.8
Phe	4.0	2.5	3.2	3.9	5.2	3.4	4.7	5.7
Thr	4.7	3.8	2.8	6.3	3.2	2.4	2.7	5.5
Tyr	2.8	1.1	2.5	2.7	3.3	1.1	3.3	3.4
Val	13.9	9.8	9.6	13.6	8.2	11.6	10.7	10.6
Non-essential								
Ala	6.3	8.8	5.7	6.1	14.6	10.6	8.8	6.1
Asp	0.7	2.3	0.8	0.4	0.7	2.9	0.7	0.7
Glu	2.8	2.5	2.6	2.4	2.7	2.7	3.6	3.0
Gly	12.3	24.5	32.8	10.5	17.2	17.6	22.1	12.2
Pro	3.8	9.0	3.1	3.2	2.8	8.9	2.8	1.7
Ser	10.7	11.3	10.8	8.9	13.3	11.2	10.1	10.7

[1] Diet 1 contained fish meal, corn gluten meal, soy protein concentrate, diet 2 contained basal ingredients as in diet 1 and gelatin, diet 3 contained basal ingredients as in diet 1 plus glycine, diet 4 contained the same ingredients as in diet 3 but supplemented with arginine, histidine, lysine, methionine, and threonine.

Table 2.10. Concentrations of muscle free amino acids in rainbow trout at 26 and 36 h after feeding the experimental diets[1] on day 17 in Experiment 2.

Amino acid	26 h after feeding				36 h after feeding			
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 1	Diet 2	Diet 3	Diet 4
	$\bar{X}\pm$SEM (μmol/g wet weight)[2]				$\bar{X}\pm$SEM (μmol/g wet weight)			
Essential								
Arg	0.370±.060	0.415±.049	0.244±.076	0.311±.035	0.134±.042	0.422±.045	0.301±.020	0.242±.013
Cys	tr[3]	tr	0.128±.000	0.155±.000	0.041±.041	tr	tr	tr
His	5.749±.460	6.389±.257	6.119±.553	7.587±.733	7.223±.952	7.091±.290	6.661±.450	7.603±.601
Ile	0.135±.040	0.119±.015	0.148±.035	0.158±.060	0.059±.028	0.096±.023	0.135±.015	0.063±.025
Leu	0.472±.127	0.312±.036	0.347±.054	0.454±.151	0.189±.049	0.244±.047	0.300±.044	0.179±.045
Lys	0.109±.025	0.128±.048	0.169±.085	0.384±.108	0.091±.091	0.048±.020	0.038±.019	0.149±.037
Met	0.218±.035	0.178±.024	0.197±.035	0.235±.007	0.088±.006	0.121±.021	0.159±.032	0.177±.019
Phe	0.187±.000	0.128±.000	0.201±.072	0.259±.000	0.081±.082	tr	0.121±.087	tr
Thr	0.820±.151	0.524±.072	0.598±.092	1.190±.082	0.466±.048	0.344±.211	0.905±.065	1.062±.111
Tyr	tr	tr	tr	0.146±.000	0.090±.090	tr	0.090±.090	tr
Val	0.287±.101	0.371±.029	0.360±.087	0.356±.131	0.138±.037	0.301±.052	0.278±.049	0.175±.053
Non-essential								
Ala	1.876±.380	1.953±.207	1.841±.359	1.465±.271	2.280±.614	2.159±.348	2.378±.360	0.956±.136
Asp	0.483±.052	0.693±.193	0.493±.128	0.285±.095	0.296±.099	0.865±.066	0.507±.069	0.281±.040
Glu	1.186±.149	1.074±.081	0.886±.070	1.065±.164	0.930±.106	1.068±.134	1.021±.103	0.998±.189
Gly	19.551±2.232	15.075±1.700	24.880±2.430	17.363±1.223	19.891±1.284	13.461±.732	24.042±1.259	17.527±2.256
Pro	1.711±.340	3.523±.591	1.397±.189	0.971±.214	0.892±.130	3.969±.680	0.950±.148	0.594±.111
Ser	2.596±.308	1.495±.100	3.340±.675	1.486±.156	1.430±.132	1.839±.252	2.398±.478	0.966±.057
TAA[4]	35.750±2.634	32.377±1.828	41.491±4.133	33.870±1.735	34.320±1.856	32.226±1.478	40.284±2.222	30.972±1.989
EAA[5]	8.347±.960	8.564±.257	8.655±.794	11.235±.731	8.600±.542	8.865±.461	8.988±.636	9.650±.716
NEAA[6]	27.403±2.420	23.813±1.227	32.837±3.420	22.635±1.866	25.719±1.551	23.361±1.227	31.296±1.950	21.322±2.331
EAA/NEAA	0.30	0.35	0.26	0.50	0.33	0.38	0.29	0.45

[1] Diet 1 contained fish meal, corn gluten meal, soy protein concentrate, diet 2 contained basal ingredients as in diet 1 and gelatin, diet 3 contained basal ingredients as in diet 1, diet 4 contained the same ingredients as in diet 3 and supplemented with arginine, histidine, lysine, methionine, and threonine.
[2] Means of four samples (four fish) and standard error of the means (n=4). [3] present in trace amount. [4] Total amino acids. [5] Total essential amino acids. [6] Total non-essential amino acids.

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Table 2.11. Relative concentrations of muscle free amino acids (expressed as a percentage) in rainbow trout at 26 and 36 h after feeding experimental diets[1] on day 17 in Experiment 2.

Amino acid	26 h after feeding				36 h after feeding			
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 1	Diet 2	Diet 3	Diet 4
Essential								
Arg	1.0	1.3	0.6	0.9	0.4	1.3	0.7	0.8
Cys	0	0	0.3	0.5	0.1	0	0	0
His	16.1	19.7	14.9	22.4	21.0	22.0	16.5	24.5
Ile	0.4	0.4	0.4	0.5	0.2	0.3	0.3	0.2
Leu	1.3	1.0	0.8	1.3	0.6	0.8	0.7	0.6
Lys	0.3	0.4	0.4	1.1	0.3	0.1	0.1	0.5
Met	0.6	0.6	0.5	0.7	0.3	0.4	0.4	0.6
Phe	0.5	0.4	0.5	0.8	0.2	0	0.3	0
Thr	2.3	1.6	1.4	3.5	1.4	1.7	2.2	3.4
Tyr	0	0	0.3	0.4	0.3	0	0.2	0
Val	0.8	1.1	0.9	1.1	0.4	0.9	0.7	0.6
Non-essential								
Ala	5.2	6.0	4.4	4.3	6.6	6.7	5.9	3.1
Asp	1.4	2.1	1.2	0.8	0.9	2.7	1.3	0.9
Glu	3.3	3.3	2.1	3.1	2.7	3.3	2.5	3.2
Gly	54.7	46.6	59.8	51.3	58.0	41.8	59.7	56.6
Pro	4.8	10.9	3.4	2.9	2.6	12.3	2.4	1.9
Ser	7.3	4.6	8.0	4.4	4.2	5.7	6.0	3.1

[1] Diet 1 contained fish meal, corn gluten meal, soy protein concentrate, diet 2 contained basal ingredients as in diet 1 and gelatin, diet 3 contained basal ingredients as in diet 1 plus glycine, diet 4 contained the same ingredients as in diet 3 but supplemented with arginine, histidine, lysine, methionine, and threonine.

Table 2.12. Correlation coefficients[1] of concentrations between plasma and muscle free amino acids in Experiment 2

Amino acid	Correlation coefficients
Arg	0.080
His	-0.475
Ile	0.845*
Leu	0.906*
Lys	0.937*
Met	0.924*
Phe	0.914*
Thr	0.436
Val	0.899*
Ala	0.560
Asp	0.795*
Glu	0.064
Gly	0.301
Pro	0.830*
Ser	0.352

[1] The values used in the calculation were the means of each dietary treatment at 26 and 36 h (n = 8). The coefficients with an asterisk were significant at $P < 0.05$.
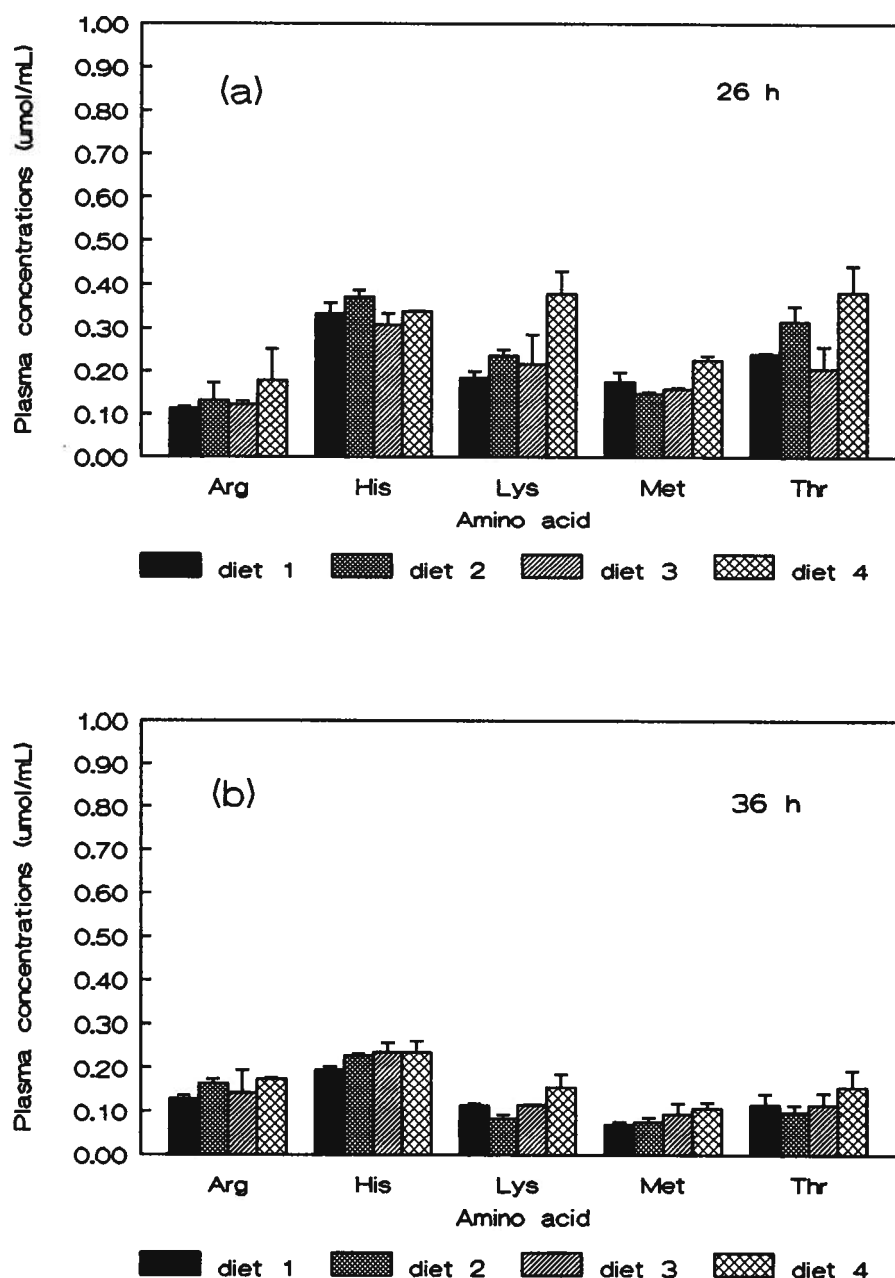
Figure 2.3. Plasma concentrations of supplementary amino acids in rainbow trout determined at 26 h (a), and 36 h (b) after feeding the experimental diets on day 17 in Experiment 2. Diet 1 = fish meal, corn gluten meal, soy protein; diet 2 = fish meal, corn gluten meal, soy protein, gelatin; diet 3 = fish meal, corn gluten meal, soy protein, and glycine; diet 4 = fish meal, corn gluten meal, soy protein, supplemented with arginine, histidine, lysine, methionine, and threonine. Each point represents a mean of two pools of plasma, two fish/pool, (n=2).
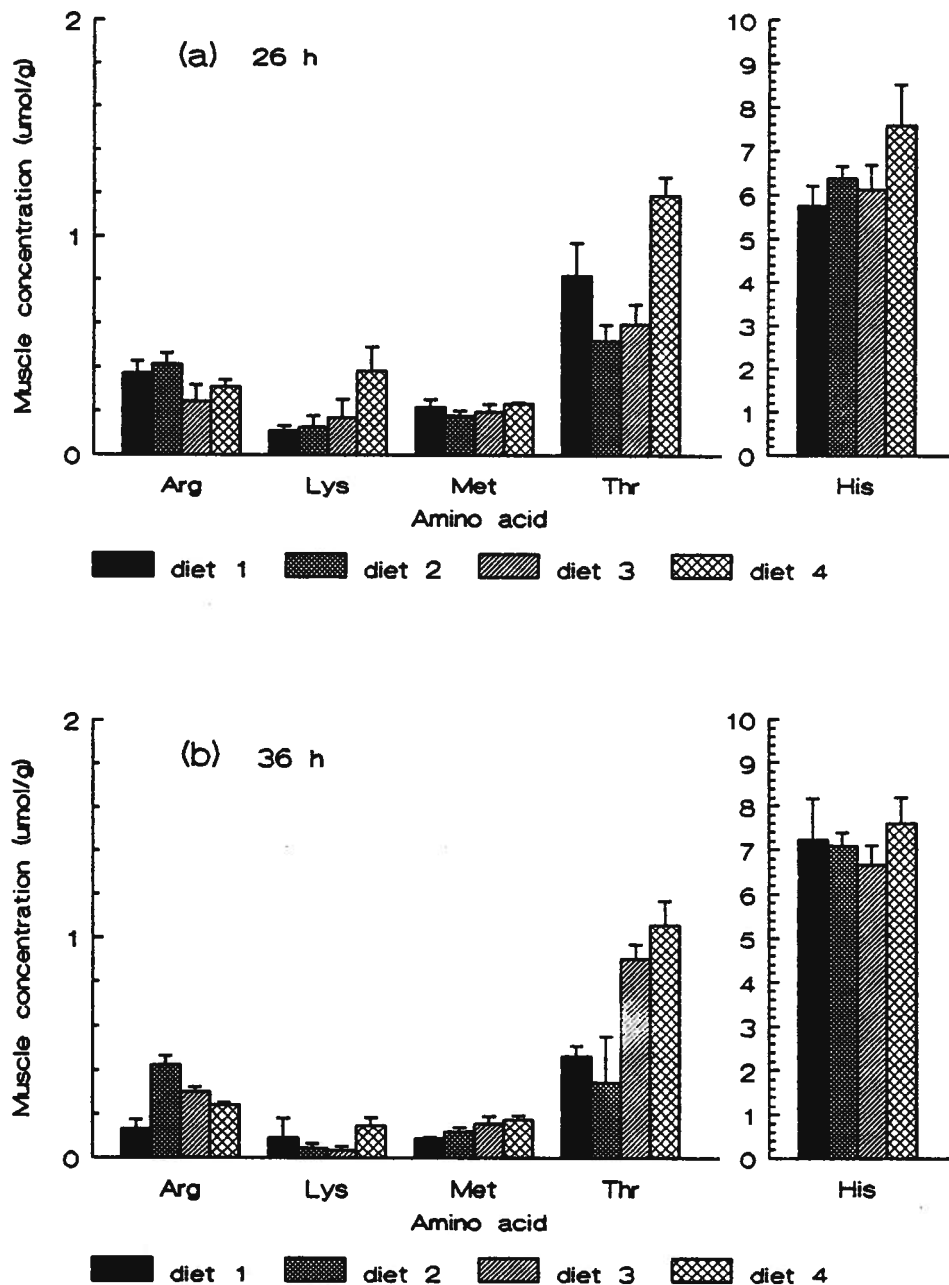
Figure 2.4. Muscle concentrations of supplementary amino acids in rainbow trout determined at 26 h (a), and 36 h (b) after feeding the experimental diets on day 17 in Experiment 2. Diet 1 = fish meal, corn gluten meal, soy protein; diet 2 = fish meal, corn gluten meal, soy protein, gelatin; diet 3 = fish meal, corn gluten meal, soy protein, and glycine; diet 4 = fish meal, corn gluten meal, soy protein, supplemented with arginine, histidine, lysine, methionine, and threonine. Each point represents a mean of four samples from four fish (n=4).

4.3.5 Comparison Between Concentrations of Plasma Amino Acids in Fish in Experiment 1 (Freshwater) and Experiment 2 (Seawater)

Diet 2 used in Experiment 1 and 2 was similar in composition. The amino acid compositions of diet 2 in the two experiments were also similar except the concentrations of particularly arginine and leucine in the diet used in Experiment 2 were higher than those for the diet used in Experiment 1 (Table 1.2, 1.21, and 2.2).

The results of the plasma concentrations of total essential (EAA), total non-essential (NEAA), and total amino acids (TAA) in fish fed this diet that were sampled at the same intervals after feeding in Experiments 1.1 and 2 are presented in Table 2.13.

The levels of plasma TAA between the two experiments did not markedly differ. The concentrations of plasma EAA in fish in Experiment 2, however, were consistently higher than those in fish in Experiment 1.1. Comparison of the concentrations of individual essential amino acids in fish between the two experiments revealed that arginine, the branched-chain amino acids, lysine, and threonine were responsible for the higher levels of EAA detected in fish in Experiment 2. The higher concentrations in the plasma, however, corresponded to the dietary levels of these amino acids in the latter experiment.

