The use of terrestrial nutrition principles and techniques to advance Australian abalone aquaculture

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Summary

The development of an abalone aquaculture industry in Australia is entirely dependent on manufactured feeds because of restrictions on the use of algae as a source of nutrients. As a consequence, there is a need to formulate feeds that can cost–effectively sustain growth rates of farmed abalone. Unlike many other aquaculture species, abalone have the capacity to consume a wide range of diets, which made it possible to apply terrestrial nutrition principles to estimate the protein and energy digestibility coefficients of a range of feed ingredients and to determine whether they are additive. The optimum digestible protein to energy ratio for growth and requirements for specific amino acids and lipids were also determined. Diets formulated according to these data improved growth rates of commercially farmed abalone from 30–85 mm/day to 70–100 mm/day and reduced diet costs from 5.00–7.00 $/kg to 2.50–3.00 $/kg in less than 10 years.

Keywords: abalone, nutrient requirements, digestibility, protein, energy, amino acids, lipids

Introduction

Abalone are slow–growing, herbivorous marine gastropods. More than 100 species exist in waters ranging from tropical to cold. Culture of abalone first began in Japan more than 50 years ago and was mainly directed at ocean ranching for stock enhancement. Currently, abalone are cultured in several countries including Australia where two species are grown: the blacklip abalone (*Haliotis rubra*) and the greenlip abalone (*Haliotis laevigata*). The greenlip species is cultured in South Australia, Victoria, Tasmania and Western Australia; the blacklip species is cultured only in Victoria and Tasmania. A hybrid of the two species, known as the Tiger abalone, is also produced in Victoria and Tasmania. Unlike other countries, where abalone are grown in deep trough–like tanks and mainly fed macroalgae, the majority of Australian abalone culture occurs in land–based tanks and manufactured feed is used. The reasons for using manufactured feeds are that it is easier to store than algae, it does not introduce pests into the growing system, its nutritional profile can be manipulated to meet the requirements of the animal and it does not vary seasonally. In addition, the harvesting of algae from the wild is prohibited in Australia and culture of algae is difficult and labour intensive.

The feeding habits and digestive physiology of abalone, a bottom dwelling, grazing herbivore, are different to those of most fish. Not surprisingly, given their diet of macroalgae, abalone possess a very long gut to aid digestion and absorption of nutrients (3 times longer than their bodies; Cui *et al*. 2001). The first section of the digestive tract is the buccal region, which contains the mouth and radula. The radula is a ribbon–like membrane containing numerous chitinous teeth and is used to scrape, pierce, tear or cut off small pieces of food that are then directed in a continuous stream toward the digestive tract by conveyor belt like movements of the membrane. The food then enters the oesophagus, which contains many mucous cells that aid movement of the food into the crop (Harris 1994). The function of the crop is mainly that of storage but some breakdown of food occurs there, as it contains a large number of mucous– and secretory cells. Digesta then enters the stomach, which contains secretory cells, mucous cells, ciliated cells and phagocytes. The stomach chamber narrows into a heavily ciliated sac and then into the intestine. A large number of secretory cells were observed in the crop and stomach of *Haliotis laeviagata* by Harris (1994), who suggested these regions are important in digestion. Another study on the digestive physiology of *Haliotis discus hannai* showed that the intestine and the rectum play an important role in digestion and that extracellular digestion may occur in the lumen of the gut (Cui *et al*. 2001).

Because the digestive physiology and feeding habits of abalone differ from those of most fish, commercial fish feeds are deemed unsuitable for abalone. As a result, diets that meet the nutritional requirements of abalone and result in appropriate growth rates were developed. This involved the application of principles and techniques used for nutrition of terrestrial animals. Research was focused on the following areas: protein and energy digestibility of feed ingredients, whether digestibility coefficients are additive when used in compound diet formulations, optimum digestible protein to energy ratio for growth, essential amino acid requirements and requirements for lipids. The outcomes from this research have resulted in an improvement in commercial growth rates from 30–85 µm/day to 70–100 µm/day and a reduction in feed costs from 5.00–7.00 $/kg to 2.50–3.00 $/kg since 1994.

