NUTRIENT DIGESTIBILITY IN SOUTH AFRICAN ABALONE *(HALIOTIS MIDAE* L.)

Submitted in fulfillment of the Requirements for the Degree of DOCTOR OF PHILOSOPHY Rhodes University

by

James Sales

July 2001

CONTENTS

ACKNOWLEDGEMENTS

This study was only possible because of the help of numerous people at the Department of Ichthyology and Fisheries Science, at Rhodes University, in Grahamstown, in South Africa and around the world. Although it is not possible to mention them all here, I would like to highlight the following sponsors and people:

The Claude Harris Leon Foundation who awarded me a post-doctoral fellowship and allowed me to submit this study as a Ph.D dissertation. Especially thanks to Mrs Maggie Sükel for all the communication.

Sea Plant Products, Hermanus, South Africa, for sponsoring the study, provision of experimental animals and useful comments. Other sponsors included: THRIP (Technology and Human Resources for Industry Programme), the South African Network for Coastal and Oceanographic Research, the National Research Foundation and Rhodes University Joint Research Committee.

Dr. P.J. Britz, my supervisor, head, landlord and friend, and his family.

Wynand Lambrechts of Western Province Cooperation, Roggebaai, South Africa, for providing me with feed ingredients.

Ingrid Lupatsh, National Center for Mariculture, Eilat, Israel, for help with laboratory techniques.

My Heavenly Father that always holds His hand above me.

ABSTRACT

The evaluation of potential alternative protein sources for the formulation of least-cost optimal diets to satisfy the nutrient requirements of South African abalone (*Haliotis midae*) has been hampered by the absence of a suitable, practical, replicable and reliable digestibility technique. A suitable low-cost faeces collection technique was developed in this study to obtain suitable quantities of excreta for analysis from this species. Acid-insoluble ash was identified as a reliable, replicable and safe internal marker in comparison to chromic oxide and crude fibre for use in nutrient digestibility studies with *H. midae*. This was validated by the consistency and repeatability of the results and by comparison to total collection of faeces. The traditional substitution method used in digestibility studies with fish to evaluate protein digestibility of feed ingredients was found to be unsuitable for *H. midae*. Apparent protein digestibility values exceeding 100 % derived through this method could be attributed to associative effects between feed ingredients, differential diet and faecal nutrient leaching, and mathematical artifacts in calculations when using substitution versus single protein diets. An ingredient particle size of less than 450 µm in comparison to particle size classes of above 450 µm was shown to enhance nutrient (dry matter, organic matter, protein, fat) digestibility and minimise dry matter leaching from diets. The dietary inclusion level of both pre-gelatinised maize starch and α-cellulose did not influence ($P > 0.05$) apparent nutrient (protein, fat, fiber, starch) digestibility. Using the above digestibility protocol amino acid availability of all plants ingredients currently used in the South African animal industry was evaluated for *H. midae*. Soybean meal (96.86 %) and lupins (96.51 %) presented the highest apparent mean amino acid availability of all plant protein ingredients evaluated with *H. midae*. Canola meal (94.21 %), faba beans (92.87 %) sunflower meal (92.77 %), peanut meal (87.39 %) and cottonseed meal (85.15 %) presented higher apparent mean amino acid availability values than fish meal (82.75 %). Apparent protein digestibility was highly correlated (r = 0.99) with mean apparent amino acid availability, while true amino acid availability was 1.88 % units higher than apparent amino acid availability for all ingredients tested. Predicted apparent protein digestibility in compound diets was within 1.1-6.5 % of determined values. Calcium phosphate mono dibasic presented the lowest ($P < 0.05$) dietary phosphorus leaching (51.51 % maximum) and highest apparent phosphorus digestibility (66.27 %) in comparison to other inorganic phosphorus sources. Based on the method of direct experimentation to determine the optimal dietary protein level using graded levels of dietary protein 28.1-35.9 % dietary protein from good quality sources is recommended for maximum growth of juvenile *H. midae*. This study provides a scientifically sound research tool including a faecal collection technique, suitable marker and assay technique that could be use in further studies to improve least-cost diet formulation for *H. midae*. Future nutritional studies in *H. midae* should primarily concentrate on reducing dietary nutrient leaching and improving the intake of nutrients in order to properly evaluate responses of this species to different dietary regimes.

INTRODUCTION

Abalone are among the most highly valued seafoods in the world, with prime demand in Asian countries where products form part of the traditional cuisine. As in other parts of the world (Japan, China, Australia, North America, Canada), a decline of South African abalone fisheries, mainly due to poaching, has stimulated the development of abalone farming to meet the demand for the product. Since 1990 an estimated US$12 million have been invested to build a South African industry of some 12 commercial farms around the coastline from Port Nolloth on the Atlantic coast to East London on the Indian Ocean. Their combined annual projected production is of 500-800 t. Abalone farming, like any form of intensive animal husbandry, for example broiler chickens and pigs, is highly capital intensive with high running costs. Animals are stocked at high densities in small shore based man-made structures and reared on seaweeds and/or artificial diets.

As with all animal industries, feed cost is considered to be the highest recurrent cost in abalone farming. Although some abalone farms in South Africa rely on the harvest of seaweed to feed abalone, the need for nutritionally complete feeds is becoming more critical for the following reasons: (1) a limited supply of seaweed, (2) logistical problems in harvesting, transporting and storing of seaweed, and (3) the possibility of obtaining higher yields and faster growth with the improvement of artificial diet formulations. In comparison to seaweed, an artificial diet is a compact product, with constant supply to any region, and can easily be handled and stored. Although total cost to manufacture an artificial diet is much higher per unit than harvesting seaweed, this is balanced by the much better feed conversion ratio that can be achieved with artificial diets (*ca* 1-1.5:1) in comparison to seaweed (*ca* 12-15:1).

A project was initiated in 1989 by Rhodes University to develop artificial diets for *Haliotis midae*. This project was successful in developing a water stable pelleted diet (Knauer, 1994; Britz, 1996a), and in identifying fish meal as the most suitable protein source for inclusion in artificial diets for *H. midae* (Britz, 1996b). However, the increasing cost of high quality fish meal, and increasing shortages as requirements for aquafeeds increase, pose real problems for cost-effective feed formulation (Hertrampf and Piedad-Pascual, 2000). This

is especially true for aquaculture in developing countries such as South Africa where a devaluating currency has dramatically increased the real cost of imported fish meal. Priority is given internationally to the search for alternatives to fish meal in animal feeds (Hardy and Kissel, 1997). Developing alternative protein sources for aquatic feeds which support rapid growth but do not increase pollution from aquaculture will require the combined efforts of all of the major scientific disciplines that collectively constitute aquaculture (Hardy, 1999). Some progress in this regard has been made with *H. midae* in that replacement of 30 % of the fish meal component in artificial abalone diets with either soybean or sunflower meal did not decrease growth rate (Shipton and Britz, 2001).

One way of reducing feed costs is through improved diet development by more effective use of available nutrients in feed ingredients to match with the requirements of the animal. By precisely measuring the availability of essential nutrients in alternative feed ingredients, it was possible to eliminate fish meal from poultry feed formulations (Scott et al., 1982). Animals do not require feed ingredients *per se*, but rather the nutrients which form part of the chemical composition of these ingredients. A nutrient may be present in an ingredient, but nutritionally worthless if it is unavailable to the animal (Akiyama, 1991). Knowledge of the availability of nutrients in a feed ingredient is not only a basic requirement for formulating least-cost diets, but is also important for the maintainance of water quality and the prevention of water pollution with species kept in an aquatic environment. Undigested nutrients excreted by *H. midae* fed on artificial diets in comparison to animals fed on seaweed have been linked to the higher rate of infection of the parasitic filter feeder sabellid polychaete, *Terebrasabella heterouncinata* in the former.

Digestibility could be described as the most important single determinant of nutrient availability (Sauer et al., 2000), and should be given priority in any nutritional study that evaluates the potential of feed ingredients for use in the diet of an animal species. Digestibility can be defined as the difference between dietary nutrient and faecal nutrient content divided by the dietary nutrient content. Although the direct method of measurement of food intake and total collection of faeces would be the ideal method to determine digestibility, the aquatic environment make these estimations very difficult. Contamination of faeces with uneaten feed (Cho et al., 1982), and leaching of nutrients

from feed and faeces (Allan et al., 1999) may lead to under-or overestimation of digestibility. Therefore, the use of markers as an indirect method of determining digestibility that only requires the collection of a subsample of faeces and does not require an accurate estimation of dietary intake is generally preferred with aquatic species (Jones and De Silva, 1997; Morales et al., 1999). The determination of digestibility by the direct method was found to be inappropriate with *Haliotis* species fed on artificial diets due to problems associated with the accurate measurement of feed ingested and faeces voided (Wee et al., 1992a; Shipton, 2000). Although Wee et al. (1992b) identified chromic oxide (Cr_3O_2) and acid-insoluble ash as suitable markers for digestibility studies in *H. rubra* and *H. laevigata*, the use of Cr_3O_2 as marker in the first digestibility studies with *H. midae* fed on compound diets (Britz, 1996a) failed to produce reliable and replicable results. Shipton (2000) concluded that Cr_3O_2 as marker could provide a reliable indication of apparent protein digestibility in *H. midae* if one of the three different types of faeces produced was collected. However, this method is labour intensive and subjective in that it requires sorting of faeces, and very small quantities of faeces are finally available for analysis. Furthermore, as the evacuation times of the three faecal types do not differ significantly, it is not possible to collect a specific type of faeces over a specific time period (Shipton, 2000). Thus, evaluation of nutrient availability of feed ingredients by means of digestibility measurement in *H. midae* has been hampered by the lack of a suitable marker that is representative in all type of faeces produced. At the outset of this study a need existed for a marker that would satisfy this requirement and enable the collection of adequate quantities of faeces in order to analyse for a range of nutrients.

While attempts at determining nutrient digestibility in abalone to date have been limited to single ingredient diets (Fleming et al., 1998; Vandepeer et al., 1999; Shipton, 2000), very few feed ingredients are fed as the sole component of a diet, and practical diets almost always consist of a mixture of feed ingredients. Therefore the determination of the digestibility values of several nutrients from feed ingredients is normally evaluated through comparison of the digestibilities of a reference and a test diet, the test diet being a mixture of the reference diet and the test ingredient (Cho et al., 1982). An advantage of this method over testing ingredients singly is that the test ingredient may be more acceptable to the

animal when fed in combination with other ingredients, which leads to a normal level of intake (NRC, 1993). This technique is based on the assumption that there is no interaction between the reference diet and the test ingredient, and that results are independent of the level of inclusion of the test ingredient (Aksnes et al., 1996). Although this technique was never previously evaluated with *Haliotis* species, Maguire et al. (1993) mentioned that nutrient associations between feed ingredients might be problematic in evaluating digestibility through substitution trials with abalone. This could be related to the identification of viable bacteria, capable of hydrolysing a variety of complex algal polysaccharides, in the gut of *H. midae* (Erasmus, 1996; Erasmus et al., 1997), and *H. laevigata* (Harris et al., 1998), that could promote associative effects with respect to nutrient digestibility. Evaluation of this technique would not only simplify further digestibility studies with *H. midae,* but would also provide an indication of the possibility of associative effects between feed ingredients in compound diets.

From the above it was evident at the outset of the present study that, in order to effectively evaluate available nutrients in alternative feed ingredients for inclusion in abalone diets, a suitable technique for faeces collection, a reliable and replicable marker, and a suitable digestibility assay technique, should be a priority.

It has been shown that factors such as dietary inclusion level of starch (NRC, 1993) and dietary fibre inclusion (Hilton et al., 1983) may have an effect on apparent nutrient digestibility in shrimp and fish. Pre-gelatinised maize starch and a-cellulose have been routinely varied in nutritional studies with *H. midae* in order to formulate diets with specific protein and energy levels (Britz and Hecht, 1997; Shipton and Britz, 2001), without knowing the possible effect on nutrient digestibility. Evaluation of the influence of these diet manipulating factors on nutrient digestibility was desirable if a proper digestibility protocol for *H. midae* was to be established.

Once a protocol has been established to determine the availability of nutrients such as dry matter, organic matter, energy, protein and amino acids through digestibility it should be extended to the possible evaluation of mineral availability. The starting point for availability of minerals through digestibility should be phosphorus. Although calcium is the main constituent of abalone shell, it is believed that most aquatic species have no dietary calcium

requirement *per se* (Coote et al., 1996), however, many studies suggest that the ratio of calcium to phosphorus should be considered in animal nutrition. The main source of phosphate for aquatic species is their feed due to the low concentration of phosphate in natural waters (NRC, 1993). The lack of information on bioavailability of phosphorus from various feed ingredients for aquatic species can lead to oversupplementation of phosphorus in formulated diets, resulting in increased feed costs, the possibility of affecting the bioavailability of other nutrients, and increased phosphorus wastes which enter the environment via effluent water (Tan et al., 2001).

Determination of nutrient availability of feed ingredients would be useless if it could not be applied to the nutrient requirements of the animal. Studies have been conducted on the dietary protein requirements of *H. midae* (Britz, 1996c), however, a limited protein range (27-47 %) and the use of a single protein ingredient (fish meal) prevented the establishment of an optimal dietary protein level.

Aims and objectives of the present study

Thus, objectives of this study were:

(1) To review and summarise aquaculture and especially nutritional work performed on *H. midae*.

(2) To identify a suitable faecal collection technique for *H. midae*.

(3) To identify a suitable marker for digestibility studies in *H. midae.*

(4) To evaluate the substitution technique used in digestibility of feed ingredients for *H. midae.*

(5) To investigate the possible effect that manipulation of diets with certain ingredients (pre-gelatinsed maize starch and a-cellulose) might have on nutrient digestibility.

(6) To evaluate nutrient digestibility of all feed ingredients utilised in the South African animal industry for *H. midae*.

(7) To evaluate the availability of different inorganic phosphorus sources for *H. midae.*

(8) To determine the optimal dietary protein requirements of *H. midae*.

This work is presented in the form of articles published and submitted for publication in various journals. A final discussion is provided in which the extent to which the objectives were met is evaluated and contextualised. Suggestions are made regarding future work.

References

Akiyama, D., 1991. The use of soy products and other plant protein supplements in aquaculture feeds. Paper presented at the VICTAM-ASIA '91 Conference, Bangkok, Thailand.

Aksnes, A., Hjertnes, T., Opstvedt, J., 1996. Comparison of two assay methods for determination of nutrient and energy digestibility in fish. Aquaculture 140, 343-359.

Allan, G.L., Rowland, S.J., Parkinson, S., Stone, D.A.J., Jantrarotai, W., 1999. Nutrient digestibility for juvenile silver perch *Bidyanus bidyanus*: development of methods. Aquaculture 170, 131-145.

Britz, P.J., 1996a. The nutritional requirements of *Haliotis midae* and development of a practical diet for abalone aquaculture. Ph.D Dissertation, Rhodes University, Grahamstown, South Africa, 150 pp.

Britz, P.J., 1996b. The suitability of selected protein sources for inclusion in formulated diets for the South African abalone, *Haliotis midae.* Aquaculture 140, 63-73.

Britz, P.J., 1996c. Effect of dietary protein level on growth performance of South African abalone, *Haliotis midae*, fed fishmeal-based semi-purified diets. Aquaculture 140, 55-61.

Britz, P.J., Hecht, T., 1997. Effect of dietary protein and energy level on growth and body composition of South African abalone, *Haliotis midae*. Aquaculture 156, 195-210.

Cho, C.Y., Slinger, S.J., Bayley, H.S., 1982. Bioenergetics of salmonid fishes: energy intake, expenditure and productivity. Comp. Biochem. Physiol. 73B, 25-41.

Coote, T.A., Hone, P.W., Kenyn, R., Maguire, G.B., 1996. The effect of different combinations of dietary calcium and phosphorus on the growth of juvenile *Haliotis laevigata*. Aquaculture 145, 267-279.

Erasmus, J.H., 1996. The role of enteric bacteria in the abalone, *Haliotis midae*. M.Sc thesis, University of Cape Town, South Africa, 83 pp.

Erasmus, J.H., Cook, P.A., Coyne, V.E., 1997. The role of bacteria in the digestion of seaweed by the abalone *Haliotis midae*. Aquaculture 155, 377-386.

Fleming, A.E., Van Barneveld, R.J., Hone, P.W., Vandepeer, M.E., Kruk, J.A., 1998. Complementary additivity of the digestibility coefficients of feed ingredients fed to juvenile greenlip abalone (*Haliotis laevigata*). J. Shellfish Res. 17, 641-647.

Hardy, R.W., 1999. Collaborative oppurtunities between fish nutrition and other disciplines in aquaculture: an overview. Aquaculture 177, 217-230.

Hardy, R.W., Kissel, G.W.M., 1997. Trends in aquaculture feeding. Feed Mix 5, 31-34.

Harris, J.O., Burke, M.C., Maguire, G.B., 1998. Characterization of the digestive tract of greenlip abalone, *Haliotis laevigata* Donovan. II. Microenvironment and bactterial flora. J. Shelfish Res. 17, 989-994.

Hertrampf, J.W., Piedad-Pascual, F., 2000. Handbook on Ingredients for Aquaculture Feeds, Kluwer Academic Publishers, Dordrecht, The Netherlands, 573 pp.

Hilton, J.W., Atkinson, J.L., Slinger, S.J., 1983. Effect of increased dietary fiber on the growth of rainbow trout (*Salmo gardneri*). Can. J. Fish. Aquat. Sci. 40, 81-85.

Jones, P.L., De Silva, S.S., 1997. Influence of differential movement of the marker chromic oxide and nutrients on digestibility estimations in the Australian freshwater crayfish *Cherax destructor*. Aquaculture 154, 323-336.

Knauer, J., 1994. Development of an artificial weaning diet for the South African abalone, *Haliotis midae* (Haliotidae: Gastropoda). M.Sc thesis, Rhodes University, Grahamstown, South Africa, 141 pp.

Maguire, G.B., Wee, K.L., Hindrum, S.M., 1993. Digestibility studies – The “ins” and “outs” of abalone guts. Austasia Aquaculture 7, 42-45.

Morales, A.E., Cardenete, G., Sanz, A., De la Higuera, M., 1999. Re-evaluation of crude fibre and acid-insoluble ash as inert markers, alternative to chromic oxide, in digestibility studies with rainbow trout (*Oncorhynchus mykiss*). Aquaculture 179, 71-79.

National Research Council (NRC), 1993. Nutrient Requirements of Fish, National Academy Press, Washington, 114 pp.

Sauer, W.C., Fan, M.Z., Mosenthin, R., Drochner, W., 2000. Methods for measuring ileal amino acid digestibility in pigs. In: D’Mello, J.P.F. (Ed.), Farm Animal Metabolism and Nutrition, CABI Publishing, Wallingford, Oxfordshire, UK, pp. 279-306.

Scott, M.L., Nesheim, M.C., Young, R.J., 1982. Nutrition of the Chicken, 3rd edn. M.L. Scott and Assoc., Ithaca, NY, 562 pp.

Shipton, T.A., 2000. The protein requirements for the South African abalone, *Haliotis midae*. Ph.D Dissertation, Rhodes University, Grahamstown, South Africa, 146 pp.

Shipton, T.A., Britz, P.J., 2001. Partial and total replacement of fishmeal with selected plant protein sources in diets for the South African abalone, *H. midae*. J. Shellfish Res. (In press).

Tan, B., Mai, K., Liufu, Z., 2001. Response of juvenile abalone, *Haliotis discus hannai*, to dietary calcium, phosphorus and calcium/phosphorus ratio. Aquaculture 198, 141-158.

Vandepeer, M.E., Van Barneveld, R.J., Hone, P.W., Havenhand, J.N. 1999. The digestibility of raw, autoclaved and phytase treated legumes in greenlip abalone, *Haliotis laevigata*. Proc. Of the 6th annual abalone aquaculture workshop, World Aquaculture Society Conference, April 1999, Sydney, April.

Wee, K.L., Maguire, G.B., Hindrum, S.M., 1992a. Methodology for digestibility studies with abalone. I. Preliminary studies on the feeding and defaecatory behaviour of blacklip abalone, *Haliotis rubra*, fed natural and artificial diets. In: G.L. Allan G.L., Dall, W. (Eds.), Proc. Aquaculture Nutrition Workshop, 15-17 April 1991, NSW Fisheries, Brackish Water Fish Culture Research Station, Salamander Bay, Australia, pp. 192-196.

Wee, K.L., Maguire, G.B., Hindrum, S.M., 1992b. Methodology for digestibility studies with abalone. 2. Comparison of markers in an artificial diet for blacklip abalone (*Haliotis rubra*) and greenlip abalone (*Haliotis laevigata*). In: Paper delivered at the Third Asian Fisheries Forum, 26-30 October 1992, Singapore.

CHAPTER ONE

Review : Research on abalone (*Haliotis midae* L.) cultivation in South Africa

Sales, J., Britz, P.J., 2001. Review: Research on abalone (*Haliotis midae* L.) cultivation in South Africa. Aquaculture Research 32, 863-874.

Sales, J., Britz, P.J., 2000. South African abalone culture succeeds through collaboration. World Aquaculture, Sept 2000, Vol. 31, No. 3, pp. 44, 45, 49, 50, 61.

Abstract

Although abalone fisheries in South Africa have existed since 1949 cultivation started by successful spawning of captured specimens in 1981 to produce spat and juveniles. Twelve abalone farms, with an estimated investment of US$12 million, have since been established on the coast of South Africa, with a projected production of 500-800 tons. While *H. midae* can reach a maximum size of about 200 mm shell length at an age of over 30 years in the wild, farms produce an average size animal of 100 mm shell length after four years. Growth rates of 0.08-4.5 % body weight/day for abalone of 10-17 mm shell length have been observed under simulated farm conditions on formulated diets, while the corresponding feed conversion ratio (FCR) was 0.9-2.4. Growth rate, FCR and protein efficiency ratio decline at water temperature above 20 °C. Anesthetics, for example magnesium sulfate, are used to prevent injury during removal from tanks. Prostrate diatoms, such as *Cocconeis sublittoralis* (Hendey), *Amphora proteoides* (Hustedt) and *Achnanthes brevipes* (Agardh) are preferred after the metamorphosis from the larval stage (5-7 days, depending on temperature). Most South African abalone growers use formulated feeds in pellet form, due to their convenience and cost benefit to farm operations and management. Although fish meal has been found to be the most suitable protein source for inclusion in formulated diets, plant proteins such as soybean meal, cottonseed meal and sunflower meal presented good growth and apparent protein digestibility. The parasite sabellid polychaete, *Terebrasabella heterouncinata*, recently named by American researchers, is indigenous to a variety of South African intertidal mollusks and impedes growth by causing irritation beneath the mantle in abalone. Prevention is possible to some degree by high standards of hygiene and husbandry of abalone in the tanks, but new techniques to control large infestations are being researched. In spite of a reputation for toughness, abalone meat frozen immediately after shucking is relatively tender in comparison to red meat. The success of abalone farming in South Africa has been due to a high degree of cooperation between the private sector and government-backed research institutions, and it is anticipated that this collaboration will continue.

1. Introduction

Aquaculture of the South African abalone (*Haliotis midae*) developed through the 1990's, in parallel with the emergence of the culture of other commercially important species in Asian countries, the USA, Australia, New Zealand and Chile. The development of South African abalone culture technology has been based on a combination of technology transfer and local innovation by industry in partnership with research institutions. Research to develop abalone culture technology has been almost entirely industry driven and funded, and the results illustrate a good example of industry-science community collaboration.

Of the six haliotid species that occur in southern African waters (*H. midae* L., *H. parvum* L., *H. spadicea* (Donovan), *H. queketti* (Smith), *H. speciosa* (Reeve), *H. pustulata* (Reeve)) only *H. midae*, known locally as "perlemoen", is of commercial significance (Muller, 1986; Hecht, 1994). Although the South African abalone fishery has existed since 1949 (reviewed by Tarr, 1992) the first attempts to cultivate *H. midae* in South Africa were made only in 1981 when captured specimens were successfully spawned to produce spat and juvenile abalone (Genade et al., 1985; 1988).

A concerted R&D effort to establish commercial abalone farming began in 1990 when programmes were initiated by the University of Cape Town, the Council for Scientific and Industrial Research and Rhodes University in partnerships with three fishing companies. Subsequently, some 12 abalone farms have since been established ranging in distribution from Port Nolloth on the Atlantic coast to East London on the Indian Ocean. It is estimated that some US$12 million has been invested in the industry and the projected production of the existing shore based farms is 500-800 tons. One farm, Port Nolloth Sea Farms, is developing abalone ranching, that is grow out of hatchery reared abalone seed in the natural environment north of the natural range of *H. midae* (Sweijd et al., 1998).

This review was written in an attempt to compile all available scientific results on the cultivation of abalone in South Africa.

2. Spawning and seed production

Some luck was involved in the first attempt to spawn *H. midae*, as the ripe abalone collected by Genade et al. (1985; 1988) spawned spontaneously in their plastic bags during transit, and the viable larvae were settled at the Fisheries Development Corporation laboratories at Knysna. Subsequent experience has revealed that, unlike other abalone species, *H. midae* will usually not spawn if collected ripe from the wild, and thus abalone hatcheries have developed broodstock conditioning protocols and maintain a hatchery broodstock population which is spawned on a regular basis. Broodstock conditioning and maintenance techniques for *H. midae* are summarised in Fleming (1999).

Apart from Genade et al.'s work, no further scientific publications have emerged on the development of hatchery technology for *H. midae*. This is partly because the basic techniques used have been adapted from technology developed elsewhere (Hahn, 1989), and because local innovations have been guarded as proprietary information. Insights into the reproductive biology of *H. midae* are however provided in ecological studies by Newman (1967), Tarr (1995) and Wood (1993). Tarr (1995), studying populations at six sites around the South African coast, showed that 100 % sexual maturity may occur at around 7.2 years in the wild, some 4 years earlier than previously estimated by Newman (1967). On the warmer east coast, and under culture conditions, abalone mature earlier and 100 % sexual maturity may occur as early as three years of age (Wood, 1993). Spawning occurs twice per year in certain areas, namely during spring and autumn. There are, however, variations due to locality. A linear relationship ($F = 0.0198\ W - 2.196$) between fecundity (F, million of eggs) and animal weight (W) has been established. Within a ripe ovary two discrete groups of eggs are found and these are released on consecutive spawning. Egg production is in the order of several million per individual and is a function of ovary volume due to the arrangement of the germinal surfaces (Newman, 1967).

3. Growth

In the wild *H. midae* reaches a maximum size of about 200 mm shell length at an age of over 30 years (Newman, 1968). However, high individual variability was found in the

growth rates of wild animals from different sites at the South African coast. Even for similar sized animals, for example animals of 68 mm length, increment ranged from 9-33 mm/year in comparison to 5-24 mm/year for animals of 82 mm shell length at one location (Tarr, 1995).

The growth rate of *H. midae* under various laboratory conditions and on various formulated diets is compared in Table 1. This comparison, however, must be evaluated with caution, as various trial conditions, such as the initial size of animals, the stocking density, temperature and duration, are only a few factors that could influence the growth rate (Fleming et al., 1996).

4. Temperature

The distribution of the South African abalone *H. midae* spans the confluence of the Atlantic and Indian Oceans, ranging from the cold waters of the Benguela upwelling system on the Western Cape coast to the warmer Eastern Cape coast which is influenced by the south flowing Agulhas current (minimum of 12-13 °C; maximum of 21 °C) (Britz et al., 1997a). An inverse relationship between maximum size and mean sea temperature was demonstrated in natural stocks (Newman, 1968; 1969; Tarr, 1995; Wood, 1993).

The preferred optimal temperature of juvenile *H. midae* (30-45 mm shell length) was found to be 24 °C and the 50 % critical thermal maximum was 27.9 °C (Hecht, 1994). However, trends in growth rate, feed consumption, mortality, protein efficiency ratio (PER) and feed conversion ratio (FCR) data indicate that a temperature range of 12-20 °C (Table 2) is physiologically optimal for *H. midae* (Britz et al., 1997a). Above 20 °C growth rates declined, FCR and PER deteriorated, and mortality increased significantly suggesting a gradual breakdown in physiological processes. This hypothesis is supported by the data of Lyon (1995) who observed that ammonia excretion and oxygen consumption rates in *H. midae*, expressed per kg feed consumed, increased significantly above 20 °C. In a practical farming context, it would be advisable to cease feeding when water temperatures exceed 20 °C. Although *H. midae* are able to survive acute exposure to extremes of 25 °C (Hecht, 1994), they probably would not grow at these temperatures (Britz et al., 1997a).

Table 1

Performance of *H. midae* when feeding on various formulated diets (temperature 12-24 ºC)

Study	Size (shell length, mm)	Duration (days)	Daily feed intake[a]	(Unit)	Growth rate	(Unit)	Feed conversion ratio[b]
Britz (1996a)	21.2	124	0.5-2.8	(% live weight)	29-65	(µm/day)	0.7-3.4
Britz (1996b)	19.9	95	1.0	(% live weight)	2.5-2.9	(mm/month)	1.04-1.15
Britz and Hecht (1997)	35-43	142	23-45	(mg)	0.08-4.5	(% live weight/day)	0.9-2.4
Britz and Hecht (1997)	10-17	72	7.2-9.7	(mg)	1.5-2.2	(% live weight/day)	1.0-1.5
Britz et al. (1997a)	17.5	90	15.7-23.5	(mg)	25-85	(µm/day)	0.97-3.02
Knauer et al. (1996)	3.22-11.29	30	5.55	(% feeding rate)[c]	59	(µm/day)	0.44
Shipton (2000)	15	316	0.67-1.36	(% live weight)	0.24-1.68	(mm/month)	1.0-7.1
Shipton(2000)	45	316	0.16-0.40	(% live weight)	0.33-2.12	(mm/month)	1.0-6.2

[a] Dry matter basis.
[b] g dry feed consumed/g wet weight gain.
[c] (g dry weight feed intake over experimental period)/{number of days x [(final wet abalone weight + initial wet abalone weight)/2]} x 100.

Table 2

Nutritional indices and mortality for abalone (17.5 mm shell length, 1.1 g live weight, *n* = two replicates of 30 animals each per treatment, means ± S.D.) reared on a formulated diet between 12 and 24 °C over three months (the loss of solids from the feed due to leaching over 24 h is included) (Britz et al., 1997a)

	Temperature (° C)						
	12	14	16	18	20	22	24
Feed intake (mg/day)[a]	15.7 ± 1.36	19.1 ± 1.21	20.5 ± 1.24	21.3 ± 1.26	23.5 ± 1.32	21.7 ± 1.37	16.2 ± 1.24
Feed conversion ratio[b]	1.37 ± 0.24	1.29 ± 0.27	1.12 ± 0.12	1.15 ± 0.22	0.97 ± 0.06	1.82 ± 0.21	3.02 ± 0.76
Protein efficiency ratio[c]	1.76 ± 0.15	2.62 ± 0.14	2.72 ± 0.11	2.97 ± 0.17	3.0 ± 0.33	1.73 ± 0.22	0.41 ± 0.09
Condition factor[d]	1.21 ± 0.02	1.11 ± 0.02	0.98 ± 0.03	1.08 ± 0.02	0.94 ± 0.01	0.91 ± 0.01	0.89 ± 0.03
Mortality (%)	2.5 ± 0.8	3.3 ± 0.0	3.3 ± 1.7	1.7 ± 1.7	0.83 ± 0.8	9.16 ± 0.8	32.5 ± 2.5
24 h dry matter leaching (% recovered)	94.3 ± 0.9	92.9 ± 0.5	91.3 ± 0.5	90.3 ± 0.6	89.4 ± 0.5	91.1 ± 0.6	89.3 ± 0.6

[a] Dry matter basis.
[b] g dry feed consumed/g wet weight gain.
[c] g wet weight gain/g protein consumed.
[d] (g wet abalone weight/mm shell length$^{2.99}$) x 5575 (Britz, 1996a).

5. Handling and transport

Abalone live in the high energy subtidal zone, and are renowned for ability to pull their shell down rapidly and tightly onto the substratum, complicating their removal and handling on abalone farms (White et al., 1996). Mechanical removal often results in injury or death due to a slow healing rate and increased probability of bacterial infection and stress (Genade et al., 1988). A study evaluating the suitability of various anaesthetics, showed that magnesium sulphate was an effective anaesthetics for removal of *H. midae*, while ethylenediamine tetra-asetic acid was unsuitable and procaine hydrochloride was unsafe. Effective concentrations of magnesium sulphate were 4, 14 and 22 g/100ml for the size classes 5-15, 20-50 and 60-90 mm shell length, respectively (White et al., 1996). Although 2-phenoxyethanol was an effective anaesthetic, significant mortalities occurred following recovery indicating its unsuitability for abalone (White, 1995).

Regarding transportation of live *H. midae* transit time should not be longer than 36 h. Juveniles can survive for longer periods (52 h), but the percentage mortality gradually increases with time. The provision of enriched oxygen levels in the transport container helps to reduce mortalities where long shipment times are envisaged. For shorter periods, oxygen enrichment is not vital but it may alleviate stress. Temperature fluctuations that the animals are exposed to must be kept to a minimum for example by correct insulation design. It is important to keep the inside very humid to prevent desiccation that can harm the gills. Animals are starved for 2-3 days before transport to prevent them from producing faeces inside the box. Acclimatisation does reduce stress but it does not make a significant difference to survival rates in transit (Cook and Ruck, 1991).

6. Nutrition

Feed makes up a major proportion of production costs on South African abalone farms, where the stock is reared on either a compound formulated diet, harvested kelp (*Ecklonia maxima*), cultured *Gracillaria* spp. *(gracilis, verrucosa*) or a combination of the above (Cook, 1998). Young newly metamorphosed abalone are reared on diatoms cultured on plates or in bags and then weaned on to seaweed or formulated feed at 4-6 mm shell length (approximately 5-6 months old). The development of abalone farming has

stimulated research into abalone digestive physiology, the application of animal feed science principles to abalone, abalone feeding behaviour, and the optimisation of the utilisation of both natural and formulated diets under intensive culture conditions (Britz et al., 1994; Fleming et al., 1996).

6.1. Feeding behaviour and digestive processes

H. midae is an entirely herbivorous species with a nocturnal pattern of grazing behaviour, tending to remain inactive by day, both in the field and laboratory tanks, and move about at night (Barkai and Griffiths, 1987). A laboratory behavioural study showed that although appearance rate of juvenile *H. midae* (5.00-8.54 mm shell length) in glass tanks was about 80 % between 1900 and 2300 h, fewer than 7 % were actively feeding (Knauer et al., 1995a). Although taking a wide range of algae the preferred food item of mature *H. midae* in nature is kelp *E. maxima*, with *Plocamium* spp. also a major element of the diet on the south coast of South Africa. The diets of abalone in smaller size classes are similar, except that *Ulva* spp. is taken in larger proportion. This might be explained by the fact that both *Ulva* spp. and small abalone tend to be more abundant in shallower water. The conclusion is that *H. midae* select from a wide group of acceptable species largely according to their abundance in the surrounding habitat (Barkai and Griffiths, 1986). Growth rates of cultured *H. midae* on *E. maxima* can be improved by addition of either *Porphyra*, *Ulva*, or *Aeodes* spp., or a mixture of all three, in a rotation diet (Simpson and Cook, 1998).

Feed intake (wet weight) in wild *H. midae* averaged 8.1 % of soft body weight/day at 14 °C and 11.4 % at 19 °C. Feeding rates are fairly rapid for a gastropod of this size, at 0.83-0.93 % body mass/h, but they are sustained for about half of the day only, so that the rate averaged over 24 h is in fact 0.45 and 0.48 %/h (Barkai and Griffiths, 1987). Both formulated feeds and preferred algal species are digested within 24 h (Britz et al., 1996; Day and Cook, 1995), however, less preferred algae are digested more slowly and may remain recognizable in the crop for 48 h (Day and Cook, 1995). When fed a formulated diet, gut fullness peaked 6 h after feed was offered, and results indicated that the bulk of feed was consumed between 1800 and 2400 h. Enzyme secretion began with the onset of feeding and continued for at least 6 h after peak gut fullness was attained. Protease

activity increased significantly following ingestion, whereas amylase activity was maintained at a fairly constant level (Britz et al., 1996). A low level of lipase activity was observed, suggesting that the ability of *H. midae* to digest fat is limited (Britz et al., 1996; Knauer et al, 1996). It was concluded that when formulated food is used, a feeding frequency of once per day is recommended (Britz et al., 1996).

It has been shown that the digestive physiology of juvenile *H. midae* (3.22-11.29 mm shell length) can readily adapt to formulated diets (Knauer et al., 1996). Formulated diets are now used to wean abalone from diatoms on most South African farms.

Viable bacteria, capable of hydrolysing a variety of complex polysaccharides (laminarin, carboxymethylcellulose, alginate, agarose, carrageenan) in algae, have been identified in the gut of *H. midae*. Endogenous polysaccharases of abalone fed either *E. maxima* or *G. verrucosa* varied in response to diet (Erasmus et al., 1994; Erasmus, 1996; Erasmus et al., 1997). A probiotic research programme to use bacteria to enhance feed digestion in abalone is underway at the University of Cape Town. Bacterial isolates included in diets containing *E. maxima* and *G. gracilis* extracts have been shown to improve growth in abalone (Doeschate et al., 2000).

6.2. Post-larval feeding

After fertilisation the egg undergoes repeated cleavage to form a trocophore larva which develops into a non-feeding veliger stage, which is also planktonic (Tarr, 1987). The larval period is 5 days at 20 °C and 7 days at 17.5 °C (Genade et al., 1985; 1988) whereafter the larvae settle down in shallow water, apparently being induced to settle by the presence of mauve coloured encrusting alga lithothammion. Once settled the larvae develop into miniature abalone and begin feeding on benthic diatoms (Tarr, 1987).

In a study on the grazing of post-larval abalone fed on cultured diatoms it was shown that prostrate diatoms such as *Cocconeis sublittoralis*, *Amphora proteoides* and *Achnanthes brevipes* were preferred. However the post-larval abalone would consume the more loosely packed, overstorey species such as *Delphineis karstenii* (Boden) Fryxell, *Diploneis placida* (Schmidt) Hustedt and *Nitzchia palea* (Kützing) Wm. Smith if their preferred food was not available. Pre-grazing of settlement surfaces by juvenile abalone

before the introduction of larvae removed most of the overstorey diatoms but left the preferred species virtually intact (Matthews and Cook, 1995).

6.3. Formulated diets

Natural feeds (cultured or harvested seaweed) are used for cultured abalone in the USA, Taiwan and Japan. However, if abalone farming is to become a substantial aquaculture industry, the development of pelleted food for abalone is seen as being fundamental to its growth. Compared to natural sources, pelleted feeds offer convenience and cost benefits to farm management (Britz et al., 1994).

One of the requirements of abalone feed is that the water-soluble nutrients remain in the feed and the food particles remain bound together for at least two days (Fleming et al., 1996). The 24-h water stability of seven different binders (five alginates in combination with two sequestrants, agar, gelatine and a mixture of agar-gelatine) revealed that a 1:3 agar:gelatine mixture retained 70.7 % of its dry weight after 24 h (Knauer et al., 1993). These hydrocolloids proved to be impractical and too costly in a commercial abalone diet, so a starch-bound dry pellet (Table 3) was developed (Britz et al., 1994).