Table 2.13. Plasma concentrations of branched-chain amino acids, lysine and threonine and total concentrations of essential amino acids, non-essential amino acids, and total amino acids in fish fed diet 2 sampled at different times after feeding in Experiment 1.1 and Experiment 2

Amino acid	0 h		3 h		9 h		15 h	
	$\bar{X} \pm SEM^1$ (μmol/mL)	$\bar{X} \pm SEM^2$ (μmol/mL)	$\bar{X} \pm SEM$ (μmol/mL)	$\bar{X} \pm SEM$ (μmol/mL)	$\bar{X} \pm SEM$ (μmol/mL)	$\bar{X} \pm SEM$ (μmol/mL)	$\bar{X} \pm SEM$ (μmol/mL)	$\bar{X} \pm SEM$ (μmol/mL)
	Exp. 1.1	Exp. 2	Exp. 1.1	Exp. 2	Exp. 1.1	Exp. 2	Exp. 1.1	Exp. 2
Ile	0.101 ± .016	0.313 ± .073	0.177 ± .025	0.242 ± .002	0.193 ± .031	0.406 ± .075	0.153 ± .010	0.382[3]
Leu	0.251 ± .045	0.837 ± .146	0.335 ± .046	0.534 ± .034	0.399 ± .058	0.897 ± .141	0.305 ± .006	0.881
Val	0.378 ± .051	0.767 ± .105	0.483 ± .071	0.561 ± .014	0.568 ± .064	0.870 ± .116	0.448 ± .034	0.829
Lys	0.223 ± .017	0.195 ± .051	0.255 ± .034	0.298 ± .040	0.237 ± .022	0.368 ± .047	0.236 ± .004	0.298
Thr	0.175 ± .013	0.279 ± .002	0.175 ± .046	0.153 ± .048	0.200 ± .034	0.357 ± .073	0.202 ± .027	0.415
TAA[4]	9.318 ± .875	9.148 ± .531	8.610 ± .803	7.337 ± .590	9.953 ± .738	11.400 ± 1.444	10.340 ± .206	11.197
EAA[5]	1.808 ± .150	3.525 ± .465	2.289 ± .338	2.861 ± .105	2.404 ± .206	4.253 ± .510	2.047 ± .147	4.162
NEAA[6]	7.510 ± .735	5.623 ± .060	6.321 ± .484	4.476 ± .485	7.550 ± .583	7.147 ± .935	8.289 ± .334	7.040

[1] Each value in Experiment 1.1 represents a mean of three pools of plasma (five fish/pool) and standard error of the mean (n=3). [2] Each value in Experiment 2 represents a mean of two pools of plasma (two fish/pool), and standard error of the mean (n=2). [3] Only one pool of plasma.
[4] Total amino acids. [5] Total essential amino acids. [6] Total non-essential amino acids

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4.4 DISCUSSION

Plasma concentrations of essential and non-essential amino acids in fish fed diets 2 and 4 started to increase, and they reached peaks earlier than observed in fish fed diets 1 and 3. More interestingly, plasma glycine in fish fed diet 2 (contained gelatin) increased sooner following meal consumption than in fish fed diet 3 (contained free glycine). This finding negated the hypothesis that glycine supplied in the free form in the diet would be absorbed more rapidly than when fed as a constituent of protein. It may be inferred from the more rapid increases in other plasma amino acids after consumption of diet 2 compared to diet 3 that gelatin present in the diet was rapidly hydrolyzed. In relation to this finding, gelatin present in the diet possibly has favorable effects on the digestive process in general. This finding was actually supported by the studies of Boge *et al.*, (1981) who found that glycine uptake by rainbow trout was more rapid from glycylglycine (a dipeptide) than from the equivalent free glycine, especially at a high concentration. A similar phenomenon that has been observed in mammals (Matthews, 1973).

The great increases in the concentrations of plasma alanine, glycine, and proline in fish fed diet 2 between 9 and 15 h after feeding corresponded to the high levels of these amino acids in diet 2 which originated from gelatin (Table 2.2). Plasma serine concentrations in fish fed diet 2 also rose to a great extent. This finding was similar to the results of Experiment 1 and is consistent with the formation of serine from glycine. The increases in the levels of plasma serine and alanine in fish fed diet 3 did not agree with the amino acid composition of this diet. Moreover, the concentrations of these two plasma amino acids were many fold higher than the concentrations observed in fish fed diet 4 which contained similar

proportions of feed ingredients. Diet 3 was supplemented with free glycine, whereas diet 4 was supplemented with free arginine, histidine, lysine, methionine, and threonine. The increased concentrations of plasma serine and alanine in fish fed diet 3 clearly resulted from the effects of the inclusion of glycine in this diet. The rise in the levels of these two amino acids indicated that excess plasma glycine in fish fed diet 3 was converted to serine and alanine. In general, serine is catabolized by three routes which are initiated by, (1) serine dehydratase which involves the non-oxidative deamination of serine to pyruvate, (2) serine pyruvate transaminase in which serine is transaminated with pyruvate to form hydroxypyruvate and alanine, and (3) serine hydroxymethyltransferase which is the reversed reaction of the glycine cleavage system to form glycine (Walton and Cowey, 1982; Bender, 1985). All three enzymes have been detected in rainbow trout liver and kidney (Walton and Cowey, 1981). Cowey and Walton (1989) suggested that the hydroxymethyltransferase is probably the main route of serine catabolism in rainbow trout as it is in mammals. The high plasma concentrations of glycine and alanine in rainbow trout in the present experiment indicated that serine was catabolized via serine pyruvate transaminase. This finding is in agreement with the high levels and low Km of this transaminase in rainbow trout liver as reported by Walton and Cowey (1981). Cowey and Walton (1989) stressed that this pathway is likely important during gluconeogenesis from serine. Dehydratase has been found at a very low level in rainbow trout tissues and, seemingly, does not have an important role in serine metabolism in this species.

The pattern of changes observed in concentrations of glycine and serine in plasma of fish fed diet 3 seemed to be followed, as well, in the muscle of the fish fed

this diet. In comparison with the concentrations of free amino acids in the muscle of fish fed other diets, fish fed diet 3 had higher levels of both glycine and serine at 26 and 36 h postprandial. The results of the present study showed that excess amounts of these two amino acids in the circulation were absorbed and retained in muscle (Christensen, 1964). The accumulation of serine, glycine, and alanine has similarly been observed in muscle of carp fed a diet supplemented with a mixture of non-essential amino acids (Murai *et al.*, 1989a).

The high levels of serine and glycine, gluconeogenic amino acids, in plasma and muscle of fish fed diet 3 suggested the possibility that the capacity of the fish for catabolism of glycine was exceeded. The depression of appetite observed with this group of fish might reflect a toxic effect of excess glycine in diet 3. The reduction of feed intake in these fish was possibly a natural mechanism to reduce the influx of glycine (Harper *et al.*, 1970). Fauconneau, (1988) also observed a decrease in voluntary food intake of rainbow trout fed a diet singly supplemented with alanine, aspartic acid, or glutamic acid. The negative effects of supplementary glycine were also observed in the studies of Todd *et al.*, (1967). They found that chinook salmon fed a diet supplemented with 10% glycine had higher liver glycogen than fish fed the control diet. Hughes (1985) also found that rainbow trout and lake trout showed inferior growth rate and feed conversion efficiency when they were fed a diet supplemented with 15% glycine (as % of diet) in comparison with 15 % glutamic acid.

Comparisons of the concentrations of plasma amino acids in fish fed the different diets showed that plasma essential amino acids in fish fed diet 1 and 4 were high. The total essential amino acid concentrations in plasma of fish fed diets 1 and

4 reached 63.4 and 68.6% of TAA at 26 h postprandial (day 17 sampling). The concentrations of essential amino acids in fish fed diets 1 and 4 later decreased to the levels lower than those in diet 2 and 3 at 36 h. This suggested that the circulating amino acids in fish fed diets 1 and 4 were removed more rapidly from the plasma pool and were associated with faster protein synthesis than in fish fed diet 2 and 3.

Higher free histidine, lysine, and threonine concentrations in muscle of fish fed diet 4 than in fish fed other diets were responsible for the higher muscle concentrations of total essential amino acids, particularly at 26 h postprandial. This implied that the concentrations of histidine, lysine, and threonine in the circulation were in excess of those required by the fish. Under this circumstance, muscle tissues absorbed these amino acids and acted as a temporary storage reservoir. In fact, the accumulation of the aforementioned amino acids in the muscle of fish in the present experiment suggested that these amino acids were still available to the fish at 26 and 36 h after feeding. The results contradicted the belief that a rapid and great increase of plasma amino acids will lead to rapid catabolism and that the excess amino acids accordingly are not available for protein synthesis over a protracted period (Cowey, 1980). The evidence for an enhanced rate of amino acid catabolism with increased levels of particular amino acids in the plasma has been reported by others (Walton *et al.*, 1984a; Cowey and Walton, 1988; Kim *et al.*, 1992) in rainbow trout. The results of different studies regarding the enzyme activity responsible for amino acid degradation in fish are, however, confusing. For instance, Cowey *et al.*, (1981) studied the effects of quality and quantity of dietary protein on activity of urocase, histidine deaminase, AMP deaminase, and serine pyruvate transaminase in

rainbow trout. Changes in enzyme activity were detected only with serine pyruvate transaminase. The results contradicted those found for carp (Sakaguchi and Kawai, 1970). Kim *et al.*, (1992) recently reported that liver histidine deaminase activity in rainbow trout was higher in fish fed a commercial diet containing 50% protein than in fish fed diets containing 10 or 35% protein. Although it is well understood that other animals adapt to changes in dietary proteins concentration through alterations in the activity of enzymes, the mechanism which controls protein metabolism in fish is still unclear. The results of the present study, however, suggested that supplementary amino acids were still available to the fish until at least 26 h after feeding based on routine feeding, once daily.

In contrast to histidine, lysine, and threonine, the levels of arginine and methionine in the muscle of fish fed diet 4 did not markedly differ from those in fish fed diet 3 or the other diets. The plasma concentrations of these two amino acids in the fish that were fed diet 4 increased to higher levels than those in fish fed diet 3 (contained similar proportions of ingredients) on day 7 after feeding (first sampling). The concentrations decreased to levels similar to those in fish fed diet 3 at 36 h after feeding. Thus, the concentrations of both arginine and methionine in the circulation of fish fed diet 4 likely met the requirements and consequently no excess accumulated in the muscle pool.

The high concentrations of free amino acids, particularly non-essential amino acids, observed in muscle relative to plasma in the present experiment agree with the results found in channel catfish (Wilson and Poe, 1974), rainbow trout (Kaushik and Luquet, 1977a; Kaushik and Luquet, 1979), carp (Ogata, 1986), Atlantic cod (Lyndon *et al.*, 1993), and Atlantic salmon (Espe *et al.*, 1993). The concentrations of

most essential amino acids in muscle were found to be correlated with those in plasma (six amino acids out of nine) whereas the relationships between muscle and plasma non-essential amino acids were inconsistent. This suggests conservation of essential amino acids for protein synthesis, and a variable role of non-essential amino acids in metabolism.

Lower concentrations of branched-chain amino acids in the muscle than in the plasma suggested that these amino acids are catabolized in the muscle of fish as in mammals. High activity of branched-chain amino acid aminotransferase has been found in kidney and red muscle of rainbow trout (Teigland and Klungsøyr, 1983). Branched-chain keto acid dehydrogenase, the enzyme in the second step of their catabolic pathway, has also been reported in red muscle in the same species of fish (Christiansen and Klungsøyr, 1987). Furthermore, Hughes *et al.*, (1983) found branched-chain amino acid aminotransferase in five tissues of lake trout fingerlings. The activity of the enzyme varied among tissues as follows: posterior kidney> skeletal muscle> gill> liver> anterior kidney.

Histidine has been reported in many studies as the most abundant essential amino acid in the muscle pool of carp and rainbow trout (Kaushik and Luquet, 1977a; Medale *et al.*, 1987; Van der Boon *et al.*, 1989). A similar phenomenon was found in the present experiment. Histidine is believed to have an important role as a buffer which relates to the imidazol group of histidine (Suyama *et al.*, 1986; Van der Boon *et al.*, 1989). The predominance of histidine in the muscle tissue pool is, however, characteristic of only a few species. Wilson and Poe (1974) did not find either histidine or a derivative of histidine as the most abundant free essential amino acid in the muscle of channel catfish. Furthermore, Lyndon *et al.*, (1993)

found that arginine was the most abundant free amino acid in the white muscle tissue of Atlantic cod. The presence of glycine as the predominant free amino acid in fish tissue seems to be a common phenomenon. Jüss (1980) suggested, on the basis of its low molecular weight, that glycine is the most suitable amino acid for use as an osmotic effector.

Comparisons between the concentrations of plasma amino acids in fish fed diet 2 in the present experiment (seawater), and Experiment 1 (freshwater) showed that there was no marked difference in the levels of TAA. The levels of branched-chain amino acids, arginine, lysine, and threonine in fish fed diet 2 in the present experiment were higher than those in fish in Experiment 1. Their increases, however, corresponded to the levels supplied in the diet. Plasma amino acid concentrations in fish did not seem to be affected by salinity of water in the present experiment.

In considering the above results, no guidance was found to support the hypothesis that free glycine is absorbed at a faster rate than glycine derived from digestion of intact protein. Furthermore, excess glycine in fish fed diet 3 may have exerted a toxic effect. Fish fed the diet supplemented with arginine, histidine, lysine, methionine, and threonine to simulate the concentrations in fish muscle protein showed higher concentrations of these amino acids in plasma and muscle free pool. The concentrations of some of these amino acids in the muscle were higher than those in muscle of fish fed the diet containing similar ingredients until at least 26 h postprandial indicating that these amino acids were still available in the tissue for protein synthesis at least at the time of feeding on the next day. Lastly, the results did not suggest any effect of water salinity on plasma amino acid concentrations in the fish.

CHAPTER 5

EXPERIMENT 3

PATTERNS AND CONCENTRATIONS OF FREE AMINO ACIDS IN THE PLASMA OF RAINBOW TROUT FED FISH MEAL AS THE PRINCIPAL SOURCE OF PROTEIN IN DIETS CONTAINING 6% OR 24% OF LIPID

5.1 INTRODUCTION

Fish, like other animals, eat to satisfy energy requirements. If diets contain inadequate amount of energy part of dietary protein will be potentially utilized as a source of energy (Walton, 1985). Dietary lipid and carbohydrates may to a limited extent spare protein (Cho, 1985; Cho and Kaushik, 1990). The benificial effects of the incorporation of such protein-sparing nutrients have been found in several species of fish (Gropps *et al.*, 1982; Lie *et al.*, 1988; De Silva *et al.*, 1991). The inclusion of dietary lipid at high levels have, however, been found to delay gastric evacuation in other animals (Gitler, 1964; Mclaughlan and Morrison, 1968). Windell *et al.*, (1972) suggested that 15% or higher dietary lipid may reduce gastric motility in rainbow trout.

The results from the previous two experiments showed that plasma concentrations of amino acids increased after consumption and digestion of protein, and were correlated with the amino acid concentrations in the dietary protein. The changes in the plasma concentrations of different amino acids also indicated that different proteins were digested at different rates. The patterns of plasma free amino acids were, therefore, used as indicators of the time required for protein digestion, and the entrance of free amino acids into the circulation when diets contained 6 or 24% lipid.

5.2 MATERIALS AND METHODS

5.2.1 Diets

The compositions of the two diets formulated for this experiment are shown in Table 3.1. Diets 1 and 2 were formulated to contain 6 and 24% lipid, respectively. The additional level of lipid in diet 2 was added at the expense of ground wheat in the formulation. The dietary concentration of protein was maintained by increasing the proportion of herring meal. Due to the mechanical problems associated with pelleting diets containing high concentrations of oil, part of the herring oil was added to the feed before pelleting and the remainder of the oil was sprayed on after pelleting. The protein concentration in both diets was 40%.

5.2.2 Fish

Rainbow trout weighing between 400 and 500 g were distributed randomly into eight 150 L tanks so that each tank contained five to seven fish. Each of the two experimental diets was then assigned randomly to four groups of fish (i.e. four replicate groups). The fish were transferred from the pre-experimental diet (EWOS) to the test diets over a period of one week. The feeding of the experimental diets was then continued for another week. The fish were fed once daily to satiation every morning.

The study was conducted at the UBC aquarium facilities, and the experimental conditions were similar to those described for experiments 1.1 and 1.2 except water temperature was 15°C and photoperiod was 14 h.

5.2.3 Sampling Procedure

At the conclusion of the experiment, blood samples were taken from the fish at 6, 12, 24, and 36 h after the last feeding. At each sampling time, two fish from one replicate tanks on each of the respective diets were caught, and anesthetized with 0.01% tricaine-methanesulfonate (MS-222). The procedures for blood sampling and for treatment of blood and plasma samples were the same as described in the previous experiments except analyses were conducted on blood samples from individual fish.

Table 3.1. Ingredient and proximate composition (air-dry basis) of diets used in Experiment 3

Ingredient	Diet 1	Diet 2
	g/kg	g/kg
Herring meal (whole steam-dried)	445.50	495.00
Ground wheat[1]	474.50	237.00
Herring oil[2]	10.00	190.00
Calcium monophosphate.H_2O	10.00	18.00
Calcium lignosulphonate	20.00	20.00
Premix[3]	40.00	40.00
Total	1000.00	1000.00
Calculated analysis[4]		
Crude protein (%)	40.00	40.00
Ether-extractable lipid (%)	6.00	24.00
Ash (%)	7.56	8.65
Gross energy	4390	5359

[1] Autoclaved at 120°C for 1.5 hours.

[2] Stabilized with 0.05% ethoxyquin

[3] The premix supplied the following per kg of diet as fed: thiamin HCl, 67.3 mg; riboflavin, 104.2 mg; niacin, 400 mg; biotin, 5 mg; folic acid, 25 mg; pyridoxine HCl, 60.8 mg; cyanocobalamine, 0.1 mg; D-calcium pantothenate, 218.3 mg; ascorbic acid, 1500 mg; choline chloride, 7680.5 mg; inositol, 2000 mg; menadione 30 mg; vitamin A, 10,000 IU; vitamin D_3, 1000 IU; vitamin E, 1000 IU; Mg (as $MgSO_4$), 380 mg; Mn (as $MnSO_4.H_2O$), 17 mg; Zn (as ZnO), 50 mg; Fe (as $FeSO_4.7H_2O$), 85 mg; Cu (as $CuSO_4.5H_2O$), 2 mg; Co (as $CoCl.6H_2O$), 0.003 mg; K (as K_2SO_4), 895 mg; I (as KIO_3), 5 mg; NaCl (as NaCl), 2830 mg; F (as NaF), 4.5 mg; Se (as $Na_2SeO_3.5H_2O$), 0.10 mg.

[4] The values for crude protein, ether-extractable lipid, and ash were calculated using values from NRC (1984). The values for gross energy were estimated by ascribing 5.65 kcal/g crude protein, 9.5 kcal/g crude lipid, 4.0 kcal/g carbohydrate (Alexis *et al.*, 1985)

5.3 RESULTS

5.3.1 Amino Acid Compositions of Experimental Diets

The calculated (NRC, 1984) amino acid composition of two diets is shown in Table 3.2. Although, the proportions of fish meal and ground wheat in the two diets differed slightly, the concentrations of most amino acids were similar, except lysine.

Table 3.2. Amino acid composition of experimental diets used in Experiment 3[1]

Amino acid	Experimental diet[2]	
	Diet 1	Diet 2
	g/16g N	
Arg	6.2	6.4
His	2.2	2.3
Ile	4.4	4.4
Leu	7.2	7.3
Lys	6.9	7.4
Met	2.6	2.7
Cys	1.1	1.1
Phe	4.1	3.9
Thr	3.9	4.0
Tyr	3.1	3.1
Val	5.7	5.9
EAA	47.4	48.5

[1] Calculated from values given in NRC handbook (1984).
[2] Diet 1 contained 6% lipid, diet 2 contained 24% lipid (as-fed basis).

5.3.2 Diet Acceptance

Fish fed the diet containing 6% lipid had poor appetite, whereas those fed the diet with 24% lipid had excellent appetite. Feed consumption of fish fed the low lipid diet was about two-thirds that of fish fed 24% lipid diet during the entire feeding trial and on the sampling day.

