2000/2003 Abalone Aquaculture Subprogram: Adaptation of nutritional technologies developed for greenlip abalone for the production of suitable manufactured feeds for blacklip abalone

Ms Meegan Vandepeer, Dr Robert van Barneveld and Dr Ann Fleming

Published by the South Australian Research and Development Institute Aquatic Sciences Centre (SARDI)

© Fisheries Research and Development Corporation and the South Australian Research and Development Institute Aquatic Sciences Centre
July 2002

COPYRIGHT

This work is copyright. Except as permitted under the Copyright Act 1968 (Cth), no part of this publication may be reproduced by any process, electronic or otherwise, without the specific written permission of the copyright owners. Neither may information be stored electronically in any form whatsoever without such permission.

DISCLAIMER

The authors do not warrant that the information in this book is free from errors or omissions. The authors do not accept any form of liability, be it contractual, tortious or otherwise, for the contents of this book or for any consequences arising from its use or any reliance placed upon it. The information, opinions and advice contained in this book may not relate to, or be relevant to, a reader's particular circumstances. Opinions expressed by the authors are the individual opinions of those persons and are not necessarily those of the publisher or research provider.

ISBN 0 7308 5273 3

ACKNOWLEDGMENTS

Thanks are owed to Annette Doonan for providing technical assistance with the project including tagging, weighing, cleaning and feeding of the abalone, to Dr Yongshun Xiao for helping with the mathematics in Objective 2 and for Steven Clarke and Dr Robert van Barneveld for critical review of the report. Thanks also to Mark Gervis from Southern Ocean Mariculture for providing the blacklip abalone used in this project.

TABLE OF CONTENTS

NON TECHNICAL SUMMARY

2000/203 Abalone Aquaculture Subprogram: Adaptation of nutritional technologies developed for greenlip abalone for the production of suitable manufactured feeds for blacklip abalone

PRINCIPAL INVESTIGATOR: Meegan Vandepeer
ADDRESS: SARDI Aquatic Sciences Centre
2 Hamra Ave
West Beach SA 5024
Ph: 08 8200 2400 Fax: 08 8200 2481

OBJECTIVES:

1. To determine the digestibility of nutrients from a range of feedstuffs to be used in artificial diets for blacklip abalone in comparison with existing data on greenlip abalone.
2. To determine the optimal digestible protein:energy ratio for growth of blacklip abalone.

NON TECHNICAL SUMMARY:

Two species of abalone are currently being cultured in Australia, greenlip abalone, *Haliotis laevigata*, and blacklip abalone, *Haliotis rubra*. Blacklips, which are favoured by the Japanese market are cultured in Tasmania and Victoria, whilst greenlips, which are favoured by the Chinese market are cultured in South Australia, Victoria and Tasmania. Due to a moratorium on the collection of algae, abalone's natural diet, a manufactured diet is used to feed abalone. The development of cost effective diets that meet the nutritional needs of abalone has been a significant output from previously funded FRDC and CRC projects. Research to date has resulted in both a reduction of the cost of manufactured diets and an increase in abalone growth rates. All research that has been done to date, however, has been conducted on the greenlip species. It is not known whether the nutritional requirements of the blacklip species are the same as the greenlips and thus whether they should be fed the same manufactured diets. If the nutritional requirements of the blacklip species do differ from the greenlips then their potential maximum growth rate will not be reached by feeding them a diet based on the nutritional requirements of the greenlips. Given that it currently takes approximately 3 years for cultured abalone to reach market size, formulating diets that enable them to grow at their maximum potential rate is highly desirable.

The aim of this project was to determine the nutritional requirements of blacklip abalone to enable feed manufacturer's to

formulate diets specifically for them as has been done for greenlip abalone. This was done by first evaluating blacklip abalone's ability to digest protein and energy from 12 ingredients and comparing them against values obtained for greenlip abalone (objective 1). The second part of the project involved using the digestibility coefficients obtained in objective 1 to formulate 10 diets containing 5 levels of digestible protein and 2 levels of digestible energy (high and low). This was done so the protein:energy ratio at which optimal growth of blacklip abalone occurs could be determined (objective 2).

Of the twelve ingredients evaluated significant differences were found between blacklips and greenlips in their ability to digest the protein from 8 and energy from 10 of them. These results indicate that blacklip and greenlip abalone have different digestive capacities and therefore should be fed different manufactured diets. Based on the results it appears that blacklip abalone have a greater capacity than greenlip abalone to digest protein from ingredients that do not contain non-starch polysaccharides (non-plant based ingredients) and a greater capacity to digest cellulose than greenlip abalone. In comparison greenlip abalone seem to have a greater capacity than blacklip abalone to digest protein from ingredients that do contain non-starch polysaccharides (lupins & semolina). The protein and energy from milk products (casein, whey and skim milk powder) and energy from pregelatinised maize starch were found to be highly digestible for both abalone species whilst fishmeal and semolina, which have been traditionally used in manufactured diets for abalone, were both poorly digested by both species.

The level of digestible protein for optimal growth of blacklip abalone was 22.2 %. This was found for diets containing both high (11.5 $MJ.kg^{-1}$) and low (9.5 $MJ.kg^{-1}$) levels of digestible energy (DE). Since no difference was found in growth rate between abalone fed the diets containing high and low levels of DE it is recommended that feed manufacturers formulate blacklip abalone diets to contain 23.4g $DP.MJDE^{-1}$ to obtain maximum growth rates at least cost. The close match of the actual protein and energy digestibility value of each of the ten diets to the values formulated using the ingredient digestibility coefficients obtained in objective 1 indicate that the digestibility coefficients determined for blacklip abalone in this study are additive. Thus ingredient inclusion levels can be modified to accommodate changes in ingredient price and availability whilst maintaining optimal growth by formulating diets to 23.4 g $DP.MJDE^{-1}$ using the digestibility coefficients obtained in this study.

Given the very low energy digestibility of semolina for both greenlip and blacklip abalone, and the fact that it has traditionally been a major constituent of commercial abalone diets, it is recommended that future studies investigate methods to improve its digestibility for abalone. Possible mechanisms include the addition of enzymes to diets containing semolina (eg. amylase) or gelatinising semolina before incorporating into diets.

KEYWORDS: Blacklip abalone, digestibility, growth, protein, energy

BACKGROUND

To date, the majority of Australian commercial abalone aquaculture efforts have concentrated on two species of abalone, greenlips (*Haliotis laevigata*) and blacklips (*Haliotis rubra*). Both species have been targeted for development as they appeal to different parts of the large market for abalone in Asia. The Chinese market favours greenlip abalone while the Japanese market favours blacklip abalone, as they are similar to the native Japanese abalone, Ezo Awabi (*Haliotis discus hannai*). Abalone aquaculture currently occurs in three states in Australia.

South Australia has 14 on-shore and one off-shore abalone farms with production at approximately 50-70 tonnes for 1999/2000. It is the most developed of states with all commercial farms only growing greenlips. Expected production is 150 tonnes by 2004/2005 (based on current stock - note it takes 3 years to reach market size) which, at the current market price of $42/kg, would be worth $6.3 m.

Tasmania currently has 5 on-shore abalone farms and 10 off-shore farms with production at approximately 3.9 tonnes for 1999/2000. Although both greenlips and blacklips are being farmed, blacklip production is much greater than that of green (approximately 80:20). Expected production is 100 tonnes by 2004/2005 which, at the current market price of $42/kg, would be worth $4.2 m.