Ingredient digestibility

Maximum nutritional efficiency is attained by matching diet specifications as closely as possible to the animal's requirements at the lowest cost. Nutritionists generally use the following procedures to achieve maximum production efficiency: (1), the nutritional requirements of the animal are defined; (2), the nutritive value of individual ingredients to be used in the compound feeds are defined by applying previously determined digestibility or availability coefficients to measurements of chemical composition; (3), least–cost diets are formulated using linear programming techniques (Fleming *et al*. 1998). The nutritive value of an ingredient is often expressed in terms of the digestibility or availability of amino acids and energy. Digestibility may be estimated by the direct method, which involves measurement of feed consumed and collection of faeces produced, or by the indirect method, which involves either the addition of an indigestible marker such as chromic oxide to the feed or the use of an indigestible marker component of the feed such as acid–insoluble ash. Because of the logistical difficulties of determining the amount of feed consumed by abalone and of collecting faeces, the indirect chromic oxide technique, for which only a representative faecal sample is required, was used (Wee *et al.* 1992, 1994; Fleming *et al*. 1998; Vandepeer 2002a, Vandepeer and van Barneveld 2003). Digestibility tanks in which faecal samples were collected by settlement were used (Figure 1). The protein and energy digestibilities of a variety of ingredients were determined using this technique (Table 1).

Ingredient additivity

Digestibility coefficients for nutrients differ between feed components. In diet formulation, it is assumed that the nutritive value of individual diet ingredients will be the same irrespective of whether they are fed individually or with other ingredients in mixed diets (Fleming *et al*. 1998). However, studies with terrestrial animals have shown that the inclusion of dietary fibre may result in a depression of digestibility coefficients of the constituent ingredients. Laplace *et al*. (1989) found that the digestibility of crude protein and amino acids in growing pigs was less for diets containing wheat bran plus soybean hulls than for diets containing either wheat bran or soybean hulls. Similarly, van Barneveld *et al*. (1995) reported that growing pigs exhibited a linear decrease in lysine and energy digestibility in response to graded levels of a lupin non–starch polysaccharide (NSP) isolate.

To investigate the additivity of digestibility coefficients for abalone, Fleming *et al*. (1998) formulated a series of isonitrogenous diets containing semolina, fishmeal, barley, lupin kernel meal and various combinations of these ingredients. These diets were fed to juvenile greenlip abalone to determine the apparent faecal digestibility of nitrogen (N), amino acids and gross energy. The digestibility coefficients of the single–ingredient diets were used to predict the digestibilities of N, amino acids and gross energy in casein and mixed diets. The apparent faecal digestibilities of N, amino acids and gross energy of the constituent ingredients were additive. Vandepeer *et al*. (1999) investigated the use of apparent amino acid– and energy digestibility coefficients of individual ingredients to formulate diets for juvenile greenlip abalone to specified digestible protein and energy levels. Diets were formulated to the same nutritional profile and differed in the level of inclusion of semolina, soy flour, casein and fishmeal. Shell–length growth rate and wet weight did not differ among diets, confirming the findings of Fleming *et al*. (1998).

Optimum digestible protein:energy ratio

Growth rate in animals is dependent on protein deposition rate, which is determined by the intake of digestible protein and energy. Studies on pigs reported a linear–plateau relationship between protein intake and protein deposition (Campbell *et al*. 1984, 1985). These results gave rise to the development of the concept of protein and energy phases in protein deposition. In the first phase, digestible protein is the limiting factor; once the intake of digestible protein required for maximal growth is attained, digestible energy becomes limiting and no further increase in growth rate is possible at that energy level. A further increase in protein intake has no beneficial effect on protein gain and growth rate may decline if excess protein is ingested (Sugahara *et al*. 1969). If an additional quantity of protein–free energy is made available, a further increase in growth may be possible beyond the level of protein that previously elicited maximal growth. The maximum protein deposition capacity is dependent on factors such as sex, genotype and age.