Table 3
Semi-purified dietary formulation used to feed abalone *H. midae* under culture conditions (Britz et al., 1994)

Ingredient	%
Casein	32.0
Dextrin	44.0
Kelp powder	5.0
Fish oil	5.0
Agar	9.0
Vitamin mixture	1.5
Mineral mixture	3.5

Dry matter leaching of this dry pellet was in the order of 5 % over 24 h (Britz and Clayden, 1996). The proximate (Table 4), amino acid (Table 5), fatty acid (Table 6) and mineral (Table 7) profiles of abalone (Knauer et al., 1994; 1995b) was used as a guideline to

formulate a practical diet. Pellets produced a significantly better increase in shell length and weight in juvenile (5.00 – 8.54 mm shell length) *H. midae* than gels (Knauer et al., 1995a).

Table 4
Proximate composition (dry matter basis, means ± S.D.) of soft body tissue and total body tissue of two size classes of *H. midae* (Knauer et al., 1994)

	Size class (shell length, mm)			
	10-20 (*n* = 558)		45-55 (*n* = 58)	
	Soft body	Total body	Soft body	Total body
Weight (g)	0.46 ± 0.04	0.72 ± 0.05	22.28 ± 2.60	30.33 ± 3.02
Moisture (%)	81.31 ± 1.52	68.69 ± 3.26	77.91 ± 1.23	62.56 ± 0.94
Fat (%)	2.40 ± 0.85	1.40 ± 0.47	0.76 ± 0.06	0.52 ± 0.26
Protein (%)	44.67 ± 4.16	22.87 ± 2.83	39.67 ± 1.33	31.33 ± 3.20
Ash (%)	11.96 ± 1.94	58.22 ± 5.66	11.88 ± 2.29	34.91 ± 4.02
Carbohydrate (%)	38.93 ± 9.71	15.60 ± 1.54	42.19 ± 4.31	29.94 ± 4.29

From studies with different size classes of abalone the following formula was develop to predict formulated feed consumption of *H. midae* within the optimal temperature zone (12-20 °C) (Britz et al., 1997a):

$$C = \frac{2.99 \times FCR \times (5.25T + 27.8)}{L}$$

where:

C is consumption (% body weight/day)
FCR is feed conversion ratio
L is abalone length (µm) for which consumption is predicted
T is ambient temperature (°C)

Table 5

Amino acid composition (% of wet weight, means ± S.D.) of juvenile (8.12-16.96 mm shell length, 0.08-0.73 g live weight, 0.04-0.50 g soft body weight, *n* = 3 samples from 279 pooled animals) *H. midae* (Knauer et al., 1995b)

	Soft body tissue	Total body tissue
Alanine	2.81 ± 0.11	1.33 ± 0.15
Arginine	3.81 ± 0.14	1.81 ± 0.20
Aspartic acid	5.08 ± 0.24	2.41 ± 0.29
Cystine	1.23 ± 0.05	0.56 ± 0.14
Glutamic acid	7.14 ± 0.25	3.38 ± 0.33
Glycine	4.03 ± 0.14	1.91 ± 0.16
Histidine	0.88 ± 0.09	0.42 ± 0.07
Isoleucine	1.96 ± 0.11	0.93 ± 0.12
Leucine	3.34 ± 0.15	1.59 ± 0.18
Lysine	3.00 ± 0.17	1.42 ± 0.19
Methionine	1.02 ± 0.03	0.48 ± 0.04
Phenylalanine	1.88 ± 0.16	0.89 ± 0.14
Proline	2.53 ± 0.11	1.20 ± 0.15
Serine	2.47 ± 0.09	1.17 ± 0.12
Threonine	2.41 ± 0.15	1.15 ± 0.15
Tryptophan	0.36 ± 0.04	0.19 ± 0.02
Valine	2.23 ± 0.15	1.06 ± 0.15

About 63 % of the energy consumed in food is lost in faeces in wild animals and a further 32 % expended on respiration. Energy losses in the form of ammonia excretion are negligible, accounting for less than 1 % of consumption. Some 5 % of energy intake, or 13 % of absorbed ration, is thus available for growth and reproductive output (Barkai and Griffiths, 1988). No information could be found on energy requirements or metabolism in animals reared on formulated diets.

Table 6

Fatty acid composition of juvenile (8.12-16.96 mm shell length, 0.08-0.73 g live weight, 0.04-0.50 g soft body weight, *n* = 3 samples from 279 pooled animals) *H. midae* (Knauer et al., 1995b)

Fatty acid	(% of total)
12:0	1.80
16:0	16.25
16:1	1.56
18:0	4.70
18:1	7.27
18:2*w*6	10.86
18:3*w*3	1.11
19:0	7.22
20:0	4.02
20:1	2.48
20:2	0.53
20:4	3.27
20:5*w*3	9.55
22:0	2.95
22:1	2.47
22:4*w*5	3.34
22:5	1.12
22:6*w*3	0.72
24:0	5.57
24:1	4.91
24:4	3.94

Table 7

Mineral composition (µg/g wet weigh, mean ± S.D.) of *H. midae.*

	Knauer et al. (1995b)[a]		Van As et al. (1975)[b]
	Soft body tissue	Total body tissue	
Calcium	187.0 ± 83.5	76452.7 ± 16920.0	
Cadmiun	0.2 ± 0.1	0.6 ± 0.1	
Cobalt	0.6 ± 0.2	3.4 ± 0.2	0.025 ± 0.007
Copper	7.2 ± 1.3	15.4 ± 2.4	
Iron	210.6 ± 48.2	410.0 ± 95.6	18 ± 14
Sodium	2100.0 ± 200.0	3800.0 ± 346.4	
Magnesium	1000.0 ± 100.0	1895.0 ± 193.2	
Manganese	0.8 ± 0.3	2.2 ±0.7	0.17 ± 0.07
Nickel	2.3 ± 0.5	7.2 ± 1.4	
Zinc	20.5 ± 0.2	46.0 ± 3.5	12 ± 2
Chromium			0.50 ± 0.25

[a] 8.12-16.96 mm shell length, 0.08-0.73 g live weight, 0.04-0.50 g soft body weight, n = 3 samples from 15 pooled animals.

[b] From south west coast of South Africa; no indication of animal history.

6.4. Protein and amino acids

While it is generally accepted that animals eat to satisfy their energetic requirements, they do not have a protein requirement *per se*. Rather, they have a requirement for essential and non-essential amino acids that, supplied in the correct ratios with the appropriate level of digestible energy, will maximise somatic growth. Non-essential amino acids are those that can be synthesised by the animal, however, essential amino acids cannot be synthesised and must therefore be supplied as a part of the diet. It was concluded from results presented in Table 8 that low-temperature fish meal and *Spirulina* algae are the most suitable proteins for inclusion in practical diets for *H. midae*. Increasing concerns about the future supply and demand for fish meal have increased efforts to reduce their use as the major protein source in commercial aquaculture feed formulations. No significant differences were found in growth rates of *H. midae* of 10.6 mm shell length over a 180 day period when 30 % of the fish meal component had been replaced by either soy or sunflower meals, or torula yeast (Shipton, 2000).

Table 8

Feed intake, specific growth rate (SGR), length increment, feed conversion ratio (FCR) and protein efficiency ratio (PER) of abalone (21.2 mm shell length, 1.76 g live weight (LW), n = 2 replicates of 15 animals each per treatment) over three months fed on formulated (single protein) and natural diets (Britz, 1996a)

	Diets						
	Fish meal	Casein	Soybean meal	Torula yeast	*Spirulina* spp.	*P. corallorhiza*	*E. maxima*
Crude protein in diet (%)	29	31	32	29	19	10	10
Feed intake (% LW/day)[a]	0.8	0.5	0.6	0.7	0.8	1.3	2.8
SGR[b]	0.9	0.6	0.5	0.6	0.8	0.4	0.6
Length increment (μm/day)	65	45	41	42	58	29	54
FCR[c]	0.8	0.7	1.0	1.0	0.8	2.8	3.4
PER[d]	3.9	4.7	3.4	3.3	6.5	2.2	3.0

[a] Dry matter basis.
[b] [(ln g final wet weight – ln g initial wet weight)/time in days] x 100.
[c] g dry feed consumed/g wet weight gain.
[d] g wet weight gain/g protein consumed.

Among two size classes of *H. midae* (35-43 mm shell length, 7-14 g live weight and 10-17 mm shell length, 0.2-1 g live weight) a dietary lipid level of 10 % at crude protein levels of 34 and 44 % produced the lowest growth rate, % protein deposited and PER in comparison to diets containing 2 and 6 % fat. These trends were, however, more marked among the small size class. In addition, the 10 % fat diets yielded poorer FCR values among the small abalone, but this trend was not evident in the larger size class which displayed a trend in improving FCR with an increasing protein to energy (PE) ratio. For the larger abalone, growth rates increased with increasing PE ratio and peaked in the 44 % protein and 6 % fat treatment. By contrast, the growth rate of the small abalone fed diets increased with PE ratio up to a level of 34 % dietary crude protein and then leveled off suggesting that smaller abalone have a lower protein requirement than larger abalone (Britz and Hecht, 1997). This is in contrast with results of Shipton (2000) that suggest a poorer performance in smaller than large animals with similar diets (Table 9).

Identification of limiting amino acids and the daily requirement for amino acids have to be determined before the supplementation of synthetic amino acids in formulated feeds for abalone can be exploited (Fleming et al., 1996). Diets supplemented with graded levels of crystalline arginine failed to produce any improvement in the growth rate of *H. midae*. Although it remains to be conclusively determined whether abalone are able to utilise arginine and other essential amino acids in crystalline form, the indication is that their use in formulated diets are not promising due to their high solubility. It seems that the proportion of arginine, relative to other essential amino acids, in abalone tissue does not provide an indication of what its dietary requirement is, and that arginine is not a limiting amino acid (Britz et al., 1997b; Shipton, 2000).

While quantitative determination of the protein, amino acid and energy requirements is a prerequisite to the development of diets that maximize feed utilization, the determination of nutrient availability from feed sources is also required if diets are to be designed that satisfy the nutritional requirements of the animals. Digestibility trials that provide an indication of nutrient bioavailability are therefore essential to the formulation of nutritionally complete diets (Shipton, 2000).

Table 9

Feed intake, length increment, feed conversion ratio (FCR) and protein efficiency ratio (PER) of small (15 mm shell length, 0.57 g live weight LW), *n* = 3 replicate groups of animals per treatment) and large (45 mm shell length, 23 g live weight, *n* = 3 replicate groups of animals per reatment) abalone over 316 days fed on formulated single protein diets (Shipton, 2000)

	Diets						
	Danish LT Fish meal	*Spirulina* spp.	Torula yeast	Sunflower meal	Cottonseed meal	Carcass meal	Brewery waste
Crude protein in diet (%)	20.0	18.7	22.2	20.6	20.8	20.7	19.3
Feed intake (% LW/day)[a]							
small	1.20	1.17	0.86	1.23	1.28	1.36	0.67
large	0.40	0.34	0.27	0.36	0.37	0.37	0.16
Length increment (mm/month)							
small	1.59	1.54	1.23	1.49	0.97	0.43	0.24
large	2.12	1.82	1.24	1.72	1.18	0.43	0.33
FCR[b]							
small	1.1	1.2	1.2	1.4	3.1	6.2	7.1
large	1.1	1.3	2.6	1.2	1.8	4.0	6.2
PER[c]							
small	5.4	5.6	3.3	4.1	2.0	0.8	0.4
large	5.5	5.8	2.3	4.8	3.3	1.4	0.6

[a] Dry matter basis.
[b] g dry feed consumed/g wet weight gain.
[c] g wet weight gain/g protein consumed.

Although total collection is theoretically the best method to use, with abalone collection of all faeces produced is difficult as there are often not discrete pellets and tend to disintegrate in water, even when measures are taken not to disturb them. Leaching of soluble nutrients from the faeces and feed is also a problem when determining the digestibility of specific nutrients, but this is a problem common to all methods (Fleming et al., 1996). Three distinguishable types of faeces namely, (A) light green amorphous faeces, (B) medium green amorphous faeces and (C) dark green discrete faecal pellets and strings were produced in *H. midae* (Shipton, 2000). Protein content of type C faeces was significantly higher than in the other two types. This was also found for chromic oxide concentration in the latter species, except that the content in type A was also significant lower than type B. Type A and B faeces contained large amounts of mucosal material that reduced the overall concentration of chromic oxide and protein in the faeces. Shipton (2000) obtained apparent protein digestibility values of 14.2, 61.3 and 74.7 % for type A, B and C faeces, respectively, in *H. midae* using a fish meal based formulated diet.

Apparent crude protein digestibility of different feedstuffs are presented in Table 10. Apparent crude protein digestibility was significantly higher at 18 °C (96.7 %) than at either 15 °C (93.9 %) or 22 °C (92.7 %) (Dixon, 1992).

7. Health

The parasite sabellid polychaete, *Terebrasabella heterouncinata,* has caused serious problems in Californian abalone farms after accidental introduction in the late 1980s from South Africa. It is indigenous to South Africa and is found in a wide range of South African intertidal mollusks. Growth of abalone is influenced because of the interference caused by larvae at the mantle-shell interface (Ruck and Cook, 1998). Improvements in hygiene, and husbandry, in tanks have considerably reduced the problems that this infection has recently caused in South African abalone farms (Cook, 1998). Research is being performed on the use of ultrasound treatment of sabellid infestations in South African abalone (Loubser and Dormehl, 2000).

Table 10

Apparent protein digestibility coefficients (%) for different feed ingredients according to the chromic oxide marker method

	Coefficient	Reference
P. corallorhiza	57.3	Dixon (1992)[a]
G. amanzii	80.0	Dixon (1992)
Semi-puried[b]	95.6	Dixon (1992)
Casein	93.8	Shipton (2000)[c]
Fish meal (Danish 999LT)	82.0	Shipton (2000)
Abalone viscera silage[d]	72.2	Shipton (2000)
Carcass meal[e]	52.1	Shipton (2000)
Torula yeast	83.4	Shipton (2000)
Spirulina	74.2	Shipton (2000)
Soybean meal (solvent extracted)	79.2	Shipton (2000)
Cottonseed meal (solvent extracted)	76.2	Shipton (2000)
Sunflower meal (solvent extracted)	68.1	Shipton (2000)

[a] Results from Dixon (1992) from six replicate groups of 10 animals (55-85 mm shell length).

[b] Calcium caseinate (30 %); dextrin (50 %), agar (9 %); dried kelp (5 %) as main ingredients.

[c] Results from Shipton (2000) from single protein test diets containing 20 % protein, 6 % lipid and 0.5 % chromic oxide, three replicate groups of six animals (69.9 mm shell length), based on chromic oxide in Type C faeces.

[d] Abalone viscera silage prepared using citric and phosphoric acids and heat treatment according to Viana et al. (1996).

[e] Carcass meal from South African red meat abattoirs.

Although abalone are not filter feeders and have not usually been considered to be affected by the toxic red tides, recent evidence suggests that at least one species of toxic algae found in South African waters may release some of its toxin into the water, and mortalities of larvae in some hatcheries have been experienced at such times. Research is currently being undertaken to understand the mechanism of this toxicity (Cook, 1998; Botes et al., 2000).

In April 1999 routine monitoring provided evidence of the presence of paralytic shellfish poisoning toxins in cultured South African abalone (Pitcher et al., 2000). A health management program has been run since March 1999 on South African abalone

production units. Findings include the occurrence of renal coccidia, the presence of an unknown rickettsia-like organism in the digestive gland, and protozon parasites affecting various sections of the gut. In general a lack of knowledge is experienced on abalone diseases (Mouton, 2000).

8. Abalone products

Abalone are among the most highly valued seafoods in the world, with the prime demand in Asian countries where abalone products form part of traditional cuisine and ceremony (Britz, 1996c). Shells with a good nacre are used as jewelry. Viscera may be sold for human consumption or may be used as crayfish bait, but in many instances they are discarded (Olley and Thrower, 1977).

8.1 Nutrient composition

The modern consumer wants to be aware of the nutrient composition of the food that is consumed. The proximate, amino acid, fatty acid and mineral composition of *H. midae* are presented in Tables 4, 5, 6 and 7. Although most of this research was conducted on small juveniles below market size, and included guts, it does serve as an indicator of the composition of *H. midae*.

8.2 Other characteristics

The onset of pH decline in abalone meat was found to be 17 h after shucking when wild adult *H. midae* were kept at 7 °C, while the corresponding figure was 13 h at 16 °C. Abalone frozen immediately after shucking at -20 °C had a significantly higher pH_{7days} than those kept at 7 and 16 °C, indicating the absence or delay of *rigor* development at a low temperature (Sales et al., 1999). When an animal dies the conversion of muscle to meat involves a number of biochemical and biophysical changes. The energy carrier of muscle, glycogen, is broken down during anaerobic respiration to lactic acid that causes a decrease in pH as *rigor* proceeds. As with all chemical reactions this process is accelerated by increasing temperature (Lawrie, 1991). Furthermore, abalone that are frozen immediately had a significantly lower (more tender) Instron value, indicating that either toughness associated with *rigor* as in red meat is absent in abalone meat (Sales et al., 1999), or that there was no *rigor* development (Lawrie, 1991).

Conclusions

Abalone farming could be described as the pioneer industry of mariculture in South Africa, a country where aquaculture is relatively unknown. The decline of South African abalone fisheries due to poaching, as in other parts of the world, has stimulated the development of abalone farming to meet the demand for the product. The success of abalone cultivation in South Africa can be attributed to cooperation between industry and research organizations who together have steered research in a practical direction. Although some research has been performed on management aspects such as optimal water temperature and handling, most published research on *H. midae* has concentrated on nutrition of the animal. A great percentage of the research effort was put into the development of a formulated diet, and the search for the most cost effective feed ingredients which promote maximal growth. Research has been conducted on protein and lipid requirements, however, information on energy and mineral metabolism is lacking. Technologies regarding aspects such as spawning, density and processing have generally been adopted from those existing in established industries from overseas (Japan, Australia). Research on genetics and diseases has just started. Continued collaboration between all involved in this industry is needed in order to improve existing techniques, prevent duplication of high cost experiments and establish a correct and concise protocol for further research.

References

Barkai, R., Griffiths, C.L., 1986. Diet of the South African abalone *Haliotis midae*. S. Afr. J. Mar. Sci. 4, 37-44.

Barkai, R., Griffiths, C.L., 1987. Consumption, absorption efficiency, respiration and excretion in the South African abalone *Haliotis midae*. S. Afr. J. Mar. Sci. 5, 523-529.

Barkai, R., Griffiths, C.L., 1988. An energy budget for the South African abalone *Haliotis midae* Linnaeus. J. Mollusc Stud. 54, 43-51.

Britz, P.J., 1996a. The suitability of selected protein sources for inclusion in formulated diets for the South African abalone, *Haliotis midae.* Aquaculture 140, 63-73.

Britz, P.J., 1996b. Effect of dietary protein level on growth performance of South African abalone, *Haliotis midae*, fed fishmeal-based semi-purified diets. Aquaculture 140, 55-61.

Britz, P.J., 1996c. The nutritional requirements of *Haliotis midae* and development of a practical diet for abalone aquaculture. Ph.D Dissertation, Rhodes University, Grahamstown, South Africa, 150 pp.

Britz, P.J., Bacela, N., Hecht, T., 1997b. Can crystalline arginine be used to quantify the arginine requirement of abalone? Aquaculture 157, 95-105.

Britz, P.J., Clayden, C., 1996. Evaluation of the growth rate and feed conversion ratio of *Haliotis midae* fed formulated practical diets. Proc. Aquacult. Assoc. S. Afr. 5, 68-73.

Britz, P.J., Hecht, T., 1997. Effect of dietary protein and energy level on growth and body composition of South African abalone, *Haliotis midae*. Aquaculture 156, 195-210.

Britz, P.J., Hecht, T., Knauer, J., 1996. Gastric evacuation time and digestive enzyme activity in abalone *Haliotis midae* fed a formulated diet. S. Afr. J. Mar. Sci. 17, 297-303.

Britz, P.J.,Hecht, T., Knauer, J., Dixon, M.G., 1994. The development of an artificial feed for abalone farming. S. Afr. J. Sci. 90, 7-8.

Britz, P.J., Hecht, T., Mangold, S., 1997a. Effect of temperature on growth, feed consumption and nutritional indices of *Haliotis midae* fed a formulated diet. Aquaculture 152, 191-203.

Botes, L. G., Pitcher, C., Cook, P.A., 2000. The potential risk of harmful algae to abalone farming on the south coast of South Africa. Poster presented at the Fourth International Abalone Symposium, 6-11 February 2000, University of Cape Town, Cape Town, South Africa.

Cook, P.A., Ruck, K., 1991. Transportation of live perlemoen. Zoology Department, University of Cape Town, South Africa.

Cook, P.A., 1998. The current status of abalone farming in South Africa. J. Shellfish Res. 17, 601-602.

Day, R., Cook, P.A., 1995. Bias towards brown algae in determining diet and food preferences: the South African abalone *Haliotis midae*. Mar. Freshwater Res. 46, 623-628.

Dixon, M.G., 1992. The effect of temperature and photoperiod on the digestive physiology of the South African abalone *Haliotis midae*. M.Sc thesis, Rhodes University, Grahamstown, South Africa, 85 pp.

Doeschate, K., Macey, B., Coyne, V., 2000. Characterisation of the enteric bacteria of the abalone *Haliotis midae*, and their role in the digestion of ingested seaweed. Poster presented at the Fourth International Abalone Symposium, 6-11 February 2000, University of Cape Town, Cape Town, South Africa.

Erasmus, J.H., 1996. The role of enteric bacteria in the abalone, *Haliotis midae*. M.Sc thesis, University of Cape Town, South Africa, 83 pp.

Erasmus, J.H., Cook, P.A., Coyne, V.E., 1997. The role of bacteria in the digestion of seaweed by the abalone *Haliotis midae*. Aquaculture 155, 377-386.

Erasmus, J., Coyne, V., Cook, P., 1994. Characteristics of gut microflora in the abalone, *Haliotis midae*. Poster presented at the Second International Symposium on abalone biology, fisheries and culture, 7-11 February 1994, Hobart, Australia.

Fleming, A.E., Van Barneveld, R.J., Hone, P.W., 1996. The development of artificial diets for abalone: A review and future directions. Aquaculture 140, 5-53.

Fleming, A.E., 1999. Conditioning of Australian abalone broodstock. A report for the Marine and Freshwater Research Institute, Queenscliff, Australia, 34 pp.

Genade, A.B., Hirst, A.L., Smit, C.J., 1985. Observations on the spawing and rearing of the South African abalone *Haliotis midae* Linn. Fisheries Development Corporation, Knysna, South Africa, 13 pp.

Genade, A.B., Hirst, A.L., Smit, C.J., 1988. Observations on the spawing, development and rearing of the South African abalone *Haliotis midae* Linn. S. Afr. J. Mar. Sci. 6, 3-12.

Hahn, K.O., 1989. Handbook of culture of abalone and other marine gastropods. CRC Press Inc., Boca Raton, Florida, 348 pp.

Hecht, T., 1994. Behavioural thermoregulation of the abalone, *Haliotis midae*, and implications for intensive culture. Aquaculture 126, 171-181.

Knauer, J., Brady, D., Duncan, J.R., Hecht, T., 1995b. Amino acid, fatty acid and mineral element profile of juvenile South African abalone, *Haliotis midae* L. Aquacult. Res. 26, 283-288.

Knauer, J., Britz, P.J., Hecht, T., 1993. The effect of seven binding agents on 24-hour water stability of an artificial weaning diet for the South African abalone, *Haliotis midae* (Haliotidae, Gastropoda). Aquaculture 115, 327-334.

Knauer, J., Britz, P.J., Hecht, T., 1996. Comparative growth performance and digestive enzyme activity of juvenile South African abalone, *Haliotis midae*, fed on diatoms and a practical diet. Aquaculture 140, 75-85.

Knauer, J., Hecht, T., Britz, P.J., 1995a. A note on the feeding behaviour and growth of juvenile South African abalone, *Haliotis midae*, fed on an artificial weaning diet. S. Afr. J. Sci. 91, 91-93.

Knauer, J., Hecht, T., Duncan, J.R., 1994. Proximate composition of the South African abalone, *Haliotis midae* (Haliotidae, Gastropoda). Aquacult. Fish. Manage. 25, 351-354.

Lawrie, R.A., 1991. Meat Science, 5th edn. Pergamon Press, Oxford, UK, 293 pp.

Loubser, N.C., Dormehl, N., 2000. The use of ultrasound in the treatment of sabellid infestations in South African abalone. Paper presented at the Fourth International Abalone Symposium, 6-11 February 2000, University of Cape Town, Cape Town, South Africa.

Lyon, G., 1995. Aspects of physiology of the South African abalone *Haliotis midae* L., and implications for intensive abalone culture. M.Sc thesis, Rhodes University, Grahamstown, South Africa, 85 pp.

Matthews, I., Cook, P. A., 1995. Diatom diet of abalone post-larvae (*Haliotis midae*) and the effect of pre-grazing the diatom overstorey. Mar. Freshwater Res. 46, 545-548.

Mouton, A., 2000. Health management and disease surveillance in abalone, *Haliotis midae*, in South Africa. Paper presented at the Fourth International Abalone Symposium, 6-11 February 2000, University of Cape Town, Cape Town, South Africa.

Muller, S., 1986. Taxonomy of the genus *Haliotis* in South Africa. Trans. Roy. Soc. S. Afr. 46, 69-77.

Newman, G.G., 1967. Reproduction of the South African abalone *Haliotis midae*. Investigational Report of the Division of Sea Fisheries, South Africa 64, 1-24.

Newman, G.G., 1968. Growth of the South African abalone *Haliotis midae*. Investigational Report of the Division of Sea Fisheries, South Africa 67, 1-24.

Newman, G.G., 1969. Distribution of the abalone (*Haliotis midae*) and the effect of temperature on productivity. Investigational Report of the Division of Sea Fisheries, South Africa 74, 1-7.

Olley, J., Thrower, S.J., 1977. Abalone – an esoteric food. Adv. Food Res. 23, 143-186.

Pitcher, G., Franco, J., Whyte, K., Viljoen, C., 2000. Abalone (*Haliotis midae*) farming and paralytic shellfish poisoning on the coast of South Africa. Paper presented at the Fourth International Abalone Symposium, 6-11 February 2000, University of Cape Town, Cape Town, South Africa.

Ruck, K.R., Cook, P.A., 1998. Sabellid infestations in the shells of South African molluscs: Implications for abalone mariculture. J. Shellfish Res. 17, 693-699.

Sales, J., Britz, P.J., Shipton, T., 1999. Quality characteristics of South African abalone, *Haliotis midae* L., meat. Aquacult. Res. 30, 799-802.

Shipton, T.A., 2000. The protein requirements for the South African abalone, *Haliotis midae*. Ph.D Dissertation, Rhodes University, Grahamstown, South Africa, 146 pp.

Simpson, B.J.A., Cook, P.A., 1998. Rotation diets: A method of improving growth of cultured abalone using natural algal diets. J. Shellfish Res. 17, 635-640.

Sweijd, N., Snethlage, Q., Harvey, D., Cook, P., 1998. Experimental abalone (*Haliotis midae*) seeding in South Africa. J. Shellfish Res. 17, 897-904.

Tarr, R., 1987. Biology and management of South African perlemoen. Underwater, 41-42.

Tarr, R.J.Q., 1992. The abalone fishery of South Africa. In: Shepherd, S.A., Tegner, M.J., Guzmand del Proo, S.A. (Eds.), Abalone of the World. Biology, Fisheries and Culture, Fishing News Books, London UK, pp. 438-447.

Tarr, R.J.Q., 1995. Growth and movement of the South African abalone *Haliotis midae*: a reassessment. Mar. Freshwater Res. 46, 583-590.

Van As, D., Fourie, H.O., Vleggaar, C.M., 1975. Trace element concentrations in marine organims from the Cape West coast. S. Afr. J. Sci. 71, 151-154.

Viana, M.T., López, L.M., García-Esquivel, Z., Mendez, E., 1996. The use of silage made from fish and abalone viscera as an ingredient in abalone feed. Aquaculture 140, 87-98.

White, H.I., 1995. Anaesthesia in abalone, *Haliotis midae*. M.Sc thesis, Rhodes University, Grahamstown, South Africa, 96 pp.

White, H.I., Hecht, T., Potgieter, B., 1996. The effect of four anaesthetics on *Haliotis midae* and their suitability for application in commercial abalone culture. Aquaculture 140, 145-151.

Wood, A.D., 1993. Aspects of the biology and ecology of the South African abalone *Haliotis midae* Linnaeus, 1758 (Mollusca: Gastropoda) along the Eastern Cape and Ciskei Coast. M.Sc thesis, Rhodes University, Grahamstown, South Africa, 161 pp.

CHAPTER TWO

Digestibility of feedstuffs in abalone (*Haliotis* species)

Sales, J., 1999. Digestibility of feedstuffs in abalone (*Haliotis* species). Afma Matrix (Quarterly Magazine of the South African Animal Feed Manufacturers Association) 8:17-21.

1. Introduction

Abalone are among the most highly valued seafoods in the world, with the prime demand in Asian countries where abalone products form part of traditional cuisine and ceremony. In the 1980's economic conditions have created a classic opportunity for the development of aquaculture, and there has been an upsurge in effort to establish abalone culture in a number of countries. Although most existing farms currently use natural algal feeds, a regular supply of large volumes of seaweed often presents logistical problems, and it would appear that the development of industrial scale abalone production in most countries will be dependent on the availability of nutritionally complete pelleted feeds (Britz, 1996).

Some work related to nutrient requirements of *Haliotis species* has been performed (Fleming *et al*., 1996). The next step will be to determine the nutritive value of feedstuffs that can be used in the formulation of a least cost optimal diet to satisfy the nutrient requirements. This will benefit the industry by optimal usage of optimal feedstuffs for optimal production.

The following communication was written in an attempt to compile all literature available on digestibility of feedstuffs specific for abalone. Although not concentrating on digestibility of feedstuffs in other aquatic species, relevant descriptive terminology was included.

2. Terminology

Bioavailability of nutrients and not simply composition determine the true nutritive value of a formulated feed. Also, digestibility of a feedstuff to an animal depends not only on the animals' digestive tract architecture and physiology and environmental conditions, but also on the feedstuff's physical and nutrient characteristics (Lee and Lawrence, 1997).

True digestibility describes the portion of the feed that is absorbed minus the materials that are lost by the gut in the process of ingestion and digestion. These losses are known collectively as metabolic faecal nitrogen losses for protein digestibility and metabolic faecal energy losses for energy digestibility. In contrast, apparent feed digestibility, the standard used in the field of animal nutrition today, describes the portion of the feed that is absorbed. It is based on the difference between the amount of feed ingested and the amount of faeces, without distinguishing

between components derived from the feed and other components such as gut mucosa cells, bacteria and digestive enzymes in the faeces (Lee and Lawrence, 1997).

3. Methods of determining digestibility

The methods used to determine feed digestibility have had a major influence on the published results. The goal of feed digestibility studies is to provide reproducible and meaningful results relating the nutritional characteristics of a feedstuff to the animal's ability to digest and assimilate a feed formulated with the feedstuff. Methods used should be as simple as possible, enabling the rapid evaluation of many feeds or feedstuffs. If proper protocols are not followed with aquatic animals, feed and faeces tend to become soluble, and uneaten food and faeces can become mixed very easily.

Total collection and chromic oxide markers are the most commonly used methods for digestibility in aquatic species, but each has its limitations (Lee and Lawrence, 1997). Although total collection is theoretically the best method to use, with abalone collection of all faeces produced is difficult as they are often not discrete pellets and tend to disintegrate in water, even when measures are taken not to disturb them. Leaching of soluble nutrients from the faeces and feed is also a problem when determining the digestibility of specific nutrients, but this is a problem common to all methods. Artificial feeds tend to disintegrate, making total collection of uneaten feed difficult and contamination with faecal material a problem (Wee et al., 1992a; Fleming et al., 1996).

The main problem with using the chromic oxide marker method, the most frequently used method for determining feed digestibility in aquatic species, is the non-homogeneous distribution of it in the faeces due to selective transport in the digestive tract (Lee and Lawrence, 1997; Fleming et al., 1996). This problem has been described in fish (Bowen, 1978), crawfish (Brown et al., 1986) and lobster (Leavitt, 1985). The result is that the digestibility of a nutrient can be under-or overestimated, depending on when the faeces are collected. If faeces are collected soon after feeding, the concentration of chromic oxide will be higher relative to faeces collected several hours later as digestion is completed (Lee and Lawrence, 1997).

Wee et al. (1992b) compared a number of internal and external markers for their suitability for Australian abalone (*H. rubra*, *H. laevigata*) digestion studies. They found that incorporating the external marker chromic oxide (0.5 g/100g) gave the least variable results. The internal marker acid-insoluble ash produced similar results, while ash and crude fibre were not suitable. However, no protocol regarding what marker, concentration of marker, feeding period, or faeces collection period has yet been established for abalone.

4. Factors influencing feed digestibility

It should not be assumed that the digestibility values for abalone will necessarily be similar to the values for other aquatic animals, as abalone have a very complex gut structure and the enzymatic capability to digest some complex carbohydrates (Wee et al., 1992a). Furthermore, it is well known that digestibility of feedstuffs in animals, as well as in abalone, is influenced by factors such as species (Wee et al., 1992b) and environmental influences (Dixon, 1992). Poor palatability, as in crustaceans, results in greater nutrient leaching and greater feed waste, increasing the chance of feed/faeces contamination during sampling (Lee and Lawrence, 1997). In a preliminary test with *H. rubra* Fleming (1995) found that approximately 18 % of nitrogen was lost from faeces during the first 14 hours in seawater, but no further loss had occurred after 24 hours.

It was found that blacklip abalone (*H. rubra*) are slow feeders, often consuming the food 24 hours or more after presentation (Wee et al., 1992a). Results indicated that in *H. midae* the bulk of pelleted feed was consumed between 1800 and 2400 h. A discrete quantity of food is ingested and then processed over a 24-h period, whereupon feeding begins again (Britz et al., 1996).

Three distinguishable types of faeces were produced in *H. rubra*, *H. laevigata* (Maguire et al., 1993) and *H. midae* (Shipton, 2000). In Australian abalone the three types could be distinguish into (A) discrete whole pellets, (B) clumps of amorphous material and (C) long, thin, dark strings (Maguire et al., 1993), while (A) light amorphous faeces, (B) medium amorphous faeces and (C) dark discrete faecal pellets and strings were identified in *H. midae* (Shipton, 2000). While crude protein content of the three types of faeces in Australian abalone did not differ

significantly (Maguire et al., 1993), protein content of type C faeces in the South African species was significantly higher than in the other two types (Shipton, 2000). This was also true for chromium concentration in the latter species, except that type A was also significantly lower than type B. Type A and B faeces contained large amounts of mucosal material that reduced the overall concentration of chromium and protein in the faeces. Shipton (2000) found apparent protein digestibility values of 14.2, 61.3 and 74.7 % for type A, B and C faeces, respectively, in *H. midae* using an extruded fish meal based diet.

The Australian species continue to produce faeces for up to seven days after feeding, with type C faeces dominant towards the end of the period. There appeared to be a diurnal pattern of faecal production. For faeces produced during the night the ratio produced on a dry-weight basis from the same aquarium was 1.9:2.1:1 (A:B:C). During the day only faeces of type A were produced in measurable quantities (Wee et al., 1992a). During starvation or low food consumption rates it was found that the incidence of type C faeces increases. All three types were pooled in the Australian experiments (Maguire et al., 1993). Shipton (2000) found that there was no significant difference in the chrome content of animals fed a chrome diet and animals not exposed to chrome.

Other observations on factors affecting digestibility have included that animals are grazing on each other's shells. Thus, organic material should be remove from the surface of the shells (Wee et al., 1992a). Furthermore, Fleming et al. (1996) stated that a single feeding followed by collection of faeces during a period of fasting may alter digestibility in comparison to daily feeding and collection. Addition of marine or vegetable oils at inclusion levels higher than 30 % in abalone diets will reduce the digestibility of nitrogen, amino acids and gross energy of feedstuffs (Van Barneveld et al., 1998).

5. Nutrient associations

The optimal method to describe associative effects of feed ingredients would be to measure the digestibility of a single feedstuff that has been added at a minimum of three levels to a reference feed. The results of the digestibility trail would then be plotted with the feed digestibility versus the percent substitution of the feedstuff. The shape and slope of the curve could then be

evaluated using multiple regression analysis to understand the associative effects of this feedstuff on feed digestibility (Lee and Lawrence, 1997).

6. Digestibility values for abalone species

Wee and co-workers observed the digestibility of dry matter in several sources of animal (fish meal, squid meal, fish oil) and plant (whole wheat, wheat pollard, wheatgerm, barley, oats, corn starch, field peas, lupin meal) to be between 51 and 65 % in Australian abalone when using chromic oxide as a marker. The corresponding crude protein digestibility was between 65 and 75 % (Fleming et al., 1996). According to Dixon (1992) *H. midae* digested a semi-purified artificial diet with a dry matter digestibility of 84 % and crude protein digestibility of 96 %. Fleming et al. (1998) has presented apparent feacal digestibility coefficients for nitrogen and amino acids of single ingredients as presented in Table 1. Apparent digestibility of protein in casein was found to be about 87 %. Fleming (1995) found that dry matter digestibility was between 21.9 and 44.9 %, organic matter digestibility between 50 and 79 %, digestible energy content from 6.3-8.8 kJ/g and digestible nitrogen between –0.5 and 13.4 mg/g in *H. rubra* when testing six different algae.

Vandepeer et al. (1999) reported digestibility values of soyflour and grain legumes determined with *H. laevigata* as presented in Table 2. All legumes (whole seed) were ground in a hammer mill and then in a centrifugal laboratory mill with a 2 mm sieve. Autoclave treatment had a negative effect on digestibility with soyflour and lupins being the most affected. Apart from an increase in dry matter digestibility for vetch, dry matter, nitrogen and gross energy digestibility values of all other legumes with added phytase were not significantly different from those values obtained without phytase addition.

According to Fleming et al. (1998) apparent faecal digestibility values of feedstuffs determined for *H. laevigata* are additive. This means that apparent faecal digestibilities are good descriptors of nutritive values and can be used with confidence in diet formulations.

Although digestibility values of feedstuffs (semolina, casein, fish meal, soyflour) for *H. laevigata* were determined by Coote (1997), this work is under commercial caveat and most cannot be published (G. Maguire – personal communication).

Table 1

Apparent feacal digestibility coefficients of nitrogen and amino acids in experimental diets containing single ingredients determined in *H. laevigata* with the chromic oxide marker method (Fleming et al., 1998)

Feedstuff	Barley[a]	Semolina	Fish meal	Lupin kernel meal
Nitrogen	0.54	0.71	0.43	0.91
Amino acids				
Aspartic acid	0.43	0.54	0.37	0.91
Threonine	0.39	0.62	0.36	0.89
Serine	0.46	0.81	0.37	0.91
Glutamic acid	0.52	0.77	0.43	0.94
Proline	0.40	0.71	0.20	0.89
Glycine	0.45	0.67	0.41	0.90
Alanine	0.43	0.63	0.42	0.88
Valine	0.43	0.69	0.33	0.88
Isoleucine	0.30	0.58	0.33	0.86
Leucine	0.40	0.65	0.36	0.88
Tyrosine	0.44	0.75	0.31	0.92
Phenylalanine	0.41	0.70	0.34	0.89
Lysine	0.45	0.61	0.42	0.91
Histidine	0.45	0.68	0.48	0.91
Arginine	0.51	0.71	0.37	0.95

[a] Clean chebec barley. No further background information (processing, type, etc.) of different feedstuffs were supplied.