Victoria has 4 on-shore abalone farms and 3 off-shore farms with production at approximately 50 tonnes for 1999/2000. It is the second most developed of states after South Australia. As for Tasmania, both greenlips and blacklips are being farmed but again production of blacklips far exceeds that of greenlips (also approximately 80:20). Expected production is 225 tonnes by 2004/2005 which, at the current market price of $48/kg, would be worth $10.8 m.

The development of cost effective diets that meet the nutritional requirement of abalone has been a significant output from the previous nutrition projects done within the Abalone Aquaculture Subprogram. Research to date has reduced feed costs from $5-7 a kilogram to around $2 to 2.50 a kilogram, with a three-fold increase in annual growth rates. Farmers have recognised the research achievements of previous FRDC funded nutrition research as being the most significant input to their new industry (Jim Morrison, pers. comm.).

The nutrition research to date that forms the basis of current manufactured diet formulations has been done entirely on the greenlip species. It has been assumed that the nutritional requirements of the blacklip species are the same as the greenlips and thus the same diets have been used for both species. There is reason to believe, however, that the digestive capability of these two species are not the same. Kemp (Adelaide University Animal Science Department, unpublished data) has observed differences in enzyme activity (carbohydrases and alkaline and acid phosphatase) between greenlip and blacklip abalone fed the same commercial diets. In general there appears to be higher levels of the enzyme sucrase in blacklip abalone compared to greenlip, while greenlip abalone appear to have more beta-galactosidase activity (Kemp, Adelaide University Animal Science Department, pers. com.). Differences in amounts/types of enzymes would mean that blacklip and greenlip abalone would vary in their ability to digest certain feedstuffs.

Differences in the digestive capacity between blacklips and greenlips could possibly occur due to the fact that they are found in different habitats (Shepherd 1973) and therefore would be adapted to eating the algal species associated with their habitat. Blacklip abalone reportedly have a wider range of acceptable food algae than greenlip (Shepherd 1975). On Victorian and New South Wales coasts it feeds extensively on the fronds of the large kelp *Phyllospora comosa* while on Tasmanian coasts it often feeds on drifting blades of the giant kelp *Macrocystis pyrifera* (Shepherd 1975). In comparison greenlip abalone rejects nearly all species of brown and green algae, preferring red. Red and brown algae differ in many respects, particularly their storage and structural polysaccharides which require different enzymes for their breakdown. Thus if blacklip abalone encounter and thus consume more brown algae than reds in comparison to greenlip abalone, it would not be surprising if they had different types/quantities of digestive enzymes.

Since blacklip abalone is the predominant species cultured in two states in Australia, it is logical to determine if greenlips and blacklips have different nutritional requirements for growth. The determination of the nutritional requirements of blacklip abalone was voted by industry as the second highest research priority at the last Abalone Aquaculture Steering Committee meeting and is the aim of this project. Results from this research will be able to be used by the feed manufacturers to produce a diet specifically for blacklip abalone. This will ensure optimal growth rates and thus help reduce the time taken to reach market size.

NEED

One of the greatest drawbacks for abalone farmers is the time taken for the abalone to reach market size which is approximately 3 years. The longer the time the animal has to be held until sale the greater the cost to produce each animal due to costs associated with its maintenance (eg. feeding & cleaning) and thus the lower the return. In addition, there is an increased risk of abalone dying before making it to sale. Thus increasing abalone growth rates and shortening the time taken to reach market size is highly desirable.

Besides enhancement through genetic techniques, which can take several years, much faster short term improvements in growth rates can be achieved through better nutrition. Research over the last 5 years on the nutrition of greenlip abalone has resulted in improvements in growth rates from 30-80 μm/day in 1994 to 80-100 μm/day in 1999 and a reduction in the cost of commercial diets from $5.00 to $7.00 a kg in 1994 (Fleming et al. 1996) to $2.00 to $2.50 a kg in 1999. At present little research has been done on the nutrition of blacklip abalone and blacklip diets are formulated using information on the nutritional requirements of greenlip abalone. Blacklip abalone are the main species grown in Tasmania and Victoria. Tasmania currently has 5 on-shore abalone farms and 10 off-shore farms with production at approximately 3.9 tonnes for 1999/2000 while Victoria has 4 on-shore abalone farms and 3 off-shore farms with production at approximately 50 tonnes for 1999/2000. There is strong industry and scientific evidence to suggest that the nutritional requirements of blacklips differ from that of greenlips. This means that the manufactured diets that they are given at present are probably producing growth rates that are lower than could be potentially achieved if fed a diet that was matched to their nutritional specifications.

OBJECTIVES

1. To determine the digestibility of nutrients from a range of feedstuffs to be used in artificial diets for blacklip abalone in comparison with existing data on greenlip abalone.

2. To determine the optimal digestible protein:energy ratio for growth of blacklip abalone.

3. To develop nutritional specifications for blacklip abalone (for different age classes) leading to new diets that outperform current greenlip diets.

Changes to original objectives

Due to the nutritional specifications of current commercial diets being confidential it was decided that little value could be gained from completing the third objective. Without knowing the amount of digestible protein and energy and digestible protein:energy ratio of the commercial diets no interpretation and

thus gain in knowledge could be made from results of a laboratory growth trial comparing commercial diets to a diet developed using the nutritional specifications established from this current study.

OBJECTIVE 1: Determination of the digestibility of nutrients from a range of feedstuffs to be used in manufactured diets for blacklip abalone in comparison with existing data on greenlip abalone

ABSTRACT In this study the digestive capacity of blacklip abalone, *Haliotis rubra*, was compared against that of the greenlip abalone, *Haliotis laevigata*. This was done by assessing each abalone species' ability to digest the protein and energy from twelve ingredients; semolina, defatted soyflour, fishmeal, casein, pregelatinised maize starch, mung beans, whey powder, skim milk powder, whole lupins (*Lupinus angustifolius* and *Lupinus luteus*), dehulled lupins (*L. angustifolius*) and bull kelp (*Durvillea potatorum*). Significant differences were found between the two abalone species in their capacity to digest the protein and energy from some of the ingredients assessed. Based on the differences observed it was hypothesised that blacklip abalone might have a higher protease activity than greenlips whilst greenlip abalone might have a greater capacity to digest soluble non-starch polysaccharides. It also appears that blacklip abalone have a greater ability to digest cellulose than greenlips.

INTRODUCTION

Two species of abalone are currently being cultured in Australia, *Haliotis rubra*, commonly known as blacklip abalone and *Haliotis laevigata*, commonly known as greenlip abalone. Due to the desire to feed highly productive manufactured diets on abalone farms in Australia considerable research has been conducted on abalone nutrition. The majority of research that has been done to determine the abalone's nutritional requirements has been conducted on the greenlip species. It is not known however, whether the nutritional requirements of the blacklip species are the same as that of the greenlip's, and thus whether it is appropriate to feed them the same manufactured diet given that the diets have been formulated based on data on the nutritional requirements of greenlips. If the nutritional requirements of blacklip abalone are not the same as for greenlip abalone, then the growth rates obtained when feeding them manufactured diets formulated to the dietary specifications of greenlips may be considerably less than what could be achieved if formulated to their nutritional requirements. Given that feeding costs represent a considerable proportion of overall farm running costs then the implications of this scenario are obvious.