The concept of an optimal digestible protein:energy ratio (DP:DE) for growth has been applied to both greenlip (Coote 1998) and blacklip abalone (Vandepeer 2002b). Coote (1998) fed juvenile

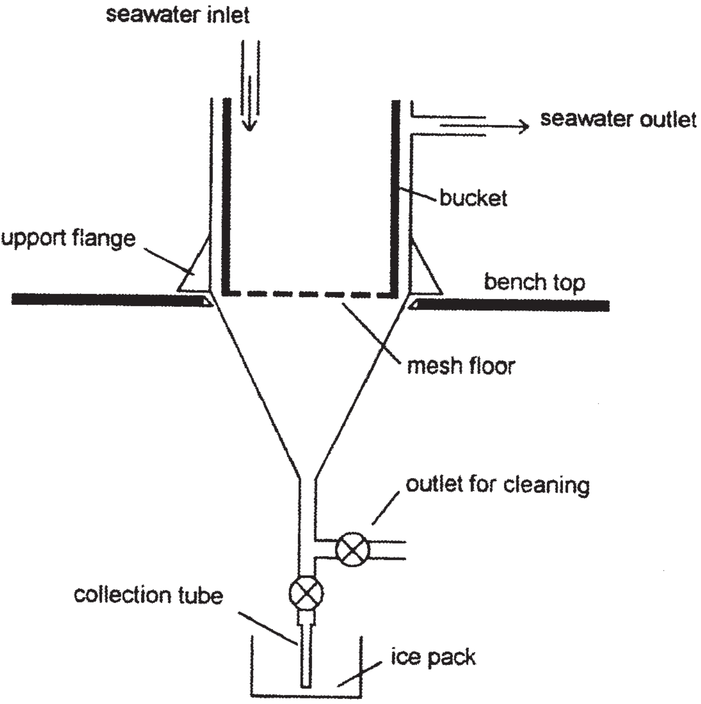

Figure 1 Tank for abalone digestibility experiments (Fleming *et al*. 1998).

Table 1 Apparent faecal protein and energy digestibility coefficients of various ingredients for greenlip abalone (Fleming 1998; Vandepeer 2002a, 2003).

Ingredient	**Protein digestibility**	**Energy digestibility**
Soy flour	0.82	0.78
Soybean meal	0.75	0.75
Field peas	0.89	0.49
Faba beans	0.95	0.65
Vetch	0.87	0.45
Canola meal	0.66	0.54
Sunflower meal	0.75	0.50
Maize meal	0.49	0.48
Whole *L. angustifolius*	0.91	0.50
Dehulled *L angustifolius*	0.92	0.82
Whole *L. luteus*	0.91	0.83
Mung beans	0.91	0.67
Meat and bone meal	0.34	0.24
Blood meal	0.08	0.10
Fishmeal	0.46	0.52
Casein	0.77	0.78
Skim milk powder	0.85	0.89
Whey powder	0.95	1.00
Pre–gelatinised maize starch	–	0.93
Semolina	0.71	0.25
Barley	0.54	0.25