Table 2

Apparent feacal digestibility coefficients of dry matter, gross energy, nitrogen and amino acids in legume grains determined in *H. laevigata* with the chromic oxide marker method (Vandepeer et al., 1999)

Feedstuff	Soyflour[a]	Yellow lupins (*Lupinus luteus*)	Field peas (*Pisum sativum* cv. Alma)	Vetch (*Vicia sativa* cv. Blanchefleur)	Faba beans (*Vicia faba* cv. Fjord)
Dry matter	0.57	0.61	0.25	0.29	0.45
Gross energy[b]	0.84	0.83	0.49	0.45	0.65
Nitrogen	0.87	0.91	0.75	0.75	0.85
Amino acids					
Aspartic acid	0.98	0.98	0.92	0.93	0.96
Threonine	0.84	0.88	0.75	0.71	0.84
Serine	0.90	0.94	0.74	0.75	0.85
Glutamic acid	0.97	0.98	0.87	0.89	0.94
Proline	0.91	0.93	0.82	0.80	0.86
Glycine	0.85	0.91	0.73	0.70	0.80
Alanine	0.85	0.89	0.76	0.71	0.84
Valine	0.85	0.86	0.71	0.70	0.84
Isoleucine	0.85	0.88	0.71	0.69	0.83
Leucine	0.87	0.90	0.71	0.68	0.70
Tyrosine	0.85	0.89	0.79	0.74	0.86
Phenylalanine	0.86	0.88	0.73	0.68	0.66
Lysine	0.91	0.92	0.80	0.76	0.88
Histidine	0.90	0.93	0.78	0.72	0.86
Arginine	0.92	0.96	0.84	0.78	0.90

[a] defatted; Baker's Nutrisoy' brand, Agribusiness (Maccles Field, South Australia, Austalia).

[b] Adjusted to account for the contribution of digestible energy from oil, pregelled starch and sodium alginate in diets.

7. Conclusions

It is clear that very little work on the digestibility of feedstuffs has been undertaken in any abalone species. At this stage it is not even possible to start setting up a database of digestibility values of different feedstuffs for abalone, and to make a comparison with other animal and aquatic species. The few studies conducted have concentrated on the influence of a treatment on the digestibility of the feedstuffs used, and not on the building up of a database of values.

Probably the main drawback is that methods used in the few digestibility studies with abalone are without any type of standardization. The approach has to be a precise correct protocol that will answer the question that is asked, namely: What is nutritive value of different feedstuffs for abalone?

References

Bowen, S.H., 1978. Chromic acid in assimilation studies – a caution. Trans. Amer. Fish. Soc. 107, 755-756.

Brown, P.B., Williams, C.D., Robinson, E.H., Akiyama, D.M., Lawrence, A.L., 1986. Evaluation of methods for determining *in vivo* digestion coefficients for adult red swamp crayfish *Procambarus clarkii*. J. World Aquacult. Soc. 17, 19-24.

Britz, P.J., 1996. The nutritional requirements of *Haliotis midae* and development of a practical diet for abalone aquaculture. Ph.D Dissertation, Rhodes University, South Africa, 150 pp.

Britz, P.J., Hecht, T., Knauer, J., 1996. Gastric evacuation time and digestive enzyme activity in abalone *Haliotis midae* fed a formulated diet. S. Afr. J. Mar. Sci. 17, 297-303.

Coote, T.A., 1997. The protein, energy, and lysine requirements of greenlip abalone (*Haliotis laevigata*). Ph.D. Dissertation. University of Tasmania, Hobart, Tasmania, Australia, 143 pp.

Dixon, M.G., 1992. The effect of temperature and photoperiod on the digestive physiology of the South African abalone *Haliotis midae*. M.Sc. thesis, Rhodes University, South Africa, 85 pp.

Fleming, A.E., 1995. Digestive efficiency of the Australian abalone *Haliotis rubra* in relation to growth and feed preference. Aquaculture 134, 279-293.

Fleming, A.E., Van Barneveld, R.J., Hone, P.W., 1996. The development of artificial diets for abalone: A review and future directions. Aquaculture 140, 5-53.

Fleming, A.E., Van Barneveld, R.J., Hone, P.W., Vandepeer, M.E., Kruk, J.A., 1998. Complementary additivity of the digestibility coefficients of feed ingredients fed to juvenile greenlip abalone (*Haliotis laevigata*). J. Shellfish Res. 17, 641-647.

Leavitt, D.F., 1985. An evaluation of gravimetric and inert marker techniques to measure digestiblity in the American lobster. Aquaculture 47,131-142.

Lee, P.G., Lawrence, A.L., 1997. Digestibility. In: D'Abramo, L.R., Conklin, D.E., Akiyama, D.M. (Eds.), Crustacean Nutrition, Advances in World Aquaculture, Vol. 6, World Aquaculture Society, Louisiana, USA, pp. 194-260.

Maguire, G.B., Wee, K.L., Hindrum, S.M., 1993. Digestibility studies – The "ins" and "outs" of abalone guts. Austasia Aquacult. 7, 42-46.

Shipton T.A., 2000. The protein requirements for the South African abalone, *Haliotis midae*. Ph.D Dissertation, Rhodes University, Grahamstown, South Africa, 146 pp.

Van Barneveld, R.J., Fleming, A.E., Vandepeer, M.E., Kruk, J.A., Hone, P.W., 1998. Influence of dietary oil type and oil inclusion level in manufactured feeds on the digestibility of nutrients by juvenile greenlip abalone (*Haliotis laevigata*). J. Shellfish Res. 17, 649-655.

Vandepeer, M.E., Van Barneveld, R.J., Hone, P.W., Havenhand, J.N. 1999. The digestibility of raw, autoclaved and phytase treated legumes in greenlip abalone, *Haliotis laevigata*. Proc. Of the 6th annual abalone aquaculture workshop, World Aquaculture Society Conference, April 1999, Sydney, April.

Wee, K.L., Maguire, G.B., Hindrum, S.M., 1992a. Methodology for digestibility studies with abalone. I. Preliminary studies on the feeding and defaecatory behaviour of blacklip abalone, *Haliotis rubra*, fed natural and artificial diets. In: G.L. Allan G.L., Dall, W. (Eds.), Proc. Aquaculture Nutrition Workshop, 15-17 April 1991, NSW Fisheries, Brackish Water Fish Culture Research Station, Salamander Bay, Australia, pp. 192-196.

Wee, K.L., Maguire, G.B., Hindrum, S.M., 1992b. Methodology for digestibility studies with abalone. 2. Comparison of markers in an artificial diet for blacklip abalone (*Haliotis rubra*) and greenlip abalone (*Haliotis laevigata*). In: Paper delivered at the Third Asian Fisheries Forum, 26-30 October 1992, Singapore.

CHAPTER THREE

Preliminary investigations to find a suitable faeces collection method in digestibility studies with South African abalone (*Haliotis midae*)

Sales, J., 2000. Study of faeces collection methods to define optimum abalone diets. Fish Farmer (International File) May/June 2000, Vol. 14, No. 3, pp. 8-9

1. Introduction

Determination of the nutritive value of feedstuffs that can be used in the formulation of least cost optimal diets to satisfy the nutrient requirements of *Haliotis* species is essential in optimal usage of optimal feedstuffs for optimal production. A starting point would be to determine apparent feed digestibility, the standard used in the field of animal nutrition today. It is based on the difference between the amount of feed ingested and the amount of faeces, without distinction between components derived from the feed and other components such as gut mucosa cells, bacteria and digestive enzymes in the faeces.

The methods used to determine feed digestibility have a major influence on the published results. The goal of feed digestibility studies is to provide reproducible and meaningful results relating the nutritional characteristics of a feedstuff to the animal's ability to digest and assimilate a feed formulated containing that feedstuff. Methods used should be as simple as possible, enabling rapid evaluation of many feeds or feedstuffs. If proper protocols are not followed with aquatic animals, the feed and faeces tend to become soluble, and uneaten food and faeces can become mixed very easily (Lee and Lawrence, 1997).

The first goal was to find a suitable method of collection. With abalone collection of faeces produced is difficult as they are often not discrete pellets and tend to disintegrate in water, even when measures are taken not to disturb them. Different methods of collection of faeces were evaluated in preliminary trials in an attempt to find the most suitable way to collect sufficient amounts for proper chemical analysis.

2. Method one

The first attempt was a conical plastic tank of 2-l capacity as illustrated in Figs. 1 and 2. It was supplied with its own biological filter with an inflow of 1 l of seawater per min. Aeration was supplied via an airstone. A mesh floor (8 x 6 mm holes) allowed faeces to drop into a collection tube supplied with a rubber stopper. Two abalone (8 cm shell length) were adapted for a period of 4 days with continual access to commercial abalone food in the digestibility tank. From the fifth morning the tank was cleaned at 0800 h and feed taken away, faeces were

collected by gently pouring the content of the collection tube on a 100 µm mesh at 1200 and 1600 h, and abalone were fed at 1600 h. Abalone were kept in the dark continually, except during collection when lights were turned on for about 15 min. Faeces were scraped off mesh and oven-dried at 50 °C.

Fig. 1 Illustration of 2-l conical plastic tank with biological filter tested in Method 1.

2.1. Suitability

The main drawback was the small size of the tank. Only two abalone of shell length 83.5 ± 2.12 mm; live weight 100.5 ± 16.56 g could be held due to overcrowding. Furthermore, it was found that faeces clotted on the sides of the funnel and also in the filter of the outflow pipe when the tank was cleaned. This illustrates that some faeces broke up and were floating around in the water. A total of 13.5 mg dry faeces (2.25mg/abalone/day) was collected over 3 days (6 collections).

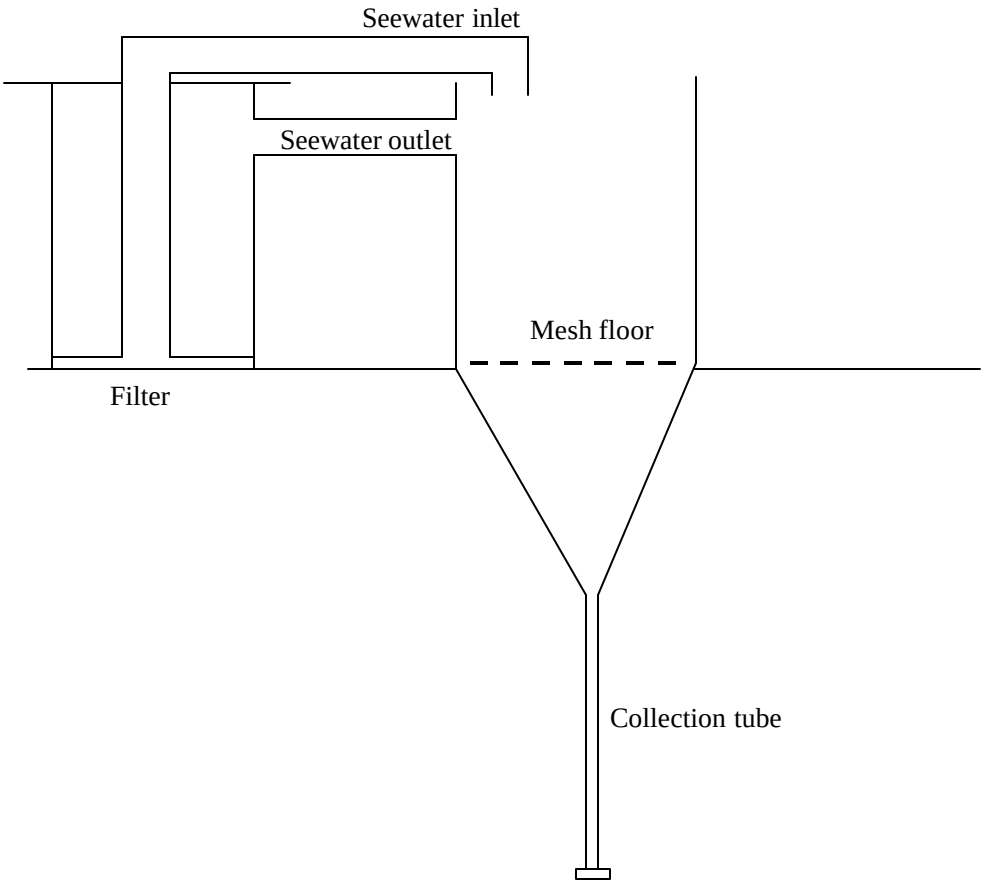

Fig. 2 Schematic layout of tank tested in Method 1.

3. Method two

A similar system as described by Vanderpeer et al. (1999) for Australian abalone (*H. laevigata)* was tested (Figs. 3 and 4).

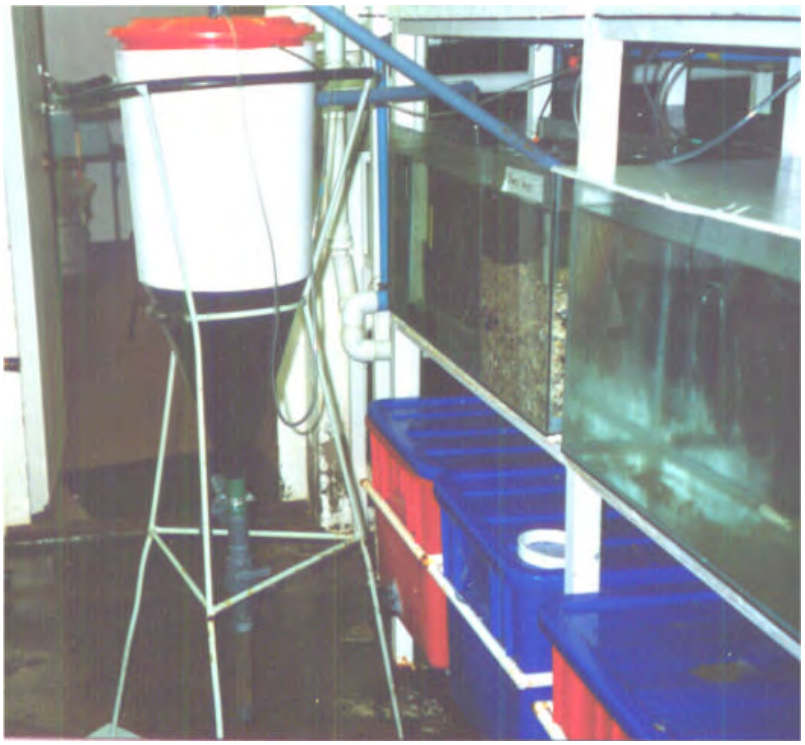

Fig. 3 Illustration of system tested in Method 2.

Nine abalone of shell length 75.8 ± 7.19 mm; live weight 77.1 ± 21.75 g were stocked into this tank. Water inflow, aeration, adaption, collection and feeding protocol was similar to Method 1. The 20-l bucket was supplied with a mesh floor (8 x 6 mm holes). During cleaning abalone were out of the water for about 3 min.

3.1. Suitability

As soon as faeces landed in the water column under the mesh floor of the bucket they broke up into small pieces and floated on the top. This was indicated by the amount of faeces clotting onto the funnel when the tank was cleaned. It might be that the faeces of South African abalone

do not form as compact pellets as that of the Australian abalone. Only 5.4 mg dry excreta (0.2 mg/abalone/day) was collected over 3 days.

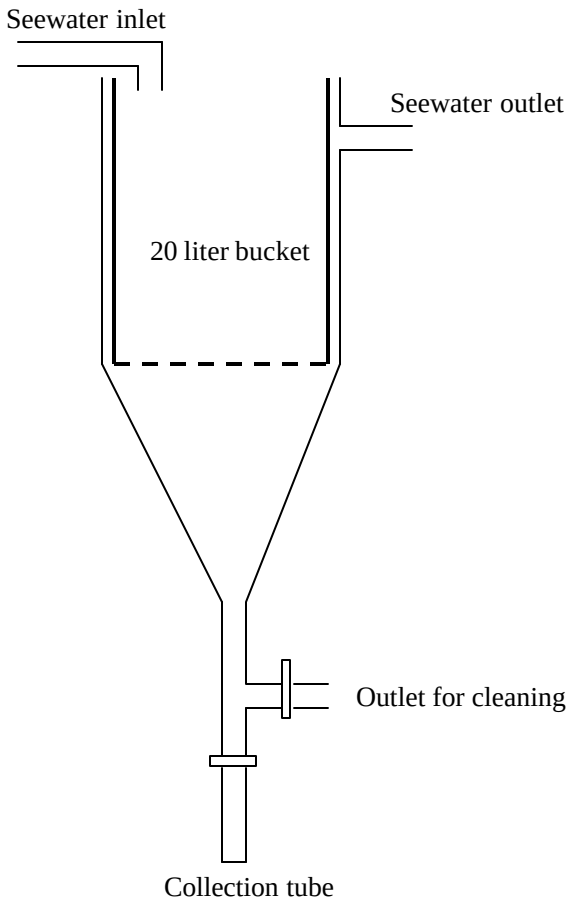

Fig. 4 Schematic layout of tank tested in Method 2.

4. Method three

A glass tank of 30 x 90 x 40 cm with its own biological filter was used. Two separate tanks were built from PVC sheets. The first inner tank was supplied with a mesh floor of 200 µm, and the second with a mesh floor of 8 x 6 mm (Figs. 5 and 6). Seven abalone of shell length 73.6 ± 6.53 mm; live weight 70.4 ± 18.71 g were kept in the second inner tank using the same protocol as in Methods 1 and 2. During cleaning abalone were out of the water for about 3 min.

Fig. 5 System used in Method 3.

4.1. Suitability

Faeces were scraped of mesh floor of the first tank. A total of 69.1 mg dry excreta (3.3 mg/abalone/day) was collected over 3 days.

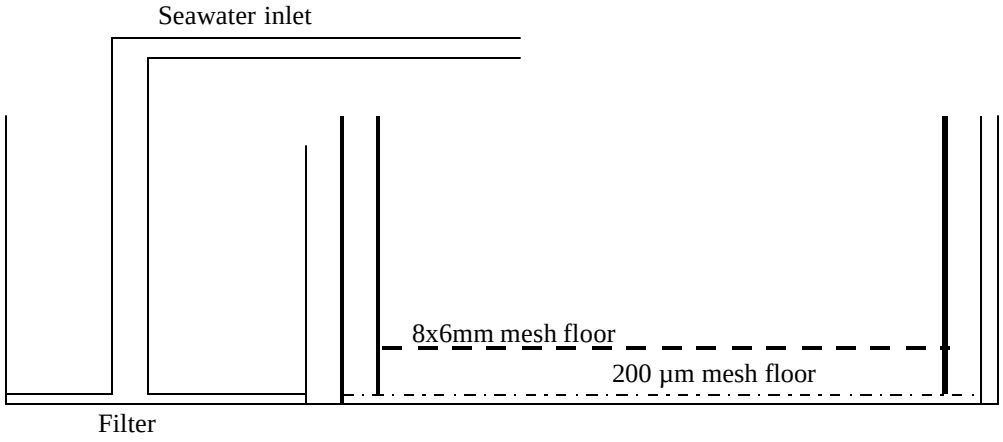

Fig. 6 Schematic layout of tank tested in Method 3.

Conclusion

The aim of this preliminary study was to find the method of faeces collection that would produce the maximum amount of faeces in order to evaluate both energy and amino acid digestibility in future experiments. Approximately 2 g of dry excreta will be required for proper analysis of energy amino acids. The experiment clearly illustrated that Method 3 is superior to Methods 1 and 2 (Table 1).

Also regarding working hours and costs on equipment this method is preferred. This method can easily be incorporated into existing filter sytems.

Table 1

Theoretical production of excreta by different systems tested

System	Excreta (mg/abalone/day)	Amount of abalone possible in one tank	Days to collect 2 g of dry excreta
1	2.25	2	444
2	0.2	10	100
3	3.3	40	15

References

Lee, P.G., Lawrence, A.L., 1997. Digestibility. In: D'Abramo, L.R., Conklin, D.E., Akiyama, D.M. (Eds.), Crustacean Nutrition, Advances in World Aquaculture, Vol. 6, World Aquaculture Society, Louisiana, USA, pp. 194-260.

Vandepeer, M.E., Van Barneveld, R.J., Hone, P.W., Havenhand, J.N. 1999. The digestibility of raw, autoclaved and phytase treated legumes in greenlip abalone, *Haliotis laevigata*. Proc. Of the 6th annual abalone aquaculture workshop, April 1999, World Aquaculture Society Conference, Sydney, April.

CHAPTER FOUR

Evaluation of different markers to determine apparent nutrient digestibility coefficients of feed ingredients for South African abalone *(Haliotis midae* L.)

Sales, J., Britz, P.J., 2001. Evaluation of different markers to determine apparent nutrient digestibility coefficients of feed ingredients for of South African abalone (*Haliotis midae* L.). Aquaculture 202, 113-129.

Abstract

Chromic oxide (Cr_2O_3), acid-insoluble ash (AIA) and crude fibre were evaluated as inert markers in digestibility studies with *Haliotis midae* by calculating apparent dry matter, organic matter, energy, protein and amino acid availibility for fish meal, soybean meal and cottonseed meal included at 30 % into a reference diet. AIA was the only marker that yielded consistent realistic apparent digestibility coefficients (ADCs), which were comparable ($P > 0.05$) to values derived through total collection. Both Cr_2O_3 and crude fibre in faeces were either lower or similar to their respective levels in feed, resulting in negative ADCs. Leaching of markers from the diets did not have an effect ($P > 0.05$) on digestibility coefficients. No difference ($P > 0.05$) was found in apparent dry matter digestibility between faeces voided either during the night or daytime. Washing of faeces with distilled water after collection had no influence ($P > 0.05$) on AIA content. In all test ingredients mean apparent amino acid digestibility reflected apparent protein digestibility. Apparent digestibility of protein in fish meal (76 %) by *H. midae* was within the range of values reported for fish species (62-91 %). Soybean meal is a promising feed ingredient in abalone feeds due to the high apparent digestibility of its organic matter (79 %), energy (83 %), protein (85 %) and both essential and non-essential amino acids (mean of 86 %).

1. Introduction

A dependable measure of the digestibility of various nutrients is one of the critical elements required for effective animal nutrition research (De la Noüe and Choubert, 1986). Feed digestibility has also become of great interest to aquaculturists due to the need for low pollution feeds (Cho et al., 1994; Lupatsch and Kissel, 1998). Technical difficulties associated with digestibility methods for aquatic species include the collection of representative faecal samples, leaching of nutrients from the faeces in contact with water (De la Noüe and Choubert, 1986), and fracturing of faecal material into small particles with time, a process promoted by aeration and movement of the experimental animals (De Silva and Anderson, 1995).

In general the direct method of total collection is not performed in fish studies due to the need for automatic, continuous faecal collection devices that are expensive to install and restrict the magnitude of the experiment. In *Haliotis* spp., total collection is not practical with formulated feeds because of the long periods that the feed remains in water, and the rasping action during feeding that increases the possibility of nutrient loss, thus resulting in overestimates of digestibility. Total collection is however appropriate with natural feeds such as seaweed (Wee et al., 1992a). The use of a marker as an indirect method of determining digestibility has generally been preferred for aquatic species (Morales et al., 1999). Chromic oxide (Cr_2O_3), proposed by Edin (1918) for use as a marker in digestibility studies with ruminants, and confirmed by Nose (1960) for fish studies, has since been the most widely used inert marker in digestibility studies with aquatic species. Although initial attempts to use Cr_2O_3 as a marker in digestibility trials for *H. midae* failed due to problems in attaining replicable and reliable results (Britz, 1995), it has been successfully applied in studies with *H. rubra* (Wee et al., 1992a) and *H. laevigata* (Wee et al., 1992a, b; Fleming et al., 1998; Van Barneveld et al., 1998) fed artificial diets. In both *H. rubra* (Wee et al., 1992a) and *H. midae* (Shipton, 2000), three different types of faeces have been observed. Shipton (2000) concluded that Cr_2O_3 could be used as marker to give a reliable indication of apparent protein digestibility in *H. midae* if one type of faeces was collected. However, as this technique only evaluates protein digestibility, and requires sorting of all faeces, it is unpractical if routine collection has to be performed with significant numbers of animals. Therefore it was desirable to determine whether an internal

marker could be identified which would be representative in all faeces of *H. midae*. Of the various inherent components (ash, acid-insoluble ash, hydrolysis resistant organic matter, crude fibre, cellulose) of dietary ingredients used as markers in digestibility studies, the insoluble mineral components of diets determined as acid-insoluble ash (AIA) has been the most popular (Bowen, 1981; De Silva and Perea, 1983; Atkinson et al., 1984; Bureau et al., 1999; Rodehutscord et al., 2000). Wee et al. (1992b) identified AIA as a suitable marker for digestibility studies in *H. rubra* and *H. laevigata.*

The primary objective of this study was to evaluate the validity of an external marker (Cr_2O_3) and two internal markers (AIA, crude fiber) for estimating the apparent digestibility of a wide range of nutrients in individual feed ingredients in abalone (*H. midae*) fed artificial diets.

2. Methods

2.1. Evaluation of different markers for determining of digestibility

2.1.1. Experimental animals

Four hundred and fifty six juvenile abalone (*Haliotis midae*) (53.96 ± 0.12 mm shell length; 31.77 ± 0.24 g live weight, mean ± S.E.) obtained from a commercial abalone farm in South Africa were acclimatised over a two month period in a recirculating system. The animals were individually weighed on a Mettler 3000 electronic scale, reading to the nearest 0.01g, and shell length was measured to the nearest 0.01 mm with the use of Vernier calipers. All mucus was brushed off of each abalone shell with a soft toothbrush.

2.1.2. Feed ingredients and diet preparation

Test ingredients (Table 1) were incorporated into a reference diet (Table 2), based on a practical commercial starch-bound dry pellet diet for culture of *H. midae* developed by Britz et al. (1994), in a ratio of 30 % test ingredient: 70 % reference diet in order to determine apparent digestibility coefficients (ADCs) of test ingredients (Cho et al., 1982).

Table 1

Chemical composition of feed ingredients tested (dry matter basis)

Component	Fish meal[a]	Soybean meal[b]	Cottonseed meal[b]
Dry matter (%)	93.67	90.68	94.46
Organic matter (%)	88.29	93.46	92.70
Gross energy (kJ/g)	23.05	20.13	20.67
Crude protein (%)	71.48	48.57	49.21
Amino acids (%): essential			
Arginine	4.21	3.49	5.22
Histidine	1.63	1.28	1.40
Isoleucine	3.36	2.41	1.75
Leucine	5.11	3.61	2.92
Lysine	5.53	3.03	2.37
Phenylalanine	2.93	2.52	2.67
Methionine	2.19	0.61	0.61
Threonine	3.09	1.79	1.64
Valine	3.92	2.60	2.37
Amino acids (%): non-essential			
Aspartic acid	5.84	4.84	4.25
Serine	2.63	1.90	1.89
Glutamic acid	8.43	7.58	8.39
Proline	2.75	2.55	1.87
Glycine	3.61	2.01	2.10
Alanine	4.10	2.14	1.95
Tyrosine	2.10	1.37	1.16

[a] Low-temperature dried fish meal (Danish 999 LT), Esbjerg Fiskeindustri a.m.b.a. (Esbjerg, Danmark).

[b] Soybean and cottonseed meal were dehulled, pre-pressed, solvent extracted, Western Province Corp., (Roggebaai, South Africa).

Table 2

Ingredient composition of reference diet used for evaluating of markers (dry matter basis)

Ingredient	%
Casein[a]	37.0
Maize starch[b]	39.0
Kelp powder[c]	5.0
Fish oil[d]	2.0
Carboxymethyl-cellulose[a]	2.0
a-Cellulose[a]	9.5
Vitamin/mineral mixture[e]	5.0
Mono calcium phosphate[f]	0.5

[a] Sigma Chemicals (St. Louis, MO, USA).
[b] Further composition and source priority Sea Plant Products (Pty) LTD (Hermanus, South Africa).
[c] Kelp Products (Simonstown, South Africa).
[d] Marinol-R, Marine Oil Refiners (Cape Town, South Africa).
[e] Per kg of feed: retinol, 12000 IU; cholecalciferol, 1800 IU; α-tocopheryl, 150 mg; menadione, 5 mg; thiamin, 20 mg; riboflavin, 25 mg; pyridoxine, 20 mg; Vit. B_{12}, 0.04 mg; niacin, 150 mg; Ca-pantothenate, 50 mg; folic acid, 5 mg; biotin, 0.8 mg; ascorbic acid, 750 mg; inositol, 200; manganese, 150 mg; iron, 25 mg; zinc, 25 mg; copper, 70 mg; cobalt, 2 mg; iodine, 1 mg.
[f] Kynoch Feeds (Pty) LTD (Randburg, South Africa).

Test ingredients were ground and sieved to a particle size between 125 and 1000 µm before incorporation into diets. All dry ingredients were mixed in a commercial food mixer for 30 min, whereafter oil was gradually added, while mixing constantly. Eighty-five ml of water per 100 g of feed was slowly blended into the mix, resulting in a suitably textured dough, as for fish food (Lovell, 1989). This was further processed through a cold-extruder. Drying was carried out in a convection oven at 35 °C for 48 h. The dry product was cut into 1 x 1 cm pieces and stored at 4 °C till used. Chromic oxide was used as a marker at 0.75 % inclusion level in all diets. The above procedures were followed to produce a reference and three test diets in which fish meal, soybean meal and cottonseed meal, respectively, were included. Dry matter loss over 16

h for these diets (each in triplicate) in the experimental tanks was determined by calculating weight loss of approximately 1.3 g of feed (dried at 65 °C for 24 h).

2.1.3. Experimental system

Filtered (10 µm) seawater was pumped through an indoor recirculating system consisting of a series of 15 glass holding tanks (40-l capacity), a settlement tank of 300-l capacity and primary and secondary biological filters. Two 100-mm PVC pipes cut in half were supplied as shelters in each tank. Inflow into each tank was through two 5-mm black pipes in order to prevent growth of algae. Room air temperature was cooled to 15 °C, while water was heated to 18 °C. Aeration was supplied via airstones in each tank.

Water temperature (17.4-17.8 ºC), pH (7.55-7.74), and dissolved oxygen (6.9-7.3 mg/l) were monitored every week with an oxygen and pH probe, total ammonia (below 0.06 mg/l) by the manual phenolhypochlorite method (Solórzano, 1969) and salinity (35-36 ‰) with a refractometer. Flow rate in the tanks was checked on a daily basis and maintained at 2 l per min, ensuring three complete water changes per h. Half of the water in the system was replaced weekly with fresh seawater. Clumping of abalone due to photophobic behaviour, which promotes chipping of shells and clotting of faeces and pedal mucus on the shells, was minimised by maintaining the experimental animals in darkness. The lights were only turned on during cleaning, feeding and sampling (0800-0900 and 1500-1600 h).

2.1.4. Feeding and fecal collection

Thirty eight abalone were placed in a grey PVC holding container with a oyster net floor with 8 x 6 mm mesh aperture. This was placed into a second plastic faecal collection container with a net floor of 200-µm mesh. These two containers were then placed into the glass holding tanks. This method was found to be more effective in collecting quantities of excreta from *H. midae* than conical tanks based on a settling effect (Sales, 2000). Abalone were adapted for a period of 10 days to the test diets (3 replicates/diet). Tanks were randomly allocated to treatments. Feeding was performed at 0.2 % of live weight, which was slightly in excess of intake. Faecal collection was performed over a 20-day period. During adaptation and collection abalone were fed at 1600 h. The next morning at 0800 h all uneaten feed and faeces were collected by

removing the tank with the 200-µm net floor. The 200-µm mesh tank was replaced at 1500 h, put in a blast freezer at –20 °C for 1 h, and the frozen faeces were then scraped off. Frozen faeces were stored until the end of the 20-day collection period whereafter they were freeze dried and analysed. Changing of tanks was also performed during the 10-day adaptation period to get animals used to the experimental protocol.

2.1.5. Analytical procedure

Dry matter was determined by drying samples at 105 °C for 24 h, ash and AIA content according to Atkinson et al. (1984), crude fibre by the Weende method, crude protein (*N* x 6.25) by using the Kjeldahl technique, energy content by direct combustion in an adiabatic calorimeter, and Cr_2O_3 spectrophometrically according to Furukawa and Tsukahara (1966). Samples were hydrolysed with 6 N HCl in a sealed tube for 24 h in an oil bath at 110 °C whereafter a Beckman amino acid analyser (Model 6300) was used for separating amino acids using sodium elution buffers. Organic matter was calculated as dry matter minus ash.

2.1.6. Digestibility coefficient calculation and statistical analyses

ADCs in experimental diets were calculated according to the formula from Maynard and Loosli (1969):

ADC of dry matter of diet (%) = 100 × [1 – (concentration of marker in diet/concentration of marker in faeces)]

ADC of nutrients and energy of diet (%) = 100 × [1- (concentration of marker in diet/concentration of marker in faeces) × (concentration of nutrient or energy in faeces/concentration of nutrient or energy in diet)]

ADC of dry matter in the test ingredients (%) was determined as follows:

ADC (%) = [ADC of test diet – (0.7 × ADC of reference diet)]/0.3

The above formula was also used to calculate the leaching rate of individual test ingredients in test diets.

ADC of organic matter, energy and protein the test ingredients (%) was calculated using the formula of Cho and Slinger (1979):

ADC (%) = [(nutrient or energy ADC of test diet) – (0.7 × nutrient or energy ADC of reference diet)]/0.3

as well as the formula applied by Sugiura et al. (1998):

ADC (%) = [(concentration of nutrient or energy in test diet × nutrient or energy ADC of test diet) – (0.7 × concentration of nutrient or energy in reference diet × nutrient or energy ADC of reference diet)]/(0.3 × concentration of nutrient or energy in test ingredient)

Results were subjected to one-way analysis of variance. Differences between means ($P < 0.05$) were evaluated by the Tukey's HSD test (Snedecor and Cochran, 1991).

2.2. Influence of time of collection on marker content of faeces

In order to evaluate differences in marker content between night and day faeces three tanks of experimental abalone (as described above) were fasted for a period of 96 h, fed a single meal of the fish meal test diet for 16 h, and starved again for 96 h during which period faeces were collected every 12 h. The experimental protocol, collection and analyses of Cr_2O_3 and AIA were as described above.

Leaching, as described in Section 2.1.2, was determined for this diet (triplicate samples) in a randomized experimental design at 1, 3, 6, 10 and 16 h after placing food in tanks. In addition, Cr_2O_3, organic matter, AIA, protein and energy contents were determined in leached samples as in Section 2.1.5.

2.3. Influence of inclusion of bentonite on AIA content of faeces

Two formulated diets (Table 3) were fed to replicate tanks of abalone in order to test the effect of the inclusion of bentonite in the diet on the AIA content of faeces. The experimental protocol was as described above, except that diets were fed for 7 days in the adaptation period and faecal collection was performed over 7 days.

Table 3

Ingredient and determined nutrient composition of diets used for evaluating of inclusion of bentonite on marker content in faeces (dry matter basis)

Ingredient[a]	Without bentonite (%)	With bentonite (%)
Fish meal	40.0	40.0
Maize starch	30.0	30.0
Kelp powder	5.0	5.0
Fish oil	1.0	1.0
Carboxymethyl-cellulose	2.0	2.0
a-Cellulose	19.0	18.0
Vitamin/mineral mixture[b]	2.5	2.5
Mono calcium phosphate	0.5	0.5
Bentonite[c]	0.0	1.0
Analysis (dry matter basis)		
Dry matter (%)	94.22	93.10
Organic matter (%)	90.89	90.51
Crude protein (%)	29.60	29.71
Gross energy (kJ/g)	18.02	17.97

[a] Origin of ingredients as in Table 2.
[b] See Table 2.
[c] Boland Base Minerals (Milnerton, South Africa).

2.4. Influence of washing of faeces with distilled water on AIA content in faeces

The diet used in Section 2.3 without bentonite was fed to replicate tanks of abalone using the experimental protocol followed as in Section 2.3. However, after faeces were scraped off they were washed with small quantities of distilled water through filter paper.

2.5. Total collection

Three conical 2-l tanks, incorporated into the recirculating experimental system described in Section 2.1.3, were stocked with three abalone (56.08 ± 0.64 mm shell length, 34.89 ± 1.82 g live weight, mean ± S.E.) each. Water flow was restricted to 200 ml/min. The outflow was sieved through a 50-µm net. Animals were fasted for 6 days, fed with a measured amount of the diet without bentonite (Table 3) for 16 h, and faeces collected thereafter at 12-h intervals for

the next 6 days while no feed was offered. The net used to filter the outflow was washed during each collection in the tank water, and all water was filtered through pre-weighted dried filter paper. The filter paper and faeces were dried for 24 h at 55 °C.

Paired and unpaired *t*-tests (Snedecor and Cochran, 1991) were used to test for differences between treatments in Sections 2.2-2.5, while leaching results in Section 2.2 were analysed according to one-way analysis of variance as described in Section 2.1.6.

3. Results

3.1. Digestibility calculations using different markers

For most diets the levels of Cr_2O_3 and crude fibre were similar to those recorded in the faeces (Table 4), leading to unrealistic, and in some instances, negative apparent dry matter digestibility coefficients.

Table 4

Organic matter, protein, energy and marker contents in diets and faeces (mean ± S.E., $n = 3$; dry matter basis)

Component	Reference	Fish meal	Soybean meal	Cottonseed meal
Diets				
Organic matter (%)	92.34	91.37	93.20	93.03
Gross energy (kJ/g)	18.54	19.52	18.49	18.76
Crude protein (%)	33.73	44.65	37.15	38.29
Chromic oxide (%)	0.71	0.75	0.71	0.76
Acid insoluble ash (%)	0.42	0.77	0.59	0.85
Crude fibre (%)	7.59	5.89	6.31	7.76
Faeces				
Organic matter (%)	$36.55^{a} \pm 1.31$	$43.95^{b} \pm 1.31$	$40.35^{ab} \pm 0.75$	$45.85^{b} \pm 1.41$
Gross energy (kJ/g)	$3.68^{a} \pm 0.69$	$6.08^{b} \pm 0.39$	$3.65^{a} \pm 0.35$	$6.02^{b} \pm 0.45$
Crude protein (%)	$9.34^{a} \pm 1.19$	$19.06^{c} \pm 0.85$	$8.65^{a} \pm 0.60$	$15.00^{b} \pm 0.70$
Chromic oxide (%)	$0.59^{ab} \pm 0.07$	$0.81^{c} \pm 0.02$	$0.78^{bc} \pm 0.06$	$0.54^{a} \pm 0.03$
Acid-insoluble ash (%)	$3.21^{b} \pm 0.13$	$2.62^{ab} \pm 0.08$	$2.52^{a} \pm 0.23$	$2.61^{ab} \pm 0.07$
Crude fibre (%)	7.54 ± 0.58	6.97 ± 0.58	7.70 ± 0.22	7.23 ± 0.15

Means in the same row with different superscripts (a, b, c) are statistically different ($P < 0.05$).

Realistic values with good replication were obtained when digestibility was calculated using AIA as marker (Table 5).

A comparison between ADCs for organic matter, energy and protein calculated according to two different formulas (Cho and Slinger, 1979; Sugiura et al., 1998) revealed substantial differences when the difference in nutrient level between reference diet and test ingredient was high (Table 6).

The apparent availability of individual amino acids in test ingredients calculated according to the formula of Sugiura et al. (1998) is presented in Table 7.

A few values of over 100 % were recorded for individual amino acids. Soybean meal presented a mean apparent digestibility of 84.10 % ± 2.49 for essential amino acids, while corresponding values were 73.08 % ± 1.80 and 72.68 % ± 2.42 for fish and cottonseed meal, respectively. Apparent availability of lysine was low in soybean meal in comparison to either fish or cottonseed meal. Availability of methionine was found to be relatively low in all test ingredients. The mean apparent availability calculated for all amino acids was reflected in the general apparent protein digestibility calculated for each of the test ingredients.