Studies done on the feeding habits of blacklip and greenlip abalone in the wild by Shepherd (1975) found that blacklip abalone *H. rubra*, has a wider range of acceptable food algae than the greenlip. On Victorian coasts it feeds extensively on the fronds of the large kelp *Phyllopsora comosa* while on Tasmanian coasts it often feeds on drifting blades of the giant kelp *Macrocystis pyrifera* as well as red algae. In comparison, the greenlip abalone is selective in the kind of algae that it eats, rejecting nearly all species of brown and green algae and preferring red algae and epiphytes on seagrass leaves (Shepherd 1975).

The structural and storage polysaccharides present in red and brown algae are quite different. The storage polysaccharides in brown algae are mannitol, a sugar alcohol, and laminaran, a glucan, while the storage polysaccharide for red algae is a starch known as floridean starch which is essentially similar to waxy starches found in land plants in that it consists almost wholly (99 %) of amylopectin. The cell wall of brown algae are two layered with an inner matrix of cellulose and microfibrils and outer layer of alginic acid and sulphated fucans (Stewart 1974). The cells walls of red algae consist of an inner rigid component made up of microfibrils and an outer more amorphous component consisting of mucilage or slime. The characteristic amorphous mucilages that make up most of the rest of the cell wall (up to 70 %) are usually sulfated galactan polymers (Schweiger 1978). The two largest groups are the agars and the carrageenans.

Since they differ in their structural and storage carbohydrates, different enzymes would be required to digest red and brown algae. If blacklip abalone consume a wider range of algae than greenlips then it would be expected that they might have a different digestive enzyme profile. If this were so, then they may also differ in their capacity to digest the nutrients from the ingredients that are used in manufactured diets, particularly different carbohydrate sources.

Results from comparative studies done on other abalone have shown there are differences between species in their utilisation of different algal species. Mercer et al. (1993) examined the nutritional value of eight algal diets for *H. tuberculata* and *H. discus hannai* by comparing feeding rates, growth and biochemical composition of the animals. The algae *A. esculenta, L. saccharina* and *U. lactuca* were found to have different dietary values for the two abalone species with quite different feeding rates and feed conversion efficiency values being reported. Significantly different responses in growth performance were also recorded from particular diets. The lowest growth rates recorded for *H. tuberculata* occurred when it was fed with *L. saccharina* or *C. crispus* while the lowest growth rates recorded for *H. discus hannai* occurred when it was fed with *U. lactuca*. Mercer et al. (1993) attributed the differences in dietary values of the algae to the two abalone species to differences in specific nutritive requirements and/or digestive physiology.

Given the differences observed in the natural diet of greenlip and blacklip abalone and the fact that the nutritive value of various algae has been shown to differ between other species of abalone, then it is possible that greenlip and blacklip abalone also have different nutrient requirements and/or digestive capacities. This has important implications given that feed costs represent a large proportion of current farm running costs in Australia and that our current manufactured diets are formulated based on results from research done to establish the nutrient requirement of the greenlip species. The objective of this experiment was to compare the protein and energy digestibility of a range of ingredients by blacklip and greenlip abalone and thus establish whether they differ in their digestive capacity.

MATERIALS & METHODS

Diet formulation and manufacture

Twelve diets were formulated (Table 1) to evaluate the protein and energy digestibility from: semolina, defatted soyflour, Tasmanian fishmeal, casein, whey powder, skim milk powder, whole mung beans (*Vigna radiata*), pregelatinised waxy maize starch, bull kelp (*Durvillea potatorum*), whole lupins (*Lupinus luteus* and *Lupinus angustifolius*) and dehulled lupins (*Lupinus angustifolius*) by greenlip and blacklip abalone. The crude protein and gross energy of each of these ingredients is reported in Table 2. Due to the wide range in crude protein levels of the ingredients being evaluated it was not practically possible to formulate the diets to be isonitrogenous. Before incorporation into diets the mung beans and lupins were crushed into a fine powder (< 500 μm) using a hammer mill. Each diet contained equivalent amount of vitamin C (ascorbic acid) and E (DL-alpha tocopheryl) and vitamin and mineral premixes as described by Uki et al. (1985). Sodium alginate was included in some diets to aid in binding. Kaolin and pregelatinised waxy maize starch were used in the diets as fillers. Chromic oxide was included at 0.5 % in each diet as an indigestible marker for use in subsequent digestibility calculations.

All diets were initially hand mixed and then mixed in a spiral action dough mixer ('Impastrice', Hill Equipment and Refrigeration, Adelaide, South Australia). The mixture was then fed through a commercial pasta machine (La Prestigiosa medium, IPA, Vicenza, Italy) where it was made into 300 mm long strips using a die with slots 18 mm x 1.5 mm. The strips were dried on mesh trays overnight in a forced draft oven at 55 °C. They were then broken into 3 pieces prior to feeding.

Diet allocation

Each diet was randomly allocated to 3 digestibility tanks to provide three replicates per diet. As there were only 18 tanks in total this meant that there was four separate collection periods, two for the blacklip and two for the greenlip abalone with 6 diets (ingredients) being evaluated in each collection period.

Abalone and feeding

Juvenile greenlip and blacklip abalone (shell length 40-60 mm) were used in the experiments. The abalone had been obtained from commercial hatcheries and raised on manufactured abalone feed. The abalone were pre-conditioned for one week on the test diet assigned to their tank. During both the pre-conditioning and experimental periods the animals were fed to excess every day at approximately 1700 hrs.

Tanks and collection system

Conical shaped digestibility tanks (44 l) were used. Abalone were housed in 20 L buckets (approximately 80 –100 per bucket) that fitted inside the tanks. All the buckets were fitted with plastic mesh bottoms (1.3 cm x 1.5 cm mesh)

allowing containment of the abalone while permitting faeces to drop into the collection tube at the base of the tank. Three 25 cm lengths of PVC pipe (8 cm in diameter) were placed in the buckets as shelters for the abalone. Attached to the bottom of each digestibility tank was a screw-on collection tube (11 cm long, 15 mm diameter). Tanks were on a flow-through water system at a rate of about 2 L/min. The seawater was filtered to 30 μm by primary sand filters, then to 10 μm by secondary composite sand filters before entering the tanks. Aeration was supplied at 0.5 L/min to each tank at all times by an air stone. Water temperature and lighting were controlled during the experiment with temperature maintained at 18.0 °C ± 1.0 and a light regime of 12 h light: 12 h dark. Salinity was 35-36 ‰ throughout the experiment.