5.3.3 Plasma Amino Acid Profile

The plasma free amino acid concentrations expressed as μmol/mL and as percentage of the total plasma free amino acids as determined at different times after meal consumption are presented in Tables 3.3-3.4. The concentrations of total amino acids (TAA), total essential amino acids (EAA), total non-essential amino acids (NEAA), and ratios of EAA/NEAA in fish fed the two diets are also included in these tables. The changes in plasma concentrations of total essential amino acids, total non-essential amino acids, and total amino acids following meal consumption are displayed in Figure 3.1. Alterations in plasma concentrations of individual amino acids at different times postprandial are also presented graphically in Figure 3.2. The plasma amino acid concentrations at 24 h postprandial were used to represent the amino acid concentrations at the time of feeding (0 h).

Plasma Total Essential Amino Acids (EAA), Total Non-Essential Amino Acids (NEAA), and Total Amino Acids (TAA)

The patterns of plasma EAA concentrations in fish fed the diets containing 6% and 24% lipid were similar (Figure 3.1a). EAA concentrations peaked between 12 and 24 h after feeding. Subsequently, they gradually declined to lower levels at 36 h. The concentrations of EAA in fish fed the high lipid diet were consistently

higher than those in fish fed the low lipid diet. Plasma NEAA concentrations in fish fed the two diets did not follow the same pattern. At the time of feeding, plasma NEAA concentrations in fish fed the diet containing 6% lipid had not declined from the last feeding 24 h previously. The level declined at 6 h after which it rose and peaked at 24 h postprandial. Plasma NEAA concentrations in fish fed the diet containing 24% lipid, on the other hand, increased after feeding and peaked as early as 6 h and then gradually diminished to the same level as that in fish fed the 6% lipid diet at 36 h postprandial (Figure 3.1b). In the case of TAA, the levels in fish that were fed the high lipid diet were fairly consistent from the time of feeding until 24 h postprandial (Fig 3.1c), whereas the level in fish fed the 6% lipid diet fluctuated and showed a clear peak at 24 h postprandial. Furthermore, the TAA in fish fed the 24% lipid diet was higher than that in fish fed the 6% lipid diet throughout the sampling period. The levels of TAA in both groups of fish approached similar concentrations by 36 h after feeding.

Plasma Concentrations of Individual Amino Acids in Fish Fed Different Diets

Essential Amino Acids

Although there were similarities in the fluctuations of plasma EAA between fish fed high and low lipid content diets, there were considerable differences in the patterns of some of the individual essential amino acids between fish fed the two diets. While plasma branched-chain amino acids in fish fed the 6% lipid diet peaked at 12 h, the concentrations of the same amino acids in fish fed the 24% lipid diet did not peak until 24 h postprandial (Figures 3.2e-f, and 3.2h). Plasma arginine, lysine, and phenylalanine in fish fed the former diet, on the other hand, peaked at 24

h, and in fish fed the latter peaked earlier between 6-12 h (Figures 3.2a, and 3.2c-d). The similar trends were noted for fluctuations in the levels of the remaining plasma essential amino acids in fish fed the two diets.

Non-Essential Amino Acids

Alanine, glycine, and serine, which contributed the most to the total for non-essential amino acid concentrations in the plasma (Tables 3.3-3.4), were largely responsible for the pattern of plasma NEAA in fish fed the two different diets (Figures 3.2j,m,o and 3.1b). The distinction in the fluctuations of these amino acids between the two groups of fish was very pronounced. For example, plasma serine concentrations in fish fed the 6% lipid diet were lowest at 6 h whereas those for fish fed the 24% lipid diet were highest. At 24 h, the opposite trend was seen. Dietary treatment did not influence the patterns for the other non-essential amino acids.

Differences in the Percent Increases of Amino acids in Fish Fed Two Diets of Different Lipid content

The differences in the concentrations of plasma amino acids at the peak and at 36 h postprandial in fish fed the two different diets are presented in Figure 3.3. Most plasma amino acids, particularly essential amino acids, in fish fed the 24% lipid diet showed greater increases in concentration compared to those in fish fed the 6% lipid diet.

Table 3.3. Concentrations of plasma amino acids in rainbow trout at different times after feeding diet 1 (6% lipid) in Experiment 3.

Amino acid	Hours after feeding							
	6		12		24		36	
	$\bar{X} \pm$ SEM[1] (µmol/mL)	%[2]	$\bar{X} \pm$ SEM (µmol/mL)	%	$\bar{X} \pm$ SEM (µmol/mL)	%	$\bar{X} \pm$ SEM (µmol/mL)	%
Essential								
Arg	0.259 ± 0.022	6.8	0.378 ± 0.001	7.9	0.458 ± 0.015	8.8	0.272 ± 0.013	8.9
Cys	0.015 ± 0.011	0.4	0.030 ± 0.003	0.6	0.060 ± 0.019	1.2	0.028 ± 0.010	0.9
His	0.169 ± 0.003	4.4	0.181 ± 0.007	3.8	0.225 ± 0.016	4.3	0.144 ± 0.020	4.7
Ile	0.248 ± 0.012	6.5	0.286 ± 0.013	5.9	0.230 ± 0.062	4.4	0.090 ± 0.008	2.9
Leu	0.440 ± 0.021	11.5	0.513 ± 0.026	10.7	0.359 ± 0.084	6.9	0.148 ± 0.012	4.8
Lys	0.139 ± 0.007	3.6	0.231 ± 0.026	4.8	0.450 ± 0.145	8.6	0.180 ± 0.048	5.9
Met	0.164 ± 0.007	4.3	0.192 ± 0.012	4.0	0.160 ± 0.025	3.1	0.078 ± 0.013	2.5
Phe	0.069 ± 0.012	1.8	0.068 ± 0.007	1.4	0.123 ± 0.036	2.4	0.051 ± 0.021	1.7
Thr	0.230 ± 0.015	6.0	0.358 ± 0.018	7.4	0.258 ± 0.024	5.0	0.138 ± 0.029	4.5
Tyr	0.025 ± 0.003	0.7	0.012 ± 0.003	0.2	0.058 ± 0.007	1.1	0.046 ± 0.024	1.5
Val	0.540 ± 0.016	14.1	0.624 ± 0.034	13.0	0.505 ± 0.100	9.7	0.213 ± 0.020	6.9
Non-essential								
Ala	0.209 ± 0.012	5.5	0.285 ± 0.003	5.9	0.475 ± 0.077	9.1	0.327 ± 0.014	10.6
Asp	0.068 ± 0.006	1.8	0.074 ± 0.007	1.5	0.044 ± 0.011	0.8	0.037 ± 0.014	1.2
Glu	0.152 ± 0.009	4.0	0.101 ± 0.017	2.1	0.089 ± 0.017	1.7	0.056 ± 0.016	1.8
Gly	0.539 ± 0.063	14.1	0.739 ± 0.108	15.4	0.975 ± 0.185	18.7	0.768 ± 0.148	25.0
Pro	0.113 ± 0.000	3.0	0.162 ± 0.005	3.4	0.063 ± 0.012	1.2	0.025 ± 0.018	0.8
Ser	0.444 ± 0.008	11.6	0.574 ± 0.003	11.9	0.679 ± 0.016	13.0	0.470 ± 0.036	15.3
TAA[3]	3.823 ± 0.010		4.808 ± 0.242		5.211 ± 0.289		3.071 ± 0.350	
EAA[4]	2.298 ± 0.065		2.873 ± 0.139		2.886 ± 0.530		1.388 ± 0.103	
NEAA[5]	1.525 ± 0.055		1.935 ± 0.103		2.325 ± 0.241		1.683 ± 0.246	
EAA/NEAA	1.51		1.48		1.24		0.82	

[1] The values are means of two fish and standard error of the means (n=2). [2] % of total amino acids. [3] Total amino acids. [4] Total essential amino acids. [5] Total non-essential amino acids

Table 3.4 Concentrations of plasma amino acids in rainbow trout at different times after feeding diet 2 (24% lipid) in Experiment 3.

Amino acid	Hours after feeding							
	6		12		24		36	
	$\bar{X}$ ± SEM[1] (μmol/mL)	%[2]	$\bar{X}$ ± SEM (μmol/mL)	%	$\bar{X}$ ± SEM (μmol/mL)	%	$\bar{X}$ ± SEM (μmol/mL)	%
Essential								
Arg	0.314 ± 0.057	5.3	0.468 ± 0.025	7.9	0.396 ± 0.004	7.0	0.322 ± 0.049	9.6
Cys	0.027 ± 0.008	0.5	0.039 ± 0.001	0.7	0.060 ± 0.003	1.1	0.059 ± 0.005	1.8
His	0.216 ± 0.009	3.7	0.231 ± 0.022	3.9	0.290 ± 0.035	5.1	0.176 ± 0.007	5.3
Ile	0.242 ± 0.020	4.1	0.264 ± 0.043	4.4	0.317 ± 0.066	5.6	0.082 ± 0.018	2.5
Leu	0.415 ± 0.037	7.0	0.466 ± 0.076	7.8	0.572 ± 0.138	10.1	0.124 ± 0.028	3.7
Lys	0.349 ± 0.048	5.9	0.334 ± 0.019	5.6	0.297 ± 0.018	5.2	0.253 ± 0.025	7.6
Met	0.234 ± 0.010	4.0	0.299 ± 0.016	5.0	0.238 ± 0.001	4.2	0.102 ± 0.029	3.0
Phe	0.142 ± 0.031	2.4	0.160 ± 0.025	2.7	0.095 ± 0.021	1.7	0.065 ± 0.031	1.9
Thr	0.446 ± 0.028	7.6	0.529 ± 0.098	8.9	0.478 ± 0.072	8.4	0.184 ± 0.012	5.5
Tyr	0.089 ± 0.013	1.5	0.054 ± 0.022	0.9	0.057 ± 0.027	1.0	0.012 ± 0.000	0.4
Val	0.565 ± 0.039	9.6	0.620 ± 0.081	10.4	0.764 ± 0.212	13.5	0.205 ± 0.048	6.1
Non-essential								
Ala	0.413 ± 0.093	7.0	0.306 ± 0.030	5.2	0.252 ± 0.013	4.4	0.308 ± 0.069	9.2
Asp	0.079 ± 0.004	1.3	0.087 ± 0.003	1.5	0.090 ± 0.011	1.6	0.029 ± 0.009	0.9
Glu	0.281 ± 0.068	4.8	0.122 ± 0.008	2.1	0.174 ± 0.010	3.1	0.120 ± 0.010	3.6
Gly	1.206 ± 0.082	20.4	1.127 ± 0.018	19.0	1.038 ± 0.025	18.3	0.863 ± 0.068	25.8
Pro	0.144 ± 0.020	2.4	0.152 ± 0.031	2.6	0.127 ± 0.024	2.2	0.043 ± 0.003	1.3
Ser	0.736 ± 0.030	12.5	0.681 ± 0.072	11.5	0.430 ± 0.094	7.6	0.399 ± 0.051	11.9
TAA[3]	5.898 ± 0.156		5.939 ± 0.588		5.675 ± 0.427		3.346 ± 0.002	
EAA[4]	3.039 ± 0.140		3.464 ± 0.425		3.564 ± 0.471		1.584 ± 0.046	
NEAA[5]	2.859 ± 0.295		2.475 ± 0.163		2.111 ± 0.044		1.762 ± 0.047	
EAA/NEAA	1.06		1.40		1.69		0.90	

[1] The values are means of two fish and standard error of the means (n=2). [2] % of total amino acids. [3] Total amino acids. [4] Total essential amino acids. [5] Total non-essential amino acids.
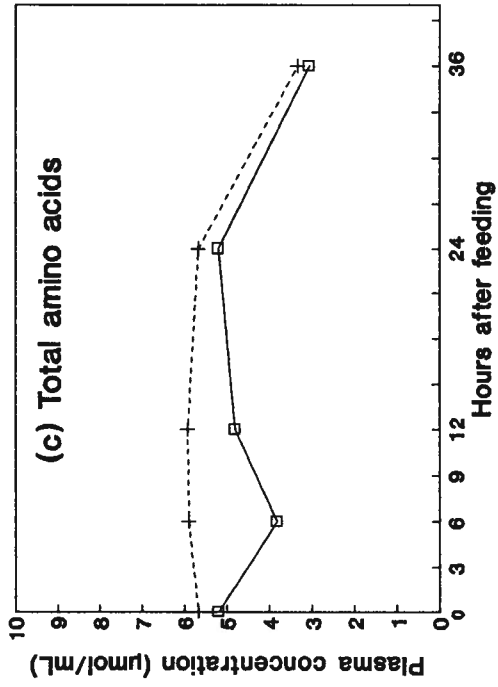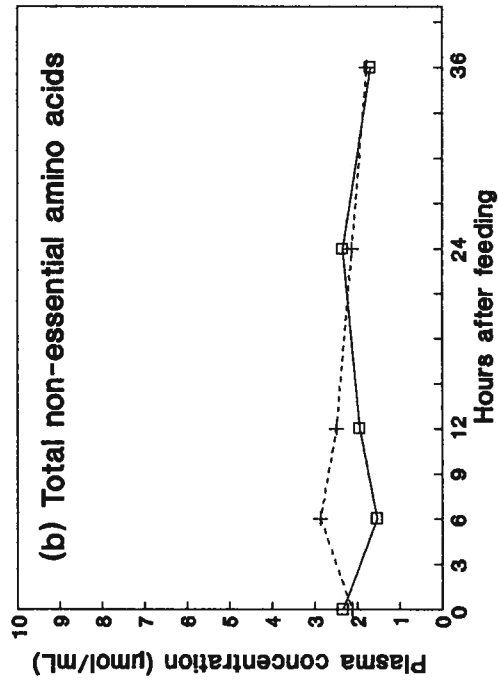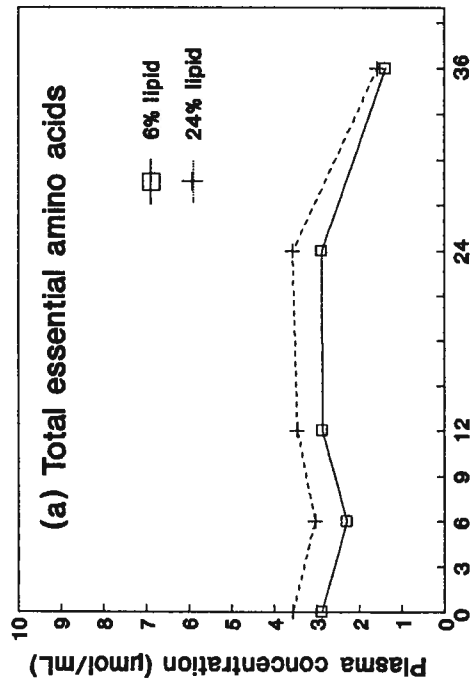
Figure 3.1. Total concentrations of plasma amino acids (essential, non-essential, and total amino acids) in rainbow trout determined at different times after feeding in Experiment 3. Diet 1 = 6% lipid, diet 2 = 24% lipid. Each point represents a mean of two fish (n=2).
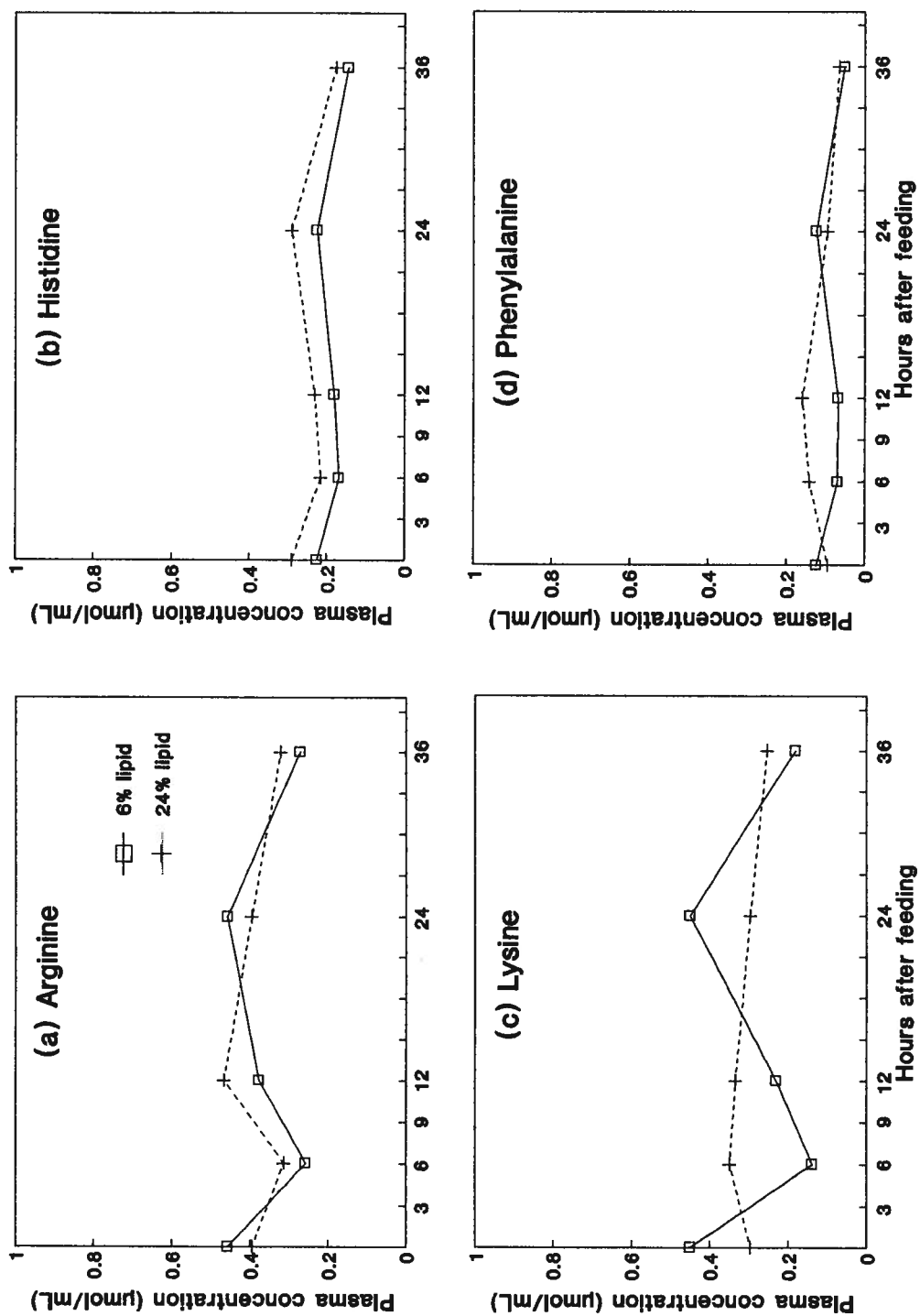
Figure 3.2. Plasma concentrations of amino acids in rainbow trout fed different diets in Experiment 3. Diet 1 = 6% lipid, diet 2 = 24% lipid. Each point represent a mean of two fish (n=2).