Calculation of leaching rates (Table 8) for individual feed ingredients in the 30 % test ingredient: 70 % reference diet ratio showed that the inclusion of either soybean or cottonseed meal at 30 % resulted in a higher ($P < 0.05$) individual contribution to leaching (25.94 % ± 1.12 and 23.73 % ± 0.25, respectively) than fish meal (15.01 % ± 0.77, mean ± S.E.).

3.2 Time of collection on marker content in faeces

No difference ($P > 0.05$) was found in the Cr_2O_3 content of faeces collected either during the night (0.43 % ± 0.04, mean ± S.E.) or day (0.55 % ± 0.10). Similarly no difference ($P > 0.05$) was found in AIA content of faeces (2.59 % ± 0.27; 2.34 % ± 0.42, respectively). However, for both markers the S.E. has increased considerably from night to day.

Table 5

Apparent dry matter, organic matter, energy and protein digestibility (%) of reference and test diets (mean ± S.E., n = 3) using acid-insoluble ash as marker

Diet	Dry matter	Organic matter	Energy	Protein
Reference	86.87^{c} ± 0.56	94.80^{c} ± 0.33	97.40^{b} ± 0.31	96.34^{b} ± 0.60
Reference + 30 % fish meal	70.57^{ab} ± 0.84	85.87^{a} ± 0.14	90.85^{a} ± 0.43	87.47^{a} ± 0.30
Reference + 30 % soybean meal	76.04^{b} ± 2.24	89.62^{b} ± 1.06	95.31^{b} ± 0.43	94.41^{a} ± 0.74
Reference + 30 % cottonseed meal	67.36^{a} ± 0.91	83.93^{a} ± 0.34	89.55^{a} ± 0.64	87.22^{a} ± 0.71

Means in the same column with different superscripts (a, b, c) are statistically different ($P < 0.05$).

Table 6

Apparent dry matter, organic matter, energy and protein digestibility (%) in test ingredients included at 30 % inclusion level in the reference diet (mean ± S.E., n = 3) using acid-insoluble ash as marker and calculated according to two different formulas

Component	Method	Fish meal	Soybean meal	Cottonseed meal
Dry matter		32.52^{ab} ± 2.79	50.75^{b} ± 7.45	21.83^{a} ± 3.04
Organic matter	Cho and Slinger (1979)	65.02^{a} ± 0.47	77.52^{b} ± 3.52	58.58^{a} ± 1.14
	Sugiura et al. (1998)	64.85^{a} ± 0.48	79.35^{b} ± 3.51	60.42^{a} ± 1.15
Difference		0.17 ± 0.02	-1.83 ± 0.01	-1.84 ± 0.00
Energy	Cho and Slinger (1979)	75.58^{a} ± 1.43	90.43^{b} ± 1.43	71.23^{a} ± 2.14
	Sugiura et al. (1998)	73.69^{b} ± 1.21	82.45^{c} ± 1.31	67.02^{a} ± 1.94
Difference		1.89 ± 0.22	7.98 ± 0.12	4.21 ± 0.20
Protein	Cho and Slinger (1979)	66.77^{a} ± 1.01	89.90^{b} ± 2.47	65.94^{a} ± 2.36
	Sugiura et al. (1998)	76.05^{a} ± 0.63	84.59^{b} ± 1.89	72.13^{a} ± 1.83
Difference		-9.28 ± 0.38	5.31 ± 0.58	-6.19 ± 0.52

Means in the same row within method with different superscripts (a, b, c) are statistically different ($P < 0.05$).

Table 7

Apparent availability (%) of individual amino acids in test ingredients included at 30 % inclusion level in the reference diet (mean ± S.E., n = 3) using acid-insoluble ash as marker

Amino acid	Fish meal	Soybean meal	Cottonseed meal
Essential			
Arginine	76.05^{b} ± 1.66	134.20^{c} ± 1.45	48.51^{a} ± 2.03
Histidine	79.92^{b} ± 1.98	103.88^{c} ± 2.31	60.74^{a} ± 2.18
Isoleucine	83.11^{b} ± 2.17	69.55^{a} ± 1.94	107.05^{c} ± 2.66
Leucine	80.54 ± 2.17	76.02 ± 2.28	84.83 ± 2.96
Lysine	86.89^{ab} ± 1.91	78.71^{a} ± 2.64	92.67^{b} ± 2.74
Phenylalanine	72.86^{a} ± 2.19	98.58^{b} ± 2.08	66.48^{a} ± 2.14
Methionine	61.08^{b} ± 1.34	58.15^{b} ± 2.63	20.73^{a} ± 2.83
Threonine	71.94^{a} ± 1.85	83.90^{b} ± 2.40	76.21^{ab} ± 1.57
Valine	77.81^{a} ± 1.74	91.65^{b} ± 2.74	81.15^{a} ± 2.33
Non-essential			
Aspartic acid	89.11^{b} ± 1.82	75.79^{a} ± 2.26	83.95^{ab} ± 2.12
Serine	55.74^{a} ± 2.46	91.69^{b} ± 1.75	70.46^{c} ± 1.73
Glutamic acid	96.53^{b} ± 2.43	95.73^{b} ± 2.43	58.84^{a} ± 2.94
Proline	74.17^{a} ± 2.50	92.41^{b} ± 3.39	113.06^{c} ± 2.99
Glycine	84.20^{b} ± 1.02	91.75^{c} ± 2.56	66.39^{a} ± 1.43
Alanine	82.37 ± 0.92	87.80 ± 2.26	79.90 ± 2.84
Tyrosine	29.46^{a} ± 2.01	53.52^{b} ± 3.11	49.93^{b} ± 2.80
Mean	74.99^{a} ± 1.81	86.32^{b} ± 2.37	72.68^{a} ± 2.31

Means in the same row with different superscripts (a, b, c) are statistically different (P < 0.05).

Table 8

Leaching of dry matter in reference and test diets (mean ± S.E.; $n = 3$)

Diet	% Leaching
Reference	$1.79^{a} \pm 0.22$
Fish meal	$5.71^{b} \pm 0.23$
Soybean meal	$8.97^{c} \pm 0.34$
Cottonseed meal	$8.31^{c} \pm 0.08$

Means in the same column with different superscripts (a, b, c) are statistically different ($P < 0.05$).

While the Cr_2O_3 content of faeces collected during a period of no feeding (0.49 % ± 0.06) was different ($P < 0.05$) to faeces collected during the feeding period (0.81 % ± 0.05, Table 4), AIA content (2.46 % ± 0.23) did not differ ($P > 0.05$) from the value derived during daily feeding (Table 4), although the S.E. was higher in the former.

Leaching of dry matter, and contents of Cr_2O_3, AIA, protein, energy and organic matter of feed after certain intervals in seawater are presented in Table 9.

Although regression analysis was not used in the present evaluation due to the limited ultimate time period of samples in seawater, it is clear that leaching of dry matter, organic matter, energy and protein contents followed a curvilinear pattern over time. While leaching of dry matter still seemed to be increasing after 16 h, both protein and energy contents did not decrease ($P > 0.05$) after 3 h in water. However, the decrease in energy content was more gradual than that of protein. No specific trends were detected in either Cr_2O_3 or AIA contents. Apparent dry matter digestibility values calculated according to Cr_2O_3 content in leached samples were still unrealistic, while dry matter digestibility calculated according to AIA content in leached samples (72.59 ± 0.78 %) did not differ ($P > 0.05$) from the value derived when using AIA content values from unleached feed (70.57 ± 0.84 %).

Table 9

Leaching of dry matter and content of organic matter, energy, protein and markers of fish meal test diet after certain time periods in water (mean ± S.E.; $n = 3$)

Time (h)	Dry matter leaching (%)	Organic matter (%)	Energy (kJ/g)	Protein (%)	Chromic oxide (%)	Acid-insoluble ash (%)
1	$2.98^{a} \pm 0.26$	$89.74^{d} \pm 0.07$	$18.58^{b} \pm 0.05$	$43.33^{b} \pm 0.42$	0.671 ± 0.03	0.734 ± 0.06
3	$3.98^{ab} \pm 0.26$	$88.84^{c} \pm 0.10$	$18.24^{ab} \pm 0.05$	$41.23^{a} \pm 0.43$	0.723 ± 0.01	0.652 ± 0.04
6	$4.74^{bc} \pm 0.20$	$88.64^{bc} \pm 0.08$	$18.16^{ab} \pm 0.04$	$41.45^{a} \pm 0.23$	0.698 ± 0.00	0.750 ± 0.05
10	$5.51^{cd} \pm 0.22$	$88.32^{ab} \pm 0.16$	$18.06^{a} \pm 0.15$	$41.17^{a} \pm 0.38$	0.703 ± 0.01	0.715 ± 0.00
16	$6.50^{d} \pm 0.38$	$88.09^{a} \pm 0.08$	$18.10^{a} \pm 0.15$	$41.00^{a} \pm 0.34$	0.666 ± 0.01	0.728 ± 0.05

Means in the same column with different superscripts (a, b, c) are statistically different ($P < 0.05$).

3.3. Influence of inclusion of bentonite on marker content in faeces

Inclusion of 1 % bentonite in the diet increased the AIA content of the diet from 0.55 to 0.66 %, but AIA content of faeces (1.97 % ± 0.10) did not differ (P > 0.05) from faeces derived from the diet without AIA (2.43 % ± 0.19). However, apparent dry matter digestibility was higher ($P < 0.05$) in the diet without bentonite due to a lower AIA content in the diet.

3.4. Influence of washing of faeces on marker content in faeces

Washing of faeces with distilled water had no influence ($P > 0.05$) on AIA content (2.22 % ± 0.22) in comparison to unwashed samples (2.43 % ± 0.19)

3.5. Total collection

Apparent dry matter digestibility for the diet without bentonite (Table 4) calculated according to the total collection method over 132 h of no feeding (58.78 % ± 8.15) did not differ ($P > 0.05$) from the value using AIA (76.98 % ± 1.72) as marker. Correction of feed intake for leaching over 16 h did not have any influence ($P > 0.05$) on apparent dry matter digestibility (56.29 % ± 8.65) calculated from the total collection method. Empty faecal strings were observed after 72 h of fasting. Apparent dry matter digestibility calculated after 72 h of fasting was 72.08 % ± 7.11.

4. Discussion

This study verified the feasibility of using AIA as an internal marker in digestibility studies with *H. midae*. Reliable and consistent estimates of digestibility of various nutrients (dry matter, organic matter, protein, energy and individual amino acids) for individual test ingredients were obtained when using AIA as a marker. Furthermore, the ADC for dry matter was not different ($P > 0.05$) to the digestibility value derived from the total collection method. It was shown that AIA content in faeces during the collection period was representative of a 24-h cycle, and leaching of the marker from the feed had no influence ($P > 0.05$) on the ADC.

The external marker Cr_2O_3 did not yield high enough concentrations in faeces to calculate realistic ADCs for dry matter, organic matter, energy, protein or individual amino acids. Reasons why Cr_2O_3 has not worked as a marker in digestibility studies with *H. midae* during

collection of all faeces remain speculative. The partitioning and preferential elimination of this substance, attributed to a differential rate of passage through the gut in comparison to the experimental diet in other aquatic species (Forster and Gabbott, 1971; Bordner et al., 1983; Leavitt, 1985; Brown et al., 1986; Jones and De Silva, 1997), might partly explain this phenomenon. However, measurement of rate of passage through the gut of *Haliotis* spp. is complicated by the complex gut structure. Another reason might be a release of nutrients during feeding, as implied by the lower AIA concentration in faeces when bentonite was included in the diet in the present study. Rasping of food during ingestion by the radula into small particles in *Haliotis* spp. promotes the release of nutrients (McLean, 1970).

Similarly, the low concentrations of crude fibre measured in abalone faeces in the present study led to unrealistic ADCs. This is not surprising, as *Haliotis* spp. is known to secrete enzymes capable of breaking down complex structural carbohydrate (Vonk and Western, 1984). Furthermore, an increase in cellulolytic activity was observed in stomach fluid of abalone when cellulose was present in the diet (Monje and Viana, 1998). While crude fibre does not represent a homogeneous group of substances (Morales et al., 1999), the value of the different components of fibre in nutrition of *Haliotis* spp. needs attention.

Dry matter leaching of experimental diets used (1.8-9 %) over 16 h was low in comparison to results obtained over 24 h (4.1-33.8 %) for commercial abalone diets (Britz and Clayden, 1996). Although a degree of leaching of individual markers and nutrients was found, this did not have a pronounced effect on digestibility estimations. Thus, leaching from the experimental diets was not taken into account in determination of digestibility coefficients. Furthermore, the experimental diets were available to the abalone for a 16-h interval, making it impossible to know the exact time at which the abalone ingested the feed offered.

Although digestibility estimates based on total collection were made in the present study for purposes of comparison, the method is not recommended with abalone fed artificial diets for several reasons: (1) the difficulty and impracticality of separation of feed particles and faeces, especially when animals are continuously fed, (2) the risk of loss of faeces that will result in an overestimation of digestibilities, (3) the limited scale that it can be applied on, and (4) the

uncertain time period for collection of faeces representing the feed consumed. In the present study faeces were still produced after 6 days of fasting, which possibly could have been due to catabolism of tissue that was not attributable to the single meal ingested. This could lead to an underestimation of digestibility, as was probably the case in the present study. The apparent dry matter digestibility value (72.08 %) obtained after 72 h when empty faecal strings were observed was close to the AIA ADC (70.57 %). A challenge for further studies would thus be to determine gut evacuation time of different feeds in *H. midae*.

No difference was found in the Cr_2O_3 or AIA contents of faeces collected either during the day or night in the present study, suggesting that the procedures followed were adequate to collect a representative sample. This is in agreement with studies on fish (De Silva and Perera, 1984; De Silva et al., 1990). However, in fish (De Silva et al., 1990) the variation in dry matter digestibility increased from day to night, contrary to the findings in the present study. This could partly be explained by the differences in feeding and digestion processes between fish and *Haliotis* spp. Washing faeces with distilled water, as practised during digestibility studies with marine shrimp (Akiyama et al., 1989), did not have any effect on AIA contents of abalone faeces. The standard errors for dry matter digestibility suggest that daily feeding and collection, in comparison to a single feeding followed by collection during a period of fasting, decreases the variance of digestibility estimations, resulting in an increase in the accuracy of measurements.

A relatively high ADC for protein in fish meal was found in the present study (76 %) in comparison to a value of 43 % for fish meal included at 70 % in a single protein diet for *H. laevigata* (Fleming et al., 1998), and values of 52.5 and 46.5 % for *H. rubra* and *H. laevigata*, respectively, with a diet containing 50 % fish meal (Wee et al., 1992b). However, the difference in quality of fish meal from different sources is a well-known fact. Dry matter, protein and energy digestibility values presently found for fish meal is in agreement to values for crustacean species (–5 to 91 %, 57-88 % and 28-83 %, respectively) (summarised by Lee and Lawrence, 1997). However, although apparent protein digestibility is within range, both dry matter and energy digestibility of fish meal are lower than corresponding values (62.0-90.8 % for protein, 68.1-88.2 % for dry matter, 83.4-91.7 % for energy) reported for fish species (summarised by Hertrampf and Piedad-Pascual, 2000).

Comparable apparent protein digestibility values for soybean meal (79.2 %) and cottonseed meal (76.2 %) for *H. midae* were reported by Shipton (2000) when using Cr_2O_3 content in one type of faeces as marker. Except for dry matter digestibility (51 %), ADCs for protein and energy of soybean meal were within the ranges reported in crustacean and fish species (56-98 %, 84-99 % and 72-91 %, respectively, summarised by Lee and Lawrence, 1997, Hertrampf and Piedad-Pascual, 2000). However, comparison of ADCs for feed ingredients between species is complicated because of differences in methodology used (Allan et al., 2000). In the past, the majority of digestibility values derived from studies with a test ingredient included in a reference diet were based on the equation of Cho and Slinger (1979). This equation assumes that the nutrient digestibility of the test diet is the average of the nutrient digestibility of the reference diet and the test ingredient weighted by the proportion of each in the test diet. This, as was also shown in the present study, does not account for the relative contribution of the nutrient from the reference diet and the test ingredient to the test diet (Forster, 1999). The latter is facilitated by the equation of Sugiura et al. (1998).

Good agreement was obtained between mean apparent availability for amino acids and apparent protein digestibility for the test ingredients evaluated. The values of over 100 % for apparent individual amino acid availability reported in this study might have been due to possible interactions between nutrients in the reference diet and test ingredients, or differential leaching of some nutrients within ingredients (Allan et al., 2000).

The present study indicated that the digestibility of nutrients in soybean meal is relatively high, which will promote the goal of formulating low pollution feeds. Although good growth rates have been achieved with soybean meal at an inclusion rates as high as 50 % with *H. midae* (Shipton, 2000), and it is included in some commercial abalone diets (Fleming et al., 1996), this ingredient has some limitations due to a poor balance of certain amino acids, and anti-nutritional factors (Hertrampf and Piedad-Pascual, 2000). Furthermore, a better understanding of the nutrient associations that occur among the most frequently used feed ingredients in aquaculture, and affect ADCs of compound practical feeds, should be one of the major goals for aquatic nutritionists (Lee and Lawrence, 1997).

In conclusion, evidence is presented in this study that AIA is a reliable and replicable internal marker for determination of digestibility of feed ingredients in *H. midae*, in contrast to external markers that are not recovered in appropriate quantities in the faeces. The present study was the first to describe apparent digestibility of nutrients of feed ingredients included at a certain inclusion level into a reference diet for *Haliotis* spp. In addition, nutrient digestibility data of commonly available feed ingredients are presented that could be used as a starting point for the least-cost formulation of diets for *H. midae*.

Acknowledgements

This study was sponsored by Sea Plant Products, Hermanus, South Africa, the South African Network for Coastal and Oceanographic Research, the National Research Foundation and Rhodes University Joint Research Committee. The first author was supported by a post-doctoral scholarship from the Claude Leon Harris Foundation. The authors gratefully acknowledge the skilled technical assistance of P. Truter, G. Clayden and D. Barnes of Sea Plant Products during providing of animals and building of the experimental system.

References

Akiyama, D.M., Coelho, S.R., Lawrence, A.L., Robinson, E.H., 1989. Apparent digestibility of feedstuffs by the marine shrimp *Penaeus vannamei* Boone. Nippon Suisan Gakkaisha 55, 91-98.

Allan, G.L., Parkinson, S., Booth, M.A., Stone, D.A.J., Rowland, S.J., Frances, J., Warner-Smith, R., 2000. Replacement of fish meal in diets for Australian silver perch *Bidyanus bidyanus*: I. Digestibility of alternative ingredients. Aquaculture 186, 293-310.

Atkinson, J.L., Hilton, J.W., Slinger, S.J., 1984. Evaluation of acid-insoluble ash as an indicator of feed digestibility in rainbow trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. 41, 1384-1386.

Bordner, C.E., D'Abramo, R., Conklin, D.E., 1983. Assimilation of nutrients by cultured hybrid lobsters (*Homarus* sp.) fed experimental diets. J. World Maricult. Soc. 14, 11-24.

Bowen, S.H., 1981. Digestion and assimilation of periphytic aggregate by *Tilapia mossambica*. Trans. Am. Fish. Soc. 110, 239-245.

Britz, P.J., 1995. The nutritional requirements of *Haliotis midae* and development of a practical diet for abalone aquaculture. Ph.D Dissertation, Rhodes University, South Africa, 150 pp.

Britz, P.J., Clayden, C., 1996. Evaluation of the growth rate and feed conversion ratio of *Haliotis midae* fed formulated practical diets. Proc. Aquacult. Assoc. S. Afr. 5: 68-73.

Britz, P.J., Hecht, T., Knauer, J., Dixon, M.G., 1994. The development of an artificial feed for abalone farming. SA J. Sci. 90, 7-8.

Brown, P.B., Williams, C.D., Robinson, E.H., Akiyama, D.M., Lawrence, A.L., 1986. Evaluation of methods for determining in vivo digestion coefficients for adult red swamp crayfish *Procambarus clarkii*. J. World Aquacult. Soc. 17, 19-24.

Bureau, D.P., Harris, A.M., Cho, C.Y., 1999. Apparent digestibility of rendered animal protein ingredients for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 180, 345-358.

Cho, C.Y., Slinger, S.J., 1979. Apparent digestibility measurement in feedstuffs for rainbow trout. In: Halver. J.E., Tiews, K. (Eds.), Finfish Nutrition and Fish Feed Technology. Proceedings of a World Symposium, Vol. 2, Heenemann, Berlin, pp. 239-247.

Cho, C.Y., Slinger, S.J., Bayley, H.S., 1982. Bioenergetics of salmonid fishes: energy intake, expenditure and productivity. Comp. Biochem. Physiol. 73B, 25-41.

Cho, C.Y., Hynes, J.D., Wood, K.R., Yoshida, H.K., 1994. Development of high-nutrient-dense, low-pollution diets and prediction of aquaculture wastes using biological approaches. Aquaculture 124, 293-305.

De la Noüe, J., Choubert, G., 1986. Digestibility in rainbow trout: comparison of the direct and indirect methods of measurement. Prog. Fish-Cult. 48: 190-195.

De Silva, S.S., Anderson, T.A., 1995. Fish Nutrition in Aquaculture. Chapman & Hall Aquaculture Series 1, London, 319 pp.

De Silva, S.S., Perea, M.K., 1983. Digestibility of an aquatic macrophyte by the cichlid *Etroplus suratensis* (Bloch) with observations in the relative merits of three indigenous components as markers and daily changes in protein digestibility. J. Fish Biol. 23, 675-684.

De Silva, S.S., Perea, M.K., 1984. Digestibility in *Saratherodon niloticus* fry: effect of dietary protein level and salinity with further observations on daily variability in digestibility. Aquaculture 38, 293-306.

De Silva, S.S., Shim, K.F., Khim Ong, A., 1990. An evaluation of the method used in digestibility estimations of a dietary ingredient and comparisons on external and internal markers, and time of faeces collection in digestibility studies in the fish *Oreochromis aureus* (Steindacher). Reprod. Nutr. Dev. 30, 215-226.

Edin, H., 1918. Orienterance försök över användbarheten av en pa 'ledkroppsprincipen' grundad metod att bestämma en foderblandings smältbarhet. Cent. Forsoksvasendet Jordbruk.. Stockholm Medd. 165, 1-28.

Fleming, A.E., Van Barneveld, R.J., Hone P.W., 1996. The development of artificial diets for abalone: A review and future directions. Aquaculture 140, 5-53.

Fleming, A.E., Van Barneveld, R.J., Hone, P.W., Vandepeer, M.E., Kruck, J.A., 1998. Complimentary additivity of the digestibility coefficients of feed ingredients fed to juvenile greenlip abalone (*Haliotis laevigata*). J. Shelfish Res. 17, 641-647.

Forster, I., 1999. A note on the method of calculating digestibility coefficients of nutrients provided by single ingredients to feeds of aquatic animals. Aquacult. Nutr. 5, 143-145.

Forster, J.R.M., Gabbott, P.A., 1971. The assimilation of nutrients from compound diets by the prawns *Palaemon serratus* and *Pandalus platyceros*. J. Mar. Biol. Assoc., U.K. 51, 943-961.

Furukawa, A., Tsukahara, H., 1966. On the acid digestion method for determination of chromic oxide as an index substance in the study of digestibility of fish feed. Bull. Jpn. Soc. Sci. Fish. 32, 502-506 (In Japanese, with English abstract).

Hertrampf, J.W., Piedad-Pascual, F., 2000. Handbook on Ingredients for Aquaculture Feeds, Kluwer Academic Publishers, Dordrecht, The Netherlands, 573 pp.

Jones, P.L., De Silva, S.S., 1997. Influence of differential movement of the marker chromic oxide and nutrients on digestibility estimations in the Australian freshwater crayfish *Cherax destructor*. Aquaculture 154, 323-336.

Leavitt, D.F., 1985. An evaluation of gravimetric and inert marker techniques to measure digestibility in the American lobster. Aquaculture 47, 131-142.

Lee, P.G., Lawrence, A.L., 1997. Digestibility. In: D'Abramo, L.R., Conklin, D.E., Akiyama, D.M. (Eds.), Crustacean Nutrition, Advances in World Aquaculture, Vol. 6, World Aquaculture Society, Louisiana, USA, pp. 194-260.

Lovell, T., 1989. Nutrition and Feeding of Fish. Von Nostrand Reinhold, New York, USA, 260 pp.

Lupatsch, I., Kissel, G. Wm., 1998. Predicting aquaculture waste from gilthead seabream (*Sparus aurata*) culture using a nutritional approach. Aquat. Living Resour. 11, 265-268.

Maynard, L.A., Loosli, J.K., 1969. Animal Nutrition, 6th edn. McGraw-Hill, New York, NY, 613 pp.

McLean, N., 1970. Digestion in *Haliotis rufescens* Swainson (Gastropoda: Prosobranchia). J. Exp. Zool. 173, 303-318.

Monje, H., Viana, M.T., 1998. Effect of cellulose on the growth and cellulolytic activity of abalone *Haliotis fulgens* when used as an ingredient in formulated artificial diets. J. Shellfish Res. 17, 667-671.

Morales, A.E., Cardenete, G., Sanz, A., De la Higuera, M., 1999. Re-evaluation of crude fibre and acid-insoluble ash as inert markers, alternative to chromic oxide, in digestibility studies with rainbow trout (*Oncorhynchus mykiss*). Aquaculture 179, 71-79.

Nose, T., 1960. On the digestion of food protein by goldfish (*Carassius auratus* L.) and rainbow trout (*Salmo irideus g*.). Bull. Freshwater Fish. Res. Lab. 10, 11-22.

Rodehutscord, M., Gregus, Z., Pfeffer, E., 2000. Effect of phoshorus intake on faecal and non-faecal phosphorus excretion in rainbow trout (*Oncorhynchus mykiss*) and the consequences for comparative phosphorus availability studies. Aquaculture 188, 383-398.

Sales, J., 2000. Study of faeces collection methods to define optimum abalone diets. Fish Farmer International, May/June 2000, Vol. 14, No. 3, pp. 8-9.

Shipton, T.A., 2000. The protein requirements for the South African abalone, *Haliotis midae*. Ph.D Dissertation, Rhodes University, Grahamstown, South Africa, 146 pp.

Snedecor, G.W., Cochran, W.G., 1991. Statistical Methods (8th edition). Iowa State University Press, Ames, USA, 503 pp.

Solórzano, L., 1969. Determination of ammonia in natural water by the phenolhypochlorite method. Limnol. Oceanogr. 14, 799-801.

Sugiura, S.D., Dong, F.M., Rathbone, C.K., Hardy, R.W., 1998. Apparent protein digestibility and mineral availabilities in various feed ingredients for salmonid feeds. Aquaculture 159, 177-202.

Van Barneveld, R.J., Fleming, A.E., Vandepeer, M.E., Kruk, J.A., Hone, P.W., 1998. Influence of dietary oil type and oil inclusion level in manufactured feeds on the digestibility of nutrients by juvenile greenlip abalone (*Haliotis laevigata*). J. Shellfish Res. 17, 649-655.

Vonk, H.J., Western, J.R.H., 1984. Comparative Biochemistry and Physiology of Enzymatic Digestion, Academic Press, London, 501 pp.

Wee, K.L., Maguire, G.B., Hindrum, S.M., 1992a. Methodology for digestibility studies with abalone. I. Preliminary studies on the feeding and defaecatory behaviour of blacklip abalone, *Haliotis rubra*, fed natural and artificial diets. In: Allan G.L., Dall, W. (Eds.), Proc. Aquaculture Nutrition Workshop, 15-17 April 1991, NSW Fisheries, Brackish Water Fish Culture Research Station, Salamander Bay, Australia, pp. 192-196.

Wee, K.L., Maguire, G.B., Hindrum, S.M., 1992b. Methodology for digestibility studies with abalone. 2. Comparison of markers in an artificial diet for blacklip abalone (*Haliotis rubra*) and greenlip abalone (*Haliotis laevigata*). In: Paper delivered at the Third Asian Fisheries Forum, 26-30 October 1992, Singapore.

CHAPTER FIVE

Evaluation of the reference diet substitution method for determination of apparent nutrient digestibility coefficients of feed ingredients for South African abalone *(Haliotis midae* L.)

Sales, J., Britz, P.J., 2001. Evaluation of the reference diet substitution method for determination of apparent nutrient digestibility coefficients of feed ingredients for South African abalone (*Haliotis midae* L.). Aquaculture (Accepted for publication, 23/07/2001).

Abstract

Apparent nutrient (dry matter, organic matter, energy, protein) digestibility of sunflower meal, canola meal and peanut meal, substituted at 20 % inclusion level into a reference diet, was determined for *Haliotis midae* in this study. In a second experiment the influence of inclusion level of a feed ingredient (soybean meal) on apparent dry matter, organic matter, energy and protein digestibility was evaluated. Apparent protein digestibility coefficients exceeding 100 % were found for sunflower meal and canola meal included into a reference diet. This also occurred when evaluating different inclusion levels of soybean meal into a fish meal reference diet, eliminating the opportunity to test the influence of inclusion level of soybean meal on apparent nutrient digestibility. Associative effects between different feed ingredients, diet and faecal nutrient leaching and mathematical artifacts in calculations when using substitution versus single protein diets might have an influence on final apparent digestibility coefficients of highly digestible feed ingredients. It was found that apparent protein digestibility coefficients determined with single protein diets predicted determined digestibility in compound diets with an error of less than 3.3 % compared to a deviation of 13 to 16 % when using coefficients determined with substitution trials. The conclusion from this study is that single protein diets should be used for determining of apparent protein digestibility coefficients of feed ingredients for *H. midae* when evaluating feed ingredients for feed composition tables.

1. Introduction

Although apparent nutrient digestibility of feed ingredients for aquatic species has successfully been determined with only the test ingredient as diet ingredient (Wilson et al., 1981; Akiyama et al., 1989; Anderson et al., 1992; Lupatsch et al., 1997; Fleming et al., 1998), very few feed ingredients are fed as the sole component of a diet. Therefore some researchers evaluate the digestibility of feed ingredients in combination with other ingredients in the test diet. An advantage of this method over testing ingredients singly is that the test ingredient may be more acceptable to the animal when fed in combination with other ingredients, which leads to a normal level of intake (NRC, 1993). All substitution trials are based on the assumption that there is no interaction between the reference diet and the test ingredient, and that results are independent of the level of inclusion of the test ingredient (Aksnes et al., 1996). Furthermore, the use of digestibility coefficients in diet formulation is based on the hypothesis that they are additive for different ingredients. Additivity of digestibility coefficients determined for individual feed ingredients in compound diets has been demonstrated using a wide range of ingredients in poultry (Sibbald et al., 1980), fish (Cho et al., 1982; Wilson and Poe, 1985; Watanabe et al., 1996; Lupatsch et al., 1997; Allan et al., 1999) and the Australian abalone *Haliotis laevigata* (Fleming et al., 1998). However, non-additivity, due to associative effects among dietary ingredients, in which the digestibility of a mixture of ingredients is greater or smaller than the mean digestibility of the individual feedstuffs composing the mixture, is a common phenomenon in terrestrial herbivore nutrition (Mould, 1988; Pond et al., 1995). Non–additivity of individually determined apparent digestibility coefficients (ADCs) in compound diets has also been reported in crayfish (Brown et al., 1989; Reigh et al., 1990) and freshwater turtles (Bjorndal, 1991).

The aim of the present study was to determine apparent nutrient digestibility coefficients of sunflower meal, canola meal and peanut meal substituted into a reference diet in order to test the validity of the substitution method for plant proteins in *H. midae*. In a second experiment the influence of inclusion level of a feed ingredient into a reference diet on apparent nutrient digestibility was evaluated. Additionally, additivity of apparent protein digestibility coefficients of individual feed ingredients in compound diets for *H. midae*, determined either with single protein diets or by means of substitution into reference diets, was evaluated.

2. Material and methods

2.1. Evaluation of apparent nutrient digestibility of different plant protein ingredients substituted into a practical reference diet

Apparent dry matter, organic matter, energy and protein digestibility of sunflower meal (crude protein 42.58 %; gross energy 18.63 MJ/kg), canola meal (crude protein 31.81 %; gross energy 20.48 MJ/kg) and peanut meal (crude protein 42.58 %; gross energy 18.63 MJ/kg), sieved to a particle size between 150 and 450 µm, and included at 20 % into a practical reference diet (Table 1) was determined.

2.2. Evaluation of apparent nutrient digestibility of an ingredient included at different levels into a reference diet

Apparent dry matter, organic matter, energy and protein digestibility of soybean meal (crude protein 47.77 %; gross energy 18.98 MJ/kg, sieved to a particle size between 150 and 450 µm), substituted at four different levels (Table 2) into a fish meal reference diet, was determined. Fish meal was chosen as protein source in the reference diet as this is currently the main source of protein in South African abalone feed.

In order to evaluate the influence of dietary nutrient leaching on apparent digestibility coefficients feed samples subjected to experimental conditions for 8 h were analysed for acid-insoluble ash and protein as described in Section 2.4.3. It was assumed that the bulk of feed was consumed within 8 h by abalone. Also, evidence was presented by Sales and Britz (2001) that the decline in protein content of feed samples subjected to experimental conditions reached a plateau after 3 h in water.

2.3. *Additivity of individually determined apparent protein coefficients in compound diets*

Apparent protein digestibility coefficients for fish meal, soybean meal and cottonseed meal, determined (1) by means of substitution into a reference diet and (2) in single protein diets were used to predict apparent protein digestibility of the compound diets used in Sections 2.1 and 2.2. Apparent protein digestibility coefficients of 76.05, 84.59 and 72.13 % obtained by Sales

and Britz (2001) for fish meal, soybean meal and cottonseed meal, respectively, substituted at 30 % in a reference diet and using acid-insoluble ash (AIA) as marker, were used.

Table 1

Ingredient and determined nutrient composition of reference diet (dry matter basis)

Ingredient	(%)
Fish meal[a]	20
Soybean meal[b]	20
Cottonseed meal[b]	10
Maize starch[c]	40
Fish oil[d]	1.0
a-Cellulose[e]	6.5
Vitamin/mineral mixture[f,g]	2.0
Mono calcium phosphate[h]	0.5
Analysis (dry matter basis)	
Crude protein (%)	28.22
Gross energy (MJ/kg)	18.41
Dry matter leaching (%)	8.59 ± 0.38

[a] Low-temperature dried fish meal (Danish 999 LT), Esbjerg Fiskeindustri a.m.b.a. (Esbjerg, Danmark).

[b] Pre-pressed, solvent extracted, dehulled, Western Province Corp. (Roggebaai, South Africa).

[c] Further composition and source priority Sea Plant Products (Hermanus, South Africa).

[d] Marinol-R, Marine Oil Refiners (Cape Town, South Africa).

[e] Sigma Chemicals (St. Louis, MO, USA).

[f] Per kg of feed: retinol, 12000 IU; cholecalciferol, 1800 IU; α-tocopherol, 150 mg; menadione, 5 mg; thiamin, 20 mg; riboflavin, 25 mg; pyridoxine, 20 mg; Vit. B_{12}, 0.04 mg; niacin, 150 mg; Ca-pantothenate, 50 mg; folic acid, 5 mg; biotin, 0.8 mg; ascorbic acid, 750 mg; inositol, 200; manganese, 150 mg; iron, 25 mg; zinc, 25 mg; copper, 70 mg; cobalt, 2 mg; iodine, 1 mg.

[g] Epol (Pretoria, South Africa).

[h] Kynoch Feeds (Randburg, South Africa).

Table 2

Ingredient and determined nutrient composition (dry matter basis) of diets where different inclusion levels of soybean meal to a fish meal based reference diet were evaluated

Ingredient (%)	Inclusion level of soybean meal			
	10	20	30	50
Fish meal	45	40	35	25
Soybean meal	10	20	30	50
Maize starch	27	24	21	15
Fish oil	0.9	0.8	0.7	0.5
Carboxymethyl-cellulose[a]	1.8	1.6	1.4	1.0
a-Cellulose	12.6	11.2	9.8	7.0
Vitamin/mineral mixture	2.25	2.00	1.75	1.25
Mono calcium phosphate	0.45	0.40	0.35	0.25
Analysis (dry matter basis)				
Crude protein (%)	36.94	37.80	39.82	42.27
Gross energy (MJ/kg)	18.86	18.93	19.11	18.91

[a] Sigma Chemicals (St. Louis, MO, USA).
Origin of sources as in Table 1

To obtain coefficients from single protein diets, fish meal (crude protein 71.48 %; gross energy 23.05 MJ/kg), soybean meal (crude protein 47.12 %; gross energy 18.82 MJ/kg) and cottonseed meal (crude protein 49.33 %; gross energy 19.32 MJ/kg), sieved to a particle size between 150 and 450 µm, were used (Table 3), and evaluated for apparent protein digestibility. The latter ingredients were from the same batches as those used by Sales and Britz (2001).

2.4 General procedures

2.4.1. Experimental animals, experimental system, feeding protocol and faeces collection

Juvenile abalone (*Haliotis midae*) (56.49 ± 0.12 mm shell length; 35.60 ± 0.24 g live weight, mean ± S.E.) obtained from a commercial South African abalone farm, and previously used in digestibility studies (Sales and Britz, 2001) in a recirculating system, were used for the present trails.

Table 3

Ingredient and determined nutrient composition (dry matter basis) of single protein diets to evaluate the apparent protein digestibility of fish meal, soybean meal and cottonseed meal

Ingredient (%)	Fish meal	Soybean meal	Cottonseed meal
Fish meal	50	0	0
Soybean meal	0	40	0
Cottonseed meal	0	0	40
Maize starch	30	40	40
Fish oil	1.0	2.0	2.0
Carboxymethyl-cellulose	2.0	1.0	1.0
a-Cellulose	14	13.5	13.5
Vitamin/mineral mixture	2.5	3.0	3.0
Mono calcium phosphate	0.5	0.5	0.5
Analysis (dry matter basis)			
Crude protein (%)	35.18	19.16	19.33
Gross energy (MJ/kg)	19.00	17.50	17.27
Dry matter leaching (%)	3.71 ± 0.26	4.11 ± 0.51	3.40 ± 0.27

Origin of ingredients as in Table 1.

The experimental system, feeding protocol and collection of faeces were as described by Sales and Britz (2001). Filtered (10 µm) seawater was pump through an indoor (room temperature at 15 °C) recirculating system consisting of a series of 15 glass holding tanks (40-l capacity), a settlement tank of 300-l capacity and primary and secondary biological filters. Aeration was supplied via airstones in each tank. Water temperature (18.3 ± 0.24 °C), pH (7.65 ± 0.03), and dissolved oxygen (6.93 ± 0.19 mg/l) were monitored every week with an oxygen and pH probe, total ammonia (0.06 ± 0.02 mg/l) by the manual phenolhypochlorite method (Solórzano, 1969) and salinity (37 ± 0.55 ‰) with a refractometer. Flow rate in the tanks was checked on a daily basis and maintained at 2 l per min. The lights were only turned on during cleaning, feeding and sampling (0800-0900 and 1500-1600 h).

Thirty seven abalone were placed in a grey PVC holding container with an oyster net floor with 8 x 6 mm mesh aperture. This was placed into a second plastic faecal collection container with

a net floor of 200-µm mesh. These two containers were then placed into the glass holding tanks (Sales, 2000). Abalone were fed at 1600 h. The next morning at 0800 h all uneaten feed and faeces were removed and the containers replaced. Faeces were collected at 1500 h by removing the collection container, placing it in a blast freezer at –20 °C for 1 h, and then scraping the frozen faeces off. Frozen faeces were stored until the end of the collection period whereafter they were freeze-dried and analysed.