Table 1
Composition of experimental diets (g/kg, air dry basis)

	Diet											
Ingredient	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Semolina	400.0	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,
Defatted soyflour	,,	625.0	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,
Tasmanian Fishmeal	,,	,,	420.8	,,	,,	,,	,,	,,	,,	,,	,,	,,
Casein	,,	,,	,,	347.6	,,	,,	,,	,,	,,	,,	,,	,,
Pregelled Starch	189.4	214.4	418.6	200.0	489.4	158.7	289.4	150.0	150.0	374.8	100.0	100.0
Mung beans™	,,	,,	,,	,,	,,	630.7	,,	,,	,,	,,	,,	,,
Bull kelp×	,,	,,	,,	,,	,,	,,	500.0	,,	,,	,,	,,	,,
Whey	,,	,,	,,	,,	,,	,,	,,	600.0	,,	,,	,,	,,
Skim milk powder	,,	,,	,,	,,	,,	,,	,,	,,	600.0	,,	,,	,,
Lupin 1∇	,,	,,	,,	,,	,,	,,	,,	,,	,,	389.6	,,	,,
Lupin 2(	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	421.1	,,
Lupin 3|	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	500.0
Jack Mackerel oil·	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	,,	20.0
Mineral premixℜ	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Vitamin premixℜ	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Vitamin C	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin E	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Sodium alginate	,,	,,	,,	,,	,,	,,	,,	,,	,,	5.0	,,	,,
Kaolin	400.0	150.0	150.0	441.8	500.0	200.0	200.0	239.4	239.4	200.0	448.4	369.4
Chromic oxide	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0

™ Whole *Vigna radiata*
× *Durvillea potatorum*
∇ Whole *L. luteus*
(Dehulled *L. angustifolius*
| Whole *L. angustifolius*
· *Trachurus declivis* (Triabunna Fish Oils, Triabunna, Tasmania)
ℜ Vitamin and mineral premixes as described by Uki et al. (1985)

Table 2
Protein (g/kg, air-dry basis) and energy (MJ/kg, air-dry basis) content of the 12 ingredients used in the experimental diets

Ingredient	Crude protein (N x 6.25)	Gross energy (MJ/kg)
Semolina	104.0	15.51
Defatted soyflour	480.0	17.45
Fishmeal	713.0	18.71
Casein	863.0	22.00
Pregelled Starch	3.1	15.65
Mung beans	253.7	16.54
Bull kelp	69.0	10.77
Whey	135.0	15.20
Skim milk powder	361.0	17.26
Whole *L. luteus*	385.0	18.03
Dehulled *L. angustifolius*	380.0	18.28
Whole *L. angustifolius*	320.0	17.74

Faecal collection

Faeces were collected by settlement every day until 5-6 g of faeces (dry weight) was collected for each replicate sample. This took approximately 2 weeks. On each day of faecal collection the buckets containing the abalone were removed and the digestibility tanks were drained of water and all fittings were cleaned of faeces and uneaten feed. Following cleaning, the tanks were refilled and the buckets replaced. Collection tubes were fitted by 0900 hrs. A small foam container was placed underneath each tube and filled with ice to keep the collecting faeces cold and thus reduce degradation by microbes. The faeces were collected from the tubes at 1630 hrs by gently pouring the contents onto a 1 mm diameter mesh. The mesh was then placed into a petri dish and frozen at -30° C. The following day the frozen faecal sample was scraped off the mesh, pooled into a composite sample, and stored in the freezer until required for analysis. Prior to analysis the samples were freeze-dried and ground with a mortar and pestle.

Chemical analyses

Gross energy was determined by a Parr 1281 bomb calorimeter (Parr Instrument Company, Moline, Illinois). Crude protein was determined by the combustion method using a LECO® CN-2000 Carbon and Nitrogen Analyser (RACI 1999).

Chromic oxide was determined using atomic absorption spectroscopy based on a modification of the methods described by Hillebrand et al. (1953). The modified methodology involved preliminary ignition of the sample at 500°C to remove organic material and the dissolution of the sample in hydrochloric acid instead of sulphuric acid (M. Frith, personal communication, University of Tasmania, Launceston, Australia).

Digestibility determination

The apparent digestibilities of nutrients in the diets were calculated using the following formula (Hardy 1997):

$$Apparent\ digestibility = 1 - \left(\frac{Cr_{diet} \times Nutrient_{faeces}}{Cr_{faeces} \times Nutrient_{diet}} \right)$$

where *Cr* is chromium content and *Nutrient* is nutrient or energy content of the diet.

Statistical Analysis

The data were analysed by analysis of variance using a generalised linear model (SAS Institute Inc. 1988) under the assumption that the dependent variables followed normal distributions with a constant variance. Within species treatment means for nutrient digestibility of the twelve ingredients were compared by least significant difference.

RESULTS

Significant differences in apparent faecal digestibility of both protein and energy were found between blacklip and greenlip abalone for some of the ingredients evaluated (Table 3). Significant differences in protein and energy digestibility were also found within each species among ingredients (Table 3).

With respect to gross energy digestibility, blacklip abalone digested the energy from whole *angustifolius* lupins, fishmeal and skim milk powder significantly better than greenlip abalone and greenlip abalone digested the energy from whey, bull kelp and dehulled *angustifolius* lupins significantly better than blacklip abalone (Table 3). No significant difference in gross energy digestibility of semolina, defatted soyflour, casein, pregelatinised maize starch, mung beans and *luteus* lupins was found between the two species (Table 3).

Greater differences were found in the species capacity to digest protein from the ingredients with statistically similar values only being obtained for mung beans, whey, and *luteus* lupins (Table 3). Blacklip abalone digested significantly more protein from defatted soyflour, fishmeal, casein, bull kelp and skim milk than greenlip abalone, whilst greenlip abalone digested significantly more protein than blacklip abalone from semolina, dehulled and whole *angustifolius* lupins (Table 3).

Comparisons among ingredients within species showed that there were significant differences in their apparent protein and energy digestibility for both species of abalone (Table 3). Whey was the most digestible ingredient, having significantly higher protein and energy digestibility than any other ingredient evaluated for both blacklip and greenlip abalone ($P < 0.05$). Bull kelp was found to be the ingredient containing the least digestible protein for both species abalone ($P < 0.001$), while semolina was found to be the ingredient containing the least digestible energy for both species of abalone ($P < 0.001$).

Table 3
Comparison of the faecal apparent digestibility coefficients of protein (PD) and energy (GED) from 12 different ingredients fed to blacklip (*Haliotis rubra*) and greenlip (*Haliotis laevigata*) abalone

Ingredient	PD blacklip abalone	PD greenlip abalone	$F_{1,4}$	P	SEM	GED blacklip abalone	GED greenlip abalone	$F_{1,4}$	P	SEM
Semolina	0.62^{h}	0.84^{c}	441	***	0.762	0.30^{h}	0.34^{g}	5.49	NS	1.265
Defatted soyflour	0.83^{f}	0.82^{c}	18.38	**	0.730	0.83^{d}	0.78^{c}	0.73	NS	1.507
Fishmeal	0.56^{i}	0.46^{e}	27.72	**	1.382	0.63^{g}	0.52^{f}	48.09	*	1.144
Casein	0.82^{g}	0.77^{d}	27.42	**	0.624	0.79^{e}	0.78^{d}	4.02	NS	0.579
Pregelled Starch	,,	,,	,,	,,	,,	0.92^{c}	0.93^{b}	1.80	NS	0.647
Mung beans	0.89^{d}	0.91^{b}	5.13	NS	0.630	0.65^{g}	0.67^{e}	2.40	NS	0.986
Bull kelp	0.46^{j}	0.23^{f}	105	***	1.600	0.75^{f}	0.81^{c}	29.45	*	0.805
Whey	0.96^{a}	0.95^{a}	1.46	NS	0.373	0.99^{a}	1.00^{a}	43.20	*	0.106
Skim milk powder	0.94^{b}	0.85^{c}	510	***	0.286	0.95^{b}	0.89^{b}	1338	***	0.101
Lupin 1$^{\nabla}$	0.91^{c}	0.91^{b}	0.03	NS	0.804	0.79^{e}	0.83^{c}	2.83	NS	1.780
Lupin 2$^{(}$	0.85^{e}	0.92^{b}	723	***	0.211	0.70^{f}	0.82^{c}	66.19	**	1.169
Lupin 3$^{\|}$	0.84^{ef}	0.91^{b}	371	***	0.284	0.63^{g}	0.50^{f}	202	***	0.682

