

1. INTRODUCTION

Energy expenditure associated with the metabolism of protein may be reduced in both homeothermic and poikilothermic animals by increasing dietary lipid. This metabolic response known as 'protein sparing effect' has been demonstrated in rainbow trout, *Salmo gairdneri*, in relation to dietary lipid content (LEE and PUTNAM, 1973). More efficient utilization of dietary protein is achieved in rainbow trout by increasing dietary lipid content provided protein intake is above a critical threshold (MEDLAND and BEAMISH, 1985).

During the late 1980s, high-energy feed containing fat of 300 g kg⁻¹ DM was introduced to the salmon feeding. Extruded feed was already in the use at that time (BERGHEIM et al., 1991) but the dietary concentration of the fat did not exceed 200 g.kg⁻¹ (DRAEGNI, 1992).

Effects of extruded high-energy diets on growth, feed conversion and body composition have been reported. Most of these experiments have been conducted with rainbow trout (ALSTED and JOKUMSEN, 1990; LANARI et al., 1995) and small- sized Atlantic salmon in sea water (JOHNSEN and WANDSWIK, 1991; JOHNSEN et al., 1993; HILLESTAD 1994).

Rainbow trout have a high potential for rapid growth (AUSTRENG et al., 1987) and high feed efficiency (STOREBAKKEN and AUSTRENG, 1987). One factor which contributes to this is the efficient mechanism possessed by fish for protein catabolism and for excretion of waste nitrogen (SMITH et al., 1978a, b). The primary waste product of protein in all animals is ammonia, that possesses no biological value and must be rapidly excreted or converted to less toxic compounds. Terrestrial homeotherms, at considerable energy cost, convert ammonia to urea and uric acid which additionally must be concentrated and excreted by the kidneys (SMITH et al., 1978a, b). Trout diets contain high levels of protein when compared with those for other species. If there is an excess amount of protein in relation to the amount of energy, the excess protein is used as an energy source (PHILLIPS, 1972). Therefore loading the water with considerable but variable amount of ammonia in which the fish live.

The feed represents a major cost for the fish farmer. Furthermore, minimizing the feed loss is the most effective way of reducing water pollution since it has deleterious effects on the environment and on the hygiene of the fish farm. Accordingly, overfeeding should be reduced to a minimum. On the other hand, feeding should not be limited that the fish will suffer from

underfeeding or that the amount and quality of trout will decrease. For these reasons, nutrient and energy supply to maximize production and minimize pollution is an important aspect of fish research. The calculations of the optimum ration level of nutrient and energy are based on knowledge of the requirements of practical fish farming. Water temperature and fish size are very important in determining these requirements. Several other factors, however, interfere. This concept should be ultimately related to the fish feeding strategy. Many studies examined the effects of feeding regimes in terms of restrictive feeding having the above mentioned concerns to improve sound feeding systems with variable fat and protein levels and to define zero growth, which is needed to be applied to find the exact maintenance energy requirements. But such attempts of restrictive feeding have never been used beyond a few feeding levels, and is always done in terms of percent body weight per day and scarcely define the meaning and impact of varying a wide range of restrictive feeding intensities. Thus, more elaborated experimental feeding methods are essential than just finding the feeding level for below, at or above maintenance levels.

In this study wide range of feeding intensities using various energy level diets and trout of various ages and sizes to evaluate and determine the energy requirements are examined.

2. LITERATURE REVIEW

2.1 Determination of energy requirements, utilization and dietary supply to fish

Bioenergetics or nutritional energetics is the study of the balance between dietary energy supply, expenditure and gain, and requires an examination of physiological processes by which energy is transformed in living organisms. An understanding and study of the nutritional energetics of any animal, including fish, is necessary as a basis for providing a defined and adequately balanced dietary regime under any particular physical environment. Full definition of the energy and nutrient requirements depends upon a knowledge of the partition of the dietary energy yielding components between catabolism as fuels and anabolism as storage in tissues.

Several approaches and methods have been developed to study the balance between energy supply, expenditure and gain. Because of their aquatic and poikilothermic nature, not all conventional nutritional energetics approaches used with homeotherms are applicable to fish.

2.1.1 Dietary sources of energy

All organic compounds in animal feed release heat upon combustion, and thus are potential sources of energy. However, only energy contained in absorbable end products of digestion can be utilized, whereas faecal energy is lost directly. Dietary carbohydrates do not seem to play such an important role. Physiologically, lipids and protein form an important part of the structure of a fish, but the need for chemical energy can preclude their incorporation into the tissues and may involve their catabolism as a source of energy. Thus, utilization of the energy and nutrients of each diet depends both on the level of intake and on the composition of the diet.

An optimal balance of energy and protein components in the diet is important because an excess or deficiency of non-protein energy (lipid and carbohydrates) may result in lower growth rates. If the diet is deficient in non-protein energy, the amino acids would be used for energetic purposes (basal metabolism and voluntary activity) rather than protein synthesis.

With the demonstration of 'protein sparing effect' of increasing dietary lipids (LEE and PUTNAM, 1973), a series of studies followed to answer how far the dietary lipids may be increased simultaneously decreasing the dietary protein not only to understand the physiological response of fish but also to lower the feed costs as much as possible. By the early 1990s 30% of the diets already consisted of lipids which was beyond realistic limits for land animals (ALSTED and JOKUMSEN, 1990; JOHNSEN and WANDSWIK, 1991; JOHNSEN et al., 1993; HILLESTAD 1994; LANARI et al., 1995).