2.4.2. Diet preparation

All dry ingredients were mixed in a commercial food mixer for 30 min, whereafter oil was gradually added, while mixing constantly. Eighty five ml of water per 100 g of feed was slowly blended into the mix, resulting in a suitably textured dough. This was further processed through a cold-extruder. Drying was carried out in a convection oven at 35 °C for 48 h. The dry product was cut into 1 x 1 cm pieces and stored at 4 °C until used. Diet dry matter leaching over time (16 h) in the experimental tanks was determined by calculating weight loss of approximately 2 g of feed (dried at 55 °C for 48 h).

2.4.3. Analytical procedure

Ash and AIA content were done according to Atkinson et al. (1984), crude protein (*N* x 6.25) by using the micro-Kjeldahl technique and gross energy content by direct combustion in an adiabatic calorimeter. Organic matter was calculated as dry matter minus ash.

2.4.4. Digestibility coefficient calculations and statistical analyses

ADCs in experimental diets were calculated according to the formula from Maynard and Loosli (1969):

ADC of dry matter of diet (%) = 100 × [1 – (% marker in diet/% marker in faeces)] (1)

ADCs of nutrients and energy of diet (%) = 100 × [1- (% marker in diet/% marker in faeces) × (nutrient or energy concentration in faeces / nutrient or energy concentration in diet)] (2)

ADC of dry matter of the test ingredients (%) was determined as follows:

ADC (%) = [ADC of test diet – (proportion of reference diet in test diet × ADC of reference diet)]/proportion of test ingredient in test diet (3)

ADC of organic matter, energy and protein of the test ingredients (%) was calculated using the formula applied by Sugiura et al. (1998) and suggested by Forster (1999):

ADC (%) = [(proportion of nutrient or energy concentration in test diet × nutrient or energy ADC of test diet) – (proportion of reference diet in test diet × nutrient or energy concentration in reference diet × nutrient or energy ADC of reference diet)]/(proportion of test ingredient in test diet × nutrient or energy concentration in test ingredient) (4)

Results for Section 2.2 were analysed using one-way analysis of variance. Differences between means ($P < 0.05$) were evaluated by the Tukey's HSD test (Snedecor and Cochran, 1991).

3. Results

3.1. Apparent nutrient digestibilities of different plant protein sources substituted into a reference diet

Apparent nutrient digestibilities of sunflower meal, canola meal and peanut meal evaluated by substitution into a reference diet are presented in Table 4.

Table 4
Apparent nutrient digestibilities (%) of plant proteins substituted into a practical reference diet (mean ± S.E.; $n = 3$)

Nutrient	Sunflower meal[a]	Canola meal[b]	Peanut meal[c]
Dry matter	70.62 ± 4.47	74.72 ± 6.97	-5.60 ± 7.44
Organic matter	83.34 ± 1.56	83.12 ± 3.44	42.90 ± 1.02
Energy	87.34 ± 0.67	83.97 ± 1.67	67.81 ± 1.23
Protein	101.54 ± 1.20	102.62 ± 2.25	78.79 ± 0.60

[a] Pre-pressed, solvent extracted, Western Province Corp. (Roggebaai, South Africa).
[b] Cold press, Pioneer Feeds (Paarl, South Africa).
[c] Solvent extracted, Meadow Feed Mills (Paarl, South Africa).

Peanut meal presented a negative apparent dry matter digestibility coefficient, while apparent protein digestibility of sunflower and canola meal exceeded 100 %.

3.2. Apparent protein digestibility of soybean meal included at different levels into a fish meal based reference diet.

Apparent nutrient digestibilities of soybean meal included at different levels into a fish meal based reference diet are presented in Table 5.

Table 5

Apparent nutrient digestibility (%) of soybean meal included at different levels into a fish meal based reference diet (mean ± S.E., $n = 3$)

Nutrient	Inclusion level (%)			
	10	20	30	50
Dry matter	112.81 ± 10.38	84.45 ± 6.76	79.11 ± 2.05	82.82 ± 8.17
Organic matter	108.63 ± 4.49	91.57 ± 5.60	90.03 ± 0.75	91.35 ± 4.89
Energy	105.19 ± 1.30	100.03 ± 6.40	100.40 ± 0.83	97.05 ± 3.33
Protein	118.87^{a} ± 4.61	97.87^{b} ± 4.20	102.73^{b} ± 0.76	99.70^{b} ± 2.73

Means in the same row with different superscripts (a, b) are statistically different ($P < 0.05$).

Digestibility values exceeding 100 % were obtained in all nutrient categories evaluated. With the exception of apparent protein digestibility at the 10 % soybean meal inclusion level, no differences ($P > 0.05$) were found in apparent digestibility of other nutrients between inclusion levels.

3.3. Additivity of apparent protein digestibility in compound diets.

The apparent protein digestibility coefficients for fish meal, soybean meal and cottonseed meal determined in single protein diets was 87.76 ± 1.15, 97.00 ± 0.11 and 90.03 ± 1.20 %, respectively.

An apparent protein digestibility coefficient of 95.16 ± 0.10 % was obtained for the reference diet used in Section 2.1. Predicted apparent protein digestibility calculated according to digestibility coefficients obtained by Sales and Britz (2001) through substitution into a reference diet was 78.68 %, in comparison to a value of 92.00 % when using coefficients determined with single protein diets in the present study.

Calculated and determined apparent protein digestibility of diets used in a substitution trial with different inclusion levels of soybean meal are shown in Table 6.

While predicted apparent protein digestibility in compound diets calculated according to apparent protein digestibility coefficients found with single protein diets (present study) were slightly lower than determined values, coefficients obtained by Sales and Britz (2001), by means of substitution, underestimated apparent protein digestibility by between 13.50 and 16.33 %.

According to unpaired *t*-tests only in the diet containing 10 % soybean meal did leaching result in a lower ($P < 0.05$) apparent protein digestibility coefficient.

4. Discussion

The present results indicated that the use of substitution of a feed ingredient into a reference diet is not a valid method to evaluate apparent nutrient digestibility *in H. midae*. Values of over 100 % were derived for apparent protein digestibility when soybean meal, sunflower meal and canola meal were substituted into a reference diet. Possible reasons for this could be (1) associative effects between feed ingredients, (2) diet nutrient leaching, (3) faecal nutrient leaching and (4) the nature of the additional mathematical calculations required when using the substitution method.

Interaction between a reference diet and feed ingredients in a test diet when using the substitution method is often observed, particularly in herbivorous animals that depend to a great extent on microbial fermentation. The addition of components to the test diets that stimulate fermentative activity, particularly starch or cellulose, often promote digestibility (Mould, 1988; Pond et al., 1995). It is likely that microbial activity in abalone could enhance digestion, as they are herbivorous archaeogastropods whose diet in nature consists mainly of macroalgae (Harris et al., 1998a). Viable bacteria, capable of hydrolysing a variety of complex polysaccharides in algae, have been identified in the gut of *H. midae* (Erasmus, 1996; Erasmus et al., 1997) and *H. laevigata* (Harris et al., 1998b) that could promote associative effects with respect to nutrient digestibility.

Table 6

Calculated and determined apparent protein digestibility coefficients and dry matter leaching of a fish meal based reference diet with different inclusion levels of soybean meal (mean ± S.E.; *n* = 3)

	Inclusion level of soybean meal			
	10	20	30	50
Determined (unleached feed samples)	92.73 ± 0.60	91.95 ± 1.06	92.83 ± 0.28	94.54 ± 1.84
Calculated from single protein diets[a]	89.42	90.81	92.01	93.95
Difference[b]	3.57	1.24	0.88	0.62
Calculated using a reference diet[c]	77.59	78.87	79.98	81.77
Difference[b]	16.33	14.23	13.84	13.50
Determined (leached feed samples[d])	89.88 ± 0.83	90.20 ± 1.29	91.27 ± 0.34	92.90 ± 2.39
Difference (unleached vs leached diets; % units)	2.85 ± 0.23	1.75 ± 0.23	1.56 ± 0.06	1.63 ± 0.55

[a] Apparent protein digestibility coefficients determined with single protein diets in this study.
[b] [(Determined-calculated)/determined] x 100.
[c] Apparent protein digestibility coefficients from Sales and Britz (2001). Ingredients substituted at 30 % in a casein based reference diet, Cr_2O_3 included in diets, AIA used as marker.
[d] Corrected for acid-insoluble ash and protein leaching after 8 h under experimental conditions.

As in the study of Sales and Britz (2001) the present study indicated that diet nutrient leaching did not have a pronounced effect on apparent nutrient digestibility. Furthermore, diet nutrient leaching will tend to be neutralised by faecal nutrient leaching (Eqs. (1) and (2)). However, it was found in *H. rubra* that approximately 18 % of nitrogen was lost from faeces during the first 14 h in seawater, with no further losses after 24 h (Fleming, 1995), while nitrogen leaching from a compound diet was only 3 % after 1 h and 7-8 % after more than 3 h in seawater (Sales and Britz, 2001). The latter illustrates that faecal nutrient loss could have an influence on apparent nutrient digestibility in abalone. In salmonids, Smith et al. (1980) showed that more than half of the faecal nitrogen was in the unrecoverable liquid fraction, and that digestibility coefficients were 10 % higher when based solely on solid faecal material. Cho et al. (1982) postulated that leaching losses would be proportionally higher in poorly digested feed ingredients which contained a substantial level of fiber and carbohydrates due to the larger quantity of faeces produced. This would result in erroneously high digestibility coefficients.

Another reason for apparent digestibility values of higher than 100 % could be the nature of additional mathematical calculations required when the substitution method is used. While diet and faecal nutrient leaching in the calculation of nutrient digestibility of a compound diet (Eqs. (1) and (2)) that is fed arises from faeces from the compound diet, the calculation of digestibility of an individual ingredient through substitution contains additional calculations (Eqs. (3) and (4)) which indirectly calculate the digestibility of a single ingredient. These additional calculations do not account for the possibility that the individual ingredients in a compound diet may leach nutrients at a differential rate.

Apparent protein digestibility coefficients determined with single protein diets were found to be additive in compound diets, while coefficients determined through substitution (Sales and Britz, 2001) underestimate digestibility in compound diets with 13-16 %. However, even with coefficients from single protein diets predicted digestibility was lower than determined digestibility in compound diets. This again emphasises the possibility of either associative effects or increased faecal nutrient leaching in compound diets.

Apparent protein digestibility coefficients of fish meal, soybean meal and cottonseed meal determined with single protein diets in the present study differed from values derived for these ingredients obtained in a previous trial (Sales and Britz, 2001) where the substitution method with a casein based reference diet was used. This was responsible for the extreme underestimate of apparent digestibility when the latter was used for prediction. Reasons for this could be either associative effects or the influence of chromic oxide (Cr_2O_3), included to evaluate different markers, in the study of Sales and Britz (2001), on digestibility. However, mean apparent protein digestibility of soybean meal substituted at 30 % inclusion in a fish meal based reference diet in the present study was 102.73 % in comparison to a value of 84.59 % obtained by Sales and Britz (2001) when substituted soybean meal from the same batch at 30 % inclusion in a casein reference diet. A value of 97 % was obtained for the same soybean meal in a single protein diet, in which associative effects were eliminated, in the present study. This would put more emphasis on Cr_2O_3 as the possible reason for the difference.

Evidence from this study is that plant protein sources are very effectively digested by *H. midae*. Any small factors that could influence apparent digestibility, such as associative effects between diet ingredients and nutrient leaching from faeces, could cause erroneously high apparent nutrient digestibility coefficients of ingredients (over 100 %) when evaluated by substitution into a reference diet because of additional mathematical calculations and the nature of these calculations when using substitution trials. Although faecal nutrient leaching will also be present in single nutrient diets, associative effects will be eliminated. Furthermore, digestibility will be determined with a direct equation, as for a compound diet, and the present study indicated that the apparent protein digestibility of compound diets can be determined additively from coefficients determined for single protein diets. It is thus concluded that single protein diets should be used to determine apparent digestibility of protein and availability of amino acids of ingredients for inclusion in feed composition tables for application in least-cost diet formulation for *H. midae*. The suitability of the substitution method to determine apparent digestibility for other nutrients, especially energy, for *H. midae*, still has to be evaluated.

Acknowledgements

This study was sponsored by Sea Plant Products, Hermanus, South Africa, the South African Network for Coastal and Oceanographic Research, the National Research Foundation and Rhodes University Joint Research Committee. The first author was supported by a post-doctoral scholarship from the Claude Leon Harris Foundation.

References

Akiyama, D.M., Coelho, S.R., Lawrence, A.L., Robinson, E.H., 1989. Apparent digestibility of feedstuffs by the marine shrimp *Penaeus vannamei* Boone. Nippon Suisan Gakkaishi 55, 91-98.

Aksnes, A., Hjertnes, T., Opstvedt, J., 1996. Comparison of two assay methods for determination of nutrient and energy digestibility in fish. Aquaculture 140, 343-359.

Allan, G.L., Rowland, S.J., Parkinson, S., Stone, D.A.J., Jantrarotai, W., 1999. Nutrient digestibility for juvenile silver perch *Bidyanus bidyanus*: development of methods. Aquaculture 170, 131-145.

Anderson, J.S., Lall, S.P., Anderson, D.M., Chandrasoma, J., 1992. Apparent and true availability of amino acids from common feed ingredients for Atlantic salmon (*Salmo salar*) reared in sea water. Aquaculture 108, 111-124.

Atkinson, J.L., Hilton, J.W., Slinger, S.J., 1984. Evaluation of acid-insoluble ash as an indicator of feed digestibility in rainbow trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. 41, 1384-1386.

Bjorndal, K.A., 1991. Diet mixing: nonadditive interactions of diet items in an omnivorous freshwater turtle. Ecology 72, 1234-1241.

Brown, P.B., Robinson, E.H., Clark, A.E., Lawrence, A.L., 1989. Apparent digestible energy coefficients and associative effects in practical diets for red swamp crayfish. J. World Aquacult. Soc. 20, 122-126.

Cho, C.Y., Slinger, S.J., Bayley, H.S., 1982. Bioenergetics of salmonid fishes: energy intake, expenditure and productivity. Comp. Biochem. Physiol. 73B, 25-41.

Erasmus, J.H., 1996. The role of enteric bacteria in the abalone, *Haliotis midae*. M.Sc thesis, University of Cape Town, South Africa, 83 pp.

Erasmus, J.H., Cook, P.A., Coyne, V.E., 1997. The role of bacteria in the digestion of seaweed by the abalone *Haliotis midae*. Aquaculture 155, 377-386.

Fleming, A.E., 1995. Digestive efficiency of the Australian abalone *Haliotis rubra* in relation to growth and feed preference. Aquaculture 134, 279-293.

Fleming, A.E., Van Barneveld, R.J., Hone, P.W., Vandepeer, M.E., Kruck, J.A., 1998. Complimentary additivity of the digestibility coefficients of feed ingredients fed to juvenile greenlip abalone (*Haliotis laevigata*). J. Shellfish Res. 17, 641-647.

Forster, I., 1999. A note on the method of calculating digestibility coefficients of nutrients provided by single ingredients to feeds of aquatic animals. Aquacult. Nutr. 5, 143-145.

Harris, J.O., Burke, M.C., Maguire, G.B., 1998a. Characterization of the digestive tract of greenlip abalone, *Haliotis laevigata* Donovan. I. Morphology and histology. J. Shellfish Res. 17, 979-988.

Harris, J.O., Burke, M.C., Maguire, G.B., 1998b. Characterization of the digestive tract of greenlip abalone, *Haliotis laevigata* Donovan. II. Microenvironment and bactterial flora. J. Shellfish. Res 17, 989-994.

Lupatsch, I., Kissel, G. Wm., Sklan, D., Pfeffer, E., 1997. Apparent digestibility coefficients of feed ingredients and their predictability in compound diets for gilthead seabream, *Sparus aurata* L. Aquacult. Nutr. 3, 81-89.

Maynard, L.A., Loosli, J.K., 1969. Animal Nutrition, 6th edn. McGraw-Hill, New York, NY, 613 pp.

Mould, F.L., 1988. Asociative effects of feed. In: Ørskov, E.R. (Ed.), Feed Science, World Animal Science B4, Elsevier Science Publishers B.V., Amsterdam, pp. 177-202.

National Research Council (NRC), 1993. Nutrient Requirements of Fish, National Academy Press, Washington DC, 114 pp.

Pond, W.G., Church, D.C., Pond, K.R., 1995. Basic Animal Nutrition and Feeding, 4th edn., John Wiley, New York, 615 pp.

Reigh, R.C., Braden, S.L., Craig, R.J., 1990. Apparent digestibility coefficients for common feedstuffs in formulated diets for red swamp crayfish, *Procambarus clarkii*. Aquaculture 84, 321-334.

Sales, J., 2000. Study of faeces collection methods to define optimum abalone diets. Fish Farmer International, May/June 2000, Vol. 14, No. 3, pp. 8-9.

Sales, J., Britz, P.J., 2001. Evaluation of different markers to determine apparent nutrient digestibility coefficients of feed ingredients for South African abalone (*Haliotis midae* L.). Aquaculture 202, 113-129.

Sibbald, I.R., Barrette, J.P., Price, K., 1980. Predicting true metabolizable energy, gross energy, carbohydrate, and proximate analysis values by assuming additivity. Poultr. Sci. 59, 805-807.

Smith, R.R., Peterson, M.C., Allred, A.C., 1980. Effect of leaching on apparent digestion coefficients of feedstuffs for salmonids. Prog. Fish-Cult. 42, 195-199.

Snedecor, G.W., Cochran W.G., 1991. Statistical Methods (8th edition). Iowa State University Press, Ames, USA, 503 pp.

Solórzano, L., 1969. Determination of ammonia in natural water by the phenolhypochlorite method. Limnol. Oceanogr. 14, 799-801.

Sugiura, S.D., Dong, F.M., Rathbone, C.K., Hardy, R.W., 1998. Apparent protein digestibility and mineral availabilities in various feed ingredients for salmonid feeds. Aquaculture 159, 177-202.

Watanabe, T., Takeuchi., T., Satoh, S., Kiron, V., 1996. Digestible energy: methodological influences and a mode of calculation. Fish. Sci. 62, 288-292.

Wilson, R.P., Poe, W.E., 1985. Apparent digestible protein and energy coefficients of common feed ingredients for channel catfish. Prog. Fish-Cult. 47, 154-158.

Wilson, R.P., Robinson, E.H., Poe, W.E., 1981. Apparent and true availability of amino acids from common feed ingredients for channel catfish. J. Nutr. 111, 923-929.

CHAPTER SIX

Influence of ingredient particle size and inclusion level of pre-gelatinised maize starch on apparent digestibility coefficients of diets in South African abalone (*Haliotis midae* L.)

Sales, J., Britz, P.J., 2001. Influence of ingredient particle size and dietary inclusion level of pre-gelatinised maize starch on apparent nutrient digestibility of diets in South African abalone (*Haliotis midae* L.). Aquaculture (Revised sent, 17/09/2001).

Abstract

The influence of ingredient particle size on dry matter leaching and apparent digestibility coefficients in *Haliotis midae* was evaluated by including soybean meal sieved to three different particle sizes (< 150, 150-450 and 450-1000 µm) in diets. No differences ($P > 0.05$) were found in dry matter leaching and apparent digestibility of dry matter, organic matter, protein and fat between ingredient particle sizes of < 150 and 150-450 µm. However, an ingredient particle size of 450-1000 µm increased ($P < 0.05$) dry matter leaching and decreased ($P < 0.05$) apparent digestibility. In a second experiment pre-gelatinised maize starch was included in experimental diets at 20, 30, 40 and 50 % replacing α-cellulose fibre. No influences ($P > 0.05$) on apparent digestibility of protein, fat, fiber and starch were observed among diets; however, apparent dry matter, organic matter, energy and nitrogen-free extract digestibility increased linearly ($P < 0.05$) as inclusion level of dietary pre-gelatinised maize starch increased. A relatively high apparent digestibility (> 60 %) of fibre was observed, indicating a need for further studies to quantify the effect of the source and type of fibre on its digestibility.

1. Introduction

The efficiency of a feed manufacturing process and the biological efficiency of a compound diet is dependent upon the initial grinding, and consequent particle size, of the basic raw feed materials used (Tacon, 1990). For the nutritionist, grinding (1) facilitates the destruction of heat labile anti-nutritional factors often present in raw materials and (2) improves nutrient digestibility by increasing the surface area of the feed particles. To the animal-feed formulator, grinding improves (1) feed acceptability to the animal, (2) pelletability by extending die life, penetration of steam within the feed particles, and increased horsepower efficiency, (3) the mixing properties of individual feed ingredients, and (4) also increases the bulk density of the feedstuff (Tacon and Jackson, 1985). Furthermore, for aquatic feeds that have to remain in water for prolonged periods, feed ingredients are finely ground to achieve satisfactory water stability of pellets (Tan and Dominy, 1997). However, particle size reduction is the most time consuming feed processing step and can account for up to 60 % of the feed production cost (Sorenson and Phillips, 1992).

In most teleost fish their ability to digest and metabolise carbohydrates is limited; therefore, highly digestible carbohydrates, such as cooked (gelatinised) starches, are used in formulated diets to increase energy availability (Kim and Kaushik, 1992). Furthermore, gelatinised starches are also good binders in compounded aquatic diets. However, protein and starch digestibility tends to be depressed as dietary carbohydrate inclusion levels increase (Steffens, 1989; NRC, 1993). Pre-gelatinised starch levels have been routinely varied in abalone nutritional experiments with *Haliotis midae* in order to formulate diets with specific protein and energy levels (Britz and Hecht, 1997; Shipton and Britz, 2001). However, it is not known whether the starch level affects digestibility. As part of a wider study to established a reliable and replicable digestibility technique for *H. midae* (Sales, 2000; Sales and Britz, 2001) there is a need to determine the influence of factors affecting digestibility in order to formulate diets and design controlled experiments in which certain measurements are valid.

The aim of the first experiment in this study was to evaluate the influence of ingredient particle size on apparent digestibility coefficients (dry matter, organic matter, protein, fat, phosphorus)

digestibility in *H. midae*. A second experiment was conducted to evaluate the effects of substitution of α-cellulose with graded levels of pre-gelatinised maize starch on apparent digestibility of diets.

2. Material and methods

2.1. Influence of ingredient particle size on apparent nutrient digestibility

Apparent digestibility coefficients (dry matter, organic matter, protein, fat and phosphorus) of experimental diets containing soybean meal of three different particle-size categories was evaluated (Table 1). After initial size reduction using an ultra-centrifugal mill (ZM 1, Retsch GmbH & Co KG, Haan, Germany) the soybean meal was sieved to the desired particle size using graded geological sieves and included in diets.

2.2. Influence of dietary pre-gelatinised maize starch inclusion on apparent digestibility

Four diets were formulated in which only the inclusion levels of pre-gelatinised maize starch was varied by replacement with α-cellulose (Table 2). Apparent digestibility was determined for dry matter, organic matter, protein, energy, fat, starch and nitrogen-free extract.

2.3. General procedures

2.3.1. Experimental animals, experimental system, feeding protocol and faeces collection

Juvenile abalone *Haliotis midae* (56.49 ± 0.12 mm shell length; 35.60 ± 0.24 g live weight, mean ± S.E.) were obtained from a commercial South African abalone farm, and conditioned to a recirculating system for 8 months.

The experimental system, feeding protocol and collection of faeces were as described by Sales and Britz (2001). Filtered (10-µm) seawater was pumped through an indoor recirculating system consisting of a series of 15 glass holding tanks (40-l capacity), a settlement tank of 300-l capacity and primary and secondary biological filters. Room air temperature (15 °C), flow rate (2 l/min), aeration were according to Sales and Britz (2001). The lights were only turned on during cleaning, feeding and sampling (0800 h to 0900 h and 1500 h to 1600 h). Water temperature (17.73 ± 0.37 °C), pH (7.62 ± 0.02), dissolved oxygen (7.70 ± 0.12 mg/l), total

ammonia (0.17 ± 0.06 mg/l) and salinity (37 ± 0.65 ‰) were measured weekly following methods described in Sales and Britz (2001).

Table 1

Ingredient and determined nutrient composition of diets in which particle size of soybean meal was varied (dry matter basis)

Ingredients (%)	Particle size		
	< 150 µm	150-450 µm	450-1000 µm
Soybean meal[a]	40	40	40
Pre-gelatinised maize starch[b]	40	40	40
Fish oil[c]	2.0	2.0	2.0
Carboxymethyl-cellulose[d]	1.0	1.0	1.0
a-Cellulose[d]	13.5	13.5	13.5
Vitamin/mineral mixture[e,f]	3.0	3.0	3.0
Mono calcium phosphate[g]	0.5	0.5	0.5
Diet analysis			
Crude protein (%)	18.87	19.16	19.91
Gross energy (MJ/kg)	17.46	17.50	17.48
Crude fat (%)	2.34	1.92	1.87
Phosphorus (%)	0.45	0.43	0.43
Soybean meal analysis			
Crude protein (%)	46.72	47.12	47.25
Gross energy (MJ/kg)	19.19	18.82	18.92
Crude fat (%)	3.42	1.56	1.33
Total phosphorus (%)	0.76	0.72	0.72

[a] Pre-pressed, solvent extracted, dehulled, Western Province Corp. (Roggebaai, South Africa).
[b] Source proprietary knowledge of Sea Plant Products (Hermanus, South Africa).
[c] Marinol-R, Marine Oil Refiners (Cape Town, South Africa).
[d] Sigma Chemicals (St. Louis, MO, USA).
[e] Per kg of feed: retinol, 12000 IU; cholecalciferol, 1800 IU; α-tocopherol, 150 mg; menadione, 5 mg; thiamin, 20 mg; riboflavin, 25 mg; pyridoxine, 20 mg; Vit. B_{12}, 0.04 mg; niacin, 150 mg; Ca-pantothenate, 50 mg; folic acid, 5 mg; biotin, 0.8 mg; ascorbic acid, 750 mg; inositol, 200; manganese, 150 mg; iron, 25 mg; zinc, 25 mg; copper, 70 mg; cobalt, 2 mg; iodine, 1 mg.
[f] Epol (Pretoria, South Africa).
[g] Kynoch Feeds (Randburg, South Africa).

Table 2

Ingredient and determined nutrient composition of diets with different inclusion levels of pre-gelatinised maize starch and α-cellulose (dry matter basis)

Ingredient	Inclusion level (%)			
Fish meal[a]	20	20	20	20
Soybean meal[b]	10	10	10	10
Cottonseed meal[c]	5.0	5.0	5.0	5.0
Sunflower meal[c]	5.0	5.0	5.0	5.0
Pre-gelatinised maize starch[b]	20	30	40	50
Fish oil[b]	2.0	2.0	2.0	2.0
a-cellulose[b]	35	25	15	5
Vitamin/mineral mixture[b]	2.5	2.5	2.5	2.5
Mono calcium phosphate[b]	0.5	0.5	0.5	0.5
Analysis				
Crude protein (%)	23.52	23.15	23.21	23.46
Gross energy (MJ/kg)	18.00	17.96	17.90	17.67
Crude fat (%)	3.77	3.71	3.40	2.99
Crude fibre (%)	29.54	21.75	13.66	6.52
Total starch (%)	22.29	31.19	44.24	45.79
Nitrogen-free extract (%)[d]	36.74	45.08	53.48	60.62

[a] Low-temperature dried fish meal (Danish 999 LT), Esbjerg Fiskeindustri a.m.b.a. (Esbjerg, Danmark).
[b] As in Table 1.
[c] Pre-pressed, solvent extracted, Western Province Corp. (Roggebaai, South Africa).
[d] By difference 100 - (ash + crude protein + crude fat + crude fibre).

Thirty seven abalone were placed in a grey PVC holding container with an oyster net floor with 8 x 6 mm mesh aperture. This was placed into a second plastic faecal collection container with a net floor of 200-µm mesh. These two containers were then placed into the glass holding tanks (Sales, 2000). Collection of faeces was performed by removing the 200-µm mesh tank as described by Sales and Britz (2001). Frozen faeces (–20 °C) were stored until the end of the collection period whereafter they were freeze-dried and analysed.

2.3.2. Diet preparation

All dry ingredients were mixed in a commercial food mixer for 30 min, whereafter oil was gradually added, while mixing constantly. Eighty five ml of water per 100 g of feed was slowly blended into the mix before processing through a cold-extruder (1.5 x 10 mm die size). After drying in a convection oven at 35 °C for 48 h the dry product was cut into 1 x 1 cm pieces and stored at 4 °C until used. Dry matter loss over 16 h for diets in the experimental tanks was determined by calculating weight loss of approximately 2 g of feed (dried at 55 °C for 48 h).

2.3.3. Analytical procedures

Ash and acid-insoluble ash (AIA) content were determined according to Atkinson et al. (1984), crude protein (*N* x 6.25) by using the micro-Kjeldahl technique, crude fat after extraction with petroleum ether (boiling point 40-60 °C) by the Soxhlet method, fibre according to the Weende method, gross energy content by direct combustion in an adiabatic calorimeter, total phosphorus after ashing the samples and using the vanado-molybdate method (AOAC, 1980), and starch after hydrolysis by heat stable alpha-amylase (Sigma Chemicals, St. Louis, MO, USA) and amylo-glucosidase (Sigma Chemicals, St. Louis, MO, USA) followed by spectrophotometric determination of glucose (Hall, 2000). Starch gelatinisation of feed was determined by the CSIR (Pretoria, South Africa) by means of an enzymatic technique (Chiang and Johnson, 1976). Nitrogen-free extract was determined by difference.

2.3.4. Digestibility coefficient calculations and statistical analyses

Apparent digestibility coefficients (ADCs) in experimental diets were calculated according to the formula from Maynard and Loosli (1969):

ADC of dry matter of diet (%) = 100 × [1 – (% marker in diet/% marker in faeces)]

ADCs of nutrients and energy of diet (%) = 100 × [1- (% marker in diet/% marker in faeces) × (nutrient or energy concentration in faeces/nutrient or energy concentration in diet)]

Results were analysed using one-way analysis of variance. Differences between means ($P < 0.05$) were evaluated by the Tukey's HSD test (Snedecor and Cochran, 1991).

3. Results

3.1. Influence of ingredient particle size on apparent digestibility coefficients

In all parameters measured, with the exception of apparent availability of phosphorus, no difference ($P > 0.05$) was found between a particle size of smaller than 150 and 150-450 µm (Table 3). However, for the particle size category of 450-1000 µm ADCs decreased ($P < 0.05$), and dry matter leaching increased ($P < 0.05$) in comparison to particle-size categories of < 150 and 150-450 µm, respectively.

Table 3

Starch gelatinisation, dry matter leaching and apparent digestibility coefficients of diets containing soybean meal of different particle size (mean ± S.E.; $n = 3$)

Diet component	< 150 µm	150-450 µm	450-1000 µm
Starch gelatinisation (%)	96	93	91
Dry matter leaching (%)	$2.65^{b} \pm 0.043$	$4.11^{b} \pm 0.507$	$6.59^{a} \pm 0.391$
Apparent digestibility (%)			
Dry matter	$90.25^{b} \pm 1.43$	$89.26^{b} \pm 0.25$	$83.52^{a} \pm 0.40$
Organic matter	$96.60^{b} \pm 0.39$	$95.94^{b} \pm 0.10$	$93.20^{a} \pm 0.16$
Protein	$97.22^{b} \pm 0.25$	$97.00^{b} \pm 0.11$	$95.34^{a} \pm 0.19$
Fat	$98.47^{b} \pm 0.20$	$97.18^{ab} \pm 0.93$	$95.65^{a} \pm 0.50$
Phosphorus	63.88 ± 5.99	67.94 ± 2.62	57.79 ± 4.64

Means in the same row with different superscripts (a, b) are statistically different (P < 0.05).

All attempts to perform gross energy determinations on excreta collected in this experiment by direct combustion in an adiabatic calorimeter failed.

3.2. Influence of different dietary inclusion levels of pre-gelatinised maize starch on apparent digestibility coefficients

No difference ($P > 0.05$) in dry matter leaching or apparent digestibility of dry matter, energy, protein, fat, fibre and starch was found as the inclusion level of pre-gelatinised maize starch increased in diets (Table 4). However, although Tukey's-test was too robust to detect specific

differences among diets for apparent digestibility of dry matter, ANOVA analysis of this parameter revealed a *P*-value of less than 0.05. As in the first experiment, no gross energy data could be obtained for excreta produced from the diet containing 50 % pre-gelatinised maize starch and 5 % α-cellulose.

Table 4

Dry matter leaching and apparent digestibility coefficients of diets with different inclusion levels of pre-gelatinised maize starch and α-cellulose (mean ± S.E., $n = 3$)

	Inclusion level (%)			
Pre-gelatinised maize starch	20	30	40	50
α-cellulose	35	25	15	5
Dry matter leaching (%)	2.00 ± 0.66	2.47 ± 0.29	2.85 ± 0.42	1.80 ± 0.11
Apparent digestibility				
Dry matter (%)	66.51 ± 0.24	67.55 ± 2.46	74.96 ± 4.40	77.26 ± 0.97
Organic matter (%)	78.12^{a} ± 1.05	79.94^{a} ± 1.65	85.48^{ab} ± 2.75	89.25^{b} ± 0.59
Energy (MJ/kg)	84.46 ± 1.45	86.79 ± 1.57	90.88 ± 1.93	-[1]
Protein (%)	92.58 ± 0.39	90.72 ± 0.86	91.46 ± 1.76	91.36 ± 0.45
Fat (%)	96.89 ± 0.09	95.59 ± 0.35	95.47 ± 0.74	96.32 ± 0.43
Fibre (%)	63.77 ± 2.35	61.00 ± 3.77	65.29 ± 6.97	65.60 ± 3.73
Starch (%)	96.95 ± 1.78	98.31 ± 0.75	99.08 ± 0.70	98.24 ± 0.77
Nitrogen-free extract (%)	78.47^{a} ± 0.64	82.26^{ab} ± 1.27	87.41^{bc} ± 2.24	90.63^{c} ± 0.35

Means in the same row with different superscripts (a, b, c) are statistically different ($P < 0.05$).
[1] Unable to obtain gross energy values on excreta.

Regression analysis of nutrient category ADCs against dietary inclusion level of pre-gelatinised maize starch revealed that apparent digestibility of dry matter, organic matter, energy and nitrogen-free extract increased ($P < 0.05$) as the inclusion level of pre-gelatinised maize starch increased, while apparent digestibility of protein, fat, fibre and starch were not ($P > 0.05$) influenced (Table 5).

Table 5

Linear regression analysis (y = a + bx) of apparent digestibility coefficients (y) against inclusion level of pre-gelatinised maize starch (x)

Diet component	a	b	R^2	*P*	*n*
Dry matter	57.69	0.40	0.5682	0.0046	12
Organic matter	69.57	0.40	0.7452	0.0003	12
Energy	77.74	0.32	0.5464	0.0229	9
Protein	92.55	-0.03	0.0420	0.5229	12
Fat	96.71	-0.02	0.0543	0.4660	12
Fibre	60.50	0.10	0.0268	0.6110	12
Starch	96.51	0.05	0.0909	0.3410	12
Nitrogen-free extract	70.13	0.42	0.8527	0.0000	12

4. Discussion

4.1. Influence of ingredient particle size on apparent digestibility coefficients

This study showed that reducing ingredient particle size to 150-450 µm resulted in a significant reduction in dry matter leaching and an increase in ADC values in comparison to a particle size above 450 µm. However, sieving diet ingredients to a particle size of smaller than 150 µm in compound abalone diets did not yield an additional benefit in terms of dry matter leaching or ADCs compared to an ingredient particle size of 150-450 µm. Factors which could account for the reduction in leaching included a reduction in the amount of air space between particles, an increase in the contact surfaces of the ingredients to allow for greater bonding, a decrease in the the number of "break" points in the feed pellet, and an increase in potential starch gelatinisation (Obaldo et al., 1998). Improved apparent nutrient digestibility can be ascribed to more rapid digestion of the smaller particles due to their much larger surface area (Smith, 1988). However, in contrast to present results, it was found in shrimp that an extremely small particle size (< 100 µm) increased nutrient leaching and decreased nutrient digestibility in comparison to an ingredient particle size of 200 µm (Palaniswamy and Ali, 1991; Obaldo et al., 1998). This was

ascribed to the harder pellet resulting from fine particles, and accelerated leaching loss of nutrients (Palaniswamy and Ali, 1991).

The failure to obtain gross energy values on excreta produced from some diets could be attribute to a high ash content (> 56 %) of samples. Ash consists of inorganic components that are not combustible (Cho et al., 1982).

4.2. Influence of inclusion levels of pre-gelatinised maize starch on apparent digestibility coefficients

The high apparent digestibility of starch observed (97-99 %) could be attributed to the use of readily digestible pre-gelatinised starch in the experimental diets. Values of 96-98 % for apparent starch digestibility of gelatinised wheat starch have been reported for rainbow trout (Kaushik and Médale, 1994; Burel et al., 2000). In contrast to studies in fish where increases in crude or cooked/gelatinised dietary starch inclusion resulted in an increase in starch digestibility levels (Inaba et al., 1963; Singh and Nose, 1967; Bergot and Brèque, 1983; Hemre et al., 1989; Kim and Kaushik, 1992; Brauge et al., 1994; Aksness, 1995; Grisdale-Helland and Helland, 1998), no differences ($P < 0.05$) were observed in the present study. A similar trend was observed for the digestibility of gelatinised wheat starch in European seabass (Dias et al., 1998). However, in the present study, dietary starch levels were manipulated only by the use of pre-gelatinised starch and α-cellulose, while dietary protein sources were kept constant. Abalone are herbivorous with high levels of carbohydrase activity (Vonk and Western, 1984), and this probably accounts for the consistantly high level of starch digestibility observed in the present study.

Similar to the present study, the level of dietary gelatinised wheat starch did not influence apparent digestibility of energy, protein and fat in rainbow trout (Brauge et al., 1994). A trend of increasing apparent digestibility of energy with increasing dietary starch level was observed in the present study, probably due to an increase in total energy contribution from pre-gelatinised maize starch. Also, apparent digestibility of nitrogen-free extract increased ($P < 0.05$) with increasing levels of pre-gelatinised maize starch, clearly demonstrating the increased availability of soluble carbohydrate. Although feeding high concentrations of digestible carbohydrate has

been found to increase liver size and glycogen in several fish species (NRC, 1993), this has not yet been evaluated in *Haliotis* spp., which are known to efficiently digest reserve carbohydrate and store reserve energy as glycogen in muscle tissue and as galactogen in the gonads (Webber, 1970). The use of gelatinised starch in aquatic feeds is restricted by its relatively high cost (Vens-Cappell, 1984), thus the utilization of other starch sources in *H. midae* needs investigation.

Increased levels of α-cellulose in diets had no influence ($P > 0.05$) on apparent digestibility of protein, fat, fibre or starch in diets evaluated in the present study. The same trend was found for protein, fat and fibre in carp (Takeuchi et al., 1979; Schwarz and Kirchgessner, 1982) and shrimp (Borrer and Lawrence, 1989; Cutacutan, 1991). However, Schwarz and Kirchgessner (1982) found that increasing dietary cellulose levels led to depression of soluble carbohydrate digestibility in carp. A decline in apparent dry matter digestibility with increased levels of dietary α-cellulose observed in the present study also has been observed in rainbow trout (Hilton et al., 1983), shrimp (Borrer and Lawrence, 1989; Cutacutan, 1991) and lobster (Koshio et al., 1992). Concomitant with this trend was the production of a larger quantity of faeces with an increase in dietary fibre. Cho et al. (1982) postulated that faecal leaching losses from poorly digested feed ingredients which contained a substantial level of fibre would be proportionally higher due to a larger quantity of faeces produced, and would result in erroneously high digestibility coefficients. This effect was not evident in the present study because apparent protein digestibility did not differ ($P > 0.05$) with an increasing level of dietary fibre. A relatively high apparent digestibility for crude fibre (> 60 %) was observed. However, no distinction was made between the type and source of fibre, and this needs to be addressed in future studies.