NS, not significant
* $P < 0.05$
** $P < 0.01$
*** $P < 0.001$
$^{a-b}$ Within a column, ingredient digestibility coefficients with different superscripts differ significantly ($p < 0.05$)
$^{\nabla}$ Whole *L. luteus*
$^{(}$ Dehulled *L. angustifolius*
$^{|}$ Whole *L. angustifolius*

DISCUSSION

The results from the current experiment demonstrate that blacklip and greenlip abalone do differ in their digestive capacity. Significant differences were found in their protein and energy digestibility of several ingredients.

With regard to protein digestibility it is interesting to note that the ingredients which blacklip abalone could digest significantly more protein from than greenlip abalone (defatted soyflour, fishmeal, bull kelp, skim milk powder and casein) are mostly non-plant derived (excluding soyflour and bull kelp). In comparison, greenlip abalone could digest significantly more protein than blacklip abalone from the ingredients that were plant derived (lupins and semolina). A characteristic of the ingredients that greenlip abalone digest significantly more protein from than blacklip abalone (dehulled and whole *L. angustifolius* and semolina) is that unlike fishmeal, defatted soyflour, bull kelp, skim milk powder and casein, they contain soluble non-starch polysaccharides. Blacklip abalone may not be able to digest the soluble non-starch polysaccharides found in terrestrial plants as well as greenlips and they may actually interfere and reduce the blacklip abalone's ability to digest nutrients (both protein and energy) from plant feedstuffs which contain them. It is possible that if the soluble non-starch polysaccharides were removed from lupins and semolina, then blacklip abalone might be able to digest the protein from these ingredients better than could greenlip abalone.

With regard to *L. angustifolius*, dehulling had no effect on protein digestibility for blacklip abalone. Although a significant increase was found in energy digestibility after dehulling, it was much less than was found for greenlip abalone (0.63 to 0.70 for blacklips compared with 0.50 to 0.83 for greenlips). After removal of the hull the energy digestibility of *L. angustifolius* for greenlips changed from being significantly less than was found for blacklips to significantly greater than was obtained for blacklips. The hull of the lupin is composed primarily of cellulose. It thus appears that blacklip abalone are not affected by cellulose to the same degree, or have a greater capacity to digest it than greenlip abalone. It should be mentioned that cellulose is a non-soluble structural polysaccharide. It would be interesting to see if the removal of the soluble polysaccharides in the lupin resulted in a more significant increase in its energy digestibility for blacklip abalone. Soluble non-starch polysaccharides from lupins may increase the viscosity of digesta hindering the action of digestive enzymes. In broiler diets, it is recommended that the inclusion of either *L. angustifolius* or *L. albus* should not exceed 10 % (van Barneveld and Hughes 1994). This is due to the incidence of wet-sticky droppings that may be promoted by high levels of lupin non-starch polysaccharides (van Barneveld and Hughes 1994).

It is clear that milk based products (casein, skim milk powder and whey) are very digestible sources of protein and energy for both blacklip and greenlip abalone. In particular, the sugar component of milk (lactose) is very digestible for abalone given the extremely high digestibility of protein and energy from whey (the residue from milk after removal of the casein and most of the fat). Lactose is a disaccharide composed of galactose and glucose. Thus it is a much simpler carbohydrate than those found in many terrestrial plant based feedstuffs such as lupins which are composed of complex structural and storage

polysaccharides. Galactose is one of the major components of carrageenan which is found in the cell walls of red algae. In addition β-galactosidase (lactase) activity, needed for the hydrolysis of lactose has been found in the abalone (Oshima 1931; Bennett et al. 1971). Thus it is not surprising that the energy from whey, skim milk and casein is highly digestible for abalone.

Pregelatinised waxy maize starch was also found to be a highly digestible source of energy for both species of abalone. Again this is not surprising as the starch found in red algae, termed floridean starch, is essentially the same as waxy starches found in terrestrial plants in that it consists almost entirely of amylopectin. In addition Elyakova et al. (1981) found evidence for amylase-α-1,4-glucanase activity against amylopectin in extracts from the hepatopancreas of *H. asinina* and *H. varia.* The fact that the starch has been gelatinised, whereby the application of moist heat brings about swelling and rupturing of the starch granules facilitating amylolysis, would also make it more digestible for the abalone.

The low protein digestibility of bull kelp by both species could be due to the presence of tannins, naturally occurring polyphenols present in some plants to protect them against herbivory. Their main characteristic is that they bind and precipitate proteins. In *vivo* studies have shown that protein digestibility is greatly reduced when tanniniferous feeds are part of animal diets (Reed 1995). Polyphenols are predominant in brown algae (Ragan and Glombitza 1986; Steinberg 1989). It should be pointed out that bull kelp has a very low crude protein content (69 g/kg) and that even though it was included in the diet at a level of 500 g/kg, the crude protein content of the diet was only 3.45 g/kg. Thus the endogenous N contribution may have resulted in a measurement artefact whereby the apparent protein digestibility appears lower than it actually is. This could have also occurred with semolina which has a crude protein content of 104 g/kg resulting in a dietary crude protein content of only 4.16 g/kg.

Clearly neither species are able to digest the energy from semolina very well, particularly blacklips. It was found in another study by Vandepeer (unpublished) that semolina actually affected the digestibility of other ingredients within a diet. The poor digestibility of semolina and its affects on the digestibility of other ingredients are a concern given that it currently constitutes one of the major ingredients used in manufactured diets. Semolina is comprised of 76.37 % starch (dry matter basis) and thus abalone's low gross energy digestibility of semolina is most likely to be due to their inability to digest semolina's starch. This could be established by comparing the digestibility of normal and gelatinised semolina. If starch was responsible for abalone's poor gross energy digestibility of semolina, their energy digestibility of gelatinised semolina should be significantly greater than non-gelatinised semolina.

Abalone's poor gross energy digestibility of semolina may also be due to the soluble non-starch polysaccharides in wheat (arabinoxylans) which are well known to cause problems in poultry. Studies on poultry have shown that soluble polysaccharides such as β-glucans present in barley and oats and arabinoxylans in wheat, elicit negative effects through increasing viscosity (Annison 1990; Bedford et al. 1991; Choct and Annison 1992a; Annison 1993; Choct et al. 1996; Dusel et al. 1997). High gut viscosity decreases the rate of diffusion of substrates and digestive enzymes and hinders their effective interaction at the mucosal surface (Choct 1997a). It has also been suggested that viscous polysaccharides might also directly complex with digestive enzymes and reduce

their activity (Ikeda and Kusano 1983). It would not be surprising if abalone are affected in the same way as poultry by soluble non-starch polysaccharides.