greenlip abalone 20 different diets (10 digestible protein levels at two different digestible energy levels) for 61 days and found the specific growth rate of abalone fed the low energy diets (10.6 MJ/kg DE) was significantly affected by the DP:DE ratio but that of abalone fed high energy diets (12.6 MJ/kg DE) was not. Body weight gain of abalone fed low energy diets increased when the DP:DE ratio (g DP per MJ DE) was increased from 3.8 g to 17.9 and then decreased when the ratio was increased to 29.2. Second order polynomial regression of the data predicted that maximal growth of greenlip abalone would occur at a DP:DE ratio of 16.3 g, which corresponds to a digestible protein level of 17.3% at a dietary digestible energy content of 10.6 MJ/kg (Figure 2). It was hypothesized by Coote (1998) that the severe growth rate reduction that occurred when abalone were fed high energy diets was due to the lipid content of the diet. Excess dietary lipid may reduce nutrient availability by reducing protein digestibility. A later study by van Barneveld *et al*. (1998) on the effects of the type and level of oil in the diet supports this hypothesis. Vandepeer (2002b) used the same approach as Coote (1998) to investigate the optimum DP:DE ratio for growth of blacklip abalone. Growth rate did not differ between the two energy levels (9.5 MJ DE and 11.5 MJ DE) at the same level of digestible protein, but differences in growth rate were found among protein levels within an energy level. The responses to the high– and low energy diets fitted a quadratic model, and the optimum digestible protein level was predicted to be 22.2% protein, regardless of energy level. This equated to optimal DP:DE ratios for growth of 23.4 g DP per MJ DE (low energy diet) and 19.3 g DP per MJ DE (high energy diet).

Amino acid requirements

Knowledge of an animal's requirements for essential amino acids ensures that dietary protein is not wasted and facilitates establishment of an ideal protein ratio or amino acid balance. As amino acid requirements are related to energy intake, it is advantageous to express amino acid needs relative to dietary energy concentration (Fleming *et al*. 1996). Consideration of needs for each of the essential amino acids would make diet formulation very complex. To overcome this, terrestrial animal nutritionists have advocated the concept of an ideal protein that contains an ideal balance of amino acids (Cole 1980; ARC 1981; Fuller and Wang 1987; Baker and Chung 1992). Applying this concept to abalone, the concentrations of amino acids in the soft tissues should reflect the balance of amino acids required in the diet (Fleming *et al*. 1996). The requirement for each amino acid is expressed as a ratio to the most limiting amino acid in the diet. The expression of requirements in terms of the quantity of 'ideal' protein needed for maintenance and lean growth takes requirements for individual amino acids into account and simplifies the formulation of diets (McDonald *et al*. 1988). Because the most limiting amino acid is used as reference, all others are expressed as relative excesses. Lysine is conventionally used as the reference amino acid because many livestock species have a high requirement for lysine and it is normally the first and major limiting amino acid in cereal–based diets (Batterham 1992). As abalone are also fed cereal–based diets, it is likely that lysine is also the first limiting amino acid in the diet of this species.

Coote (1998) determined the dietary requirement of greenlip abalone for lysine from the data illustrated in Figure 2. Coote (1998) investigated the lysine requirement of abalone using diets supplemented with free lysine or covalently bound lysine. The free and covalently bound lysine were added at increasing levels to a basal diet at the expense of diatomaceous earth to create two series of eight diets each plus a control diet. Growth rate after 100 days was affected by supplementation with the free lysine–HCL, but the response was linear and thus an optimal dietary lysine requirement could not be established. Supplementation with covalently bound lysine resulted in a curvilinear growth rate response. A second order quadratic model was fitted to the data and maximal growth was predicted

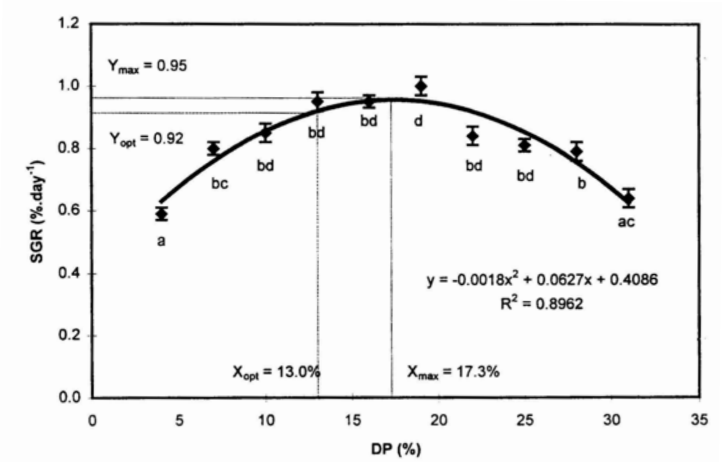

Figure 2 Specific growth rate (SGR) of abalone fed low energy (10.6 MJ/kg) diets containing various levels of digestible protein (Coote 1998).

to occur at a lysine content of 3.9% of dietary protein (Figure 3).