The gross energy value of the feed depends on its composition (mean heat of combustion values for carbohydrate, protein and lipids are 17.2, 23.6, 39.5 kJ.g⁻¹, respectively; BRAFIELD and LEWELLYN, 1982). But the measurements of the dietary energy value need to be assessed biologically. Because digestion, absorption, and utilization of the carbohydrates, lipids and protein derived from the feed are associated with various biological losses of undigested and unutilized nutrients and with energy expenditure, the different losses need to be measured, if biological processes are to be understood.

2.1.2 Measurement of energy metabolism

The free energy changes which occur in animals as the chemical energy of the diet is used to support life processes cannot be measured directly. However, ultimately the dietary energy is either voided as faeces and metabolic wastes, dissipated as heat, or retained in tissues. Measurement of the energy values of the diet and wastes by combustion of samples in calorimeters allows calculation of digestible energy.

The ultimate usefulness of the dietary energy in promoting an increase in the energy value of the fish can be determined by either measuring the fraction of the energy intake liberated as heat, or by measuring the fraction retained by the fish over part of its growth period.

Energy expended as heat by animals is measured by calorimetry, which is a general technique for measuring heat flux between two bodies.

The stoichiometry of the oxidation of the classical compounds of energy supply (proteins, fats and carbohydrates) allows calculation of the energy released as heat from measurements of respiratory exchange, oxygen consumption and carbon dioxide production, along with

measurement of urinary excretion. This method of measuring heat production is referred to as indirect calorimetry (CHO and KAUSHIK (1985, 1990).

There have been reports on studies of energy utilization and expenditure for several species, but among them particularly CHO et al. (1976), SMITH et al. (1978a, b), CHO and KAUSHIK (1985, 1990) and KAUSHIK and MEDALE (1994), worked with trout. Many of these investigations with fish have been concerned with the energy cost of swimming and have largely ignored the level of feeding and the type of the diet. Heat production was calculated from oxygen consumption measured under these conditions, in which an undefined fraction of the heat would be due to catabolism of feed components, with the balancing being provided by breakdown of body tissue.

2.1.3 Methods to measure digestibility

The first thing in evaluating the potential of any feedstuff for inclusion in a diet is the measurement of its digestibility. It is difficult to separate fish faeces from the water and to avoid contamination of the faeces by the uneaten feed. NOSE (1960) collected samples of rectal contents by manually stripping the fish, WINDELL (1978) obtained samples of rectal contents by suction to the anus or by dissecting the fish. The faeces obtained by both of these techniques involved handling the fish and expose them to considerable stress. Forced evacuation of their rectum would result in the addition of physiological fluid and intestinal epithelium to rectal contents which will force the results.

SMITH (1971) confined the fish in metabolic chambers and collected the faeces that were voided naturally into the water. CHO et al. (1975, 1982) developed 'Guelph system', in which the faecal material is collected in a settling column. Settling columns are flushed out to remove rest feed residues from the system after the last meal of the day, and faecal samples are collected the following morning before feeding. The faeces are centrifuged and the supernatant is discarded. Faeces are then freeze-dried and ground for determination of the chemical (dry matter concentration, lipid concentration, protein concentration), and energy analyses and concentration of digestion indicator. Its advantage is that there is no need to handle fish and it allows repeated determinations.

2.1.4 Branchial and urinary (non-faecal) losses

Intake of protein and the eventual catabolism of its amino acids yield ammonia, carbon dioxide and water. The excretion of the ammonia, or its detoxification product, urea, leads to the loss of combustible material by fish. Most of these nitrogenous losses occur by excretion of ammonia through the gills in form of ammonia and to a lesser extent with some loss through the kidneys in form ammonia and urea. The direct measurements of these non-faecal losses create practical and technical difficulties. The necessity of measuring the levels of ammonia and urea in relation to total faecal and non-faecal losses is complicated, and gives room for many experimental errors, and therefore questionable for the evaluation of diets. There is a method for estimating the non-faecal loss indirectly, using comparative carcass analyses. So non-faecal nitrogen excretion may be calculated by difference between digested N and retained N. Since the major part (>85%) of non-faecal N is excreted as ammonia (CHO and KAUSHIK, 1990) and since energy concentration of urea is not much different from ammonia (23.1 vs. 24.9 kJ.g⁻¹N, ELLIOT and DAVISON, 1975), may be assumed as being excreted per g of digestible N not retained by the fish. Metabolisable Energy (ME) may be calculated as DE minus energy excreted as non-faecal N (RODEHUTSCORD and PFEFFER, 1999).

2.1.5 Measurement of heat production: fish calorimetry

The energy released by catabolism of the nutrients is ultimately released as heat, so that the energy balance can be determined by measuring heat production, or by estimating the change in body energy content from weight and carcass composition changes. A direct calorimeter has been designed to measure the heat production of fish (SMITH et al. 1978a), but the high heat capacity of the calorimeter made it less sensitive to changes in metabolic rate than methods based on measurement of oxygen consumption (BRETT and GROVES, 1979). The direct determination of heat production requires the measurement of small temperature changes in the water as a result of changes in the metabolic rates from the feed ingested.

An indirect method of estimating heat production uses consumption of oxygen and production of carbon dioxide and other end-products, such as nitrogenous compounds. The heat