The results from this experiment demonstrate that greenlip and blacklip abalone have different digestive capacities. Future experiments should determine whether they have different nutritional requirements in terms of protein and energy and their optimum ratio of digestible protein to energy for growth as has already been done for greenlip abalone.

OBJECTIVE 2: Determination of the optimal digestible protein:energy ratio for growth of blacklip abalone

ABSTRACT: In this study the effect of dietary digestible protein (DP):digestible energy (DE) ratio on growth of juvenile blacklip abalone was investigated to determine the optimal ratio for growth. Abalone were fed ten different protein:energy ratio diets (5 different levels of digestible protein at low and high levels of digestible energy) for 62 days. Diets were formulated to the desired digestible protein and energy levels based on digestibility coefficients determined in Objective 1. Whole wet body weights were measured at the start and end of the experiment and specific growth rates calculated for each dietary treatment. Growth did not differ significantly between the two energy levels for each level of digestible protein, however significant differences were found among protein levels within an energy level. The level of digestible protein for optimal growth of blacklip abalone was found to be 22.2 % (irrespective of energy level). Based on second order polynomial regressions of the data this equated to optimal DP:DE ratios for growth of 23.4 g $DP.MJDE^{-1}$ (low energy diets) and 19.3 g $DP.MJDE^{-1}$ (high energy diets). Given that the optimal growth rate of blacklip abalone was not affected by energy level it is recommended that for least cost formulation feed manufacturers formulate their diets to 23.4 g $DP.MJDE^{-1}$

INTRODUCTION

Animals have a maximum capacity for protein deposition (growth) which is dependent on their sex, age and genotype. Whether this maximum capacity for growth is reached is primarily dependent on the amount of protein and energy made available for growth and the balance between the two. At a constant energy intake, protein accretion increases linearly with increasing protein intake until the required protein/energy ratio has been reached. A further increase in protein intake has no beneficial effect on protein gain and growth rate may actually 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 response of protein accretion to energy intake has been described as linear, curvilinear and linear-plateau (Bikker 1991). In pigs Close et al. (1983) reported a linear increase in protein deposition with increasing energy intake, whilst Campbell et al. (1983, 1985) and Dunkin et al. (1986) reported a linear-plateau relationship and Schneider et al. (1982) reported a curvilinear response. With regard to abalone dietary protein, requirements have been estimated using both broken-line and quadratic regression models. Mai et al. (1995) found the broken-line model gave a closer fit to the data for *H. discus hannai*, whereas the quadratic model was more suited to the data of *H. tuberculata* (Mai et al. 1995) and *H. laevigata* (Coote 1998).

The aim of this experiment was to determine the optimal digestible protein:digestible energy ratio for growth of blacklip abalone, *H. rubra* as has been previously done for the other cultured Australian abalone species *H. laevigata* (Coote 1998).

MATERIALS & METHODS

Diet formulation and manufacture

Using the ingredient digestibility coefficients determined (Objective 1, Table 3) two pairs of diets were formulated on the basis of high (HE) and low (LE) digestible energy levels. Each pair consisted of one diet high in digestible protein (HP) and one with minimal digestible protein (LP) (Table 4). The composition of the four diets and the nutrient contribution from their component ingredients can be seen in Tables 4 and 5 respectively. The two isocaloric pairs of diets (HP & LE + LP & LE) and (HP & HE + LP & HE) were blended to produce 10 diets (5 different levels of digestible protein x 2 levels of digestible energy) (Table 6). Fishmeal, casein and skim milk powder were used as sources of protein in the diets while pregelatinised waxy maize starch and Jack Mackerel oil were used as energy sources (Tables 4 and 5). The level of Jack Mackerel oil used was no greater than 2.8 % in any diet. Sodium alginate (0.5 %) was used as a binder in each diet and kaolin was used as a non-nutritive filler. Each diet contained 0.5 % chromic oxide as an indigestible marker for use in the determination of nutrient digestibility. Diets were made as outlined in Objective 1. These were produced as small chips for the growth experiment (experiment 1) and longer 30 cm strips for the digestibility experiment (experiment 2).

Table 4
Diet composition (g/kg dry weight)

Ingredient	Low energy/Low protein	Low energy/High protein	High energy/Low protein	High energy/High protein
Protein Sources				
Fishmeal	0.00	100.00	0.00	150.00
Casein	0.00	370.26	0.00	245.93
Skim milk powder	0.00	0.00	0.00	200.00
Energy sources				
Jack Mackerel oil	23.97	10.95	30.00	19.57
Pregelatinised starch	600.00	100.00	724.45	100.00
Additives				
Sodium alginate	5.00	5.00	5.00	5.00
Vitamins	3.00	3.00	3.00	3.00
Minerals	2.00	2.00	2.00	2.00
Chromic oxide	5.00	5.00	5.00	5.00
$CaSO_4$	2.00	2.00	2.00	2.00
Kaolin	359.03	401.79	228.55	267.50
TOTAL	1000	1000	1000	1000

Table 5
Protein (g/kg) and energy (MJ/kg) contributions to the four basal diets from each ingredient

Ingredient	**Low energy/Low protein**		**Low energy/High protein**		**High energy/Low protein**		**High energy/High protein**	
	DP	**DE**	**DP**	**DE**	**DP**	**DE**	**DP**	**DE**
Protein Sources								
Fishmeal	0.00	0.00	39.41	1.18	0.00	0.00	59.12	1.77
Casein	0.00	0.00	260.59	6.45	0.00	0.00	173.09	4.29
Skim milk powder	0.00	0.00	0.00	0	0.00	0.00	67.80	3.27
Energy sources								
Jack Mackerel oil	0.00	0.87	0.00	0.40	0.00	1.08	0.00	0.71
Pregelled starch	0.00	8.59	0.00	1.43	10.37	0.00	0.00	1.43
Additives								
Sodium alginate	0.00	0.04	0.00	0.04	0.00	0.04	0.00	0.04
Vitamins	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Minerals	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Chromic oxide	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
$CaSO_4$	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Kaolin	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
TOTAL DP	0.00		300.00		0.00		300.00	
TOTAL DE		9.50		9.50		11.50		11.50

Table 6
The digestible protein (DP) and energy (DE) level and digestible protein:energy ratio (DP:DE) of the 10 diets

Diet	DP (%)	DE (MJ/kg)	DP:DE
1	5	9.50	5.26
2	10	9.50	10.53
3	15	9.50	15.79
4	20	9.50	21.05
5	25	9.50	26.32
6	5	11.50	4.35
7	10	11.50	8.70
8	15	11.50	13.04
9	20	11.50	17.39
10	25	11.50	21.74

Experiment 1: Growth

Abalone

Juvenile blacklip abalone purchased from a commercial abalone farm in Victoria were used in this experiment. These animals had been raised on a commercially manufactured feed from the time they were 0.5 mm in shell length. All abalone were weighed to an accuracy of 0.001 g in wet weight. When the experiment started the mean weight of abalone (± SE) was 2.273 ± 0.0270 g. Before being assigned to tanks, each animal was tagged using the method described by Coote et al. (1996). Thirty three randomly chosen abalone were sacrificed at the start of the experiment so that meat weight and shell weight as a percentage of total body weight could be established.