(e) Isoleucine

(f) Leucine

(g) Methionine+Cystine

(h) Valine

6% lipid

24% lipid

Plasma concentration (μmol/mL)

Hours after feeding

Figure 3.2. (Continued)

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Figure 3.2. (Continued)

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Figure 3.2. (Continued)

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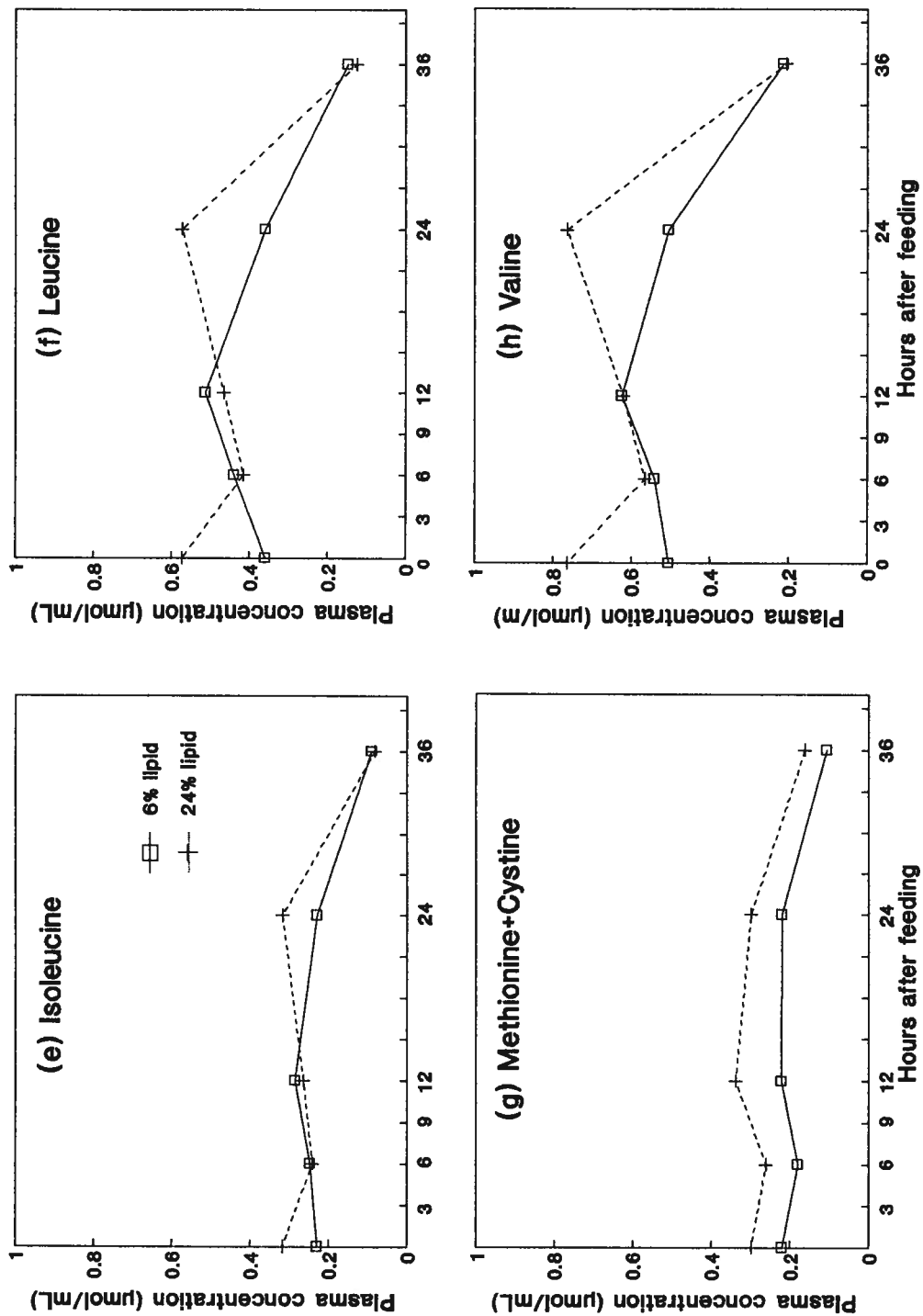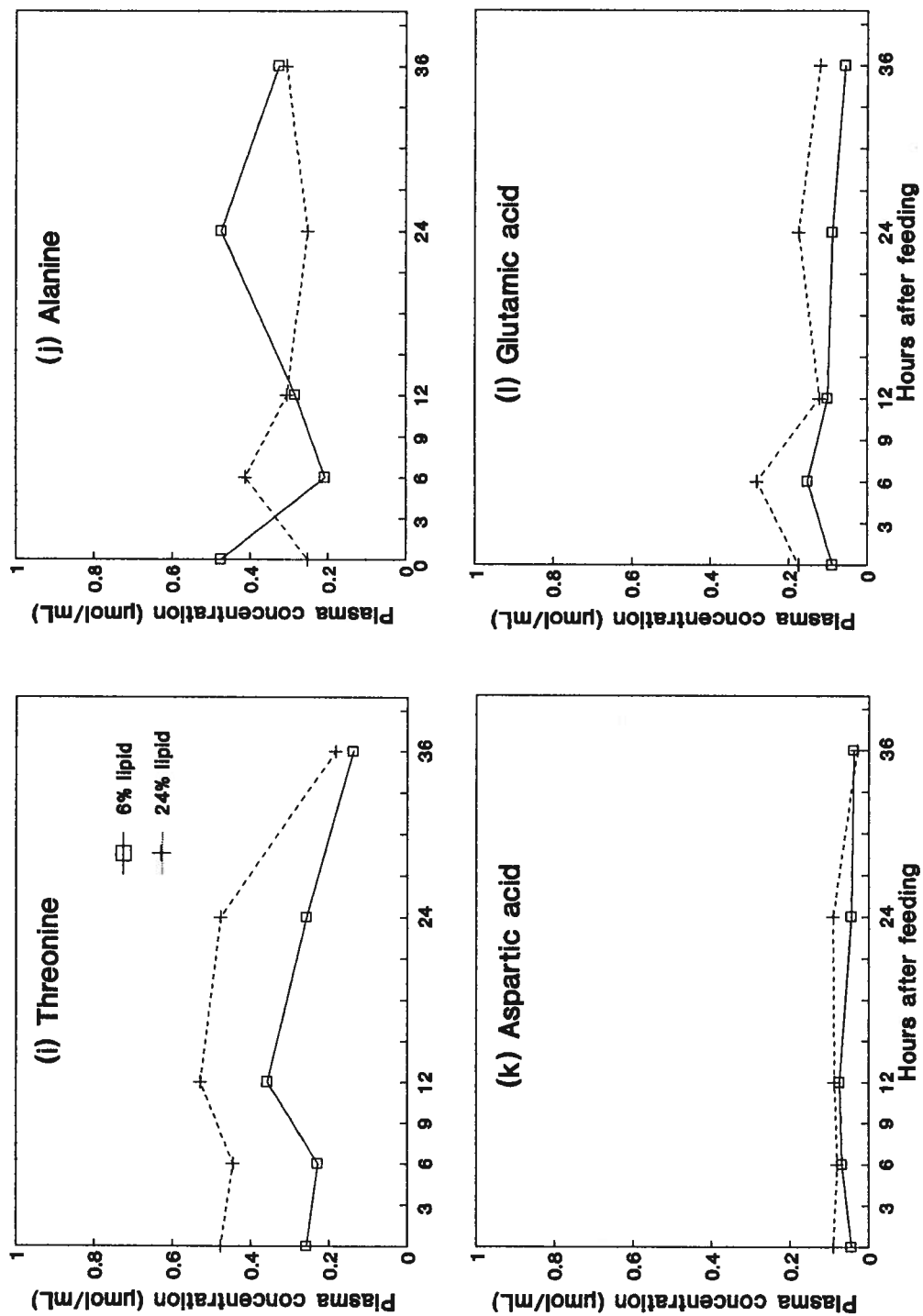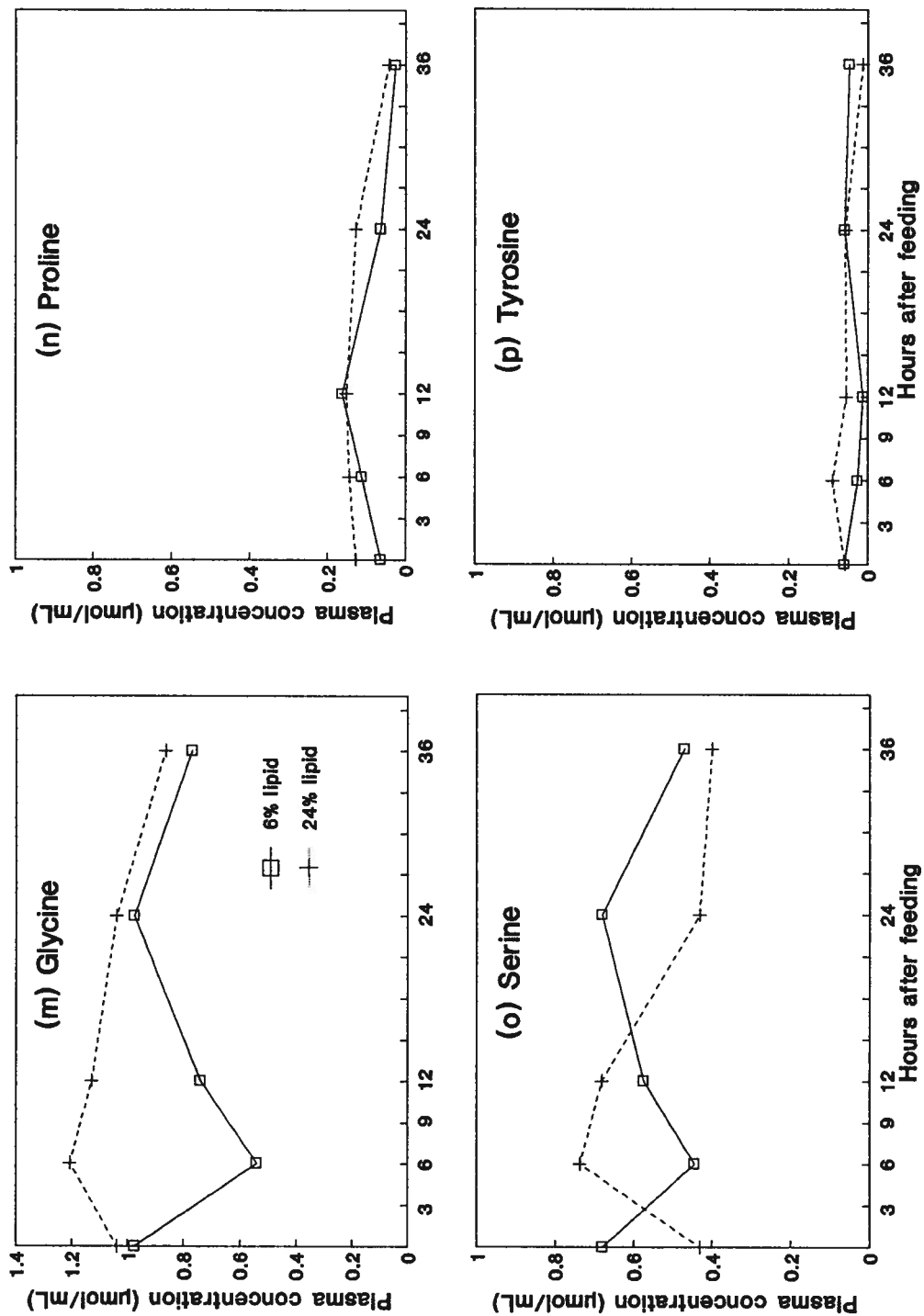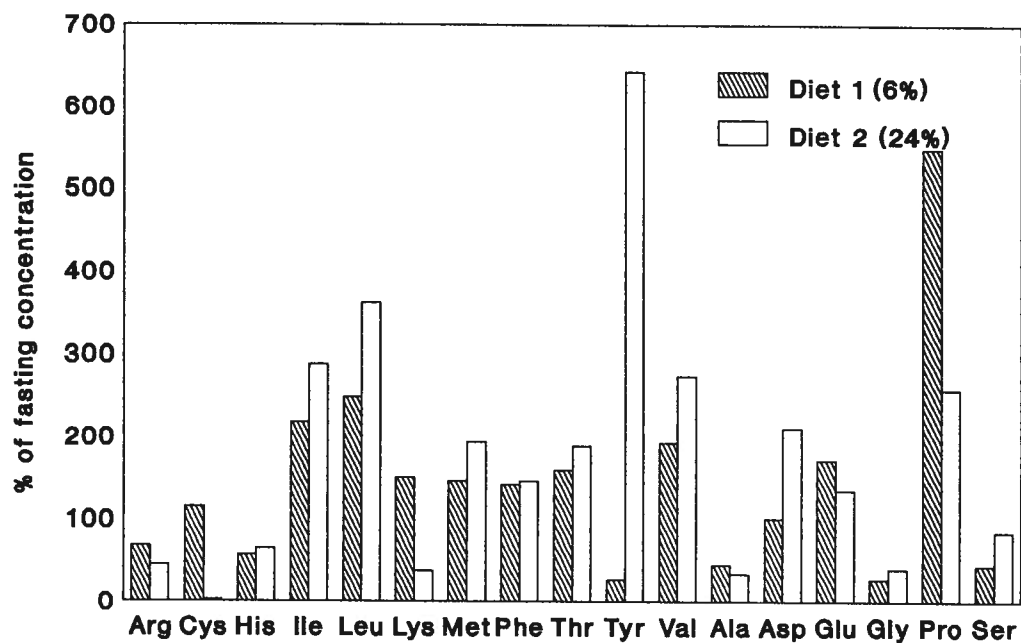
Figure 3.3. Percentage increases of plasma amino acids at peak time in comparison with the concentrations at 36 h after feeding in rainbow trout fed two different diets in Experiment 3. Diet 1 = 6% lipid, diet 2 = 24% lipid.

5.4 DISCUSSION

It is well known that animals eat to satisfy their energy need. This has been demonstrated in fish by several researchers (Cho *et al.*, 1976; Jobling, 1980; Jobling, 1983; Wilson *et al.*, 1985; Davies, 1989). The results on feed consumption in the present experiment, however, did not agree with this general trend since feed consumption declined when the diet contained only 6% lipid. This was believed to be due to the low palatability of the diet. It was observed that fish refused to accept the 6% lipid diet early in the acclimation period, whereas fish that were fed the 24% lipid diet were eager to consume food within 1-2 days. Beamish and Medland (1986) also observed in their studies, that large rainbow trout (250-500 g) fed diets low in energy were unable to consume the same amount of food as those fed diets of higher energy content. Moreover, the higher level of ground wheat in the 6% lipid diet in the present experiment may have reduced diet palatability.

Inclusion of lipid at high levels (> 15% of diet) in fish diets has been shown to improve fish growth by its protein sparing action (Takeuchi *et al.*, 1978; Beamish and Thomas, 1984). Windell *et al.*, (1972) stated that a high level of dietary lipid might reduce gastric motility. This would directly affect the rate of digestibility of other food components; protein being the most important. The temporal changes in the levels of plasma essential amino acids in fish fed diet 1 (6% lipid), paralleled those of fish fed diet 2 (24% lipid) in the present experiment. Thus, the protein in the two diets was probably digested at similar rate.

The constancy of the plasma TAA concentrations, and the consistently higher levels of plasma EAA concentrations in fish fed the high lipid diet than in fish fed the low lipid diet possibly reflected the higher food intake in the former group of

fish. Although the temporal changes in plasma EAA concentrations in the two groups of fish were similar, there were differences observed in the patterns of arginine, lysine, phenylalanine, and branched-chain amino acids. While plasma arginine, lysine and phenylalanine concentrations in fish fed the 6% lipid diet were very low at 6 h and did not reach their peaks until 24 h after feeding, the changes in the concentrations of the same amino acids in fish fed the 24% lipid diet were in reverse order. From this it may be inferred that the amounts and the rate at which amino acids were absorbed in fish fed the low lipid diet were lower than in fish fed the high lipid diet. The increase of these amino acids at 6 h in fish that were fed the high lipid diet, on the other hand, showed that the availability of these amino acids for protein synthesis in the peripheral tissues was greater. Wilson *et al.*, (1985), however, did not find any differences in the patterns for serum amino acids in catfish fed diets containing dissimilar protein to energy ratios.

Branched-chain amino acid (BCAA) metabolism mainly occurs in the muscle of salmonids (Hughes *et al.*, 1983). Therefore, changes in the concentration of plasma BCAA will reflect their availability from the digested food for protein synthesis. Differences in the pattern of change of this group of amino acids in the plasma of fish fed low and high lipid diets were noted. Both groups of fish exhibited increases in plasma BCAA concentrations after feeding. In fish that were fed the low lipid diet, BCAA concentrations reached their peaks earlier at 12 h, while the concentrations of these amino acids in fish that were fed the high lipid diet continued to increase and reached higher peaks later at 24 h after feeding. This again suggested that the availability of these amino acids to the fish was higher in fish fed the high lipid diet.

The different levels of dietary lipid also appeared to affect the metabolism of other amino acids in the present experiment. The differences in the time of appearance of alanine, glycine, and serine in the plasma between the two groups of fish were very pronounced. Alanine, serine, and glycine have been shown to be incorporated into glucose or glycogen in various fish species (Nagai and Ikeda, 1973; French *et al.*, 1981; Walton and Cowey, 1982; Moon *et al.*, 1985). The sharp reduction in the plasma levels of these amino acids after 6 h in fish fed the high lipid diet suggest that they were being catabolized at a faster rate than in fish fed the low lipid diet. With a higher level of food intake of the same dietary protein concentration (40%) than fish fed the 6% lipid diet, fish fed the 24% lipid diet would have had an adequate supply of energy for metabolism. The carbon skeletons derived from the transamination of the preceding amino acids probably served mainly as substrates for glycogenesis or lipogenesis instead of being oxidized for energy. Higuera *et al.*, (1977) found increased activity of glucose-6-phosphatase, an enzyme converting glucose-6-phosphate to glucose, in rainbow trout fed a high lipid diet. Furthermore, the reduction in the concentrations of alanine, glycine, and serine at a faster rate than that of essential amino acids in fish fed the high lipid diet in the present experiment suggests that the fish conserved the essential amino acids for protein synthesis. This finding supports results of several studies which have shown that rainbow trout utilize amino acids more efficiently for protein synthesis when diets contain high levels of lipid or energy (Atherton and Aitken, 1970; Lee and Putnam, 1973; Higuera *et al.*, 1977; Gropp *et al.*, 1982).

A confounding factor that might have affected the appearance and concentrations of plasma amino acids in fish fed the 6% lipid diet was the higher proportion of ground wheat in this diet. The higher level of this ingredient with its high content of carbohydrate could have reduced protein digestibility and amino acid absorption. Comparisons of the patterns of change of plasma amino acid concentrations in fish fed the 6% lipid diet in the present experiment with those for fish that were fed the fish meal based diet in Experiment 1.2 (large fish) showed that the patterns were similar. The inclusion of ground wheat at a high level (47% of the diet) in the 6% lipid diet in the present experiment did not seem to have affected the digestibility of the dietary protein.