This study presents evidence that different levels of both pre-gelatinised maize starch and α-cellulose do not influence the apparent digestibility of protein, fat, fibre or starch in *H. midae*. This has practical value for further digestibility studies with this species, as different levels of pre-gelatinised maize starch as binder and α-cellulose as filler would not have an influence on digestibility of the above mentioned nutrients. However, as *Haliotis* spp. are known to secrete enzymes capable of breaking down complex structural carbohydrates (Vonk and Western, 1984), and an increase in cellulolytic activity was observed in stomach fluid of abalone when

cellulose was present in the diet (Monje and Viana, 1998), further studies should concentrate on the possibility of digestion of α-cellulose in *H. midae*.

Acknowledgements

This study was sponsored by Sea Plant Products, Hermanus, South Africa, the South African Network for Coastal and Oceanographic Research, the National Research Foundation and Rhodes University Joint Research Committee. The first author was supported by a post-doctoral scholarship from the Claude Leon Harris Foundation.

References

Aksness, A., 1995. Growth, feed efficiency and slaughter quality of salmon, *Salmo salar* L., given feeds with different ratios of carbohydrate and protein. Aquacult. Nutr. 1, 241-248.

AOAC, 1980. Official Methods of Analysis of the Association of Official Analytical Chemists, 13th edn. AOAC, Arlington, VA, USA.

Atkinson, J.L., Hilton, J.W., Slinger, S.J., 1984. Evaluation of acid-insoluble ash as an indicator of feed digestibility in rainbow trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. 41, 1384-1386.

Bergot, F., Brèque, J., 1983. Digestibility of starch by rainbow trout: effects of physical state of starch and of the intake level. Aquaculture 34, 203-212.

Britz, P.J., Hecht, T., 1997. Effect of dietary protein and energy level on growth and body composition of South African abalone, *Haliotis midae*. Aquaculture 156, 195-210.

Borrer, S., Lawrence, A., 1989. Effect of lipids and cellulose on the digestibility of panaeid shrimp diets. J. World Aquacult. Soc. 20, 18A (abstract).

Brauge, C., Medale, F. , Corraze, G., 1994. Effect of dietary carbohydrate levels on growth, body composition and glycaemia in rainbow trout, *Oncorhynchus mykiss*, reared in seawater. Aquaculture 123, 109-120.

Burel, C., Boujard, T., Tulli, F., Kaushik, S.J., 2000. Digestibility of extruded peas, extruded lupin, and rapeseed meal in rainbow trout (*Oncorhynchus mykiss*) and turbot (*Psetta maxima*). Aquaculture 188, 285-298.

Chiang, B.-Y., Johnson, J.A., 1976. Measurement of total and gelatinized starch by glucoamylase and s-toluidine reagent. Cereal Chem. 54, 429-435.

Cho, C.Y., Slinger, S.J., Bayley, H.S., 1982. Bioenergetics of salmonid fishes: energy intake, expenditure and productivity. Comp. Biochem. Physiol. 73B, 25-41.

Cutacutan, M.R., 1991. Apparent digestibility of diets with various carbohydrate levels and the growth response of *Penaeus monodon*. Aquaculture 95, 89-96.

Dias, J., Alvarez, M.J., Diez, A., Arzel, J., Corraze, G., Bautista, J.M., Kaushik, S.J., 1998. Regulation of hepatic lipogenesis by dietary protein/energy in juvenile European seabass (*Dicentrarchus labrax*). Aquaculture 161, 169-186.

Grisdale-Helland, B., Helland, S.J., 1998. Macronutrient utilization by Atlantic halibut (*Hippoglossus hippoglossus*): diet digestibility and growth of 1 kg fish. Aquaculture 166, 57-65.

Hall, M.B., 2000. Neutral detergent-soluble carbohydrates. Nutritional Relevance and Analysis. A laboratory manual. Florida Cooperative Extension Service, Bulletin 330, University of Florida, Gainsville, FL, USA, 77 pp.

Hemre, G-I., Lie, ? , Lied, E., Lambertsen, G., 1989. Starch as an energy source in feed for cod (*Gadus morhua*): digestibility and retention. Aquaculture 80, 261-270.

Hilton, J.W., Atkinson, J.L., Slinger, S.J., 1983. Effect of increased dietary fiber on the growth of rainbow trout (*Salmo gardneri*). Can. J. Fish. Aquat. Sci. 40, 81-85.

Inaba, D.C., Ogino, C., Takamatsu, T.U., Kurokawa, K., 1963. Digestibility of dietary components in fishes. II. Digestibility of dietary protein and starch in rainbow trout. Bull. Jpn. Soc. Fish. 29, 242-247.

Kaushik, S.J., Médale, F., 1994. Energy requirements, utilization and dietary supply to salmonids. Aquaculture 124, 81-97.

Kim, J.D., Kaushik, S.J., 1992. Contribution of digestible energy from carbohydrates and estimation of protein/energy requirements for growth of rainbow trout (*Oncorhynchus mykiss*). Aquaculture 106, 161-169.

Koshio, S., Castell, J.D., O'Dor, R.K., 1992. The effect of different dietary energy levels in crab-protein-based diets on digestibility, oxygen consumption, and ammonia excretion of bilaterally eyestalk-ablated and intact juvenile lobsters, *Homarus americanus*. Aquaculture 108, 285-297.

Maynard, L.A., Loosli, J.K., 1969. Animal Nutrition, 6th edn. McGraw-Hill, New York, NY, 613 pp.

Monje, H., Viana, M.T., 1998. Effect of cellulose on the growth and cellulolytic activity of abalone *Haliotis fulgens* when used as an ingredient in formulated artificial diets. J. Shellfish Res. 17, 667-671.

National Research Council (NRC), 1993. Nutrient Requirements of Fish, National Academy Press, Washington, 114 pp.

Obaldo, L.G., Dominy, W.G., Terpstra, J.H., Cody, J.J., Behnke, K.C., 1998. The impact of ingredient particle size on shrimp feed. J. Appl. Aquacult. 8, 55-66.

Palaniswamy, R., Ali, S.A., 1991. The impact of particle size of ingredients in compound diets on water stability and performance in *Penaeus indicus* (H. Milne Edwards). J. Aquacult. Tropics 6, 119-127.

Sales, J., 2000. Study of faeces collection methods to define optimum abalone diets. Fish Farmer International, May/June 2000, Vol. 14, No. 3, pp. 8-9.

Sales, J., Britz, P.J., 2001. Evaluation of different markers to determine apparent nutrient digestibility coefficients of feed ingredients for South African abalone (*Haliotis midae* L.). Aquaculture 202, 113-129.

Schwarz, F.J., Kirchgessner, M., 1982. Zur Bestimmung der Nährstoffverdaulichkeit beim Karpfen (*Cyprinus carpio* L.). 3. Mitt. Zum Einfluss unterschiedlicher Protein-, Fett- und Cellulosegehalte. Bayer. Landwirtsch. Jahrb. 59, 434-440.

Shipton, T.A., Britz, P.J., 2001. Partial and total replacement of fishmeal with selected plant protein sources in diets for the South African abalone, *H. midae*. J. Shellfish Res. (In press).

Singh, R.P., Nose, T., 1967. Digestibility of carbohydrates in young rainbow trout. Bull. Freshwater Fish. Lab. Tokyo 17, 21-25.

Smith, L.S., 1988. Digestive functions in teleost fishes. In: Halver, J.E. (Ed.), Fish Nutrition, 2nd edn, Academic Press, New York, pp. 332-421.

Snedecor, G.W., Cochran W.G., 1991. Statistical Methods (8th edition). Iowa State University Press, Ames, USA, 503 pp.

Sorenson J., Phillips, D., 1992. Fine grinding for shrimp feed. Feed International December, 28-32.

Steffens, W., 1989. Principles of Fish Nutrition. Ellis Horwood Limited Publishers, Chichester, UK, 384 pp.

Tacon, A.G.J., 1990. Standard Methods for the Nutrition and Feeding of Farmed Fish and Shrimp. Argent Laboratories Press, Redmond, Washington, USA, 208 pp.

Tacon, A.G.J., Jackson, A.J., 1985. Utilization of conventional and unconventional protein sources in practical fish feeds. In: Cowey, C.B., Mackie, A.M., Bell, J.G. (Eds.), Nutrition and Feeding in Fish, Academic Press, New York, pp. 119-146.

Takeuchi, T., Arai, S., Watanabe, T., 1979. Optimum ratio of energy to protein for carp. Bull. Jpn. Soc. Sci. Fish. 45, 983-987.

Tan, R.K.H., Dominy, W.G., 1997. Commercial pelleting of crustacean feeds. In: D'Abramo, L.R., Conklin, D.E., Akiyama, D.M. (Eds.), Crustacean Nutrition, Advances in World Aquaculture, Vol. 6, World Aquaculture Society, Louisiana, USA, pp. 520-549.

Vens-Cappell, B., 1984. The effects of extrusion and pelleting of feed for trout on the digestibility of protein, amino acids and energy and on feed conversion. Aquacult. Eng. 3, 71-89.

Vonk, H.J., Western, J.R.H., 1984. Comparative Biochemistry and Physiology of Enzymatic Digestion, Academic Press, London, 501 pp.

Webber, H.H., 1970. Changes in metabolic composition during the reproductive cycle of the abalone *Haliotis cracheroidii* (Gastropoda: Prosobranchiata). Physiol. Zool. 43, 213-231.

CHAPTER SEVEN

Apparent and true availability of amino acids from common feed ingredients for South African abalone *(Haliotis midae* L.)

Sales, J., Britz, P.J., 2001. Apparent and true availability of amino acids from common feed ingredients for South African abalone (*Haliotis midae* L.). Aquaculture Nutrition (Submitted for publication, 20/10/2001).

Abstract

Apparent (APD) and true (TPD) protein digestibility and apparent (AAAA) and true (TAAA) amino acid availability of fish meal and plant protein ingredients utilised in the South African animal feed industry (corn gluten meal, soybean meal, cottonseed meal, sunflower meal, canola meal, peanut meal, lupins and faba beans), incorporated at 45 % into single protein diets, were determined with juvenile South African abalone (*Haliotis midae*). Soybean meal (96.34 %) and lupins (96.51 %) presented the highest APD values, with corn gluten meal (76.08 %) the lowest value, while values for sunflower meal (92.21 %), canola meal (93.94 %), and faba beans (93.17 %) were above 90 %. APD values for cottonseed meal (86.28 %) and peanut meal (86.73 %) were comparable to that of fish meal (82.94 %). A correlation coefficient (r) of 0.99 was found between ADP and mean AAAA. Furthermore, amino acid availabilities were relatively consistent within various feed ingredients. The mean amino acid secretion from a protein-free diet was 19.08 mg/100 dry diet, resulting in a mean increase of 1.88 % units over all feed ingredients when comparing TAAA to AAAA. Calculated APD according to APD coefficients determined with single protein diets underestimated determined APD in several compound diets by 1.10-6.50 %, while mean AAAA was underestimated with 6.93 %.

1. Introduction

Fish meal, because of its high biological value, is well recognized as the best source of protein for most fish species (Seneriches and Chiu, 1988). Presently fish meal is the main protein source in South African abalone diets (Fleming et al., 1996). However, the increasing cost of high quality fish meal, and shortages as requirements for aquafeeds increases, poses problems for cost-effective feed formulation (Seneriches and Chiu, 1988; Hertrampf and Piedad-Pascual, 2000). Priority is given internationally to the search for alternatives to fish meal in animal feeds (Hardy and Kissel, 1997). The determination of digestibility is the first step in evaluating the potential of an ingredient for use in the diet of an aquaculture species (Allan et al., 2000).

Apparent protein digestibility (APD) of several feed ingredients was evaluated for *Haliotis midae* using both chromic oxide (Cr_2O_3) as marker and *in vitro* techniques (Shipton, 2000). However, the partitioning and preferential elimination of Cr_2O_3 in *H. midae* (Shipton, 2000) hindered the collection of adequate quantities of faeces for determination of amino acid availabilities. Evidence was presented by Sales and Britz (2001a) that acid-insoluble ash is a reliable internal marker in digestibility studies with *H. midae*, resulting in a yield of sufficient faeces for analysis of several nutrients. Apparent amino acid availability (AAAA) of barley, fish meal, semolina, and lupin kernel meal (Fleming et al., 1998), and several legumes (Vandepeer et al., 1999), was determined for Australian greenlip abalone (*H. laevigata*) using the Cr_2O_3 marker method. However, no such information is available for other abalone species.

Since very few feed ingredients are fed as the sole component of a diet, the whole diet-substitution technique of Cho et al. (1982) is normally used in determination of nutrient digestibility coefficients for feed ingredients (NRC, 1993). However, the problems with this technique in determining APD in a slow feeder like abalone was demonstrated by Sales and Britz (2001b).

The present study was conducted to evaluate apparent and true protein digestibility (TPD) and amino acid availability of all plant protein ingredients utilised in animal feeds in South Africa for *H. midae*. Furthermore, the additivity of calculated apparent digestibility coefficients was evaluated in compound diets.

2. Methods

2.1. Experimental animals, feed ingredients and diet preparation

Experimental animals were 456 juvenile abalone (*Haliotis midae*) (56.49 ± 0.12 mm shell length; 35.60 ± 0.24 g live weight, mean ± S.E.) reared on a commercial abalone farm in South Africa, and previously used in digestibility trials (Sales and Britz, 2001a; b; c).

Test ingredients (Table 1) were ground and sieved to a particle size smaller than 450 μm before incorporation as single protein sources at 45 % (dry weight basis) into test diets (Table 2). A protein-free diet was made by incorporating 45 % α-cellulose in place of the test protein in order to determine endogenous faecal amino acids as g/100 g dry diet. Food was made and dried as described by Sales and Britz (2001a; b; c). The dry product was cut into 1 x 1 cm pieces and stored at 4 °C till used. Dry matter leaching over 16 h for these diets (each in triplicate) in the experimental tanks was determined by calculating weight loss of approximately 2 g of feed (dried at 55 °C for 48 h).

2.2. Experimental system, feeding and faecal collection

An indoor recirculating system consisting of a series of 15 glass holding tanks (40-l capacity), a settlement tank of 300-l capacity and primary and secondary biological filters, as described by Sales and Britz (2001a; b), were used. Room air temperature was cooled to 15 °C, while water was heated to 18 °C. Aeration was supplied via airstones in each tank.

Water temperature (17.78 ± 0.21 ºC), pH (7.65 ± 0.01), dissolved oxygen (8.25 ± 0.10 mg/l), total ammonia (0.04 ± 0.02 mg/l) and salinity (37.50 ± 0.29 ‰) were monitored on a weekly basis, as described by Sales and Britz (2001a; b). Flow rate in the tanks was maintained at 1.98 ± 0.03 l/min and lights were only turned on during cleaning, feeding and sampling (0800-0900 and 1500-1600 h).

Table 1
Chemical composition of feed ingredients tested (dry matter basis)

Component	Fish meal[a]	Corn gluten meal[b]	Oils seeds					Legumes	
			Soybean meal[c]	Cottonseed meal[c]	Sunflower meal[c]	Canola meal[d]	Peanut meal[e]	Lupins[f] (*Lupineus albus* kief)	Faba beans[g] (*Vicia faba* ascot)
Gross energy (kJ/g)	20.77	21.91	18.71	19.16	18.60	20.11	18.43	20.59	17.57
Crude protein (%)	70.59	61.72	47.85	44.68	42.44	32.35	43.19	33.53	25.59
Crude fat (%)	9.16	2.38	1.43	3.81	2.46	9.36	3.01	11.03	1.63
Ash (%)	13.00	2.76	6.46	8.45	8.68	6.06	6.90	3.91	3.01
Amino acids (%)									
Essential									
Arginine	4.50	1.97	3.99	5.01	3.70	2.07	4.75	3.58	2.34
Histidine	1.53	1.19	1.42	1.40	1.18	0.93	1.03	0.88	0.72
Isoleucine	3.47	2.44	2.59	1.70	1.99	1.49	1.72	1.53	1.17
Leucine	5.57	9.45	4.12	3.00	2.90	2.43	2.94	2.55	1.99
Lysine	5.87	0.99	3.43	2.29	1.78	2.08	1.58	1.94	1.84
Phenylalanine	2.88	3.60	2.71	2.71	2.05	1.36	2.26	1.35	1.13
Methionine	2.27	1.11	0.52	0.62	0.73	0.44	0.36	0.18	0.15
Threonine	3.14	1.82	2.00	1.48	1.52	1.54	1.29	1.17	0.85
Valine	3.98	2.56	2.86	2.49	2.50	1.92	2.05	1.60	1.37
Non-essential									
Aspartic acid	7.64	3.72	6.36	4.41	4.14	2.75	5.63	3.53	2.89
Serine	2.78	2.61	2.38	1.73	1.58	1.41	2.02	1.39	1.02
Glutamic acid	11.39	13.49	10.50	9.79	8.84	6.70	9.46	6.85	4.52
Proline	3.44	5.79	3.17	2.24	2.18	2.53	2.40	1.62	1.35
Glycine	4.46	1.56	2.23	2.04	2.61	1.77	2.82	1.40	1.23
Alanine	4.58	4.50	2.25	1.90	1.87	1.57	1.85	1.13	1.03
Tyrosine	2.01	2.65	1.44	1.11	0.95	0.78	1.36	1.19	0.63

[a] Low-temperature dried fish meal (Danish 999 LT), Esbjerg Fiskeindustri a.m.b.a. (Esbjerg, Danmark).
[b] Western Province Corp Aqua Feeds (Malmesbury, South Africa).
[c] Pre-pressed, solvent extracted, Western Province Corp. (Roggebaai, South Africa).
[d] Cold press, Pioneer Feeds (Paarl, South Africa).
[e] Solvent extracted, Meadow Feed Mills (Paarl, South Africa).
[f] Agricol (Brackenfell, South Africa).
[g] Department of Agriculture Western Cape (Elsenburg, South Africa).

Table 2

Ingredient composition of test diets (dry matter basis)

Ingredient	%
Pre-gelatinized maize starch[a]	40.0
Fish oil[b]	2.5
α-Cellulose[c]	9.5
Vitamin/mineral mixture[d,e]	2.5
Mono calcium phosphate[f]	0.5
Test protein	45.0

[a] Source priority Sea Plant Products (Hermanus, South Africa).
[b] Marinol-R, Marine Oil Refiners (Cape Town, South Africa).
[c] Sigma Chemicals (St. Louis, MO, USA).
[d] Per kg of feed: retinol, 12000 IU; cholecalciferol, 1800 IU; α-tocopherol, 150 mg; menadione, 5 mg; thiamin, 20 mg; riboflavin, 25 mg; pyridoxine, 20 mg; Vit. B_{12}, 0.04 mg; niacin, 150 mg; Ca-pantothenate, 50 mg; folic acid, 5 mg; biotin, 0.8 mg; ascorbic acid, 750 mg; inositol, 200; manganese, 150 mg; iron, 25 mg; zinc, 25 mg; copper, 70 mg; cobalt, 2 mg; iodine, 1 mg.
[e] Epol (Pretoria, South Africa).
[f] Kynoch Feeds (Randburg, South Africa).

Thirty eight abalone were placed in a grey PVC holding container with an oyster net floor with 8 x 6 mm mesh aperture. This was placed into a second plastic faecal collection container with a net floor of 200-µm mesh. This two containers were then placed into the glass holding tanks (Sales, 2000). Abalone were adapted for a period of 8 days to the test diets (3 replicates per diet). Tanks were randomly allocated to treatments. The study was divided into two separate experiments because there was a limited number of tanks available. Feeding was performed at 0.2 % of live weight, which was slightly in excess of intake. Faecal collection was performed over a 10 day period according to the protocol described by Sales and Britz (2001a; b). Frozen faeces were stored until the end of the 10 day collection period whereafter they were freeze-dried and analysed.

2.3. Analytical procedure

Ash and AIA content were determined according to Atkinson et al. (1984), crude protein (*N* x 6.25) by using the Kjeldahl technique, energy content by direct combustion in an adiabatic calorimeter, and crude fat after extraction with petroleum ether (boiling point 40-60 °C) by the Soxhlet method. Samples were hydrolysed with 6 N HCl in a sealed tube for 24 h in an oil bath at 110 °C whereafter a Beckman amino acid analyser (Model 6300) was used for separating amino acids using sodium elution buffers. Organic matter was calculated as dry matter minus ash.

2.4. Digestibility coefficient calculation

APD and AAAA in experimental diets was calculated according to the formula from Maynard and Loosli (1969):

ADP or AAAA (%) = 100 × [1- (concentration of marker in diet/concentration of marker in faeces) × (concentration of nutrient in faeces/concentration of nutrient in diet)]

TPD and true amino acid availability (TAAA) was determined using the formula of Kim (1974):

TPD or TAAA (%) = 100 × {(concentration of nutrient in diet/concentration of marker in feed)-[(concentration of nutrient in faeces/concentration of marker in faeces)-(g metabolic faecal nutrient per 100 g dry feed/concentration of marker in feed)]}/(concentration of nutrient in diet/concentration of marker in feed)

2.5. Additivity of apparent digestibility coefficients in compound diets

Determined APD of different diets (Table 3) evaluated in previous studies (Sales and Britz, 2001b; c) were compared to calculated APD according to APD values for ingredients obtained in the present study. In addition, a comparison was made between determined and calculated AAAA of a compound diet.

Table 3

Ingredient (%) and determined nutrient composition of different diets evaluated for additivity (dry matter basis)

Ingredient	Diet 1[a]	Diet 2[a]	Diet 3[a]	Diet 4[b]	Diet5[b]
Fish meal	35	20	20	16	16
Soybean meal	30	20	10	16	16
Cottonseed meal	0	10	5	8	8
Sunflower meal	0	0	5	0	0
Canola meal	0	0	0	20	0
Peanut meal	0	0	0	0	20
Pre-gelatinised maize starch	21	40	5	36	36
Fish oil	0.7	1.0	2.0	0.8	0.8
Carboxymethyl-cellulose[c]	1.4	0	0	0	0
α-Cellulose	9.8	6.5	35	5.2	5.2
Vitamin/mineral mixture	1.75	2.0	2.5	1.6	1.6
Mono calcium phosphate	0.35	0.5	0.5	0.4	0.4
Analysis (dry matter basis)					
Crude protein (%)	39.82	28.22	23.52	30.16	32.17
Gross energy (MJ/kg)	19.11	18.41	18.00	18.77	18.42
Dry matter leaching (%)	16.90 ± 1.17	8.59 ± 0.38	2.00 ± 0.66	11.23 ± 0.44	7.72 ± 0.69

Sources of ingredients as in Table 1 and 2.

[a] From Sales and Britz (2001b).

[b] From Sales and Britz (2001c).

[c] Sigma Chemicals (St. Louis, MO, USA).

3. Results

3.1. Dry matter leaching of test diets containing different protein sources

Dry matter leaching of diets containing different protein sources is presented in Table 4. Fish meal and corn gluten meal presented the lowest dry matter leaching when incorporated into test diets, with legumes (lupins and faba beans) leading to the highest leaching. Of the

oil seeds the lowest leaching was obtained with sunflower meal, and the highest with canola meal.

Table 4
Dry matter leaching (%) of test diets containing different protein sources after 16 h in seawater (mean ± S.E., *n* = 3)

Diet	%
Fish meal	1.61 ± 0.15
Corn gluten meal	1.07 ± 0.30
Soybean meal	5.43 ± 0.25
Cottonseed meal	2.40 ± 0.18
Sunflower meal	1.76 ± 0.25
Canola meal	7.90 ± 0.50
Peanut meal	4.31 ± 0.55
Lupins	12.01 ± 0.60
Faba beans	12.83 ± 0.18

3.2. Endogenous amino acids

The endogenous faecal amino acid pattern and of *H. midae* fed on a protein-free diet is compared in Table 5 to that of other species.

Values obtained for *H. midae* were similar to those derived for rainbow trout, but considerably lower than results presented for channel catfish, common carp and rats.

3.3. Apparent and true protein digestibility and amino acid availability of different protein ingredients

APD, TPD, AAAA and TAAA of different protein ingredients are presented in Tables 6, 7 and 8.

Table 5

Faecal amino acid pattern of *H. midae* (mg/100 dry diet; mean ± S.E., *n* = 3) fed a protein-free diet as compared to that of other species

Amino acid	*H. midae*	Channel catfish[a]	Common carp[b]	Rainbow trout[c]	Rats[d]
Essential					
Arginine	23.13 ± 4.87	38	121	11	40
Histidine	5.25 ± 0.84	42	-	7	16
Isoleucine	15.21 ± 2.30	33	43	19	23
Leucine	19.26 ± 2.83	56	65	21	46
Lysine	25.31 ± 3.95	80	69	15	34
Phenylalanine	11.80 ± 2.16	38	49	11	46
Methionine	5.37 ± 0.87	16	31	4	19
Threonine	18.89 ± 3.12	56	65	25	42
Valine	16.07 ± 2.63	40	62	16	32
Non-essential					
Aspartic acid	41.13 ± 7.17	100	109	30	91
Serine	14.30 ± 2.35	82	58	21	46
Glutamic acid	43.32 ± 6.78	89	113	23	84
Proline	14.46 ± 2.65	50	42	19	45
Glycine	28.89 ± 4.40	53	65	16	41
Alanine	17.00 ± 2.54	38	51	15	37
Tyrosine	5.85 ± 0.95	42	38	11	33
Mean	19.08 ± 2.90	-	-		-
Nitrogen	72.70 ± 10.99	-	-	82	151

[a] Wilson et al. (1981).
[b] Hossain and Jauncey (1989).
[c] Yamamoto et al. (1998).
[d] Keith and Bell (1988).

Table 6

Apparent (AAAA) and true (TAAA) availability (%) of individual amino acids in fish meal and corn gluten meal (mean ± S.E., *n* = 3)

Amino acid	Fish meal		Corn gluten meal	
	AAAA	TAAA	AAAA	TAAA
Essential				
Arginine	82.97 ± 1.56	84.16 ± 1.79	79.40 ± 4.59	81.80 ± 4.10
Histidine	83.70 ± 1.69	84.44 ± 1.78	79.58 ± 0.90	80.63 ± 0.77
Isoleucine	80.87 ± 1.77	81.84 ± 1.92	76.58 ± 1.26	77.86 ± 1.16
Leucine	83.82 ± 1.53	84.59 ± 1.64	78.06 ± 1.34	78.49 ± 1.30
Lysine	85.40 ± 1.49	86.34 ± 1.64	79.93 ± 0.60	84.84 ± 0.30
Phenylalanine	81.43 ± 1.63	82.31 ± 1.79	77.38 ± 1.32	78.06 ± 1.26
Methionine	82.45 ± 1.51	83.04 ± 1.61	76.69 ± 1.39	77.77 ± 1.29
Threonine	81.57 ± 1.80	83.05 ± 2.05	77.02 ± 1.03	79.20 ± 0.71
Valine	82.84 ± 1.33	83.66 ± 1.45	79.24 ± 1.18	80.36 ± 1.09
Non-essential				
Aspartic acid	82.41 ± 1.25	83.59 ± 1.46	75.50 ± 1.20	76.85 ± 1.31
Serine	81.37 ± 1.43	82.89 ± 1.68	78.29 ± 1.06	78.72 ± 0.99
Glutamic acid	85.04 ± 1.47	85.88 ± 1.60	78.27 ± 1.22	78.51 ± 1.20
Proline	83.92 ± 1.36	84.81 ± 1.50	82.11 ± 1.23	82.78 ± 1.19
Glycine	84.08 ± 1.39	85.45 ± 1.59	74.50 ± 0.83	77.79 ± 0.48
Alanine	84.87 ± 1.46	85.67 ± 1.58	74.96 ± 1.49	75.48 ± 1.44
Tyrosine	77.29 ± 2.61	78.23 ± 2.75	69.87 ± 2.01	70.49 ± 1.94
Mean	82.75 ± 1.54	83.75 ± 1.69	77.35 ± 1.04	78.73 ± 0.83
Protein	82.94 ± 1.70	84.35 ± 1.91	76.08 ± 1.93	78.79 ± 1.30

Table 7

Apparent (AAAA) and true (TAAA) availability (%) of individual amino acids in oil seeds (mean ± S.E., *n* = 3)

Amino acid	Soybean meal		Cottonseed meal		Sunflower meal		Canola meal		Peanut meal	
	AAAA	TAAA	AAAA	TAAA	AAAA	TAAA	AAAA	TAAA	AAAA	TAAA
Essential										
Arginine	97.83 ± 0.02	99.07 ± 0.35	92.21 ± 0.47	93.24 ± 0.35	96.49 ± 0.25	97.91 ± 0.39	96.56 ± 0.45	98.79 ± 0.89	93.84 ± 0.66	94.87 ± 0.81
Histidine	96.88 ± 0.20	97.80 ± 0.27	87.51 ± 0.63	88.36 ± 0.50	93.57 ± 0.33	94.57 ± 0.47	95.93 ± 0.67	97.14 ± 0.54	87.72 ± 1.29	88.80 ± 1.15
Isoleucine	96.96 ± 0.16	98.30 ± 0.26	82.46 ± 1.14	84.46 ± 0.89	92.53 ± 0.37	94.21 ± 0.62	93.05 ± 1.26	95.31 ± 1.09	86.49 ± 0.95	88.53 ± 1.00
Leucine	96.97 ± 0.17	98.07 ± 0.22	84.23 ± 1.00	85.67 ± 0.80	92.44 ± 0.43	93.87 ± 0.64	94.37 ± 0.99	96.09 ± 0.84	88.75 ± 1.14	90.20 ± 1.10
Lysine	97.08 ± 0.13	98.84 ± 0.36	82.41 ± 1.19	85.12 ± 0.87	91.36 ± 0.47	94.71 ± 0.99	94.03 ± 1.09	96.79 ± 0.92	83.09 ± 2.13	86.31 ± 1.99
Phenylalanine	97.36 ± 0.07	98.37 ± 0.21	87.14 ± 0.73	88.16 ± 0.56	93.12 ± 0.37	94.36 ± 0.60	94.62 ± 0.96	96.51 ± 0.83	91.90 ± 0.91	93.03 ± 1.00
Methionine	95.36 ± 0.16	97.52 ± 0.45	85.45 ± 1.25	87.32 ± 0.95	95.15 ± 0.28	96.58 ± 0.49	95.02 ± 0.89	97.46 ± 0.66	83.04 ± 1.88	86.10 ± 1.74
Threonine	96.26 ± 0.17	98.35 ± 0.43	82.43 ± 1.14	85.19 ± 0.73	90.78 ± 0.49	93.42 ± 0.92	92.75 ± 1.44	95.58 ± 1.12	80.93 ± 1.78	84.90 ± 1.53
Valine	96.30 ± 0.17	97.85 ± 0.17	84.88 ± 0.94	86.33 ± 0.74	92.79 ± 0.38	94.18 ± 0.61	92.75 ± 1.35	94.60 ± 1.20	88.92 ± 0.51	90.49 ± 0.58
Non-essential										
Aspartic acid	97.27 ± 0.05	98.76 ± 0.29	86.21 ± 0.75	87.32 ± 0.74	92.65 ± 0.43	93.85 ± 0.63	92.35 ± 1.60	95.72 ± 1.20	89.23 ± 1.02	90.99 ± 0.93
Serine	97.48 ± 0.15	98.82 ± 0.25	85.95 ± 0.93	86.59 ± 0.83	92.28 ± 0.38	92.97 ± 0.48	94.10 ± 1.26	96.39 ± 1.04	85.05 ± 1.20	87.32 ± 1.08
Glutamic acid	97.95 ± 0.11	98.90 ± 0.23	89.01 ± 0.69	89.36 ± 0.63	95.19 ± 0.27	95.56 ± 0.32	95.79 ± 0.81	97.26 ± 0.65	92.40 ± 0.59	93.47 ± 0.63
Proline	97.60 ± 0.09	98.65 ± 0.24	88.10 ± 0.68	90.11 ± 0.44	94.40 ± 0.40	96.28 ± 0.67	94.14 ± 1.17	95.44 ± 1.17	91.35 ± 0.73	92.69 ± 0.87
Glycine	94.79 ± 0.33	97.80 ± 0.73	82.95 ± 0.92	85.76 ± 0.72	91.19 ± 0.59	93.31 ± 0.92	93.30 ± 1.25	96.93 ± 0.87	77.41 ± 2.65	79.68 ± 2.47
Alanine	96.39 ± 0.19	98.10 ± 0.37	81.24 ± 1.21	82.65 ± 1.00	91.06 ± 0.51	92.41 ± 0.76	93.97 ± 1.13	96.41 ± 0.93	86.55 ± 1.09	88.60 ± 1.11
Tyrosine	97.46 ± 0.13	98.54 ± 0.30	80.26 ± 1.79	81.68 ± 1.56	89.30 ± 0.58	90.99 ± 0.83	94.61 ± 0.97	96.58 ± 0.82	91.58 ± 1.10	92.65 ± 1.10
Mean	96.86 ± 0.12	98.36 ± 0.30	85.15 ± 0.95	86.71 ± 0.73	92.77 ± 0.40	94.32 ± 0.64	94.21 ± 1.07	96.44 ± 0.90	87.39 ± 1.20	89.29 ± 1.16
Protein	96.34 ± 0.13	98.52 ± 0.43	86.28 ± 0.65	88.43 ± 0.44	92.21 ± 0.47	94.60 ± 0.82	93.94 ± 1.02	97.03 ± 0.72	86.73 ± 1.34	89.04 ± 1.24

Table 8

Apparent (AAAA) and true (TAAA) availability (%) of individual amino acids in legumes (mean ± S.E., *n* = 3)

Amino acid	Lupins		Faba beans	
	AAAA	TAAA	AAAA	TAAA
Essential				
Arginine	99.15 ± 0.09	100.47 ± 0.37	96.96 ± 0.31	98.92 ± 0.65
Histidine	96.56 ± 0.35	97.98 ± 0.52	93.63 ± 0.82	95.19 ± 0.68
Isoleucine	96.50 ± 0.32	98.67 ± 0.64	92.50 ± 1.03	95.24 ± 0.97
Leucine	96.78 ± 0.34	98.45 ± 0.57	93.39 ± 0.88	95.40 ± 0.81
Lysine	96.43 ± 0.36	99.71 ± 0.85	94.36 ± 0.73	97.52 ± 0.76
Phenylalanine	95.92 ± 0.42	97.89 ± 0.77	91.69 ± 1.10	94.17 ± 1.10
Methionine	94.64 ± 0.56	99.96 ± 1.37	89.50 ± 1.37	96.04 ± 1.37
Threonine	95.72 ± 0.38	99.24 ± 0.92	91.23 ± 1.09	95.83 ± 0.96
Valine	96.00 ± 0.39	98.23 ± 0.73	92.97 ± 0.88	95.42 ± 0.87
Non-essential				
Aspartic acid	96.74 ± 0.33	98.14 ± 0.61	93.26 ± 0.86	94.97 ± 1.12
Serine	96.57 ± 0.29	97.36 ± 0.38	93.18 ± 0.88	94.19 ± 0.75
Glutamic acid	97.92 ± 0.21	98.40 ± 0.28	94.13 ± 0.78	94.84 ± 0.76
Proline	97.31 ± 0.30	99.84 ± 0.64	94.61 ± 0.64	97.66 ± 0.68
Glycine	95.36 ± 0.47	99.34 ± 1.06	90.91 ± 1.09	95.42 ± 1.15
Alanine	95.27 ± 0.45	97.57 ± 0.86	91.89 ± 0.94	94.22 ± 0.94
Tyrosine	96.76 ± 0.33	98.06 ± 0.52	91.72 ± 0.99	93.69 ± 0.92
Mean	96.48 ± 0.35	98.71 ± 0.68	92.87 ± 0.90	95.54 ± 0.88
Protein	96.51 ± 0.26	99.55 ± 0.71	93.17 ± 0.91	96.80 ± 0.80

While APD of both fish meal and corn gluten meal was in the order of 80 % (Table 6), values were 86-96 % for oilseeds (Table 7), and around 95 % for legumes (Table 8). Soybean meal (96.34 %) and lupins (96.51 %) presented the highest APD, and corn gluten meal (76.08 %) the lowest value of all feed ingredients evaluated. Of the oil seed meals the lowest values were obtained with cottonseed meal (86.28 %) and peanut meal (86.73 %).

Amino acid availability within feed ingredients was relatively consistent, especially in soybean meal and sunflower meal. However, in corn gluten meal the variation was from 69.87 % for apparent availability of tyrosine to 82.11 % for apparent availability of proline, and in peanut meal from 77.41 % for apparent availability of glycine to 93.84 % for that of arginine.

Correcting amino acid availabilities for metabolic amino acid excretion did not have a notable effect on digestibility. Differences between apparent and true digestibility varied from 0.24 % units for availability of glutamic acid in corn gluten meal to 6.55 % units for availability of methionine in faba beans. The mean difference for all amino acids in all ingredients was 1.88 % units. Mean AAAA and TAAA was highly correlated ($r = 0.99$, $n = 27$) with APD and TPD.

3.4. Additivity of determined apparent protein digestibility and amino acid availability in compound diets

Determined APDs from experimental compound diets were 92.83 ± 0.28, 95.16 ± 0.10, 92.58 ± 0.39, 94.55 ± 0.48 and 89.20 ± 0.16 % for diets 1, 2, 3, 4 and 5, respectively, while predicted values calculated from APDs determined with single protein diets were 89.12, 88.97, 87.87, 90.63 and 91.24 %, respectively. This result in differences of 4.00, 6.50, 5.09, 4.15 and 1.10 %, respectively.

Calculated and determined AAAAs of Diet 2 are presented in Table 9. Determined AAAA of the compound diet was underestimated with a mean of 6.93 % by predicting values from apparent availability values determined from single protein diets for individual feed ingredients. This varied from 5.74 % for the availability of arginine to 8.75 % for availability of tyrosine.

Table 9

Calculated and determined amino acid availability (%) of Diet 2 (mean ± S.E., *n* = 3)

Amino acid	Calculated	Determined	Difference[a]
Essential			
Arginine	90.76	96.29 ± 0.42	5.74 ± 0.41
Histidine	89.74	96.37 ± 0.39	6.88 ± 0.38
Isoleucine	87.62	95.12 ± 0.55	7.88 ± 0.53
Leucine	89.16	95.36 ± 0.50	6.50 ± 0.49
Lysine	89.47	96.07 ± 0.46	6.86 ± 0.44
Phenylalanine	88.95	95.75 ± 0.47	7.10 ± 0.46
Methionine	88.21	94.62 ± 0.74	6.75 ± 0.73
Threonine	87.62	94.94 ± 0.57	7.71 ± 0.55
Valine	88.63	95.33 ± 0.50	7.02 ± 0.49
Non-essential			
Aspartic acid	89.11	95.61 ± 0.49	6.79 ± 0.48
Serine	88.73	95.52 ± 0.47	7.10 ± 0.45
Glutamic acid	91.00	96.15 ± 0.44	5.35 ± 0.43
Proline	90.23	95.99 ± 0.49	6.00 ± 0.48
Glycine	88.14	95.17 ± 0.40	7.38 ± 0.39
Alanine	88.75	95.54 ± 0.48	7.10 ± 0.46
Tyrosine	85.95	94.21 ± 0.91	8.75 ± 0.87
Mean	88.88	95.66 ± 0.49	6.93 ± 0.50

[a] Difference = [(Determined-calculated)/determined] x 100.