Experimental Conditions

Four replicate tanks of each diet to be tested were randomly assigned to 8-L plastic tanks of the dimensions 18 x 21 x 31 cm. Each tank contained 10 abalone randomly chosen and had a piece of PVC as a shelter. Tanks were on a flow through water system. The seawater was filtered to 30 μm by primary sand filters and then to 10 μm by secondary composite sand filters. Temperature was maintained at 16 ± 1 °C. Salinity was 36 ‰. Water flow was at rate which resulted in approximately 1 tank exchange every 15 minutes. Aeration was provided to all tanks. The light regime was 12-h light: 12-h dark.

Feeding & Cleaning

Abalone were fed to excess and tanks were cleaned every second day.

Measurements

Total wet body weight (BW) was measured at the start and end of the experiment to estimate specific growth rates. The experiment lasted 62 days. The specific growth rate (SGR) was calculated as:

$$SGR*100\ \% = \frac{\ln G(f) - \ln G(i)}{\otimes t}$$

where G(i) is the BW (g) at the start of the experiment, G(f) is the BW (g) at its end, and ⊗t is the experimental duration (d) (see above).

In addition to SGR, shell weight and meat weight (muscle plus epipodium) as a percentage of total wet body weight were calculated.

Statistical Analyses

Differences in percent shell and meat weight among the 10 diets and differences in specific growth rate among protein levels within the two energy levels and between energy levels for each level of digestible protein, were analysed by analysis of variance of the completely randomised design, utilising a

generalised linear model (Genstat 5, version 4.1 1998). For significant p values, treatment means were compared by least significant difference. Prior to analysis residual versus fitted values were plotted to establish that the data were in fact normally distributed, which was the case. The optimal level of protein for abalone growth (Xmax) was calculated from results of the regression of the SGR on energy level using the 2nd order polynomial of the form:

$$y = ax^2 + bx$$

$$X\max = -\frac{b}{2a}$$

$$V[X\max] = \frac{1}{4a^2}\left\{\left(\frac{b}{a}\right)^2 V[a] + V[b] - \frac{2b}{a}Cov[a,b]\right\}$$

$$SE[X\max] = \frac{1}{2|a|}\sqrt{\left(\frac{b}{a}\right)^2 V[a] + V[b] - \frac{2b}{a}Cov[a,b]}$$

$$Y\max = aX\max^2 + bX\max$$

$$V[Y\max] = X\max^2\left\{X\max^2 V[a] + V[b] + 2X\max Cov[a,b]\right\}$$

$$SE[Y\max] = X\max\sqrt{X\max^2 V[a] + V[b] + 2X\max Cov[a,b]}$$

Experiment 2: Digestibility

To determine whether the true protein and energy digestibility of each diet matched expected (calculated) digestibilities based on the individual ingredient digestibility coefficients from experiment one, the protein and energy digestibility of each diet was assessed. The methodology for the digestibility experiment is as described in Objective 1, the only difference being the temperature was 16 ± 1 ºC and not 18 ± 1 ºC.

Statistical Analyses

The mean protein and energy digestibility of each diet was calculated. Protein digestibilities were compared between each corresponding pair of high and low energy diets by analysis of variance using a generalised linear model (Genstat 5, version 4.1 1998). Prior to analysis residual versus fitted values were plotted to establish that the data were in fact normally distributed, which was the case.

RESULTS

Experiment 1: Growth

Specific growth rate

The SGR of abalone wet body weight was significantly affected by the level of digestible protein for both high and low energy diets (LE: $F_{4,12} = 17.50$, $p = < 0.001$; HE: $F_{4,12} = 5.60$, $p = 0.009$) (Figure 1). For the diets containing low levels of digestible energy no significant difference was found in SGR among the diets containing 15, 20 and 25 % digestible protein ($p > 0.05$), however the SGRs produced by abalone fed the diets containing 15, 20 and 25 % digestible protein were significantly higher than those of abalone fed the 5 and 10 % digestible protein diets ($p < 0.05$). As for the low energy diets, no significant difference was found in SGR of abalone fed the high energy diets containing 15, 20 and 25 % digestible protein ($p > 0.05$).

In general, energy level had no affect on SGR of abalone wet body weight with no significant difference in SGR being found between the high and low levels of digestible energy at digestible protein levels of 5 % ($F_{1,3} = 0.27$, $p = 0.640$), 15 %, ($F_{1,3} = 5.80$, $p = 0.095$), 20 % ($F_{1,3} = 0.02$, $p = 0.898$) or 25 % ($F_{1,3} = 0.85$, $p = 0.425$) (Figure 1). The only protein level at which a significant difference in SGR was found between high and low levels of digestible energy was 10 % ($F_{1,3} = 11.16$, $p = 0.044$) (Figure 1).

Optimal protein:energy ratio for growth

Second order polynomial regression of the SGR data predicted a maximal SGR of 0.47 ± 0.045 at a digestible protein level of 22.2 ± 4.749 %, equating to an optimal DP:DE ratio of 19.3 g $DP.MJDE^{-1}$ for the high energy diets. For the low energy diets, second order polynomial regression predicted a maximal SGR of 0.52 ± 0.004 also at a digestible protein level of 22.2 ± 0.248 %, equating to an optimal DP:DE ratio of 23.4 g $DP.MJDE^{-1}$ (Figure 1).

% shell and meat weight

The mean percentage shell weight and percentage meat weight (±SE) of 33 abalone at the start of the experiment was 28.9 ± 1.206 % and 47.5 ± 2.134 % respectively. Varying dietary protein to energy ratio had no effect on the final percent shell weight or percent wet meat weight produced by abalone (Figures 2 and 3). No significant differences were found among the 10 diets with respect to the percent shell weight ($F_{9,27} = 1.98$; $p = 0.082$) and percent wet meat weight ($F_{9,27} = 1.50$; $p = 0.198$) they produced in abalone. At the end of the experiment meat constituted 49.6 % and shell 29.1 % of abalone total wet body weight (averages of the 10 diets).

Experiment 2: Digestibility

The actual apparent protein and energy digestibility values of the 10 diets closely matched the predicted values (Table 7). There were no significant differences in mean protein digestibility between the high and low energy diets at all the digestible protein levels evaluated: 5 % ($F_{1,2}$ = 4.96; p = 0.156), 10 % ($F_{1,2}$, =7.59; p = 0.110), 15 % ($F_{1,2}$, = 1.08; 0.408), 20 %($F_{1,2}$ = 0.15; p = 0.733) or 25 % ($F_{1,2}$ = 1.48; p = 0.348).

Table 7
Predicted versus actual levels of digestible protein (DP) and digestible energy (DE) for each of the 10 diets. Values are means ± SE, n = 3.