In conclusion, as far as the appearance of amino acids in the plasma of fish was concerned, a higher concentration of dietary lipid in the present experiment did not appear to delay protein digestion. The higher levels of plasma amino acids in fish fed the high lipid diet than in fish the low lipid diet suggested that the supply of amino acids in the circulation was adequate for protein synthesis. The rapid removal of alanine, glycine, and serine (glucogenic amino acids) from the plasma of the former group of fish suggests that excess amino acids were converted to other intermediates in metabolism.

CHAPTER 6

EXPERIMENT 4

EXAMINATION OF PATTERNS AND CONCENTRATIONS OF FREE AMINO ACIDS IN THE PLASMA OF RAINBOW TROUT AS CRITERIA OF AMINO ACID AVAILABILITY FROM HERRING MEALS SUBJECTED TO HEAT TREATMENT

6.1 INTRODUCTION

High quality proteins are those that are well digested and contain all the essential amino acids necessary for protein synthesis in the proportions that support good growth (Stahman and Woldegiorgis, 1974). A protein source with a well balanced amino acid pattern such as fish meal, therefore, does not always provide sufficient amino acids for maximum protein synthesis and growth of animals if the digestibility of that protein is poor. Heat damage during processing of the feedstuff is one of several factors that alters the digestibility of dietary protein. The amino acid analysis of heat-treated proteins may represent the potential nutritive quality but, their solubility and digestibility are usually altered.

In order to investigate the effect of heat treatment on digestibility of fish meal, temporal changes in plasma amino acid concentrations were followed in rainbow trout fed diets in which herring meal had been subjected to heat treatments. To corroborate the data regarding plasma amino acid patterns in fish on the different dietary treatments, the experimental fish meals were also analyzed for the in vitro pepsin digestibility of their proteins.

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6.2 MATERIALS AND METHODS

6.2.1 Test Protein Sources

A commercial herring meal processed at low temperature (courtesy of Moore Clarke Co) was used as the control protein source in this experiment. The proximate analysis of the meal is shown in Table 4.1.

Table 4.1. Proximate composition of low-temperature dried herring meal

	% of dry matter
Protein	76.3
Ether-extract	10.5
Ash	13.1
Crude fiber	< 1.0

6.2.2 Fish

Rainbow trout used in this experiment ranged in weight from 163.9-199.0 g. They were distributed randomly (11 fish per tank) into sixteen 150 L tanks located at the UBC aquarium facility. Each dietary treatment was assigned at random to four tanks of fish. The fish were fed the experimental diets to satiation once daily at 10:00 hour for 16 days. The daily amounts of food consumed were recorded. The water supply was dechlorinated Vancouver city water. During the experiment, temperature varied between 8.5 and 9.5°C. Water flow rate was 2 L/min, and dissolved oxygen was 8 ppm. Photoperiod was 14 h.

6.2.3 Diets

Four diets were formulated. The control diet, diet 1, contained untreated low-temperature dried herring meal. Diets 2, 3, and 4 contained low-temperature dried herring meal that was further heated for different periods of time. The low-temperature herring meal was weighed out for diets 2, 3, and 4 into the enamel pans (1.5 kg each) and autoclaved at 127°C for 45, 90, and 180 minutes, respectively. All diets were formulated to provide 35% protein, and all other ingredients were the same as shown in Table 4.2.

Table 4.2. Ingredient (air-dry basis) and proximate (dry matter basis) composition of diets used in Experiment 4.

Ingredient	
	g/kg
Herring meal[1]	394.00
Ground wheat[2]	300.00
Dextrin	140.00
Herring oil[3]	81.00
Calcium monophosphate	15.00
Calcium lignosulfonate	30.00
Premix[4]	40.00
Total	1000.00
Proximate analysis[5]	
Crude protein (%)	36.5
Ether-extractable lipid (%)	13.4
Ash (%)	7.0

[1] An equal amount of herring meal which was heat-treated at different time intervals was substituted for low temperature dried herring meal in the basal diet

[2] Autoclaved at 121°C for 1.5 h

[3] Stabilized with 0.05% ethoxyquin

[4] The premix supplied the following per kg of diet as fed: thiamin HCl, 67.3 mg; riboflavin, 104.2 mg; niacin, 400 mg; biotin, 5 mg; folic acid, 25 mg; pyridoxine HCl, 60.8 mg; cyanocobalamine, 0.1 mg; D-calcium pantothenate, 218.3 mg; ascorbic acid, 1500 mg; choline chloride, 4000 mg; inositol, 2000 mg; menadione 30 mg; vitamin A, 10,000 IU; vitamin D_3, 1000 IU; vitamin E, 1000 IU; Mg (as $MgSO_4$), 380 mg; Mn (as $MnSO_4.H_2O$), 17 mg; Zn (as ZnO), 50 mg; Fe (as $FeSO_4.7H_2O$), 85 mg; Cu (as $CuSO_4.5H_2O$), 2 mg; Co (as $CoCl.6H_2O$), 0.003 mg; K (as K_2SO_4), 895 mg; I (as KIO_3), 5 mg; NaCl (as NaCl), 2836 mg; F (as NaF), 4.5 mg; Se (as $Na_2SeO_3.5H_2O$), 0.10 mg.

[5] The values were averaged from the analyses of four diets.

6.2.4 Sampling Procedure

Fish were bled at 3, 6, 12, 18, and 24 h on day 16 after the morning feeding of the experimental diets. At each sampling time (except the last sampling time), four fish from one of the replicate tanks for diets 1, 2, 3, and 4 were sampled. At the last sampling time, fish were taken from the first of the respective replicate tanks sampled at 3 h. This procedure was followed to minimize the stress associated with sampling since only one of the replicate tanks for each treatment was subjected to disturbance. The fish were anesthetized with 0.01% tricaine-methanesulfonate (MS-222) and weighed. The blood was withdrawn from the caudal vein artery complex into 4 mL heparinized vacutainers, and was immediately centrifuged at 750xg for 15 min. The resultant plasma from two fish was pooled, i.e. two plasma samples/tank. The plasma samples were frozen immediately in liquid nitrogen. The plasma was deproteinized soon after blood collection as described in the previous experiments. The deproteinized plasma was kept at -70°C for subsequent analysis for free amino acids.

6.2.5 Pepsin Digestibility of Fish Meal

The effect of the heat-treatment on digestibility of the fish meal protein was assessed by the in vitro pepsin digestibility test. The procedure was similar to the AOAC (1984) method except the concentration of pepsin (activity 1:10,000) in the digestion mixture was reduced to 0.00005% to increase the sensitivity of the test (March and Biely, 1967; Johnston and Coon, 1978; March and Hickling, 1982; Han and Parsons, 1991; Anderson *et al.*, 1993). Moreover, the incubated temperature was 35°C.

Prior to the pepsin digestibility test, samples (of the fish meal from each heat treatment and the control fish meal were extracted with diethyl ether using the Goldfisch apparatus. The pepsin digestibility test was then performed as follows. Samples of approximately 0.3 g of the defatted fish meals were placed in the digestion bottles. The digestion bottles and the end-over-end agitator employed were as described in the AOAC (1984) procedure. A total of 150 mL of 0.00005% pepsin solution[1] was added to the bottles. The bottles were capped, clamped to the agitator, and incubated with constant agitation at 35°C for 16 h. The digestibility assay was performed in duplicate with a sample of each fish meal incubated in HCl solution without pepsin to ascertain the amount of protein solubilized in HCl without the action of the enzyme.

At the end of the incubation, the insoluble residue in each bottle was filtered and dried (AOAC, 1984). Protein present in the insoluble residue was then determined according to the Kjeldahl procedure. The calculation of % indigestible protein was based on the ether-extracted sample weight. The results represented % indigestible protein in the sample. The calculation of percent digestibility of fish meal protein was based on percent crude protein present in the HCl-insoluble residue.

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[1]/ The 0.00005% pepsin solution was prepared as follows: One g of pepsin (activity 1:10,000) was dissolved in 100 mL 0.075N HCl. One hundred μL of the stock solution was then diluted to 2000 mL with freshly prepared 0.075N HCl in a volumetric flask.

6.2.6 Amino Acid Analysis

The determination of plasma amino acid concentrations was conducted as described in the previous experiments.

6.2.7 Statistical Analysis

The data for initial and final weights, weight gain, and feed consumption of fish were subjected to one way analysis of variance. The arcsin transformation was applied to the percentage values for pepsin digestibility prior to analysis of variance. The analysis was performed using Systat (Wilkinson, 1990).

6.3 RESULTS

6.3.1 Pepsin Digestibility of Fish Meal

The values obtained for the pepsin solubilization of protein present in fish meal heated at 127°C for different periods of time are given in Table 4.3 and Figure 4.1. These data were tested statistically by analysis of variance, and the results were tabulated in Appendix 6.

The values for percent digestibility of fish meal protein clearly showed the damaging effects of heat treatment. As fish meal protein was subjected to heat treatment for increasing periods of time, the percent of pepsin digestibility significantly decreased ($P < 0.05$). When fish meal proteins were incubated with HCl alone, percentages of protein left in the residue of control fish meal and in the fish meals that were heated for 45 and 90 min were similar (81.0-81.9% of crude protein). The HCl-insoluble protein of fish meal heated for 180 min was, however, lower (77.2% of crude protein).

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Table 4.3. Pepsin digestibility [1] of protein in low temperature herring meal heated at 127°C for different periods of time in Experiment 4.

	Duration of heat treatment[2] (min)			
	0	45	90	180
% of crude protein not solubilized by pepsin	14.9[3]	23.5	29.2	45.5
% of crude protein not solubilized by HCl alone	81.4	81.9	81.0	77.2
% of crude protein not solubilized by pepsin based on the HCl-insoluble residue	18.3	28.7	36.0	59.0
% of crude protein solubilized by pepsin based on the HCl-insoluble residue	81.7[a4]	71.3 [b]	64.0 [c]	41.0 [d]

[1] Pepsin concentration = 0.00005% (activity 1:10,000)
[2] Autoclaved at 127°C.
[3] Each value is a mean of two pepsin digestibility tests (n=2).
[4] Values within the same row with the same superscript were not significantly different (Tukey HSD test, $P > 0.05$).
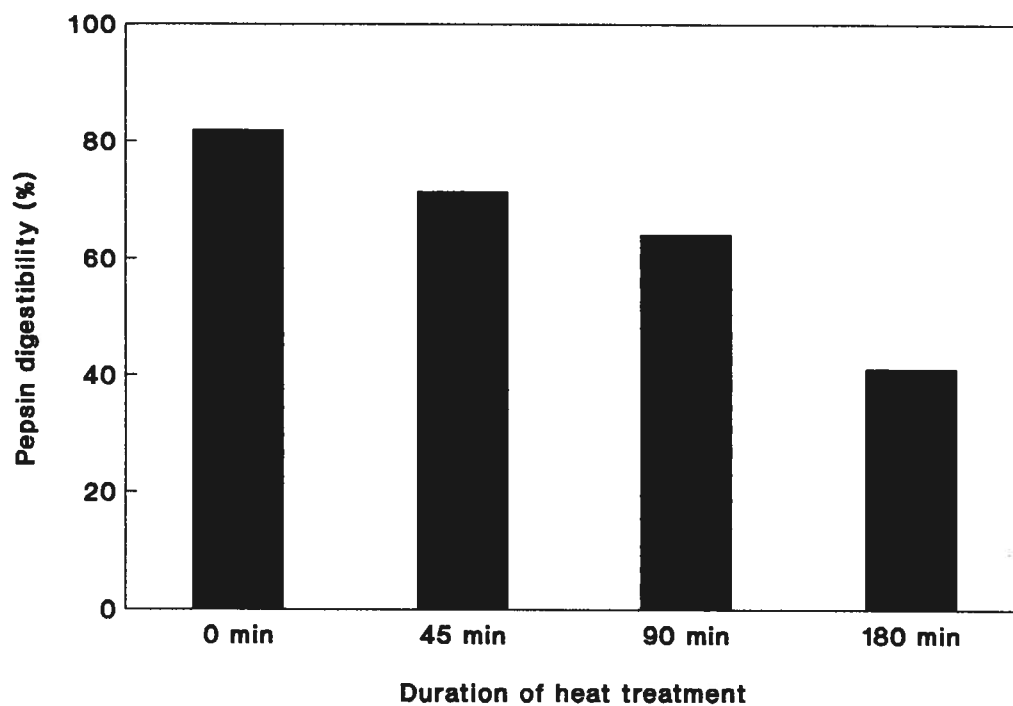
Figure 4.1. Pepsin digestibility of protein in fish meal heated for different periods of time in Experiment 4. Percent digestibility was calculated on the basis of the protein not solubilized by HCl alone.

Table 4.4. Body weight gains and feed consumptions of rainbow trout over a 16-day period in Experiment 4.

Diet[1]	Initial weight	Final weight	Weight gain	Feed consumption
	(g)	(g)	(g/fish)	(g/fish/day)
1	192.2 ± 4.8[2]	237.1 ± 6.7	44.9 ± 4.8	3.8 ± 0.35
2	180.2 ± 6.5	216.8 ± 4.8	36.6 ± 3.1	3.1 ± 0.11
3	179.6 ± 5.8	212.2 ± 10.4	32.6 ± 5.1	3.1 ± 0.21
4	193.7 ± 1.4	227.8 ± 5.9	34.1 ± 5.7	3.1 ± 0.28

[1] Diet 1 = unheated fish meal (control diet); Diet 2 = 45 min-heated fish meal diet; Diet 3 = 90 min-heated fish meal diet; Diet 4 = 180 min-heated fish meal diet.

[2] Each value represents a mean of four tanks, eleven fish/tank, (n=4). No significant difference among means in the same column was detected.

Table 4.5. Specific growth rates, and feed conversion efficiencies for rainbow trout fed different diets in Experiment 4.

Diet[1]	Specific Growth Rate[2]	Feed Efficiency[3]
	%	
1	1.31[4]	0.74
2	1.16	0.74
3	1.03	0.67
4	1.01	0.69
Pooled SEM	0.135	0.074

[1] Diet 1 = unheated fish meal (control diet); Diet 2 = 45 min heated fish meal diet; Diet 3 = 90 min heated fish meal diet; Diet 4 = 180 min heated fish meal diet.

[2] $= (lnW2-lnW1)*100/(T2-T1)$

[3] = Gain in body weight/feed consumption

[4] Each value represents a mean of four tanks, eleven fish/tank, (n=4). No significant difference among means in the same column was detected.

6.3.2 Responses of Fish to Experimental Diets

Data for initial and final weights, weight gains and feed consumptions of fish fed the different diets are provided in Table 4.4. Values for specific growth rates and feed efficiencies of the fish are given in Table 4.5. These data were subjected to one-way analysis of variance, and the statistical results are shown in Appendix 7-8. Dietary treatment did not have a significant effect on any of the parameters mentioned above.

6.3.3 Plasma Amino Acid Profile

The free amino acid levels in plasma expressed as μmol/mL and as percentages of the total plasma amino acids of fish as determined at different times postprandial are shown in Table 4.6-4.9. The levels of total amino acids (TAA), total essential amino acids (EAA), total non-essential amino acids (NEAA), and the ratios of EAA/NEAA of fish fed different diets were also calculated and included in these tables. The fluctuations in plasma concentrations of total essential amino acids, total non-essential amino acids, and total amino acids are displayed in Figure 4.2. Furthermore, the changes in plasma concentrations of individual amino acids at different times postprandial are illustrated in Figure 4.3. Assuming that the plasma amino acid concentrations at 24 h after meal consumption represented the concentrations at the time of feeding when the fish were fed once daily, plasma amino acid concentrations at 24 h were used to represent the concentrations at 0 h (feeding time).

Plasma Total Essential Amino Acid (EAA), Total Non-Essential Amino Acids (NEAA), and Total Amino Acids (TAA)

As shown in Figure 4.2a, the fluctuations in plasma concentrations of EAA in fish fed diets 1, 2, and 3 did not differ markedly from each other. The concentrations increased after feeding and peaked only once, between 6-12 h depending upon the diets. The pattern for changes of plasma EAA concentrations in fish fed diet 4 (fish meal heated for 180 min) differed notably from those of fish fed the other three diets. Plasma EAA concentrations in fish of this group peaked twice, once at 3 h and again at 18 h postprandial. The magnitude of the fluctuations of plasma NEAA in fish fed different diets was less than those noted for the EAA concentrations. With this in mind, differences in the alterations of plasma NEAA in fish fed diets 1, 2 and 3, and that in fish fed diet 4 were noticeable. The concentrations of NEAA in fish fed the former three diets increased and peaked between 6-12 h after feeding where they gradually subsided (Figure 4.2b). In the fish that were fed diet 4, the concentrations increased at 3 h, and then the levels declined, and remained low until 24 h after feeding.

Plasma Concentrations of Individual Amino Acids in Fish Fed Different Diets

Essential Amino Acids

The fluctuations of most individual plasma essential amino acids in fish fed diets 1, 2 and 3 followed the overall patterns of EAA in that the levels increased after feeding and reached peak values between 6 and 12 h depending upon the amino acid under consideration (Figure 4.3). Some variations from this pattern in fish fed these three diets were found with respect to changes in the concentrations of branched-chain amino acids. The levels of these amino acids in fish fed diet 1

increased from the time of feeding and stayed at a high level between 6-12 h after feeding. The levels of the same group of amino acids in fish fed diet 2, on the other hand, rose from the time of feeding and reached the peak at 6 h after which time the concentrations dropped sharply. In the fish that were fed diet 3, the concentrations of branched-chain amino acids remained low at 3 h after which time they increased and peaked between 6 and 12 h after feeding. The plasma concentrations of arginine, phenylalanine, and tyrosine in fish fed diets 1, 2, and 3 fluctuated only slightly (Figures 4.3a,d,p).

The fluctuations in the plasma concentrations of isoleucine, leucine, lysine, methionine + cystine, and valine in fish fed diet 4 were similar to those of EAA with respect to the same diet, i.e they peaked twice, once at 3 h and again at 18 h after feeding. Plasma concentrations of arginine, histidine, phenylalanine, threonine, and tyrosine, however, showed very small variations (Figures 4.3a-i).

Non-Essential Amino Acids

As shown in Figures 4.3j,l,n,o, the alterations in the levels of plasma alanine, glutamic acid, proline, and serine in fish fed diets 1, 2 and 3 were comparable to the overall pattern of NEAA with respect to the diets, i.e. peak values were found between 6 and 12 h after feeding, and then the levels gradually subsided. Plasma glycine concentrations in fish fed the foregoing three diets, nevertheless, behaved differently from each other over the sampling period. The responses of plasma non-essential amino acids in fish fed diet 4 were interesting. While the changes in plasma alanine and serine concentrations showed two peaks, one at 3 h and the other at 18 h, resembling those for EAA, plasma glycine concentrations fluctuated

from time to time during the sampling period. Plasma concentrations of aspartic acid, glutamic acid, and proline remained low and invariable. Moreover, the data from Tables 4.6-4.9 indicated that levels of most non-essential amino acids, particularly glutamic acid, in fish fed diet 4 were notably lower than in fish fed the other three diets.