4. Discussion

Metabolic faecal nitrogen concentration obtained in this study for *H. midae* (72.70 mg/100 g dry diet) is comparable to values of 39.3-185.2 mg/100 g diet for *Palaemon serratus* (Forster and Gabbott, 1971), 10-151 mg/100 g diet (Nose, 1967) and 83 mg/100 g diet (Yamatomo et al., 1998) for rainbow trout, and 87 mg/100 g diet (Kim, 1974) and 111 mg/100 g diet (Yamatomo et al., 1998) for common carp. However, higher values were reported for *Penaeus indicus* (326.4 mg/ 100 g dry diet; Ahamad Ali, 1988) and red sea bream (206 mg/100 g diet; Yamatomo et al., 1998). Different methods used to

determine metabolic protein and amino acid losses are: (1) feeding of a protein-free diet, (2) the regression analysis technique and (3) the N isotope dilution technique (Sauer et al., 2000). Generally, metabolic faecal nitrogen in fish has been determined by extrapolation to zero intake (Ogino et al., 1973; Kim, 1974), force-feeding of a protein-free diet (Nose, 1967; Wilson et al., 1981; Anderson et al., 1992), or by means of including feed stimulants to enhance intake of protein-free diets (Masumoto et al., 1996; Yamatomo et al., 1998). Although intake was low, it was found that *H. midae*, unlike fish species, adapted readily to a protein-free diet. This may be due to its high carbohydrate natural diet. Endogenous amino acid secretion was low in comparison to values reported for channel catfish (Wilson et al., 1981) and common carp (Hossain and Jauncey, 1989), but comparable to values derived for rainbow trout (Yamatomo et al., 1998). Many factors, including dietary conditions, physiological state and differences in the method of determination may affect the estimation of faecal metabolic nitrogen (Sauer et al., 2000). Also, by feeding a protein-free diet the animal can no longer be regarded as physiologically normal in the absence of a dietary nutrient such as protein (Low, 1980; Fuller, 1988). Differences in digestive enzymes and intestinal microbial activity between species might also have contributed to the comparative differences (Wilson et al., 1981).

This study verifies the suitability of plant protein ingredients (oil seeds and legumes) for use in compound diets for *H. midae*. Soybean meal and lupins presented APD and mean AAAA values of above 96 %, while that of sunflower meal, canola meal and faba beans were above 92 %.

APD of fish meal in the present study (82.94 %) was slightly lower than 87.76 % reported by Sales and Britz (2001b). Shipton (2000) reported a comparable value of 82.0 % for APD of fish meal (Danish 999 LT) in *H. midae*, while a value of 43 % (source of fish meal not mentioned) was obtained for *H. laeviagata* (Fleming et al., 1998). APD values of 57-88 % for fish meal have been reported in crustacean species (summarised by Lee and Lawrence, 1997), and 62.0-90.8 % in different fish species (summarised by Hertrampf and Piedad-Pascual, 2000). In contrast to present results, AAAA varied from 20 % for proline to 48 % for histidine in fish meal evaluated with *H. laevigata* (Fleming et al., 1998). While mean TAAA in menhaden fish meal was lower in channel catfish

(72.5 %; Wilson et al., 1981) than obtained with *H. midae* in the present study (83.75 %), high values were reported for flame-dried whole herring meal in common carp (93.1 %; Hossain and Jauncey, 1989), Norse-LT94 fish meal in Atlantic salmon (94.3 %; Anderson et al., 1992) and white fish meal in rainbow trout (96.4 %; Yamatomo et al., 1998). APD of corn gluten meal with a corresponding crude protein content (> 60 %) was found to be high in *Palaemon serratus* (93 %; Forster and Gabbott, 1971), common carp (93.7-96.5 %; Pongmaneerat and Watanabe, 1991) and silver perch (95.4 %; Allan et al., 2000). Although mean TAAAs of 80.8 % and 81.9 % for corn gluten meal have been reported in common carp and red sea bream (Yamatomo et al., 1998), respectively, that is comparable to the value (78.73 %) obtained in this study, mean TAAAs of 50.9 % were obtained for this feed ingredient in yellowtail (Masumoto et al., 1996), 91.9 % in Atlantic salmon (Anderson et al., 1992) and 96.5 % in rainbow trout (Yamatomo et al., 1998). Anatomical and physiological differences in the digestion systems between species, and differences in pH of corn gluten meal, might be factors related to differences in digestibility between species (Masumoto et al., 1996).

The APD of soybean meal in the present study (96.34 %) was in accordance with a value of 97.00 % reported by Sales and Britz (2001b), but higher than the value of 79.2 % presented by Shipton (2000). APDs of 84-99 % for soybean meal have been reported in crustacean and fish species (summarised by Lee and Lawrence, 1997, Hertrampf and Piedad-Pascual, 2000). Lower mean TAAAs of 84.2 % and 79.4-84.4 % for soybean meal have been obtained in channel catfish (Wilson et al., 1981) and Atlantic salmon (Anderson et al., 1992), respectively, while Allan et al (2000) reported a value of 95.7 % for AAAA of this ingredient in silver perch. It might be that abalone may not be as sensitive to the antinutritional factors present in soybean meal than fish species. Shipton (2000) reported a considerable lower APD value of 76.2 % for cottonseed meal in *H. midae* than found (86.28 %) in the present study. In agreement with present results, APD of cottonseed meal in different fish species (channel catfish, red drum, silver perch) and crayfish has been found to be around 83 % (Wilson and Poe, 1985; Reigh et al., 1990; Gaylord and Gatlin, 1996; Allan et al., 2000). Wilson et al. (1981) obtained a mean TAAA of 78.3 % for cottonseed meal with channel catfish, while the corresponding value was 92.4 % for peanut meal. APD of peanut meal was 76 % for channel catfish (Wilson

and Poe, 1985) and 98.2 % for silver perch (Allan et al., 2000). Anderson et al. (1992) reported a TPD of 91.4 % and a mean TAAA of 92.5 % for canola meal evaluated with Atlantic salmon, while a value of 88.9 % for AAAA was presented by Allan et al. (2000) with silver perch. A considerable lower ADP of 68.1 % was reported for sunflower meal in *H. midae* by Shipton (2000).

Lower APDs of 91 and 85 % have been obtained for lupins (*L. luteus*) and faba beans, respectively, evaluated with *H. laevigata* (Vandepeer et al., 1999), than that presented for *H. midae* (96.51 and 93.17 %, respectively) in this study. Corresponding mean AAAAs presented by Vandepeer et al. (1999) were 91 and 85 %, respectively, compared to present values of 96.48 and 92.87 %, respectively. A comparable mean AAAA of 97.4 % was reported for lupins (*L. albus*) in silver perch, while the corresponding value was 84.8 % for faba beans (Allan et al., 2000).

Although it seems from the above that there might be some agreement as well as some contradiction regarding protein digestibility and amino availability of feed ingredients between species, comparisons between studies is complicated because of differences in methodology used (Allan et al., 2000), chemical composition of ingredients, and processing of ingredients (Masumoto et al., 1996; Yamatomo et al., 1998).

The AAAA of plant proteins evaluated in the present study did not vary much between individual amino acids. Similar results have been obtained with plant proteins in other species, for example, linseed and mustard oil cake evaluated with common carp (Hossain and Jauncey, 1989), soybean meal and corn gluten meal with rainbow trout (Yamatomo et al., 1998) and soybean meal with silver perch (Allan et al., 2000). This might be a reflection of relatively good protein quality. Furthermore, similar to present results, small differences (3 %) between AAAA and TAAA were found by Hossain and Jauncey (1989) and Yamatomo et al. (1998), while there was good agreement between apparent protein digestibility and the mean for AAAA. This tendency was also found in channel catfish (Wilson et al., 1981), yellowtail (Masumoto et al., 1996) and silver perch (Allan et al., 2000). However, Anderson et al. (1992) reported limited use of apparent protein digestibility to predict amino acid availability of feed ingredients for Atlantic salmon.

While APD for the compound diets calculated according to APD coefficients determined for individual ingredients, underestimated determined APD with 1.10-6.50 %, a mean underestimation of 6.93 % was found for AAAA. Although dietary nitrogen leaching was not conducted in this study, it seems that, from the dry matter leaching (Table 3), differences in prediction of digestibility were not related to diet nutrient leaching. Thus, the evaluation of faecal nutrient leaching warrants investigation, a task complicated by the difficulties in obtaining suitable amounts of faeces produced from *H. midae*. Different feed ingredients have been shown to affect the composition of the faeces in salmonids, that could be responsible for differences in faecal nutrient leaching between diets. For example, water content increases in faeces when fish meal is replaced by plant protein ingredients with high soluble fibre content, such as soybean meal (Refstie et al., 1997, 1998). This has still to be evaluated in abalone.

In conclusion, protein and amino acids in oil seeds and legumes are highly digestible by *H. midae*. Of the plant proteins soybean meal has the highest content of lysine (3.43 %) with an apparent availability of 97.08 %, while cottonseed meal has the highest (5.01 %) concentration of arginine with an apparent availability of 92.21 %. Although fish meal did not present the highest digestibility values, it contains high levels and a good balance of essential amino acids, while this is not true of plant protein ingredients. Data presented in this study could be utilised in least cost formulation to find the optimal combination of plant protein ingredients to stimulate a similar balanced pattern of amino acids in compound diets for optimal growth in *H. midae*. Protein digestibility as indicator of mean amino acid availability could be utilised to identify future potential protein ingredients for compound diets. However, for inclusion of the most suitable protein ingredients in feed evaluation tables information on amino acid availability will be needed. Furthermore, palatability, and nutrient leaching are factors to take into account in addition to nutrient digestibility when formulating diets for abalone.

Acknowledgements

This study was sponsored by Sea Plant Products, Hermanus, South Africa, THRIP (Technology and Human Resources for Industry Programme), the South African Network for Coastal and Oceanographic Research, the National Research Foundation and Rhodes

University Joint Research Committee. The first author was supported by a post-doctoral scholarship from the Claude Leon Harris Foundation. We thank Drs. R.P. Wilson and J.S. Anderson for useful comments during the planning of this experiment and W. Lambrechts (Western Province Corp.) for supplying of feed ingredients.

References

Ahamad Ali, S., 1988. Studies on the evaluation of different sources of proteins, carbohydrates and mineral requirements for juvenile penaeid prawn *Panaeus indicus* H. Milne Edwards. Ph.D Dissertation, Cochin University of Science and Technology, Cochin, India, 243 pp.

Anderson, J.S., Lall, S.P., Anderson, D.M., Chandrasoma, J., 1992. Apparent and true availability of amino acids from common feed ingredients for Atlantic salmon (*Salmo salar*) reared in sea water. Aquaculture 108, 111-124.

Allan, G.L., Parkinson, S., Booth, M.A., Stone, D.A.J., Rowland, S.J., Frances, J., Warner-Smith, R., 2000. Replacement of fish meal in diets for Australian silver perch *Bidyanus bidyanus*: I. Digestibility of alternative ingredients. Aquaculture 186, 293-310.

Atkinson, J.L., Hilton, J.W., Slinger, S.J., 1984. Evaluation of acid-insoluble ash as an indicator of feed digestibility in rainbow trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. 41, 1384-1386.

Cho, C.Y., Slinger, S.J., Bayley, H.S., 1982. Bioenergetics of salmonid fishes: energy intake, expenditure and productivity. Comp. Biochem. Physiol. 73B, 25-41.

Fleming, A.E., Van Barneveld, R.J., Hone, P.W., 1996. The development of artificial diets for abalone: A review and future directions. Aquaculture 140, 5-53.

Fleming, A.E., Van Barneveld, R.J., Hone, P.W., Vandepeer, M.E., Kruck, J.A., 1998. Complimentary additivity of the digestibility coefficients of feed ingredients fed to juvenile greenlip abalone (*Haliotis laevigata*). J. Shellfish Res. 17, 641-647.

Forster, J.R.M., Gabbott, P.A., 1971. The assimilation of nutrients from compounded diets by prawns *Palaemon serratus* and *Pandalus platyceros*. J. Mar. Biol. Ass. UK 51, 943-961.

Fuller, M.F., 1988. Methods of protein evaluation for nonruminants. In: Ørskov, E.R. (Ed.), Feed Science, World Animal Science, B4, Elsevier Science Publishers B.V., Amsterdam, pp. 81-101.

Gaylord, T.G., Gatlin III, D.M., 1996. Determination of digestibility coefficients of various feedstuffs for red drum (*Sciaenops ocellatus*). Aquaculture 139, 303-314.

Hardy, R.W., Kissel, G.W.M., 1997. Trends in aquaculture feeding. Feed Mix 5, 31-34.

Hertrampf, J.W., Piedad-Pascual, F., 2000. Handbook on Ingredients for Aquaculture Feeds, Kluwer Academic Publishers, Dordrecht, The Netherlands, 573 pp.

Hossain, M.A., Jauncey, K., 1989. Studies on the protein, energy and amino acid digestibility of fish meal, mustard oilcake, linseed and sesame meal for common carp (*Cyprinus carpio* L.) Aquaculture 83, 59-72.

Keith, M.O., Bell, J.M., 1988. Digestibility of nitrogen and amino acids in selected protein sources fed to mice. J. Nutr. 118, 561-568.

Kim, Y.K., 1974. Determination of true digestibility of dietary proteins in carp with chromic oxide containing diet. Bull. Jpn. Soc. Sci. Fish. 40, 651-653.

Lee, P.G., Lawrence, A.L., 1997. Digestibility. In: D'Abramo, L.R., Conklin, D.E., Akiyama, D.M. (Eds.), Crustacean Nutrition, Advances in World Aquaculture, Vol. 6, World Aquaculture Society, Louisiana, USA, pp. 194-260.

Low, A.G., 1980. Nutrient absorption in pigs. J. Sci. Food Agric. 31, 1087-1130.

Masumoto, T., Ruchimat, T., Ito, Y., Hosokawa, H., Shimeno, S., 1996. Amino acid availability values for several protein sources for yellowtail (*Seriola quinqueradiata*). Aquaculture 146, 109-119.

Maynard, L.A., Loosli, J.K., 1969. Animal Nutrition, 6th edn. McGraw-Hill, New York, NY, 613 pp.

National Research Council (NRC), 1993. Nutrient Requirements of Fish, National Academy Press, Washington, 114 pp.

Nose, T., 1967. On the metabolic fecal nitrogen in young rainbow trout. Bull. Freshwater Fish. Lab. Tokyo 17, 97-105.

Ogino, T., Kakino, J., Chen, M.S., 1973. Protein nutrition in fish. II. Determination of metabolic fecal nitrogen and endogenous nitrogen excretion of carp. Bull. Jpn. Soc. Sci. Fish. 39, 519-523.

Pongmaneerat, J., Watanabe, T., 1991. Nutritive value of protein of feed ingredients for carp. Nippon Suisan Gakkaishi 57, 503-510.

Refstie, S., Helland, S.J., Storebakken, T., 1997. Adaptation to soybean meal in diets for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 153, 263-272.

Refstie, S., Storebakken, T., Roem, A.J., 1998. Feed consumption and conversion in Atlantic salmon (*Salmo salar*) fed diets with fish meal, extracted soybean meal or soybean meal with reduced content of oligosaccharides, trypsin inhibitors, lectins and soya antigens. Aquaculture 162, 301-312.

Reigh, R.C., Braden, S.L., Craig, R.J., 1990. Apparent digestibility coefficients for common feedstuffs in formulated diets for red swamp crayfish, *Procambarus clarkii*. Aquaculture 84, 321-334.

Sales, J., 2000. Study of faeces collection methods to define optimum abalone diets. Fish Farmer International, May/June 2000, Vol. 14, No. 3, pp. 8-9.

Sales, J., Britz, P.J., 2001a. Evaluation of different markers to determine apparent nutrient digestibility coefficients of feed ingredients for South African abalone (*Haliotis midae* L.). Aquaculture 202, 113-129.

Sales, J., Britz, P.J., 2001b. Evaluation of the reference diet substitution method for determination of apparent nutrient digestibility coefficients of feed ingredients for South African abalone, *(Haliotis midae* L.). Aquaculture (accepted for publication, 23 July 2001).

Sales, J., Britz, P.J., 2001c. Influence of ingredient particle size and dietary inclusion level of pre-gelatinised maize starch on apparent nutrient digestibility of diets in South African abalone, (*Haliotis midae* L.) Aquaculture (MS 1680N).

Sauer, W.C., Fan, M.Z., Mosenthin, R., Drochner, W., 2000. Methods for measuring ileal amino acid digestibility in pigs. In: D'Mello, J.P.F. (Ed.), Farm Animal Metabolism and Nutrition, CABI Publishing, Wallingford, Oxfordshire, UK, pp. 279-306.

Seneriches, M.L.M., Chiu, Y.N., 1988. Effect of fishmeal on the growth, survival and feed efficiency of milkfish (*Chanos chanos*) fry. Aquaculture 71, 61-69.

Shipton, T.A., 2000. The protein requirements for the South African abalone, *Haliotis midae*. Ph.D Dissertation, Rhodes University, Grahamstown, South Africa, 146 pp.

Vandepeer, M.E., Van Barneveld, R.J., Hone, P.W., Havenhand, J.N. 1999. The digestibility of raw, autoclaved and phytase treated legumes in greenlip abalone, *Haliotis laevigata*. Proc. Of the 6th annual abalone aquaculture workshop, April 1999, World Aquaculture Society Conference, Sydney, Australia.

Wilson, R.P., Poe, W.E., 1985. Apparent digestible protein and energy coefficients of common feed ingredients for channel catfish. Prog. Fish-Cult. 47, 154-158.

Wilson, R.P., Robinson, E.H., Poe, W.E., 1981. Apparent and true availability of amino acids from common feed ingredients for channel catfish. J. Nutr. 111, 923-929.

Yamatomo, T., Akimoto, A., Kishi, S., Unuma, T., Akiyama, T., 1998. Apparent and true availabilities of amino acids from several protein sources for fingerling rainbow trout, common carp, and red sea bream. Fisheries Sci. 64, 448-458.

CHAPTER EIGHT

Dietary phosphorus leaching and apparent phosphorus digestibility from different inorganic phosphorus sources for South African abalone *(Haliotis midae* L.)

Sales, J., Britz, P.J., Viljoen, J., 2001. Influence of different inorganic phosphorus sources on dietary phosphorus leaching and apparent phosphorus digestibility for South African abalone (*Haliotis midae* L.). Aquaculture Nutrition (Submitted for publication, 17/10/2001).

Abstract

The objective of this study was to determine phosphorus leaching of diets supplemented with various inorganic phosphorus sources (sodium phosphate mono basic, calcium phosphate mono basic, calcium phosphate mono dibasic, calcium phosphate dibasic), and the apparent phosphorus digestibility of these sources to *Haliotis midae*. A trend of increased dietary phosphorus leaching from diet pellets with increased water solubility of sources was observed. Sodium phosphate mono basic presented the highest ($P < 0.05$) maximum phosphorus leaching (58.84 %), while a value of 14.90 % was obtained with calcium phosphate dibasic. An unexpectedly high phosphorus leaching (48.71 % maximum) was obtained for a reference diet consisting of natural feed ingredients (fish meal, soybean meal, cottonseed meal) without any phosphorus supplementation. Calcium phosphate dibasic presented a lower ($P < 0.05$) apparent phosphorus digestibility (27.92 %) than sodium phosphate mono basic (66.45 %), calcium phosphate mono basic (72.56 %) and calcium phosphate mono dibasic (66.27 %). When dietary phosphorus leaching and apparent phosphorus digestibility were take into account, calcium phosphate mono dibasic seems to be the most promising inorganic phosphorus source for inclusion in abalone diets. Further research should concentrate on minimizing dietary phosphorus leaching through feed preparation techniques.

1. Introduction

Phosphorus is an important constituent of nucleic acids and cell membranes in all living species, and is directly involved in all energy-producing cellular reactions. The main source of phosphate for aquatic species is feed because of the low concentration of phosphate in natural waters. Feed ingredients that originate from seeds contain phosphorus primarily as the calcium-magnesium salt of phytic acid known as phytin. Phytin phosphorus is unavailable to animals with simple stomachs because they lack the enzyme phytase in the gastrointestinal tract. Therefore supplementation of feeds with inorganic phosphorus sources is a common practice. Mono basic phosphates of sodium, potassium, and calcium appear to be highly available sources to aquatic species, while dibasic and tribasic calcium phosphates vary in their availabilities and is generally less available than the mono basic form (NRC, 1993). Dietary calcium impairs the utilization of phosphorus due to chemical binding so that less phosphorus is available than from diets which are low or lacking in calcium (Nakamura, 1982). Phosphorus overformulation is a common practice because inorganic phosphorus availability from ingredients is not clearly known and feed manufacturers prefer to provide excess phosphorus to avoid growth problems. Inorganic phosphorus availability is difficult to determine but at the same time plays a critical role in determining the amount of phosphorus used by the animals and the amount of phosphorus wastes that enter the environment via effluent water (Montoya et al., 2000). Apparent absorption studies (digestibility) appear to be a suitable alternative for the determination of phosphorus availability as they require less time and labour than retention measurements, animals do not need to be killed, and a comparison of phosphorus sources based on retention or growth data can be biased by other nutritional factors influencing growth (Rodehutscord et al., 2000).

Although the influence of dietary phosphorus and calcium levels on growth of abalone has received attention (Coote et al., 1996; Tan et al., 2001), no information of phosphorus availability of feed ingredients is available for this species. This study was designed to determine dietary phosphorus leaching and apparent phosphorus digestibility of a practical diet for *Haliotis midae* supplemented with various inorganic phosphate sources.

2. Material and methods

2.1. Experimental animals, feed ingredients and diet preparation

The experimental animals were 456 juvenile abalone (*H. midae*) (56.49 ± 0.12 mm shell length; 35.60 ± 0.24 g live weight, mean ± SE) reared on a commercial abalone farm in South Africa, and adapted to a recirulating system for about 10 months (Sales and Britz, 2001a; b).

Test ingredients (Table 1) were ground with a mortar and pestle before incorporation in a reference diet (Table 2) to supply 1.0 % phosphorus to the expense of α-cellulose. Food was made and dried as described by Sales and Britz (2001a; b). The dry product was cut into 1 x 1 cm pieces and stored at 4 °C till used.

Table 1
Determined phosphorus content and inclusion level of inorganic phosphorus sources (dry matter basis)

Inorganic sources[a]	Phosphorus (%)	Inclusion (%)
Sodium phosphate mono basic, feed grade ($Na(H_2PO_4)_2.xH_2O$)	22.83	4.38
Calcium phosphate mono basic, monohydrate, feed grade ($Ca(H_2PO_4)_2.xH_2O$)	20.74	4.82
Calcium phosphate mono dibasic, feed grade[b]	20.66	4.84
Calcium phosphate dibasic, dihydrate, feed grade ($CaHPO_4.2H_2O$)	18.84	5.31

[a] Kynoch Feeds (Umbogintwini, South Africa).
[b] Calcium phosphate mono basic ($Ca(H_2PO_4)_2.xH_2O$) and calcium phosphate dibasic ($CaHPO_4.2H_2O$) in the ratio of approximately 3:1.

2.2. Experimental system, feeding and faecal collection

An indoor recirculating system consisting of a series of 15 glass holding tanks (40-l capacity), a settlement tank of 300-l capacity and primary and secondary biological filters, as described by Sales and Britz (2001a; b), was used. Room air temperature was cooled to 15 °C, while water was heated to 18 °C. Aeration was supplied via airstones in each tank.

Table 2
Ingredient and determined nutrient composition of reference diet (dry matter basis)

Ingredient	(%)
Fish meal[a]	20
Soybean meal[b]	20
Cottonseed meal[b]	10
Maize starch[c]	40
Fish oil[d]	1.0
α-Cellulose[e]	6.5
Vitamin/mineral mixture[f, g]	2.0
Analysis (dry matter basis)	
Crude protein (%)	27.64
Gross energy (MJ/kg)	17.33
Phosphorus (%)	1.24

[a] Low-temperature dried fish meal (Danish 999 LT), Esbjerg Fiskeindustri a.m.b.a. (Esbjerg, Danmark).
[b] Pre-pressed, solvent extracted, dehulled, Western Province Corp. (Roggebaai, South Africa).
[c] Further composition and source priority Sea Plant Products (Hermanus, South Africa).
[d] Marinol-R, Marine Oil Refiners (Cape Town, South Africa).
[e] Sigma Chemicals (St. Louis, MO, USA).
[f] Per kg of feed: retinol, 12000 IU; cholecalciferol, 1800 IU; α-tocopherol, 150 mg; menadione, 5 mg; thiamin, 20 mg; riboflavin, 25 mg; pyridoxine, 20 mg; Vit. B_{12}, 0.04 mg; niacin, 150 mg; Ca-pantothenate, 50 mg; folic acid, 5 mg; biotin, 0.8 mg; ascorbic acid, 750 mg; inositol, 200; manganese, 150 mg; iron, 25 mg; zinc, 25 mg; copper, 70 mg; cobalt, 2 mg; iodine, 1 mg.
[g] Epol (Pretoria, South Africa).

Water temperature (18.05 ± 0.15 ºC), pH (7.62 ± 0.01), dissolved oxygen (8.15 ± 0.15 mg/l), total ammonia (0.08 ± 0.03 mg/l) and salinity (37 ‰) were monitored on a weekly basis, as described by Sales and Britz (2001a; b). Flow rate in the tanks was maintained

at 2 l/min and lights were only turned on during cleaning, feeding and sampling (0800-0900 and 1500-1600 h).

Thirty eight abalone were placed in a grey PVC holding container with a oyster net floor with 8 x 6 mm mesh aperture. This was placed into a second plastic faecal collection container with a net floor of 200 µm mesh. These two containers were then placed into the glass holding tanks (Sales, 2000). Abalone were adapted for a period of 8 days to the test diets (3 replicates per diet). Tanks were randomly allocated to treatments. Feeding was performed at 0.2 % of live weight, which was slightly in excess of intake. Faecal collection was performed over a 10 day period according to the protocol described by Sales and Britz (2001a; b). Frozen faeces were stored until the end of the 10 day collection period whereafter they were freeze-dried and analysed.

2.3. Dietary phosphorus leaching

Leaching of dry matter and phosphorus from the experimental diets after 1, 3, 6, 12 and 24 h (each in triplicate) in the experimental tanks was determined by calculating dry matter and phosphorus leaching of approximately 2 g of feed (dried at 55 °C for 48 h).

2.4. Analytical procedure

Ash and acid insoluble ash (AIA) content were determined according to Atkinson et al. (1984), crude protein (*N* x 6.25) by using the Kjeldahl technique, energy content by direct combustion in an adiabatic calorimeter and total phosphorus after ashing the samples and using the vanado-molybdate method (AOAC, 1980). Organic matter was calculated as dry matter minus ash.

2.5. Regression analyses, digestibility coefficient calculation and statistical analyses

Phosphorus leaching (*p*) over time (*t*) was described by the following exponential model (Ørskov and McDonald, 1979):

$p = a + b(1\text{-}e^{-ct})$

where *a* is an intercept at time (*t*) = 0,

a+*b* is the asymptotic maximum phosphorus leaching, and

c is a measurement of the rate of phosphorus leaching.

In the application of this model *a* was set to 0.

Apparent digestibility coefficients (ADCs) in experimental diets were calculated according to the formula from Maynard and Loosli (1969):

ADC of dry matter of diet (%) = 100 × [1 – (concentration of marker in diet / concentration of marker in faeces)]

ADC of phosphorus of diet (%) = 100 × [1- (concentration of marker in diet/concentration of marker in faeces) × (concentration of phosphorus in faeces/concentration of phosphorus in diet)]

ADC of phosphorus of the test ingredients (%) was calculated using the formula of Sugiura et al. (1998):

ADC (%) = [(phosphorus concentration in test diet × phosphorus ADC of test diet)-(proportion of reference diet in test diet × phosphorus concentration in reference diet × phosphorus ADC of reference diet)]/(proportion of test ingredient in test diet × phosphorus concentration in test ingredient)

Results for dry matter and phosphorus leaching of diets over time and apparent nutrient digestibility were subjected to one-way analysis of variance. Differences between means ($P < 0.05$) were evaluated by the Tukey's HSD test. Unpaired *t*-tests were used to compare dry matter and phosphorus leaching at different time intervals, and parameters derived from non-linear regressions, between treatments (Snedecor and Cochran, 1991).

3. Results

3.1. Dietary phosphorus leaching

Unpaired *t*-tests revealed that after 1 h in water dry matter leaching in the calcium phosphate dibasic treatment was lower ($P < 0.05$) than in the calcium phosphate mono basic and calcium phosphate mono dibasic treatments (Table 3). After 24 h in water differences in dry matter leaching of the calcium phosphate mono basic treatment in comparison to the sodium phosphate mono basic and calcium phosphate mono dibasic treatments were not significant. After both 1 and 24 h in water phosphorus leaching between the sodium phosphate mono basic and calcium phosphate mono basic treatments were similar (Table 4; $P > 0.05$).

Table 3

Dietary dry matter leaching (%) after certain time periods in water (mean ± S.E.; n = 3)

Time (h)	Reference	Sodium phosphate mono basic	Calcium phosphate mono basic	Calcium phosphate mono dibasic	Calcium phosphate dibasic
1	$1.97^{a} \pm 1.08$	$2.31^{a} \pm 0.58$	$2.30^{a} \pm 0.21$	$3.64^{a} \pm 0.48$	$0.93^{a} \pm 0.12$
3	$2.81^{a} \pm 0.42$	$5.98^{b} \pm 0.61$	$5.77^{b} \pm 0.31$	$6.48^{b} \pm 0.28$	$3.25^{a} \pm 0.52$
6	$3.38^{a} \pm 0.94$	$8.18^{bc} \pm 0.69$	$9.09^{c} \pm 0.23$	$9.15^{c} \pm 0.42$	$5.54^{b} \pm 0.25$
12	$5.00^{a} \pm 0.15$	$9.44^{c} \pm 0.33$	$10.85^{d} \pm 0.21$	$9.98^{c} \pm 0.24$	$6.07^{b} \pm 0.68$
24	$9.99^{b} \pm 0.20$	$16.64^{d} \pm 0.76$	$14.24^{e} \pm 0.54$	$13.23^{d} \pm 0.21$	$11.51^{c} \pm 0.29$

Means in the same column with different superscripts (a, b, c, d ,e) are statistically different ($P < 0.05$).

Table 4

Dietary phosphorus leaching (%) after certain time periods in water (mean ± S.E.; n = 3)

Time (h)	Reference	Sodium phosphate mono basic	Calcium phosphate mono basic	Calcium phosphate mono dibasic	Calcium phosphate dibasic
1	$43.50^{a} \pm 0.71$	$33.44^{a} \pm 0.34$	$33.15^{a} \pm 0.98$	$29.87^{a} \pm 0.13$	$8.21^{a} \pm 1.84$
3	$47.79^{b} \pm 0.42$	$55.61^{b} \pm 0.54$	$49.68^{b} \pm 0.54$	$43.79^{b} \pm 1.24$	$12.43^{ab} \pm 1.41$
6	$48.54^{bc} \pm 0.44$	$56.35^{b} \pm 0.54$	$53.32^{c} \pm 0.51$	$49.20^{c} \pm 0.15$	$13.74^{ab} \pm 1.46$
12	$48.43^{bc} \pm 0.26$	$58.34^{bc} \pm 0.40$	$55.97^{c} \pm 0.68$	$51.48^{cd} \pm 1.15$	$14.28^{ab} \pm 0.49$
24	$49.98^{c} \pm 0.26$	$60.61^{c} \pm 1.60$	$56.36^{c} \pm 0.58$	$54.26^{d} \pm 0.11$	$16.55^{b} \pm 1.92$

Means in the same column with different superscripts (a, b, c, d) are statistically different ($P < 0.05$).

Trends ($P < 0.05$) in phosphorus leaching over time followed more or less the same pattern in all treatments (Table 4). This is also illustrated by analysis of data according to non-linear regression (Table 5). Only the rate of phosphorus leaching (*c*) for the reference diet was outside the 95 % confidence intervals of all the other treatments. Maximum phosphorus leaching, describe by *b*, was the lowest ($P < 0.05$) for the treatment containing calcium phosphate dibasic, and the highest ($P < 0.05$) for the diet supplemented with sodium phosphate mono basic. However, data for the diet supplemented with calcium phosphate dibasic did not present a good fit to the non-linear model ($R^2 = 0.52$).

3.1 Apparent phosphorus digestibility

With the exception of the diet supplemented with calcium phosphate dibasic, faecal phosphorus, apparent digestibility of dry matter, organic matter and phosphorus were similar ($P > 0.05$) between different treatments (Table 6). This was also true for apparent phosphorus digestibility from different phosphorus sources.

However, correction of apparent phosphorus digestibility for maximum dietary phosphorus leaching resulted in the highest ($P < 0.05$) value (58.20 %) for calcium phosphate dibasic in comparison to the lowest ($P < 0.05$) value (27.92 %) obtained from this source for the uncorrected values. The variation in apparent phosphorus digestibility of calcium phosphate dibasic was considerably more than in other sources.

4. Discussion

This study emphasizes the inherent problem of potential nutrient leaching from feed with slow feeders like abalone where feed pellets have to stay for prolonged periods in water. In accordance with the present results, Tan et al. (2001) obtained phosphorus leaching of 14-28, 20-48 and 37-69 % of diets supplemented with potassium phosphate dibasic and calcium phosphate dibasic after 2, 6 and 12 h, respectively, in water. As in other studies (Davis and Arnold, 1994; Velasco et al., 1998), phosphorus leaching in the present study was correlated with the water solubility of the inorganic dietary phosphorus sources used. An unexpected high phosphorus leaching of 43.50 % for the reference diet without any phosphorus supplementation was obtained after 1 h in water. However, previous studies evaluating phosphorus leaching of diets in slow feeders such as abalone (Tan et al., 2001) and shrimp (Davis and Arnold, 1994) included a reference diet consisting of mainly casein, leading to

low phosphorus leaching. Thus, comparative results of phosphorus leaching from diets containing practical feed ingredients are unavailable.

Apparent phosphorus digestibility of sodium phosphate mono basic (66.45 %) and calcium phosphate dibasic (27.92 %) is in accordance with values of 69 and 24 %, respectively, obtained for shrimp (Davis and Arnold, 1994), but considerable lower than values of 90-98% and 46-71 %, respectively, reported for fish (Lovell, 1978; Ogino et al., 1979). The value of 72.56 % for calcium phosphate mono basic is higher than a value of 49 % obtained in shrimp (Davis and Arnold, 1994).

The influence of dietary phosphorus leaching on apparent phosphorus digestibility is clearly illustrated in this study. However, when apparent phosphorus digestibility is calculated taking dietary phosphorus leaching into account, but not faecal phosphorus leaching, unrealistic values are obtained. For example, when dietary phosphorus is corrected for leaching, calcium phosphate dibasic presents the highest ($P < 0.05$) apparent phosphorus digestibility, although it still contains the highest ($P < 0.05$) faecal phosphorus concentration. Phosphorus leaching from faeces might be variable in comparison to that in feed pellets, as was found in Atlantic salmon (Phillips et al., 1993). Thus, dietary phosphorus leaching and faecal phosphorus leaching should be taken into account in attempts to corrected digestibility for leaching.

While total phosphorus is of primary importance in estimating the delivery of nutrients to the animal, dissolved reactive phosphorus is essential in evaluating the environmental impact of phosphorus leaching (Davis and Arnold, 1994). From the present study the conclusion could be drawn that calcium phosphate mono dibasic would present an apparent phosphorus digestibility similar ($P > 0.05$) to that of mono basic phosphorus sources, but will have a lower ($P < 0.05$) load of dissolved reactive phosphorus to the aquatic environment. However, further studies on digestibility of phosphorus and minerals in abalone nutrition should firstly concentrate on reducing dietary phosphorus leaching through feed preparation techniques.

Table 5

Dietary phosphorus leaching (p) over time described by $p = a + [b(1\text{-}e^{-ct})]$, a set to 0; n = 15)

Parameter	Reference	Sodium phosphate mono basic	Calcium phosphate mono basic	Calcium phosphate mono dibasic	Calcium phosphate dibasic
b (maximum phosphorus leaching)					
Mean ± S.E.	48.71^{b} ± 0.29	58.84^{e} ± 0.64	55.03^{d} ± 0.54	51.51^{c} ± 0.91	14.90^{a} ± 0.83
95 % Confidence interval	48.07-49.35	57.46-60.21	53.85-56.20	49.54-53.49	13.08-16.71
c (rate of phosphorus leaching)					
Mean ± S.E.	2.23^{b} ± 0.12	0.86^{a} ± 0.05	0.88^{a} ± 0.04	0.78^{a} ±0.06	0.68^{a} ± 0.19
95 % Confidence interval	1.96-2.50	0.76-0.96	0.79-0.98	0.65-0.92	0.26-1.09
R^2	0.8282	0.9663	0.9674	0.9278	0.5203
Absolute sum of squares	13.44	49.88	36.72	81.16	69.18
S*y.x*	1.017	1.959	1.681	2.601	2.401

Means in the same row with different superscripts (a, b, c, d ,e) are statistically different ($P < 0.05$).

Table 6

Phosphorus content (dry matter basis) and apparent digestibility coefficients of different diets and ingredients (mean ± S.E.; *n* = 3)

Parameter	Reference	Sodium phosphate mono basic	Calcium phosphate mono basic	Calcium phosphate mono dibasic	Calcium phosphate dibasic
Phosphorus content (%)					
Diet	1.24	1.93	2.01	1.97	1.80
Faeces	0.53[a] ± 0.03	0.67[a] ± 0.06	0.77[a] ± 0.03	0.74[a] ± 0.02	1.50[b] ± 0.09
Apparent digestibility (%)					
Diet					
Dry matter	65.17 ± 3.81	61.34 ± 3.46	62.90 ± 0.37	58.48 ± 2.78	65.46 ± 3.31
Organic matter	82.83 ± 2.17	80.70 ± 1.17	80.83 ± 0.49	79.73 ± 1.50	82.28 ± 2.10
Phosphorus	85.01[b] ± 1.84	86.72[b] ± 0.81	85.75[b] ± 0.60	84.36[b] ± 1.44	70.94[a] ± 4.18
Ingredient					
Phosphorus		66.45[b] ± 1.56	72.56[b] ± 1.21	66.27[b] ± 2.84	27.92[a] ± 7.51
Corrected[1]		10.76[a]	19.13[a]	22.04[a]	58.20[b]

Means in the same row with different superscripts (a, b) are statistically different ($P < 0.05$).

[1] Diet phosphorus corrected for maximum leaching (*a* value from Table 5).

Evidence has been presented in fish that phosphorus availability decreases with increasing dietary phosphorus concentration (Riche and Brown, 1996; Satoh et al., 1997) and it was recommended that when different sources of phosphorus are to be compared regarding its availability the dietary concentration of available phosphorus must be near the requirement (Riche and Brown; Rodehutscord et al., 2000). This requirement is not yet known for *H. midae* and warrants investigation.

Acknowledgements

This study was sponsored by Sea Plant Products, Hermanus, South Africa, the South African Network for Coastal and Oceanographic Research, the National Research Foundation and Rhodes University Joint Research Committee. Inorganic phosphorus sources were supplied by Kynoch Feeds (Umbogintwini, South Africa). The first author was supported by a post-doctoral scholarship from the Claude Leon Harris Foundation.