Lipid Requirements

van Barneveld *et al.* (1998) investigated the influence of oil inclusion level and type of oil on the faecal digestibility of N, amino acids and gross energy in greenlip abalone because Coote (1998) observed reduced growth in abalone fed high energy diets. Three levels of oil (30, 60 and 90 g/kg) and two types of oil were investigated (Jack Mackerel oil and olive oil). Inclusion of Jack Mackerel oil at levels greater than 60 g/kg decreased the digestion of dietary N and amino acids, and inclusion at levels greater than 30 g/kg reduced gross energy digestion of juvenile greenlip abalone. A decrease in N digestibility was observed with more than 30 g/kg of olive oil. The N digestibilities for diets containing 60 and 90 g/kg olive oil were lower than those for diets containing Jack Mackerel oil at the same levels. As with Jack Mackerel oil, gross energy digestibility was reduced in diets containing more than 30 g/kg of olive oil. These results are in agreement with those of Dunstan *et al.* (1994) who reported that dietary lipid levels greater than 4% suppressed growth of greenlip abalone. Dunstan (1994) also reported that the ratio of ω–3 to ω–6 fatty acids should be greater than 1.2 and that the eicosapentaenoic acid and docosahexanoic acid levels should both be greater than 0.3% of dry matter. Arachidonic acid supplementation gave better results than diets containing no long–chain polyunsaturated fatty acids but was not as good as diets containing long–chain ω–3 fatty acids.

Conclusions

With the development of an abalone aquaculture industry in Australia, a new feed was required that would meet the animal's nutritional requirements and provide satisfactory growth rates. Principles and practices from terrestrial nutrition research were applied to expedite the development of the diet and ensure that the most worthwhile research was conducted with the available funding. As a result, information now exists on the digestibility of a wide range of ingredients and their additivity in compound diets, the optimum digestible protein:energy ratio for growth of abalone, requirements for lysine and the optimum level and type of oil for growth. This research has resulted in significant improvements in manufactured feeds for abalone and improved growth rates, better feed conversion efficiency and a reduction in diet costs.

Future research is aimed at further improvement of growth performance of farmed abalone through the use of novel ingredients, improving water stability of diets and alternative diet production procedures.

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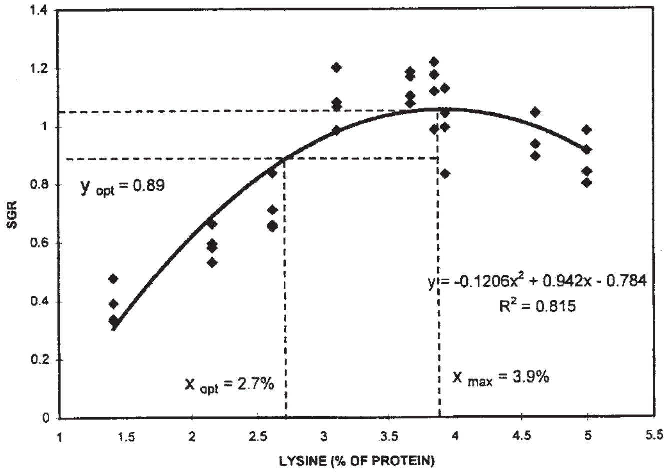

Figure 3 Specific growth rate (SGR) of abalone that were fed diets supplemented with covalently bound lysine (Coote 1998).

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