Table 4.6. Concentrations of plasma amino acids in rainbow trout at different times after feeding diet 1 (control) in Experiment 4.

Amino acid	Hours after feeding									
	3		6		12		18		24	
	$\bar{X} \pm SEM$[1] (μmol/mL)	%[2]	$\bar{X} \pm SEM$ (μmol/mL)	%	$\bar{X} \pm SEM$ (μmol/mL)	%	$\bar{X} \pm SEM$ (μmol/mL)	%	$\bar{X} \pm SEM$ (μmol/mL)	%
Essential										
Arg	0.207 ± 0.011	4.9	0.159 ± 0.003	2.7	0.186 ± 0.008	3.7	0.189 ± 0.007	4.7	0.170 ± 0.004	6.2
Cys	0.018 ± 0.001	0.4	0.035 ± 0.009	0.6	0.044 ± 0.027	0.9	0.017 ± 0.001	0.4	tr[3]	
His	0.227 ± 0.028	5.4	0.349 ± 0.036	5.9	0.272 ± 0.031	5.4	0.255 ± 0.011	6.4	0.205 ± 0.005	7.5
Ile	0.203 ± 0.003	4.8	0.397 ± 0.105	6.7	0.360 ± 0.144	7.2	0.198 ± 0.005	5.0	0.088 ± 0.017	3.2
Leu	0.356 ± 0.009	8.5	0.694 ± 0.187	11.8	0.666 ± 0.248	13.3	0.375 ± 0.001	9.4	0.159 ± 0.030	5.8
Lys	0.338 ± 0.032	8.0	0.356 ± 0.029	6.1	0.252 ± 0.068	5.0	0.192 ± 0.013	4.8	0.133 ± 0.020	4.8
Met	0.128 ± 0.013	3.0	0.217 ± 0.010	3.7	0.175 ± 0.034	3.5	0.162 ± 0.003	4.1	0.093 ± 0.008	3.4
Phe	0.137 ± 0.009	3.3	0.119 ± 0.003	2.0	0.167 ± 0.051	3.3	0.156 ± 0.030	3.9	0.169 ± 0.003	6.2
Thr	0.244 ± 0.022	5.8	0.339 ± 0.002	5.8	0.275 ± 0.123	5.5	0.252 ± 0.062	6.3	0.129 ± 0.030	4.7
Tyr	0.080 ± 0.004	1.9	0.110 ± 0.008	1.9	0.087 ± 0.016	1.8	0.070 ± 0.006	1.8	0.057 ± 0.001	2.1
Val	0.447 ± 0.012	10.6	0.888 ± 0.206	15.1	0.812 ± 0.275	16.2	0.529 ± 0.005	13.2	0.260 ± 0.031	9.5
Non-essential										
Ala	0.435 ± 0.015	10.3	0.469 ± 0.017	8.0	0.461 ± 0.130	9.2	0.356 ± 0.027	8.9	0.300 ± 0.068	10.9
Asp	0.044 ± 0.004	1.1	0.042 ± 0.001	0.7	0.041 ± 0.015	0.8	0.044 ± 0.006	1.1	0.035 ± 0.002	1.3
Glu	0.083 ± 0.022	2.0	0.149 ± 0.013	2.5	0.134 ± 0.035	2.7	0.096 ± 0.007	2.4	0.088 ± 0.011	3.2
Gly	0.681 ± 0.100	16.2	0.770 ± 0.181	13.1	0.518 ± 0.009	10.3	0.656 ± 0.013	16.4	0.523 ± 0.017	19.1
Pro	0.101 ± 0.000	2.4	0.117 ± 0.028	2.0	0.112 ± 0.056	2.2	0.072 ± 0.019	1.8	tr	
Ser	0.475 ± 0.051	11.3	0.667 ± 0.010	11.5	0.444 ± 0.071	8.9	0.375 ± 0.050	9.4	0.333 ± 0.066	12.1
TAA[4]	4.203 ± 0.346		5.877 ± 0.589		5.007 ± 1.342		3.995 ± 0.013		2.743 ± 0.386	
EAA[5]	2.385 ± 0.104		3.663 ± 0.778		3.297 ± 1.026		2.395 ± 0.017		1.462 ± 0.187	
NEAA[6]	1.819 ± 0.243		2.214 ± 0.189		1.710 ± 0.316		1.600 ± 0.005		1.281 ± 0.199	
EAA/NEAA	1.31		1.65		1.93		1.50		1.14	

[1] Mean of two pools of plasma (two fish/pool) and standard error of the mean (n=2). [2] % of total amino acids. [3] Present in a very small amount. [4] Total amino acids. [5] Total essential amino acids. [6] Total non-essential amino acids.

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Table 4.7. Concentrations of plasma amino acids in rainbow trout at different times after feeding diet 2 (45 min-heated fish meal) in Experiment 4.

Amino acid	Hours after feeding									
	3		6		12		18		24	
	$\bar{X} \pm SEM$[1] (μmol/mL)	%[2]	$\bar{X} \pm SEM$ (μmol/mL)	%	$\bar{X} \pm SEM$ (μmol/mL)	%	$\bar{X} \pm SEM$ (μmol/mL)	%	$\bar{X} \pm SEM$ (μmol/mL)	%
Essential										
Arg	0.261 ± 0.019	5.3	0.149 ± 0.002	2.5	0.223 ± 0.029	4.7	0.174 ± 0.002	4.6	0.163 ± 0.008	4.2
Cys	0.020 ± 0.001	0.4	0.021 ± 0.006	0.4	0.037 ± 0.015	0.7	0.016 ± 0.000	0.4	0.014 ± 0.002	0.4
His	0.246 ± 0.002	5.0	0.296 ± 0.008	5.1	0.315 ± 0.039	6.4	0.209 ± 0.017	5.5	0.265 ± 0.002	6.8
Ile	0.263 ± 0.003	5.3	0.393 ± 0.071	6.7	0.242 ± 0.062	4.9	0.182 ± 0.056	4.8	0.184 ± 0.004	4.7
Leu	0.456 ± 0.007	9.2	0.703 ± 0.123	12.0	0.439 ± 0.131	8.9	0.345 ± 0.110	9.0	0.338 ± 0.004	8.7
Lys	0.342 ± 0.019	6.9	0.352 ± 0.025	6.0	0.226 ± 0.034	4.6	0.175 ± 0.013	4.6	0.161 ± 0.042	4.1
Met	0.159 ± 0.004	3.2	0.214 ± 0.021	3.6	0.133 ± 0.030	2.7	0.188 ± 0.004	4.9	0.163 ± 0.017	4.2
Phe	0.183 ± 0.028	3.7	0.114 ± 0.006	2.0	0.179 ± 0.037	3.6	0.157 ± 0.030	4.1	0.105 ± 0.009	2.7
Thr	0.245 ± 0.018	5.0	0.297 ± 0.026	5.1	0.264 ± 0.009	5.4	0.241 ± 0.001	6.3	0.239 ± 0.059	6.1
Tyr	0.113 ± 0.010	2.3	0.097 ± 0.010	1.7	0.102 ± 0.011	2.1	0.084 ± 0.016	2.2	0.073 ± 0.008	1.9
Val	0.565 ± 0.006	11.4	0.869 ± 0.143	14.8	0.555 ± 0.162	11.3	0.468 ± 0.102	12.2	0.492 ± 0.000	12.6
Non-essential										
Ala	0.461 ± 0.048	9.3	0.552 ± 0.043	9.4	0.461 ± 0.080	9.3	0.304 ± 0.012	7.9	0.290 ± 0.037	7.4
Asp	0.054 ± 0.001	1.1	0.058 ± 0.002	1.0	0.055 ± 0.002	1.1	0.033 ± 0.003	0.9	0.034 ± 0.005	0.9
Glu	0.101 ± 0.009	2.1	0.116 ± 0.003	2.0	0.107 ± 0.001	2.2	0.080 ± 0.011	2.1	0.095 ± 0.005	2.4
Gly	0.751 ± 0.006	15.2	0.857 ± 0.039	14.6	0.897 ± 0.200	18.2	0.696 ± 0.071	18.2	0.792 ± 0.221	20.3
Pro	0.126 ± 0.004	2.5	0.158 ± 0.033	2.7	0.087 ± 0.007	1.8	0.052 ± 0.005	1.4	0.050 ± 0.003	1.3
Ser	0.592 ± 0.029	12.0	0.618 ± 0.057	10.5	0.597 ± 0.133	12.1	0.429 ± 0.055	11.2	0.452 ± 0.058	11.6
TAA[3]	4.938 ± 0.230		5.864 ± 0.861		4.919 ± 0.157		3.833 ± 0.123		3.910 ± 0.662	
EAA[4]	2.853 ± 0.144		3.505 ± 0.624		2.715 ± 0.260		2.239 ± 0.271		2.197 ± 0.197	
NEAA[5]	2.085 ± 0.086		2.359 ± 0.237		2.204 ± 0.417		1.594 ± 0.148		1.713 ± 0.466	
EAA/NEAA	1.37		1.49		1.23		1.40		1.28	

[1] Mean of two pools of plasma (two fish/pool) and standard error of the mean (n=2). [2] % of total amino acids. [3] Total amino acids. [4] Total essential amino acids. [5] Total non-essential amino acids.

Table 4.8. Concentrations of plasma amino acids in rainbow trout at different times after feeding diet 3 (90 min-heated fish meal) in Experiment 4.

Amino acid	Hours after feeding									
	3		6		12		18		24	
	X̄ ± SEM[1] (μmol/mL)	%[2]	X̄ ± SEM (μmol/mL)	%	X̄ ± SEM (μmol/mL)	%	X̄ ± SEM (μmol/mL)	%	X̄ ± SEM (μmol/mL)	%
Essential										
Arg	0.223 ± 0.009	5.7	0.197 ± 0.007	3.7	0.194 ± 0.014	3.7	0.170 ± 0.012	4.4	0.146 ± 0.008	4.1
Cys	0.024 ± 0.005	0.6	0.038 ± 0.000	0.7	0.034 ± 0.006	0.7	0.022 ± 0.001	0.6	0.018 ± 0.000	0.5
His	0.193 ± 0.016	4.9	0.283 ± 0.009	5.2	0.286 ± 0.020	5.5	0.235 ± 0.003	6.1	0.225 ± 0.013	6.3
Ile	0.175 ± 0.035	4.5	0.324 ± 0.010	6.0	0.355 ± 0.039	6.8	0.174 ± 0.030	4.5	0.192 ± 0.007	5.4
Leu	0.315 ± 0.068	8.1	0.581 ± 0.024	10.8	0.647 ± 0.066	12.3	0.316 ± 0.056	8.2	0.353 ± 0.013	9.9
Lys	0.211 ± 0.029	5.4	0.347 ± 0.007	6.4	0.271 ± 0.062	5.2	0.199 ± 0.003	5.2	0.180 ± 0.005	5.0
Met	0.131 ± 0.009	3.3	0.208 ± 0.009	3.9	0.180 ± 0.046	3.4	0.180 ± 0.026	4.7	0.148 ± 0.008	4.1
Phe	0.201 ± 0.004	5.1	0.149 ± 0.007	2.8	0.146 ± 0.012	2.8	0.177 ± 0.018	4.6	0.084 ± 0.012	2.4
Thr	0.144 ± 0.032	3.7	0.294 ± 0.030	5.5	0.281 ± 0.018	5.4	0.206 ± 0.026	5.4	0.224 ± 0.009	6.3
Tyr	0.097 ± 0.001	2.5	0.106 ± 0.011	2.0	0.111 ± 0.022	2.1	0.130 ± 0.049	3.4	0.058 ± 0.041	1.6
Val	0.402 ± 0.083	10.3	0.730 ± 0.039	13.5	0.771 ± 0.066	14.7	0.428 ± 0.037	11.2	0.497 ± 0.036	13.9
Non-essential										
Ala	0.393 ± 0.036	10.1	0.501 ± 0.031	9.3	0.432 ± 0.076	8.2	0.315 ± 0.017	8.2	0.339 ± 0.024	9.5
Asp	0.033 ± 0.001	0.9	0.050 ± 0.003	0.9	0.037 ± 0.010	0.7	0.044 ± 0.002	1.2	0.044 ± 0.003	1.2
Glu	0.071 ± 0.002	1.8	0.108 ± 0.007	2.0	0.102 ± 0.011	2.0	0.088 ± 0.005	2.3	0.099 ± 0.005	2.8
Gly	0.778 ± 0.089	19.9	0.741 ± 0.002	13.7	0.686 ± 0.028	13.1	0.642 ± 0.052	16.7	0.580 ± 0.078	16.2
Pro	0.053 ± 0.009	1.4	0.144 ± 0.001	2.7	0.130 ± 0.046	2.5	0.079 ± 0.003	2.1	0.053 ± 0.002	1.5
Ser	0.461 ± 0.025	11.8	0.596 ± 0.065	11.0	0.583 ± 0.048	11.1	0.428 ± 0.023	11.2	0.339 ± 0.004	9.5
TAA[3]	3.905 ± 0.362		5.397 ± 0.118		5.246 ± 0.398		3.833 ± 0.144		3.579 ± 0.162	
EAA[4]	2.116 ± 0.392		3.257 ± 0.634		3.276 ± 0.278		2.237 ± 0.246		2.125 ± 0.003	
NEAA[5]	1.789 ± 0.034		2.140 ± 0.017		1.970 ± 0.120		1.596 ± 0.102		1.454 ± 0.160	
EAA/NEAA	1.18		1.52		1.66		1.40		1.46	

[1] Mean of two pools of plasma (two fish/pool) and standard error of the mean (n=2). [2] % of total amino acids. [3] Total amino acids. [4] Total essential amino acids. [5] Total non-essential amino acids.

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Table 4.9. Concentrations of plasma amino acids in rainbow trout at different times after feeding diet 4 (180 min-heated fish meal) in Experiment 4.

Amino acid	Hours after feeding									
	3		6		12		18		24	
	X̄ ± SEM[1] (μmol/mL)	%[2]	X̄ ± SEM (μmol/mL)	%	X̄ ± SEM (μmol/mL)	%	X̄ ± SEM (μmol/mL)	%	X̄ ± SEM (μmol/mL)	%
Essential										
Arg	0.212 ± 0.004	4.4	0.168 ± 0.002	3.8	0.215 ± 0.013	6.1	0.197 ± 0.005	4.2	0.139 ± 0.002	3.7
Cys	0.075 ± 0.030	1.6	0.036 ± 0.002	0.8	0.051 ± 0.029	1.5	0.041 ± 0.006	0.9	0.057 ± 0.001	1.5
His	0.241 ± 0.006	5.0	0.253 ± 0.005	5.7	0.223 ± 0.003	6.3	0.244 ± 0.019	5.2	0.204 ± 0.018	5.5
Ile	0.270 ± 0.022	5.6	0.274 ± 0.035	6.1	0.139 ± 0.002	3.9	0.300 ± 0.043	6.4	0.167 ± 0.024	4.5
Leu	0.466 ± 0.039	9.7	0.482 ± 0.057	10.8	0.233 ± 0.004	6.6	0.544 ± 0.084	11.6	0.295 ± 0.043	7.9
Lys	0.306 ± 0.001	6.4	0.333 ± 0.024	7.5	0.205 ± 0.016	5.8	0.268 ± 0.009	5.7	0.250 ± 0.070	6.7
Met	0.180 ± 0.017	3.7	0.152 ± 0.020	3.4	0.119 ± 0.038	3.4	0.219 ± 0.017	4.7	0.155 ± 0.004	4.2
Phe	0.143 ± 0.004	3.0	0.110 ± 0.003	2.5	0.139 ± 0.004	3.9	0.136 ± 0.003	2.9	0.118 ± 0.023	3.2
Thr	0.240 ± 0.053	5.0	0.199 ± 0.017	4.5	0.154 ± 0.005	4.3	0.224 ± 0.019	4.8	0.221 ± 0.039	5.9
Tyr	0.116 ± 0.016	2.4	0.093 ± 0.017	2.1	0.119 ± 0.015	3.3	0.113 ± 0.027	2.4	0.076 ± 0.005	2.0
Val	0.583 ± 0.032	12.1	0.594 ± 0.053	13.3	0.326 ± 0.006	9.2	0.684 ± 0.089	14.6	0.417 ± 0.044	11.2
Non-essential										
Ala	0.451 ± 0.001	9.4	0.425 ± 0.063	9.5	0.318 ± 0.105	9.0	0.434 ± 0.018	9.2	0.340 ± 0.087	9.1
Asp	0.035 ± 0.006	0.7	0.045 ± 0.000	1.0	0.037 ± 0.004	1.1	0.038 ± 0.001	0.8	0.051 ± 0.004	1.4
Glu	0.080 ± 0.008	1.7	0.078 ± 0.008	1.8	0.067 ± 0.001	1.9	0.083 ± 0.011	1.8	0.086 ± 0.030	2.3
Gly	0.726 ± 0.016	15.1	0.634 ± 0.064	14.2	0.666 ± 0.042	18.8	0.584 ± 0.040	12.5	0.634 ± 0.117	17.0
Pro	0.123 ± 0.015	2.6	0.100 ± 0.022	2.2	0.079 ± 0.006	2.2	0.100 ± 0.027	2.1	0.111 ± 0.028	3.0
Ser	0.555 ± 0.003	11.6	0.485 ± 0.080	10.9	0.457 ± 0.026	12.9	0.485 ± 0.010	10.3	0.397 ± 0.061	10.7
TAA[3]	4.802 ± 0.262		4.461 ± 0.670		3.547 ± 0.009		4.694 ± 0.276		3.718 ± 0.776	
EAA[4]	2.832 ± 0.196		2.694 ± 0.333		1.923 ± 0.001		2.970 ± 0.250		2.099 ± 0.314	
NEAA[5]	1.970 ± 0.066		1.767 ± 0.337		1.624 ± 0.008		1.724 ± 0.027		1.619 ± 0.463	
EAA/NEAA	1.44		1.52		1.18		1.72		1.30	