References

AOAC, 1980. Official Methods of Analysis of the Association of Official Analytical Chemists, 13th edn. AOAC, Arlington, VA, USA.

Atkinson, J.L., Hilton, J.W., Slinger, S.J., 1984. Evaluation of acid-insoluble ash as an indicator of feed digestibility in rainbow trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. 41, 1384-1386.

Coote, T.A., Hone, P.W., Kenyn, R., Maguire, G.B., 1996. The effect of different combinations of dietary calcium and phosphorus on the growth of juvenile *Haliotis laevigata*. Aquaculture 145, 267-279.

Davis, D.A., Arnold, C.R., 1994. Estimation of apparent phosphorus availability from inorganic phosphorus sources for *Penaeus vannamei*. Aquaculture 127, 245-254.

Lovell, R.T., 1978. Dietary phosphorus requirement of channel catfish (*Ictalurus punctatus*). Trans. Am. Fish. Soc. 107, 617-621.

Maynard, L.A., Loosli, J.K., 1969. Animal Nutrition, 6th edn. McGraw-Hill, New York, NY, 613 pp.

Montoya, R.A., Lawrence, A.L., Grant, W.E., Velasco, M., 2000. Stimulation of phosphorus dynamics in an intensive shrimp culture system: effects of feed formulations and feeding strategies. Ecol. Model. 129, 131-142.

Nakamura, Y., 1982. Effects of dietary phosphorus and calcium content on the absorption of phosphorus in the digestive tract of carp. Bull. Jpn. Soc. Sci. Fish. 48, 409-413.

National Research Council (NRC), 1993. Nutrient Requirements of Fish, National Academy Press, Washington, 114 pp.

Ogino, C., Takeuch, L., Takeda, H., Watanabe, T., 1979. Availability of dietary phosphorus in carp and rainbow trout. Bull. Jpn. Soc. Sci. Fish. 44, 1105-1108.

Ørskov, E.R., McDonald, I., 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci. (Camb.) 92, 499-503.

Phillips, M.J., Clarke, R., Mowat, A., 1993. Phosphorus leaching from Atlantic salmon diets. Aquacult. Eng. 12, 47-54.

Riche, M., Brown, P.B., 1996. Availability of phosphorus from feedstuffs fed to rainbow trout,*Oncorhynchus mykiss.* Aquaculture 142, 269-282.

Rodehutscord, M., Gregus, Z., Pfeffer, E., 2000. Effect of phosphorus intake on faecal and non-faecal phosphorus excretion in rainbow trout (*Oncorhynchus mykiss*) and the consequences for comparative phosphorus availability studies. Aquaculture 188, 383-398.

Sales, J., 2000. Study of faeces collection methods to define optimum abalone diets. Fish Farmer International, May/June 2000, Vol. 14, No. 3, pp. 8-9.

Sales, J., Britz, P.J., 2001a. Evaluation of different markers to determine apparent nutrient digestibility coefficients of feed ingredients for South African abalone (*Haliotis midae* L.). Aquaculture 202, 113-129.

Sales, J., Britz, P.J., 2001b. Evaluation of the reference diet substitution method for determination of apparent nutrient digestibility coefficients of feed ingredients for South African abalone, *(Haliotis midae* L.). Aquaculture (accepted for publication, 23 July 2001).

Satoh, S., Viyakarn, V., Takeuchi, T., Watanabe, T., 1997. Availability of phosphorus in various phosphates to carp and rainbow trout determined by a simple fractionation method. Fisheries Sci. 62, 297-300.

Snedecor, G.W., Cochran W.G., 1991. Statistical Methods (8th edition). Iowa State University Press, Ames, USA, 503 pp.

Sugiura, S.D., Dong, F.M., Rathbone, C.K., Hardy, R.W., 1998. Apparent protein digestibility and mineral availabilities in various feed ingredients for salmonid feeds. Aquaculture 159, 177-202.

Tan, B., Mai, K., Liufu, Z., 2001. Response of juvenile abalone, *Haliotis discus hannai*, to dietary calcium, phosphorus and calcium/phosphorus ratio. Aquaculture 198, 141-158.

Velaco, M., Lawrence, A.L., Neill, W.H., 1998. Effects of dietary phosphorus level and inorganic source on survival and growth of *Penaeus vannamei* postlarvae in zero-water exchange culture tanks. Aquat. Living Resour. 11, 29-33.

CHAPTER NINE

Optimum dietary crude protein level for growth in South African abalone *(Haliotis midae* L.)

Sales, J., Truter, P.J., Britz, P.J., 2001. Optimum dietary crude protein level for growth in South African abalone (*Haliotis midae* L.). Aquaculture Nutrition (Submitted for publication, 02/04/2001).

Abstract

A 95-day feeding trial was conducted to evaluate the effects of dietary crude protein level on live weight gain and protein gain of juvenile (4.89 ± 0.57 g) South African abalone (*Haliotis midae*). Six semi-purified diets containing casein, fish meal and cottonseed meal as protein sources, and with crude protein levels ranging from 5.48-47.92 % on a dry matter basis, were fed to four tanks containing 30 abalone each in a continuous flow system. No differences ($P > 0.05$) were found in moisture, ash or lipid content of soft-body tissue as dietary crude protein level increased, indicating that differences ($P < 0.05$) in soft-body protein content was solely due to dietary crude protein level. The relationships between dietary crude protein level and live weight and protein gain were analysed by broken-line and second-order polynomial regression models. Based on live weight gain, 35.87 % dietary protein from good quality sources is recommended for maximum growth of juvenile *H. midae*, while, if dietary protein is reduced to 28.07 %, growth will be depressed by 5 %.

1. Introduction

Protein has been given priority in nutritional requirement studies because it is the principal diet component for animal growth, and is the highest cost consideration in commercial feeds (Lim et al., 1979; Mai et al, 1995). Requirement is most commonly understand to mean a minimal percentage of protein needed for optimal growth, however, requirement should rather be termed "optimal level" because a "true" requirement is the minimal amount of protein needed per animal per day (Guillaume, 1997). Investigations into the quantitative dietary crude protein requirements of abalone have been limited to the use of sole protein sources in the experimental diets (Ogino and Kato, 1964; Uki et al., 1986; Taylor, 1992; Britz, 1996), although compound diets have been used by Mai et al. (1995) and Coote et al. (2000). Most previous studies attempted to use diets with an amino acid profile similar to the abalone soft-body tissue profile, and some included crystalline amino acids or used gelatine to compensate for arginine deficiency. However, crystalline amino acids are prone to leaching in abalone diets which remain for prolonged periods in water and there is doubt about the acceptability of gelatine by abalone (Mai et al., 1995; Coote et al., 2000; Shipton, 2000).

This study was designed to examine the effect of dietary crude protein levels on live weight gain, protein gain and carcass composition of juvenile *H. midae* with diets containing several highly digestible protein sources (casein, fish meal, cottonseed meal) without supplementation of diets with crystalline amino acids.

2. Material and methods

2.1. Experimental diets

Six diets (Table 1) were obtained by blending a basal diet (low in crude protein) with a summit diet (high in crude protein) in appropriate proportions on the principles described by Pilbrow and Morris (1974) in order to get final determined crude protein contents of 5.48, 13.41, 21.78, 28.58, 39.38 and 47.92 % on a dry matter basis. The basal and summit diets were formulated to contain similar gross energy and crude lipid contents. Casein, assumed to be highly digestible by *H. midae*, was used as the main protein source.

Table 1
Ingredient and nutrient composition of the basal and summit diets used to obtain the six experimental diets (dry matter basis)

Ingredient (%)	Basal	Summit	Ratio[a]
Casein[b]	4.3	47.9	
Fish meal[c]	1.0	7.0	
Cottonseed meal[d]	0.9	0.0	
Pre-gelatinized maize starch[e]	87.6	25.7	
Fish oil[f]	1.7	0.0	
Vitamin/mineral mixture[g,h]	4.0	4.0	
Mono calcium phosphate[i]	0.5	0.5	
Bentonite[j]	0.0	14.9	
Determined nutrient analysis (%)			
Crude protein	5.5	47.9	
Gross energy (MJ/kg)	16.1	16.9	
Crude lipid	0.3	0.3	
Ash	3.9	17.8	
Calculated amino acid composition (%)			
Essential			
Arginine	0.2	2.0	127
Histidine	0.2	1.4	29
Isoleucine	0.3	3.0	66
Leucine	0.5	4.7	111
Lysine	0.4	4.0	100
Phenylalanine	0.3	2.6	63
Methionine	0.1	1.0	34
Threonine	0.2	1.9	80
Valine	0.3	3.3	74
Non-essential			
Aspartic acid	0.4	3.9	
Serine	0.2	1.8	
Glutamic acid	1.2	11.7	
Proline	0.5	5.2	
Glycine	0.1	1.1	
Alanine	0.2	1.7	
Tyrosine	0.2	2.4	

[a] Ratio of essential amino acids relative to lysine in soft-body tissue of *H. midae* (Knauer et al., 1995).
[b] Sigma Chemicals (St. Louis, MO, USA).
[c] Low-temperature dried fish meal (Danish 999 LT), Esbjerg Fiskeindustri a.m.b.a. (Esbjerg, Danmark).
[d] Pre-pressed, solvent extracted, dehulled, Western Province Corp. (Roggebaai, South Africa).
[e] Source priority Sea Plant Products (Hermanus, South Africa).
[f] Marinol-R, Marine Oil Refiners (Cape Town, South Africa).
[g] Per kg of feed: retinol, 12000 IU; cholecalciferol, 1800 IU; α-tocopherol, 150 mg; menadione, 5 mg; thiamin, 20 mg; riboflavin, 25 mg; pyridoxine, 20 mg; Vit. B_{12}, 0.04 mg; niacin, 150 mg; Ca-pantothenate, 50 mg; folic acid, 5 mg; biotin, 0.8 mg; ascorbic acid, 750 mg; inositol, 200; manganese, 150 mg; iron, 25 mg; zinc, 25 mg; copper, 70 mg; cobalt, 2 mg; iodine, 1 mg.
[h] Epol (Pretoria, South Africa).
[i] Kynoch Feeds (Randburg, South Africa).
[j] Boland Base Minerals (Milnerton, South Africa).

The essential amino acid profile of diets relative to lysine was, with the exception of arginine and threonine, in agreement with the essential amino acid profile of soft-body tissue of *H. midae* (Knauer et al., 1985). Shipton (2000) concluded that lysine was probably the first limiting amino acid in *H. midae*.

Food preparation was done as described by Sales and Britz (2001). All dry ingredients were mixed in a commercial food mixer for 30 min, whereafter oil was gradually added, while mixing constantly. Eight five ml of water/100 g of feed was slowly blended into the mix, resulting in a suitable textured dough. This was further processed through a cold-extruder. Drying was carried out in a convection oven at 35 °C for 48 h. The dry product was cut into 1 x 1 cm pieces and stored at 4 °C until used.

2.2. Animal rearing

A continuous flow system, connected to the main water supply of a commercial South African abalone farm, was used. The system comprised three rows of eight 45 L (39 x 39 x 30 cm) HDPE tanks. Seawater was filtered to 40 μm using a drumfilter and delivered to each tank at a flow rate of 1 l/min. One airline in each tank was used to aerate water. Water temperature during the experiment was 15.7 ± 2.2 ^{0}C. Similar size juvenile *H. midae* (4.89 ± 0.57 g) from the same cohort were selected from the grow-out system on the farm and assigned to the rearing system using a randomised block design with four blocks, six treatments and four replicates per treatment. These animals had been reared on commercial diets for about 16 months. Fifty abalone were selected randomly at the start of the experiment and stored at -20 ^{0}C for analysis for carcass composition. The abalone were starved for two days and then fed 10 % of their body weight every second day for the duration of the experiment. Tanks were cleaned twice a week. The feeding experiment was run for 95 days.

2.3. Carcass and chemical analysis of abalone and diets

Fifteen animals from each replicate were frozen at the end of the experiment for chemical analysis. Initial and final samples were slightly thawed, and shell and soft-body were separated. Soft-body to shell ratio (SB/S ratio, w/w) was computed to provide an index of nutritional status for abalone (Mai et al., 1995). Soft-body tissue was ground through a meat mincer, whereafter a sample from each replicate was dried at 105 °C for 24 h. Ashing was

performed thereafter at 550 °C for 12 h. The rest of each soft-body sample was freeze-dried and ground into fine powder for analyses of crude protein and crude lipid. Crude protein (*N* x 6.25) was done using the micro-Kjeldahl technique and crude lipid after extraction with petroleum ether (boiling point 40-60 °C) by the Soxhlet method. Gross energy content of diets was performed by direct combustion in an adiabatic calorimeter. The mean gains in live weight and protein were calculated according to Mai et al. (1995):

$$\text{MWG (g/abalone)} = W_t - W_i$$

$$\text{MPG (g/abalone)} = SB_t\,(1-M_t)\,Pt - SB_i\,(1-M_i)\,P_i$$

Where MWG is mean live weight gain; W_i, W_t is initial or final mean live weight (g); MPG is mean protein gain; SB_i, SB_t is initial or final soft-body weight (g); $SB_{i,t} = (R_{i,t} \times W_{i,t})/(1+R_{i,t})$; R_i, R_t is initial or final soft-body to shell ratio (SB/S ratio); M_i, M_t is initial or final moisture level in soft-body (%); P_i, P_t is initial or final protein level in soft-body (%).

2.4. Statistical analysis

Percentage data for survival were square-root arcsine transformed prior to analysis. Data from each treatment were subjected to two way ANOVA. Tukey's test was used to compare mean values between individual treatments where appropriated. Protein requirements were estimated from live weight gain and protein gain using the broken-line model (Robbins et al., 1979) and the second-order polynomial regression analysis model (Lovell, 1989).

3. Results

Live weight gain and percent survival of abalone fed the experimental diets are presented in Table 2.

Live weight gain reached a maximum value at a protein level of 21.78 % whereafter it was not influenced ($P > 0.05$) by dietary crude protein level. Survival was not related to dietary crude protein level, and mostly resulted from abalone escaped from the experimental system.

Table 2

Effect of dietary crude protein level (dry matter basis) on growth (wet weight) of South African abalone, *H. midae* (means ± S.E., $n = 4$)

Protein level (%)	Initial live weight (g/abalone)	Final live weight (g/abalone)	Total live weight gain (g/abalone)	Survival (%)
5.48	4.81 ± 0.07	5.93^{a} ± 0.09	1.12^{a} ±0.03	99.17 ± 0.83
13.41	4.88 ± 0.03	7.45^{b} ± 0.09	2.58^{b} ± 0.11	98.33 ± 0.96
21.78	4.99 ± 0.05	8.58^{c} ± 0.08	3.59^{c} ± 0.10	100.00 ± 0.00
28.58	4.79 ± 0.07	8.94^{cd} ± 0.17	4.14^{d} ± 0.11	95.83 ± 2.10
39.38	4.99 ± 0.03	9.12^{d} ± 0.16	4.13^{d} ± 0.15	99.17 ± 0.83
47.92	4.90 ± 0.04	8.72^{cd} ± 0.09	3.82^{cd} ± 0.12	100.00 ± 0.00

Means in the same column with different superscripts (a, b) are statistically different ($P < 0.05$).

Live weight, soft-body weight and shell weight of animals analysed for chemical composition followed the same patterns over dietary crude protein level (Table 3). However, soft-body to shell ratios (SB/S ratio, w/w) did not show any differences ($P > 0.05$) among different dietary crude protein levels.

Table 3

Effect of dietary crude protein level (dry matter basis) on carcass characteristics (wet weight) of South African abalone, *H. midae* (means ± S.E., $n = 4$)

Protein level (%)	Total live weight (g/abalone)	Soft-body weight (g/abalone)	Shell weight (g/abalone)	SB/S Ratio
5.48	5.08^{a} ± 0.15	3.76^{a} ± 0.13	1.31^{a} ± 0.02	2.90 ± 0.07
13.41	6.31^{b} ± 0.17	4.76^{b} ± 0.13	1.55^{b} ± 0.05	3.11 ± 0.08
21.78	6.96^{bc} ± 0.23	5.27^{bc} ± 0.23	1.69^{bc} ± 0.05	3.15 ± 0.19
28.58	7.25^{c} ± 0.12	5.51^{c} ± 0.17	1.75^{c} ± 0.02	3.16 ± 0.08
39.38	7.47^{c} ± 0.17	5.62^{c} ± 0.12	1.85^{c} ± 0.06	3.07 ± 0.08
47.92	6.77^{bc} ± 0.03	5.08^{bc} ± 0.02	1.69^{bc} ± 0.03	3.01 ± 0.05

Means in the same row values with different superscripts (a, b, c) are statistically different ($P < 0.05$).

Of the chemical components of soft-body weight only protein was influenced ($P < 0.05$) by dietary crude protein level (Table 4).

Table 4

Effect of dietary crude protein level on carcass composition of South African abalone, *H. midae* (dry matter basis; means ± S.E.; $n = 4$)

Protein level (%)	Moisture (%)	Ash (%)	Lipids (%)	Protein (%)	Protein gain (g/abalone)
5.48	75.00 ± 0.66	10.32 ± 0.21	2.00 ± 0.05	56.07^{a} ±0.91	0.15^{a} ± 0.02
13.41	76.82 ± 0.32	10.92 ± 0.13	1.88 ± 0.01	57.86^{ab} ± 0.67	0.26^{b} ± 0.01
21.78	76.90 ± 0.75	10.78 ± 0.50	1.87 ± 0.04	61.03^{bc} ± 1.11	0.37^{c} ± 0.01
28.58	77.30 ± 1.13	11.08 ± 0.83	1.91 ± 0.05	63.26^{cd} ± 0.55	0.42^{cd} ± 0.03
39.38	76.21 ± 0.46	10.55 ± 0.23	1.84 ± 0.02	64.85^{de} ± 0.23	0.49^{d} ± 0.03
47.92	76.82 ± 0.79	11.14 ± 0.25	1.85 ± 0.02	68.19^{e} ± 0.35	0.43^{cd} ± 0.02

Means in the same column with different superscripts (a, b, c, d, e) are statistically different ($P < 0.05$).

On the basis of live weight gain, the results estimated by the broken-line model presented a optimum dietary crude protein requirement of 25.54 % for *H. midae*, while analysis according to protein gain revealed a value of 26.91 % (Table 5). However, the second-order polynomial regression analysis showed a maximum live weight gain at 35.87 % dietary crude protein and maximum protein gain at 40.69 %. A correlation coefficient of 0.90 (n = 24; $P < 0.05$) was found between live weight gain and protein gain.

4. Discussion

Comparison of the estimates for different tests of goodness of fit for the two mathematical models used to determine optimum dietary crude protein requirements of *H. midae* indicated the second-order polynomial regression model was the more appropriate method to analyse data. Also, quite different optimum protein requirements were derived from the different models. From Tables 2 and 3 maximum growth in *H. midae* was obtained between 28.58 to 39.38 % dietary crude protein, indicating that the broken-line model could have underestimated requirements.

Table 5

Comparison of the dietary crude protein requirements of *H. midae* determined by the broken-line and second-order polynomial regression models

Statistical model	Based on weight gain	Based on protein gain
Broken-line analysis	25.54 %	26.91 %
Goodness of fit		
R^2	0.9633	0.8525
Absolute sum of squares	1.059	0.0512
$S_{y.x}$	0.2301	0.0506
Quadratic regression (X_1 - X_{max})	28.07–35.87 %	20.47–40.69 %
Goodness of fit		
R^2	0.9696	0.8723
Absolute sum of squares	0.8772	0.0443
$S_{y.x}$	0.2044	0.0459

Broken-line analysis, the most widely used method of evaluating dose-response data in nutrient requirement studies with aquatic species, frequently underestimates the requirement (Shearer, 2000). A reason stated by Morris (1983) is that broken-line analysis does not account for differences in maintenance needs and growth potentials among individuals in the experimental population. The second-order polynomial regression model has the advantage of being more accurate than other models when the relationship between dietary nutrient and growth data is curvilinear, and additionally can yield nutrient requirements for maximum and less than maximum rates of growth for both physiological and economic considerations (Lovell, 1989; Gurure et al., 1995; Mai et al., 1995). Thus, it is believed that the second-order polynomial regression model would yield more reliable and realistic values than the broken-line model in the present study.

Live weight gain is a reliable indicator for growth as long as the experimental variable is not expected to be affected by the composition of gain in the animal (Lovell, 1989). Only body protein was influenced ($P < 0.05$) by dietary crude protein levels in the present study, while moisture, ash and lipid were not affected ($P > 0.05$) by treatment. Furthermore, the R^2-values for both models indicated that a better fit was obtained for the data points when live weight gain, rather than protein gain was used. Therefore the wider

range between X_1 and X_{max} when protein gain was used. The conclusion thus made from this study is that live weight gain, rather than protein gain could be used as a non-destructive method to determine optimal dietary crude protein levels in *H. midae*.

The optimum dietary crude protein requirement according to live weight gain found in the present study (28.07–35.87 %) agrees well with values of 22.3–32.3 % and 23.3–35.6 % reported for *H. tuberculata* and *H. discus hannai*, respectively (Mai et al., 1995), 20 % found for *H. discus* (Ogino and Kato, 1964), 20-30 % for *H. discus hannai* (Uki et al., 1986), 30 % for *H. kamtschatkana* (Taylor, 1992) and 27 % for *H. laevigata* (Coote et al., 2000), but is lower than 47 % found for *H. midae* by Britz (1996). However, as stated by Mai et al. (1995), it is difficult to compare these values directly because of different diets, experimental animals and management regimes.

The diets used in the present were limiting in arginine when compared to the abalone soft-body amino acid profile, and could result in an overestimate of dietary crude protein requirements. However, evidence has been provided by Shipton (2000) that lysine, and not arginine, could be the first limiting amino acid in *H. midae*. Further studies should concentrate on identifying limiting amino acids for optimum growth of abalone and the correct proportions of essential amino acids in formulated feeds.

Acknowledgements

This study was sponsored by Sea Plant Products, Hermanus, South Africa, the South African Network for Coastal and Oceanographic Research, the National Research Foundation and Rhodes University Joint Research Committee. The first author was supported by a post-doctoral scholarship from the Claude Leon Harris Foundation.

References

Britz, P.J., 1996. Effect of dietary protein level on growth performance of South African abalone, *Haliotis midae*, fed fishmeal-based semi-purified diets. Aquaculture 140, 55-61.

Coote, T.A., Hone, P.W., Van Barneveld, R.J., Maguire, G.B., 2000. Optimal protein level in a semipurified diet for juvenile greenlip abalone *Haliotis laevigata*. Aquacult. Nutr. 6, 213-220.

Guillaume, J., 1997. Protein and amino acids. In: D'Abramo, L.R., Conklin, D.E., Akiyama, D.M. (Eds.), Crustacean Nutrition., Advances in World Aquaculture, Vol. 6, World Aquaculture Society, Louisiana, USA, pp. 26-50.

Gurure, R.M., Moccia, R.D., Atkinson, J.L., 1995. Optimal protein requirements of young Arctic charr (*Salvelinus alpinus*) fed practical diets. Aquacult. Nutr. 1, 227-234.

Knauer, J., Brady, D., Duncan, J.R., Hecht, T., 1995. Amino acid, fatty acid and mineral element profile of juvenile South African abalone, *Haliotis midae* L. Aquacult. Res. 26, 283-288.

Lim, C., Sukhawongs, S., Pascual, F.P., 1979. A preliminary study on the protein requirements of *Chanos chanos* (Forskal) fry in a controlled environment. Aquaculture 17, 195-201.

Lovell, T., 1989. Nutrition and Feeding of Fish. Von Nostrand Reinhold, New York, USA, 260 pp.

Mai, K., Mercer, J.P., Donlon, J., 1995. Comparative studies on the nutrition of two species of abalone, *Haliotis tuberculata* L. and *Haliotis discus hannai* Ino. IV. Optimum dietary protein level for growth. Aquaculture 136, 165-180.

Morris, T.R., 1983. The interpretation of response data from feeding trials. In: Haresign, W. (Ed.), Recent Advances in Animal Nutrition, Butterworths, London, pp. 1-11.

Ogina, C., Kato, N., 1964. Studies on the nutrition of abalone. II. Protein requirements of abalone, *Haliotis discus*. Bull. Jpn. Soc. Sci. Fish 30, 523-526 (In Japanese, with English abstract).

Pilbrow, P.J., Morris, T.R., 1974. Comparison of lysine requirements amongst 8 stocks of laying fowl. Brit. Poultr. Sci. 15, 51-73.

Robbins, K.R., Norton, H.W., Baker, D.H., 1979. Estimation of nutrient requirements from growth data. J. Nutr. 109, 1710-1714.

Sales, J., Britz, P.J., 2001. Evaluation of different markers to determine apparent nutrient digestibility coefficients of feed ingredients for South African abalone (*Haliotis midae* L.). Aquaculture 202, 113-129.

Shearer, K.D., 2000. Experimental design, statistical analysis and modelling of dietary nutrient requirement studies for fish: a critical review. Aquacult. Nutr. 6, 91-102.

Shipton, T.A., 2000. The protein requirements for the South African abalone, *Haliotis midae.* Ph.D Dissertation, Rhodes University, Grahamstown, South Africa, 146 pp.

Taylor, B., 1992. Abalone nutrition: optimum protein levels in artificial diets for *Haliotis kamtschatkana.* J. Shellfish Res. 11, 556.

Uki, N., Kemuyama, A., Watanabe, T., 1986. Optimum protein level in diets for abalone. Bull. Jpn. Soc. Sci. Fish 52, 1005-1012 (In Japanese, with English abstract).

CONCLUDING DISCUSSION

The main purpose of this study was to establish a suitable protocol for conducting further nutrient digestibility studies in *Haliotis midae*. Experience has shown that while the theory of digestibility is relatively simple, the technique can be problematic in producing reliable and replicable results. This is especially true for a slow feeding aquatic species such as *Haliotis* where the feed has to remain in water for prolonged periods before being consumed, leading to leaching of feed nutrients in water. Problems that needed to be overcome at the outset of this study included difficulties in accurately determining food intake and total collection of all faeces voided by *H. midae*. This limited the possibility of using the direct method as an accurate practical means of determining digestibility on a routine basis. Furthermore, faeces are produced in very small quantities by *H. midae*, which do not present discreet pellets or strings, and can be categorised into three different types. Evacuation times of the three faecal types overlap, eliminating the possibility of collecting a specific type of faeces over a specific time period. Also, previous use of the most commonly used marker in digestibility studies, chromic oxide (Cr_3O_2), was hampered by partitioning and preferential elimination (Shipton, 2000).

The first step in the evaluation of a suitable digestibility protocol for *H. midae* was to identify a suitable method to collect faeces in order to obtain adequate quantities for chemical analysis. Because of the very small quantities of faeces voided, faeces had to be collected from groups of animals held under the same conditions. As abalone are kept together in groups under farming conditions, this is arguably a better approach. Conical tanks, as used with fish digestibility studies, were found to be inappropriate for *H. midae* because of the occurrence of floating faeces that did not settle. The most suitable collection technique to maximize the amount of faeces collected and minimize disturbance of faeces during collection was found to be collection on an easily removable false mesh bottom. Adequate amounts of faeces (*ca* 2 g dry matter) for chemical analysis could be collected through this procedure in a relatively short time period (*ca* 14 days). The experimental setup is easily and cheaply replicable and can be operated in any small laboratory. If the

operator maintains very hygienic conditions and is consistent in the application of collection protocols, this collection method should produce comparable results in future studies.

Because of the problems associated with Cr_3O_2 as marker, markers that would possibly be representative of all types of faeces produced were evaluated. Acid-insoluble ash (AIA) was found as a reliable and replicable internal marker for digestibility studies with *H. midae*. The benefit of using this internal marker is that all faeces can be collected for analysis, thus more faeces is available for analysis, and faeces from different animals can be pooled, eliminating the time consuming process of sorting faeces. The technique was validated by a comparison to data obtained with total collection. Acid-insoluble ash presented realistic digestibility values in comparison to values obtained with other aquatic species, resulted in small variation between samples, and showed repeatability between studies. This can be regarded as the main contribution of this study, since sufficient faeces can be sampled with the use of the above technique to analyse a wide range of nutrients. While Cr_2O_3 was only evaluated for protein digestibility, AIA in this study was validated for the digestibility of dry matter, organic matter, protein, energy, phosphorus, lipid, starch and amino acids. Furthermore, a relatively simple analysis is required to quantify AIA, which does not require harmful chemicals, and there is no need to mix the marker into the feed. The use of the traditional Cr_2O_3 marker used in digestibility studies is now limited in most laboratories because of the need for expensive hoods to prevent explosions from the chemicals used in analysis (Austreng et al., 2000). No difference was observed in nutrient digestibility when faeces were washed in distilled water, indicating that the possibility of salt contamination from sea water did not have an influence on AIA content. However, a degree of skill and consistency is required during analysis to obtain replicable measurements. For example, it was found that the amount of water used for washing during analysis could have an influence on results.

The substitution technique used in fish digestibility studies (Cho et al., 1982) to evaluate nutrient digestibility of feed ingredients was unsuccessful when applied to *H. midae*. The nature of additional mathematical calculations required when using the substitution technique do not account for the possibility of differential nutrient leaching from feed or faeces. Another contributing factor to the failure of the substitution technique in *H. midae*

could be related to the possibility of associative effects between feed ingredients. Interactions, both synergistic and antagonistic, among feed ingredients with respect to nutrient availability once the ingredients are combined into a feed are poorly understood, but potentially critical to aquatic animal health (Hardy, 1999), and should be one of the major future goals of aquaculture nutrition studies (Lee and Lawrence, 1997).

The benthic nature of abalone imposes the additional requirements that the feed should sink rapidly and be water stable to promote maximum consumption. One way of improving water stability is by reducing ingredient particle size. However, grinding feed ingredients is time consuming and costly. Furthermore, the possible influence of ingredient particle size on the availability of nutrients to the animal has to be determined in establishment of a digestibility protocol. This study showed that an ingredient particle size of less than 450 µm in comparison to a size class less than 150 µm did not alter apparent nutrient digestibility or feed nutrient leaching in *H. midae*. However, a particle size of more than 450 µm decreased nutrient digestibility and increased nutrient leaching. This result is of direct value to feed manufacturers in that costs can be reduced by not having to grind feed ingredients to a particle size smaller than 450 µm.

The most important way to obtain good water stability in aquatic feeds is with the use of binders. Binders should not interfere with the animals' ability to digest the feed nor allow the nutritive constituents to be altered or destroyed. The most commonly used binder in manufacture of abalone feeds is pre-gelatinised starch. This ingredient is also used, together with a-cellulose, in most nutritional studies to manipulate diet composition. This study demonstrated the suitability of this technique in *H. midae* in that replacing 20, 30, 40 and 50 % of a-cellulose with pre-gelatinised starch did not have an influence on apparent digestibility of protein, fat, fibre or starch. Thus, pre-gelatinised starch and a-cellulose can be used as dietary fillers in further studies on protein digestibility and protein requirements of *H. midae*. However, although apparent fibre digestibility was over 60 %, this study did not quantify whether this fibre originated from the a-cellulose included in the experimental diets. Although apparent energy digestibility decreased as a-cellulose content increased, the utilization of a-cellulose as energy source in *H. midae* still remains unclear. Thus, the

possible energy contribution from a-cellulose should be investigated before this substance is used as filler in diets when energy is evaluated.

The nutritive value of protein in feed ingredients for monogastric animals is determined not only by the amino acid composition, but also by the bioavailability of the amino acids. Although growth assays (slope-ratio assays) are the most direct approach for the estimation of amino acid availability of protein in feed ingredients, since they provide a combined estimation of digestibility and post-absorptive utilisation of amino acids at the tissue level, these assays are expensive, time-consuming and provide an estimate of the availability of only one amino acid per assay. Measurement of digestibility is probably the most important determinant of amino acid availability (Sauer et al., 2000). By using the AIA protocol for *H. midae* digestibility studies established above, the availability of protein and amino acids of potential plant protein ingredients for use in *H. midae* feeds in South Africa was evaluated and compared to that of fish meal. As with other aquaculture species, soybean meal seems the most promising ingredient with a mean apparent amino acid availability of 96.86 %. The legumes lupins and faba beans presented values of above 93 % for mean apparent amino acid availability, while canola meal (94.21 %), sunflower meal (92.77 %), peanut meal (87.39 %) and cottonseed meal (85.15 %) presented values higher than that obtained for fish meal (82.75 %). These values are in general in accordance with values obtained with other aquatic species. This study not only identified the most suitable plant protein ingredients according to amino acid availability for inclusion in abalone feeds, but also provided values for inclusion in feed tables for use in least-cost computer programs. Furthermore, this technique can now be successfully applied as a research tool in further scientific studies on protein digestibility and amino acid availability in order to develop least-cost diet formulations for *H. midae.*

However, limitations on the use of plant protein ingredients in diet formulations because of imbalance amino acid composition and the presence of anti-nutritional factors are a well-known fact. While the problem of anti-nutritional factors can be solved through collaborative research efforts involving aquatic physiologists, nutritional biochemists, processing scientists, and aquaculture nutritionists (Hardy, 1999), the problem limitating

amino acids is complicated by the lack of response obtained in adding synthetic amino acids in abalone feeds due to leaching of these nutrients in water (Shipton, 2000).

Differences between apparent and true protein digestibility and amino acid availability were small, and of no practical value. Apparent protein digestibility was a good indicator ($r = 0.99$) of mean apparent amino acid availability of feed ingredients. This relation could be utilised in further studies to identify further protein ingredients in abalone feeds, but feed tables will necessitate the identification of amino acid availability. Although protein digestibility and amino acid availability values determined for single ingredients underestimated predicted values in compound diets, it is still within an acceptable range (1-7 % difference) for the formulation of diets. This underestimation again accentuates the possibility of differential feed, and especially faecal, nutrient losses in water, and the possibility of nutrient interactions between ingredients.

Phosphorus leaching is important for both nutritional applications and for assessing the potential impact of phosphorus releases into the environment. This study clearly demonstrated the impact of phosphorus losses through leaching. As much as 43.50 % of the natural phosphorus content of feed ingredients was lost after a compound formulated diet was subjected to leaching for 1 h under simulated farm conditions. The present results indicate that the less water-soluble calcium mono dibasic seems, according to dietary phosphorus leaching and apparent phosphorus availability, as the most suitable inorganic phosphorus source for inclusion in compound diets. However, the extremely high dietary phosphorus leaching implies a need for at least a two-fold over supply of phosphorus in the diet, which may lead to environmental pollution in sheltered bays. Urgent research is needed into techniques to reduce dietary phosphorus leaching, and thus improved intake by the animal. Once this is established, further research could be conducted on phosphorus availability of ingredients and phosphorus requirements of abalone.

Knowledge on the availability of nutrients from feed ingredients would be of no value if it could not be utilised to fulfill in the dietary requirements of the animal. Nutritional studies, especially in aquatic species, often start by investigation into an optimal dietary protein level. Therefore the traditional method of direct experimentation with graded levels of dietary crude protein was used in this study to establish the dietary protein necessary for

optimal growth in *H. midae*. The optimum crude protein level (28-36 %) agreed well with values obtained for *H. tuberculata* (22-32 %) and *H. discus hannai* (23-36 %; Mai et al., 1995). Assuming an apparent protein digestibility of 95 % for casein, and utilising values determined for fish meal (82 %) and cottonseed meal (86 %) in this study, this could be translated into an optimum digestible dietary protein content of 26-34 %. Since monogastric animals such as abalone require a balanced ratio of individual amino acids for optimal growth, determination of essential amino acid requirements should be considered to be the highest priority in further nutritional studies on abalone. However, attention should be given to ensure maximum intake of these nutrients by the animal before growth studies are conducted. Technologies applied in shrimp nutrition, such as pre-coating the amino acid mixture and use of efficient diet binders to reduce leaching losses of amino acids in amino acid test diets (Millamena et al, 1999), needs to be evaluated or tested in abalone nutrition studies.

In conclusion, this study was successful in developing a suitable protocol for digestibility studies in *H. midae* by fulfillment of the following objectives as stated in the introduction:

(1) Identification of a suitable faecal collection technique.

(2) Identification of a reliable, replicable and practical marker.

(3) Elimination of the substitution technique for use in evaluation of digestibility of protein and availability of amino acids in feed ingredients.

(4) Elimination of the possible influence of the use of pre-gelatinised maize and a-cellulose to manipulate diet composition on nutrient digestibility.

By establishment of a proper digestibility protocol it was possible to evaluate protein digestibility and amino acid availability of all plant protein ingredients currently utilised in the South African animal feed industry for *H. midae*. This lays a foundation for development of least-cost feed formulation for this species. In addition, the digestibility protocol also provides a basis for conducting scientifically sound digestibility studies with *H. midae*.

Suggestions for further research to reduce feed cost by effective use of feed ingredients should be:

(1) Techniques to improve dietary intake through the reduction of dietary leaching of especially amino acids and phosphorus.

(2) Identification of a suitable protocol to determine digestible energy content of individual feed ingredients for *H. midae*.

(3) Investigation of the possibility of associative effects between feed ingredients in compound diets.

(4) The influence on growth rate of formulating diets on a total versus a digestible nutrient content.

(5) The digestibility and utilisation of fibre in nutrition of *H. midae*.

References

Austreng, E., Storebakken, T., Thomassen, M.S., Refstie, S., Thomassen, Y., 2000. Evaluation of selected trivalent metal oxides as inert markers used to estimate apparent digestibility in salmonids. Aquaculture 188, 65-78.

Cho, C.Y., Slinger, S.J., Bayley, H.S., 1982. Bioenergetics of salmonid fishes: energy intake, expenditure and productivity. Comp. Biochem. Physiol. 73B, 25-41.

Hardy, R.W., 1999. Collaborative opportunities between fish nutrition and other disciplines in aqauculture: an overview. Aquaculture 177, 217-230.

Lee, P.G., Lawrence, A.L., 1997. Digestibility. In: D'Abramo, L.R., Conklin, D.E., Akiyama, D.M. (Eds.), Crustacean Nutrition, Advances in World Aquaculture, Vol. 6, World Aquaculture Society, Louisiana, USA, pp. 194-260.

Mai, K., Mercer, J.P., Donlon, J., 1995. Comparative studies on the nutrition of two species of abalone, *Haliotis tuberculata* L. and *Haliotis discus hannai* Ino. IV. Optimum dietary protein level for growth. Aquaculture 136, 165-180.

Millamena, O., Teruel, M.B., Kanazawa, A., Teshima, S., 1999. Quantitative dietary requirements of postlarval tiger shrimp, *Penaeus monodon*, for histidine, isoleucine, leucine, phenylalanine and tryptophan. Aquaculture 179, 169-179.

Refstie, S., Helland, S.J., Storebakken, T., 1997. Adaptation to soybean meal in diets for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 153, 263-272.

Refstie, S., Storebakken, T., Roem, A.J., 1998. Feed consumption and conversion in Atlantic salmon (*Salmo salar*) fed diets with fish meal, extracted soybean meal or soybean meal with reduced content of oligosaccharides, trypsin inhibitors, lectins and soya antigens. Aquaculture 162, 301-312.

Sauer, W.C., Fan, M.Z., Mosenthin, R., Drochner, W., 2000. Methods for measuring ileal amino acid digestibility in pigs. In: D'Mello, J.P.F. (Ed.), Farm Animal Metabolism and Nutrition, CABI Publishing, Wallingford, Oxfordshire, UK, pp. 279-306.

Shipton, T.A., 2000. The protein requirements for the South African abalone, *Haliotis midae*. Ph.D Dissertation, Rhodes University, Grahamstown, South Africa, 146 pp.