[1] Mean of two pools of plasma (two fish/pool) and standard error of the mean (n=2). [2] % of total amino acids. [3] Total amino acids. [4] Total essential amino acids. [5] Total non-essential amino acids.
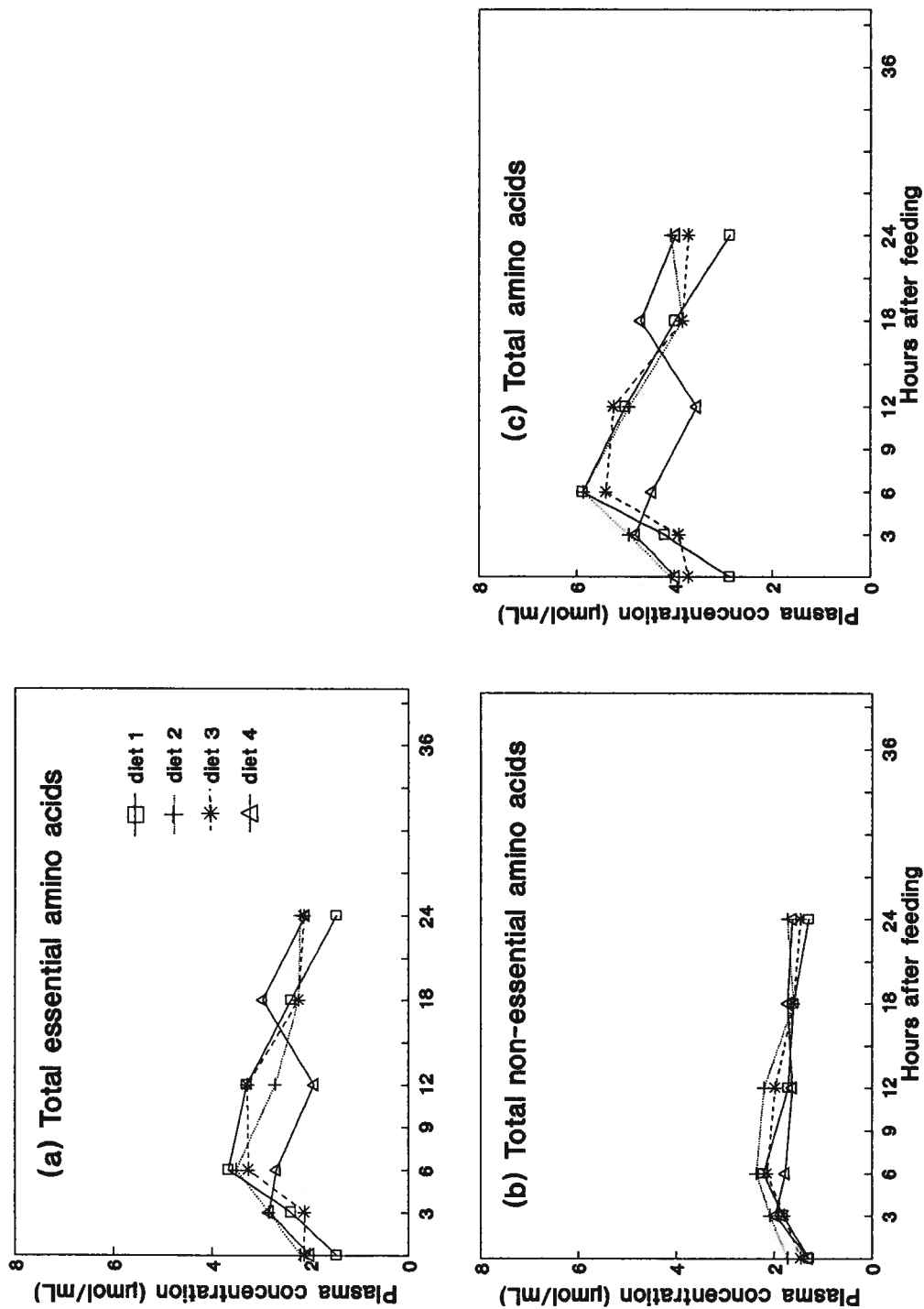
Figure 4.2. Total concentrations of plasma amino acids (essential, non-essential, and total amino acids) in rainbow trout determined at different times after feeding diets containing herring meal heated for different periods of time in Experiment 4. Diet 1 = control, diet 2 = 45 min-heated fish meal, diet 3 = 90 min-heated fish meal, diet 4 = 180 min-heated fish meal. Each points represents a mean of two pools of plasma, two fish/pool, (n=2).

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Figure 4.3. Plasma concentrations of amino acids in rainbow trout fed diets containing herring meal heated for different periods of time in Experiment 4. Diet 1 = control, diet 2 = 45 min heated fish meal, diet 3 = 90 min heated fish meal, diet 4 = 180 min heated fish meal. Each point represents a mean of two pools of plasma, two fish/pool, (n = 2).

(e) Isoleucine

(f) Leucine

(g) Methionine+Cystine

(h) Valine

diet 1
diet 2
diet 3
diet 4

Figure 4.3. (Continued)

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(i) Threonine

(j) Alanine

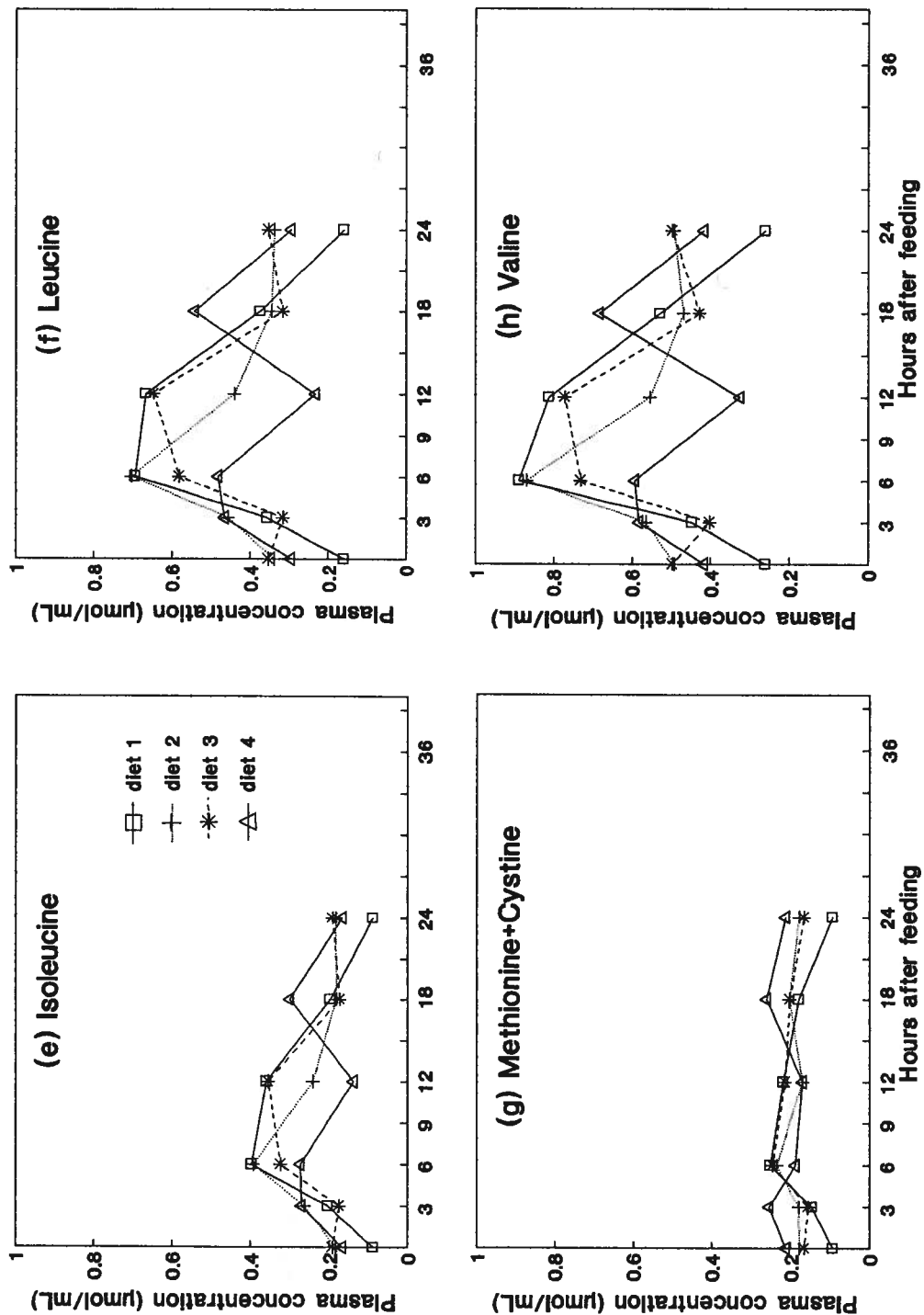(k) Aspartic acid

(l) Glutamic acid
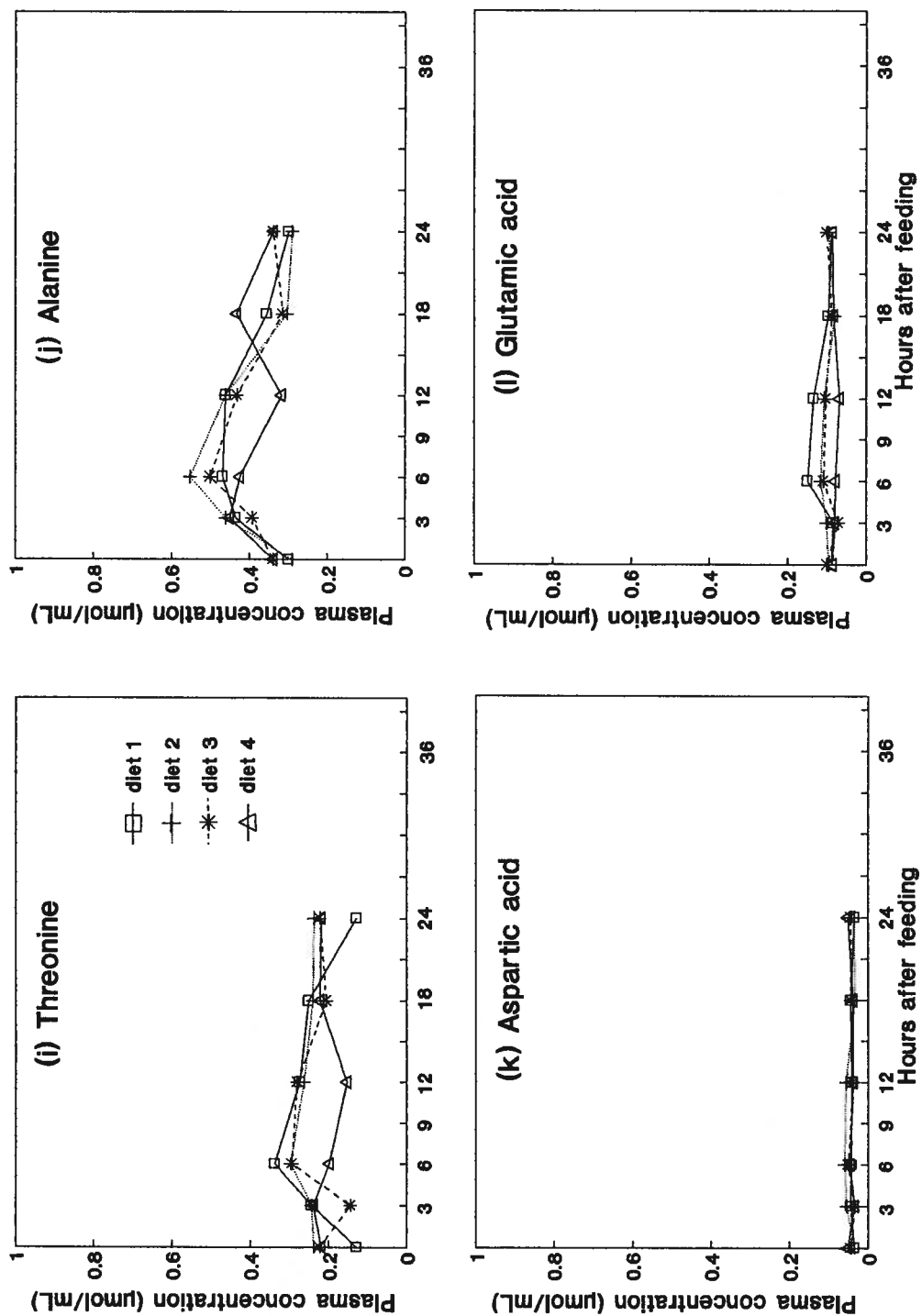
diet 1
diet 2
diet 3
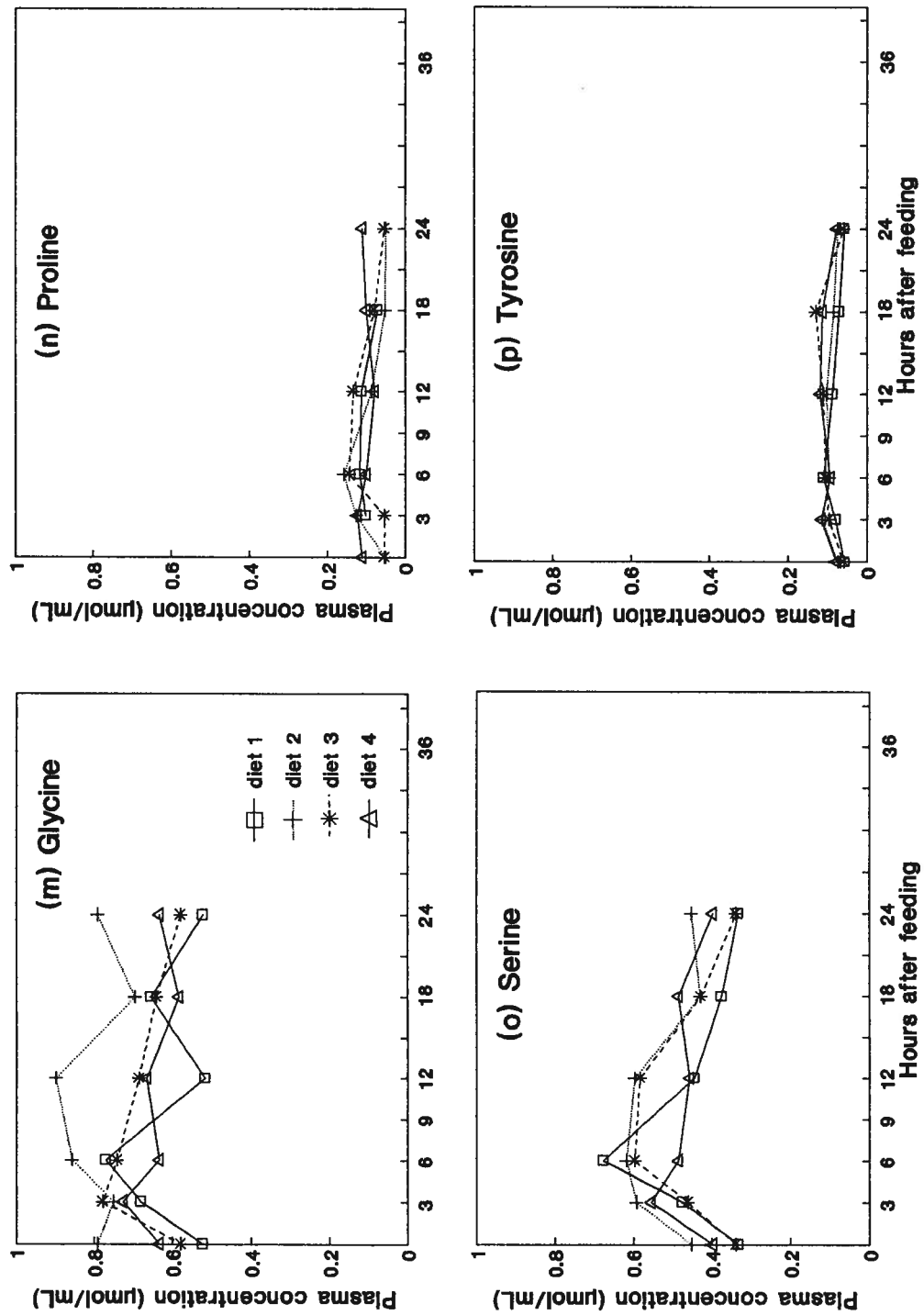diet 4

Figure 4.3. (Continued)

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Figure 4.3. (Continued)

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Percentage Changes in Plasma Essential Amino Acid Concentrations in Fish Fed Different Diets

The maximum levels of individual essential amino acids in the plasma of fish fed the different diets were taken from Tables 4.6-4.9, and listed in Table 4.10. The percentage changes in plasma essential amino acids in fish fed the different diets as compared to those in fish fed the control diet were then calculated. The results are presented in Table 4.10, and Figure 4.4. It was found that the levels of most plasma essential amino acids in fish fed diets 2, 3, and 4 that contained fish meal subjected to heat treatment were lower than those in fish fed the control meal. The extent to which they differed from the control fish varied with the periods of heating. In most cases, the longer the heating period the lower the peak concentration reached after meal consumption. The greatest negative percentage changes of most essential amino acids occurred in fish fed diet 4 (the most severe heat-treatment). The plasma concentrations of histidine, threonine, branched-chain amino acids, and phenylalanine in fish fed this diet were remarkably lower than those in the fish fed the control diet. The largest relative change occurred in the plasma level of threonine, and second largest in the level of histidine. Plasma methionine and cystine concentrations in fish fed diet 4, however, did not seem to be depressed.

Table 4.10. Comparisons of peak concentrations of plasma essential amino acids in fish fed diets[1] containing fish meal heated for different periods of time.

Amino acid	Diet 1 (A)	Diet 2 (B)	Diet 3 (C)	Diet 4 (D)	$\frac{(B-A)*100}{A}$	$\frac{(C-A)*100}{A}$	$\frac{(D-A)*100}{A}$
	μmol/mL				%	%	%
Arg	0.207(3h)[2]	0.261(3h)	0.223(3h)	0.215(12h)	26.1	7.7	3.9
Cys	0.044(12h)	0.037(12h)	0.038(6h)	0.057(24h)	-15.9	-13.6	29.5
His	0.349(6h)	0.315(12h)	0.286(12h)	0.253(6h)	-9.7	-18.1	-27.5
Ile	0.397(6h)	0.393(6h)	0.355(12h)	0.300(18h)	-1.0	-10.6	-24.4
Leu	0.694(6h)	0.703(6h)	0.647(12h)	0.544(18h)	1.3	-6.8	-21.6
Lys	0.356(6h)	0.352(6h)	0.347(6h)	0.333(6h)	-1.1	-2.5	-6.5
Met	0.217(6h)	0.214(6h)	0.208(6h)	0.219(18h)	-1.4	-4.1	0.9
Phe	0.167(12)	0.183(3h)	0.201(3h)	0.143(3h)	9.6	20.4	-14.4
Thr	0.339(6h)	0.297(6h)	0.294(6h)	0.240(3h)	-12.4	-13.3	-29.2
Tyr	0.110(6h)	0.113(3h)	0.130(18h)	0.119(12h)	2.7	18.2	8.2
Val	0.888(6h)	0.869(6h)	0.771(12h)	0.684(18h)	-2.1	-13.2	-23.0

[1] Diet 1 = unheated fish meal (control); diet 2 = 45 min-heated fish meal; diet 3 = 90 min-heated fish meal; diet 4 = 180 min-heated fish meal.

[2] Maximum plasma concentrations of different amino acids and the sampling time (in parenthesis) at which the peak was observed.
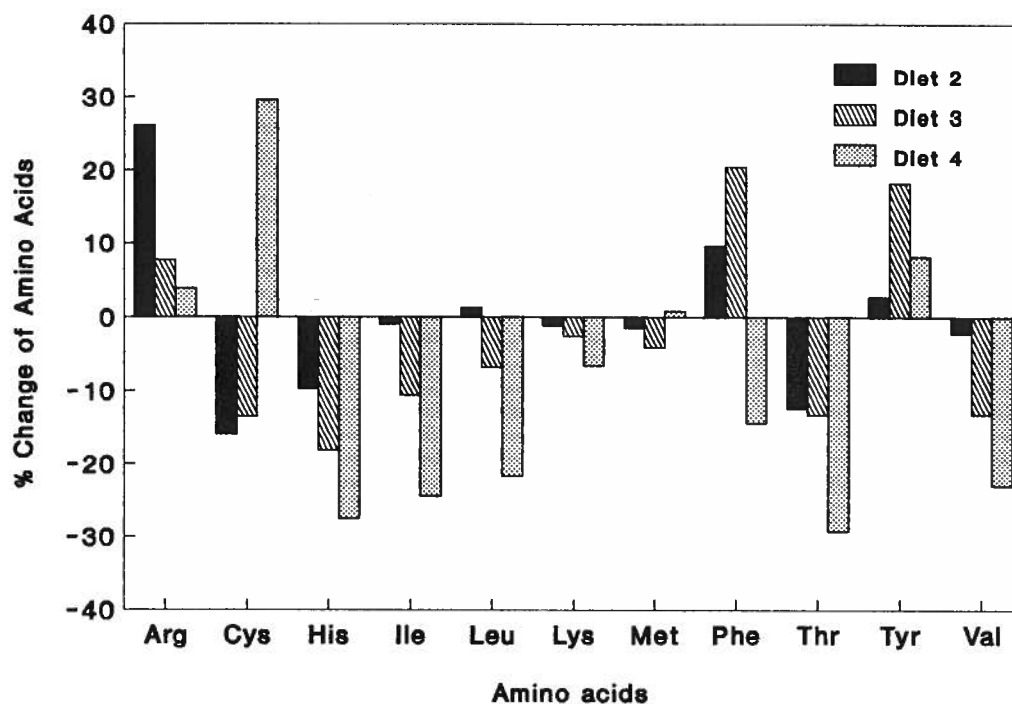
Figure 4.4. Percentage changes in plasma amino acid concentrations in rainbow trout fed heated fish meal diets relative to the control (diet 1) in Experiment 4. Diet 2 = 45 min-heated fish meal, diet 3 = 90 min-heated fish meal, diet 4 = 180 min-heated fish meal.

6.4 DISCUSSION

Although heat processing may cause little or no change in the amino acid composition of a food protein, it may profoundly influence the course of its digestion (Ford and Salter, 1966). The steric hindrance of the structure of proteins caused by the peptide cross-linkages formed during heating prevents the access of proteolytic enzymes, and this may cause marked changes in the susceptibilities of different amino acids to enzymic liberation (Ford, 1973). Consequently, the rate and proportion at which different amino acids are released from protein during the course of digestion will be different. Effects of heat treatment on the patterns of change of plasma amino acids in fish fed experimental diets in the present experiment were observed. Although, most plasma free amino acids in fish fed diets 1 (control), 2 (45 min-heated fish meal), and 3 (90 min-heated fish meal) exhibited similar patterns of change, some differences were found in the plasma concentrations of branched-chain amino acids. These amino acids in fish fed diet 1 increased and reached their peaks at 6 h at which time they plateaued or gradually declined. Those in fish fed diet 2 sharply rose to their highest level at the same time as above, but suddenly subsided. The levels in fish fed diet 3 showed a lag period between the time of feeding and 3 h, after which they increased and attained their peak values at 12 h after feeding. The dissimilarity in the time of appearance in the plasma of these amino acids was attributed to the difference in the rate of digestion and absorption of these amino acids in fish. In contrast to the pattern of plasma amino acids observed with fish fed diets 1, 2, and 3 which peaked only once, those in fish fed diet 4 (contained the most severely heated fish meal) exhibited two peaks, one at 3 h and the other at 18 h. This pattern of plasma amino acids possibly

reflected the damaging effects of heat treatment on the digestibility of protein present in the over heated fish meal used in diet 4. The denaturation of protein by heat treatment may accelerate the solubility and digestion of some proteins in fish meal causing the rise of plasma amino acid concentrations at 3 h after feeding. On the other hand, peptide cross-linkages, which form as a result of heat treatment, are resistant to hydrolysis by the proteases of the gut and release of free amino acids for absorption is slower. As a consequence, the faster rate of uptake by the tissues, compared with the absorption rate from the gut, likely accounted for the decline in the concentrations of most amino acids in fish fed diet 4 at 12 h after feeding.

When the maximum levels of each of the essential amino acids in fish fed the different diets were listed together with their peak time in Table 4.10, it was found that the degree of synchronization of essential amino acids varied with dietary treatment. Most plasma essential amino acids in fish fed diet 1 peaked at 6 and 12 h, whereas those in fish fed diet 2 peaked at 3, 6 and 12 h. Peak times for fish fed diet 3 were 3, 6, 12, and 18 h, and those for fish fed diet 4 were 3, 6, 12, 18, and 24 h post-feeding. The foregoing findings provide evidence that different amino acids in the heated-damaged protein were released at widely different rates during the digestive process (Ford, 1973).

The percentage changes in plasma amino acid concentrations in fish fed the diets containing over heated fish meals compared to those in fish fed the diet with the control meal revealed, further, that the maximum levels of most plasma amino acids in fish fed diets containing heated fish meal were lower (Figure 4.4). Moreover, the degree of the reduction in the plasma levels of amino acids clearly depended on the heating time. These results suggested that the availability of

amino acids to the fish was depressed when the fish were fed diets containing heated fish meal. In accordance with this conclusion, Anderson *et al.*, (1992) found that availabilities of amino acids to Atlantic salmon smolts from low temperature fish meal were significantly higher than from steam-dried fish meal. Growth of juvenile chinook salmon has also been shown to be depressed when the fish were fed a diet containing herring meal processed at 120-150°C (McCallum, 1985; McCallum and Higgs, 1989).

Among plasma amino acids in fish fed diets containing heated fish meal, the amino acids that showed the largest negative changes as compared to those in fish fed the control diet were threonine and histidine. Indeed, these amino acids appeared to be the most adversely affected by the heat treatments. This result disagrees with the general finding that lysine and the sulfur amino acids are the most vulnerable amino acids in fish meal protein during processing or storage (Waibel and Carpenter, 1972; El-Lakany and March, 1974; Opstvedt *et al.*, 1984; Plakas *et al.*, 1985; Plakas *et al.*, 1988). The reduction in the level of plasma threonine in fish fed heated fish meal diets in the present experiment, however, supports the chemical score of fish meal protein (Tacon and Jackson, 1985). Tacon and Jackson (1985) calculated chemical scores based on mean essential amino acid requirements of rainbow trout and carp, and listed threonine as the first limiting amino acid in herring meal. The result relating to decrease of plasma histidine was similar to those noted for chickens. Smith and Scott (1965), for instance, found that chicks fed 2 h heated fish meal showed the greatest reduction in plasma histidine as compared to those fed unheated fish meal. Bjarnason and Carpenter (1970) studied the chemical changes in pure protein, and found that at high temperature (145°C)

histidine, serine, and threonine were prone to destruction as well. Although, lysine is most prone to damage by heat treatment, the availability of this amino acid in the plasma in relation to the requirement of the fish was still better than that of threonine and histidine in the present experiment.

There were noticeable effects of heat treatment on reduction of plasma glutamic acid levels in fish fed diet 4. The concentrations of this amino acid in fish fed diet 4 were consistently the lowest. The possible causes of the low concentrations of glutamic acid may have been decreased digestibility due to (1) an ester link between the carboxyl group of glutamic acid and hydroxyl group of a hydroxy-amino acid (threonine and serine), or (2) an imide link between glutamic acid and glutamine or asparagine (Ford, 1973).

The above results on the plasma amino acid profiles supported the in vitro pepsin digestibility results for the fish meals. Graded reductions of protein digestibility in herring meal subjected to heat treatments for 45, 90, and 180 min in the present experiment were observed. These findings were in accord with those found by March and Hickling (1982). Moreover, Miyazono and Inoue (1989) reported high correlations between pepsin digestibility values of fish meal incorporated into diets for rainbow trout and growth rate and feed efficiency values. This trend appeared similarly in the present experiment. Fish fed diets containing herring meal with a low percentage of pepsin-digestible protein, in comparison with those fed the control fish meal, showed depressed growth rate and feed efficiency. The depression was not, however, statistically significant, because of the variability among replicate groups.

The magnitude and patterns of change of plasma amino acids in fish fed the experimental diets in the present study suggested that the biological availability of amino acids to the fish varied inversely with the severity of heating of the fish meal. The least available amino acids to the fish appeared to be threonine and histidine. Although, this finding was contradictory to the results found in vitro by other investigators, it may indicate the true availability of amino acids in relation to the requirements of the fish.

CHAPTER 7

CONCLUSIONS

An attempt was made in this thesis to investigate the changes in the patterns of plasma amino acids in rainbow trout in relation to dietary effects, feeding regimen, and processing conditions for the protein sources used in fish diets. The results of the studies provided a better understanding of protein nutrition in fish.

The results of growth studies in Experiment 1.1 demonstrated that fish fed a diet containing fish meal as the principal protein source had growth rates superior to those of fish fed diets containing a mixture of fish meal, soybean protein concentrate, corn gluten meal, and gelatin, either supplemented or unsupplemented with free lysine, methionine, and tryptophan. Growth rates of the fish fed the test diets were not improved when feeding frequency was increased from once to five times daily. Although statistically insignificant, the results of Experiment 1.2 demonstrated that the growth rate of fish tended to improve when the diet was also supplemented with isoleucine. The inferior growth rates of fish fed experimental diets in comparison with those fed the control may have been due to amino acid imbalances in the diets used in Experiment 1.1. Comparison between the amino acid composition of diets and the requirement values indicated that the levels of all essential amino acids in diet 3 (supplemented with lysine, methionine, and tryptophan) were equivalent to or above the requirement values. This demonstrated that chemical analyses can only be used as guide when formulating a diet for fish. The true availability of amino acids to the fish for maximum growth depends on both the amino acid balance of the diet and the digestibility of the protein sources.

Feeding fish five times per day in the present experiment, nevertheless, resulted in better utilization of absorbed amino acids for protein synthesis. Carcass composition of fish in this group, particularly of fish that were fed a diet supplemented with free essential amino acids, contained higher concentrations of protein and lower lipid than fish fed once daily. In comparison with fish that were fed once daily to satiation, fish fed five times daily showed lower but more constant concentrations of amino acids in the plasma. According to the substrate relationship of amino acids and catabolizing enzymes, the rate at which plasma amino acids were catabolized in fish that were fed five times daily was lower than in fish that were fed once daily. Amino acids in the free pool were utilized more efficiently in the former group of fish for protein synthesis than in the latter group. The effect of frequent feeding on body composition was greater than the effect on over all body weight gain.

When the fish were fed a diet containing free glycine in Experiment 2, plasma concentrations of glycine started to rise and peaked later than in fish fed a diet containing gelatin (a rich source of glycine). This finding was contradictory to the expectations, and showed that amino acids supplemented in free form do not always cause an immediate increase in plasma concentrations.

The results of Experiments 1 and 2 also showed that the peak plasma concentrations of specific amino acids in trout following ingestion of diets supplemented with amino acids in the free form were as much as double those of fish fed diets containing similar amounts of the amino acids but derived solely from intact protein. This finding is similar to those of other studies in fish and other animals. Clearance of the higher concentrations of amino acids in the plasma of fish

fed the diets supplemented with free amino acids was not completed until 24 to 36 h after feeding. Some of the amino acids that were in excess (histidine, lysine, and threonine) had accumulated in the muscle tissue pool at 26 and 36 h after feeding in Experiment 2. This finding suggests that the amino acids are still available to fish when they are fed a diet supplemented with free essential amino acids to satiation once daily. Although amino acid toxicity has often been reported with regard to essential amino acids, the present results suggested that excess glycine may also be toxic. Elevated concentrations of glycine in both plasma and muscle pools were accompanied by reductions in feed intake. Non-essential amino acids are frequently used as substitutes for intact protein when formulating isonitrogenous diets. The above finding suggests that caution should be exercised when glycine is used for this purpose, although the mechanism of the observed toxicity is unknown.

The peaks of plasma arginine, alanine, histidine and lysine concentrations at 36 h postprandial in fish fed diets containing similar ingredients in Experiment 1.2 indicates that the rates of digestion of proteins from some sources may be slower than from others. Apart from the amino acid profile of the diet, this factor should be borne in mind when feeding fish diets containing different protein sources.

A high level of dietary lipid (24%) did not alter the rate of absorption of essential amino acids into the plasma. There was, however, a considerable difference in the pattern of plasma non-essential amino acid concentrations. The rapid reduction in the concentrations of plasma non-essential amino acids after feeding fish the high lipid diet provides evidence of conversion of these amino acids to intermediates in gluconeogenesis and lipogenesis.

When fish were fed diets containing fish meal that had been subjected to heat treatment, plasma amino acid profiles and in vitro pepsin digestibility values indicated that the longer the duration of heating, the lower the protein digestibility. Moreover, the plasma amino acid profiles in fish fed the diet containing fish meal which was heated for the longest time (180 min) indicated slow release of amino acids from dietary protein. The least available amino acids to the fish appeared to be threonine and histidine. The plasma amino acid profile appears to be a good indicator, and can thus be used for assessment of protein quality in products subjected to heating during manufacture.

The changes in the plasma concentrations of branched-chain amino acids in every experiment were noteworthy in their resemblance to one another. Elevated concentrations of plasma leucine and valine as a result of addition of isoleucine in the diet were also observed which might indicate an antagonistic effect of isoleucine on degradative enzymes. Since these amino acids share the same enzymes in the first two steps of the degradation, the increase in the concentration of any one of the branched-chain amino acids can be mutually competitive to the others. The mechanism of inhibitory effects was not, however, explored in the present studies.

Higher concentrations of plasma taurine in fish fed a diet supplemented with methionine than in fish fed the basal diet were observed. In addition, the concentrations of taurine increased when methionine concentrations in the plasma decreased. This observation demonstrated the existence of degradation pathways from methionine to taurine in rainbow trout.

A very prominent phenomenon that was observed in every experiment was the change in plasma non-essential amino acid concentrations. The patterns of

change of these amino acids indicated their roles in intermediary metabolism. An example was the relationship among plasma glycine, alanine and serine concentrations in Experiments 1, 2 and 3. The dramatic increase of serine in the plasma of fish that were fed a diet containing a high concentration of glycine reveals that serine possibly play an important role as an effective glucogenic amino acid. Further studies in relation to the metabolic role of non-essential amino acids will be useful for better understanding the metabolism of amino acids, and they will provide more scientifically based information for fish diet formulation.

The results of the studies indicate that plasma amino acid concentrations are sensitive to nutritional changes and they can be used to evaluate the protein nutritive value of formulated diets for fish.

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APPENDICES

Appendix 1. ANOVA of initial weight, final weight, weight gain, and feed consumption of rainbow trout in Experiment 1.1

Source	df	Initial weight Mean square	P	Final weight Mean square	P	Weight gain Mean square	P	Feed consumption Mean square	P
Diet (D)	2	0.013	0.978	35.641	0.001*	33.897	0.004*	0.00057	0.00214*
Feeding frequency (F)	1	1.227	0.213	0.339	0.525	0.000	0.985	0.00222	0.00022*
Size (S)	2	4.801	0.036*	262.618	0.000*	49.847	0.002*	0.09629	0.00001*
D*F	2	0.267	0.652	1.059	0.324	1.209	0.435	0.00004	0.17361
D*S	4	0.956	0.308	1.303	0.119	2.551	0.234	0.00821	0.00001*
F*S	2	0.977	0.285	2.562	0.268	0.329	0.768	0.00002	0.30864
Error	4	0.560		0.700		1.169		0.00001	
Total	17								

* Significantly different

Appendix 2. ANOVA of specific growth rate (SGR), feed efficiency, productive protein value (PPV), and energy efficiency in Experiment 1.1

Source	df	SGR Mean square	P	Feed efficiency Mean square	P	PPV Mean square	P	Energy efficiency Mean square	P
Diet (D)	2	0.306	0.015*	0.036	0.004*	0.0218	0.001*	0.016	0.007*
Feeding frequency (F)	1	0.001	0.952	0.001	0.396	0.0005	0.040*	0.001	0.229
Size (S)	2	0.012	0.608	0.001	0.406	0.0013	0.007*	0.003	0.119
D*F	2	0.016	0.522	0.001	0.653	0.0003	0.067	0.000	0.947
D*S	4	0.027	0.414	0.000	0.824	0.0082	0.013*	0.001	0.388
F*S	2	0.007	0.743	0.000	0.807	0.00001	0.801	0.000	0.947
Error	4	0.085		0.005		0.00006		0.001	
Total	17								

* Significantly different

Appendix 3. ANOVA of protein gain and lipid gain of rainbow trout in Experiment 1.1

Source	df	Protein gain		Lipid gain	
		Mean square	P	Mean square	P
Diet (D)	2	2968.14	0.001*	3475.61	0.001*
Feeding frequency (F)	1	5.26	0.783	1595.56	0.013*
Size (S)	2	4910.46	0.001*	4432.57	0.001*
Error	12	66.28		188.49	
Total	17				

* Significantly different

Appendix 4. ANOVA of dry matter, protein, lipid and ash content of rainbow trout in Experiment 1.1

Source	df	Dry matter		Protein		Lipid		Ash	
		Mean square	P	Mean square	P	Mean square	P	Mean square	P
Diet (D)	2	1.736	0.002*	0.356	0.532	9.544	0.156	0.019	0.828
Feeding frequency (F)	1	0.867	0.042*	4.611	0.012*	26.541	0.030*	0.207	0.177
Size (S)	2	0.217	0.307	0.630	0.341	6.323	0.273	0.253	0.123
Error	12	0.167		0.534		4.374		0.110	
Total	17								

* Significantly different

Appendix 5. ANOVA of specific growth rate (SGR), and feed conversion efficiency in Experiment 1.2

Source	df	SGR		Feed efficiency	
		Mean Square	P	Mean Square	P
Diet	2	0.0476	0.28	0.0107	0.29
Error	12	0.1732		0.0370	
Total	14				

Appendix 6. ANOVA of pepsin digestibility in Experiment 4.

Source	df	Pepsin digestibility	
		Mean Square	P
Diet	3	215.744	0.000*
Error	4	0.284	
Total	7		

* Significantly different

Appendix 7. ANOVA of initial weight, final weight, weight gain, and feed consumption of rainbow trout in Experiment 4.

Source	df	Initial weight		Final weight		Weight gain		Feed consumption	
		Mean square	P	Mean square	P	Mean square	P	Mean square	P
Diet	3	229.206	0.130	502.917	0.120	120.253	0.313	0.522	0.164
Error	12	100.116		210.591		90.981		0.258	
Total	15								

Appendix 8. ANOVA of specific growth rate (SGR), and feed conversion efficiency in Experiment 4

Source	df	SGR		Feed efficiency	
		Mean Square	P	Mean Square	P
Diet	3	0.079	0.392	0.007	0.814
Error	12	0.073		0.022	
Total	15				