Diet	Predicted DP (%)	Actual DP (%)	Predicted DE (%)	Actual DE (%)
1 LE	5	4.7 ± 0.139	9.50	9.17 ± 0.095
2 LE	10	10.1 ± 0.205	9.50	9.38 ± 0.221
3 LE	15	14.8 ±0.134	9.50	9.45 ± 0.076
4 LE	20	19.8 ± 0.473	9.50	9.82 ± 0.157
5 LE	25	23.3 ± 0.891	9.50	9.19 ± 0.218
6 HE	5	5.1 ± 0.059	11.50	11.62 ± 0.037
7 HE	10	10.4 ± 0.166	11.50	11.70 ± 0.102
8 HE	15	15.3 ± 0.377	11.50	11.74 ±0.156
9 HE	20	19.6 ±0.505	11.50	11.59 ±0.122
10 HE	25	24.5 ± 0.163	11.50	11.24 ± 0.039

DISCUSSION

The level of digestible protein which produces optimal growth of juvenile blacklip abalone when fed diets containing either 11.5 or 9.5 $MJ.kg^{-1}$ of digestible energy is 22.2 %, equating to optimal digestible protein:energy ratios of 19.3 g $DP.MJDE^{-1}$ and 23.4 g $DP.MJDE^{-1}$ respectively. This was predicted from second order polynomial regression of SGR data (Figure 1). Because specific growth of abalone (wet meat weight) does not differ significantly when fed either high (11.5 $MJ.kg^{-1}$) or low levels (9.5 $MJ.kg^{-1}$) of digestible energy for equivalent amounts of digestible protein (Figure 1) no difference in growth of abalone is expected when fed diets containing either 19.3 g $DP.MJDE^{-1}$ or 23.4 g $DP.MJDE^{-1}$. A comparison of the DP:DE ratio for optimal growth of juvenile blacklip abalone can not be made with that obtained for juvenile greenlip abalone (Coote 1998) due to intellectual property issues associated with that data.

The plateau in specific growth rate of the abalone at DP levels above 22.2% when fed high and low energy diets indicates that they have a maximum capacity for protein deposition that can not be increased by adding more energy to the diet. Thus, there is no value in formulating diets with a DP higher than 22.2 % as the abalone will be unable to utilise the additional protein for growth and it will be excreted as waste. It in fact may be detrimental to feed the abalone diets which have much higher levels of DP as they will need to expend energy to process and eliminate the excess protein they can not use and it will end up polluting their tanks providing a source of nutrients for the growth of bacteria. It should be noted that the optimum digestible protein level for growth of abalone may need revision in future years due the abalone developing an increased capacity for protein deposition as a result of selective breeding for faster growth.

Although no significant difference was observed in specific growth rates of the abalone when fed either high or low energy diets for the same level of digestible protein, it is recommended that for least cost formulation feed manufacturers should formulate diets to the lowest DE level (9.5 $MJ.kg^{-1}$), equating to the DP:DE ratio of 23.4 g $DP.MJDE^{-1}$. It is possible that the DE level of the diet could be reduced below 9.5 $MJ.kg^{-1}$ without compromising growth however 9.5 $MJ.kg^{-1}$ was the lowest energy level evaluated in this experiment and so an additional experiment would need to be done to verify this.

No explanation can be offered as to why a significant difference in specific growth rate was found between the abalone fed the low and high DE diets at 10 % DP but not at any other DP level. It would be expected that if the low and high DE diets were to differ in the specific growth rates they produced, it would occur at the high DP levels (20 & 25 %), as the more protein there is, the more energy that is required to deposit it.

Several studies have been done on other abalone species looking at the optimal crude protein level for growth, most without regard for energy. Because the authors looked at crude protein, rather than digestible protein it is difficult to make proper comparisons among these studies. To overcome this problem optimal digestible protein levels for growth of these other species were calculated using data on the amount and types of protein sources used in their diets and on the greenlip and blacklip digestibility values calculated for the same ingredients in Objective 1. This information has been collated in Table 8. The digestible protein level found to produce optimal growth of blacklip abalone in this study lies within the range calculated for other species (Table 8). Calculation of the average from these studies

indicates that the optimal digestible protein for growth of abalone is approximately 20.8 %.

Shell weight and wet meat weight of blacklip abalone as a percentage of total wet body weight were not affected by dietary protein or energy content in this study. Mai et al. (1995) also found that the soft body to shell ratio of small *H. discus hannai* (0.38 – 0.97 g) and *H. tuberculata* (0.17 – 0.55 g) did not differ significantly when fed diets containing 20 – 50 % protein. Interestingly, the results from Mai et al. (1985) and this current study on blacklip abalone differ to results reported by Coote (1998) for greenlip abalone. Coote (1998) found that the relationship of muscle, viscera and shell to total weight was greatly affected by the protein content of the diet and to a lesser extent, by the energy content. The diets that produced fastest growth rate also yielded the highest percentage of foot muscle, were mid-range in viscera content, low in moisture and lowest in shell as a proportion of whole weight.

The digestibility coefficients determined for blacklip abalone are additive. This is because actual digestible protein and energy levels closely matched predicted levels calculated based on ingredient inclusion level and digestibility values determined in Objective 1. This means that feed manufacturers can use the ingredient digestibility coefficients determined in Objective 1 to formulate diets to the DP:DE ratio determined in this study to produce optimal growth for blacklip abalone. It should be possible to change dietary formulations to suit fluctuations in ingredient prices without a decrease in growth rate provided the diets are formulated to the same DP:DE ratio. It should be mentioned that this may not hold true when using semolina. Semolina has been observed to affect nutrient digestion in greenlip abalone (Vandepeer 2002) and it is possible it may affect nutrient digestion in blacklip abalone. In addition, the digestibility of energy from pregelatinised waxy maize starch by greenlip abalone decreases at high inclusion levels (Vandepeer 2002) and may also do so for blacklips. It is unlikely, however, that the inclusion level at which a decrease in gross energy was observed for starch (above 50 %) would be used in commercial abalone diets.

It should be noted that the optimal DP:DE ratio found for growth of blacklip abalone in this study might only apply to the size class that was studied (2.3 g, 24.0 mm). It is possible that larger blacklip abalone have a different DP:DE ratio for optimal growth. Differences in optimal protein level for growth of *H. midae* among different size classes were observed by Britz and Hecht (1997). Maximum growth of larger abalone (7.0 – 14.0 g) was achieved at a higher dietary protein level (44 %) than for smaller abalone (34 % for 0.2 – 1.0 g). They concluded that larger abalone had a higher protein requirement than smaller abalone.

Table 8
Optimal crude protein levels recommended for growth of different abalone species and calculated optimal digestible protein levels based on the amount and the type of the protein sources used in the studies and the protein digestibility values obtained for these ingredients with blacklip and greenlip abalone (Objective 1)

Abalone species	Abalone size	Protein source used in study	Optimal CP (%) for growth –based on data from study	Optimal DP (%) for growth (calculated using blacklip digestibility data)	Optimal DP (%) for growth (calculated using greenlip digestibility data)	Reference
H. kamtschatkana	?	Casein	30	23.1	24.6	Taylor (1992)
H. asinina	0.6 g weight 15 mm length	Fishmeal, shrimp meal and soybean meal	27	16.8	14.3	Bautista-Teruel and Millamena (1999)
H. laevigata	15-25 mm length	Casein	27	-	19.4	Coote et al. (2000)
H. discus hannai	3.4 – 4.8 g	White fishmeal	43	24.1	19.8	Uki et al. (1986)
H. discus hannai	3.4 – 4.8 g	Casein	20-30	16.4 – 24.6	15.4 – 23.1	Uki et al. (1986)
H. discus	0.05 g	White fishmeal	44.3	24.8	19.9	Ogino and Kato (1964)
H. discus	1.1 g	White fishmeal	42.7	23.9	19.2	Ogino and Kato (1964)
H. midae	10.5 mm length	Fishmeal	34	19.0	15.6	Britz and Hecht (1997)
H. midae	36.3 mm length	Fishmeal	44	24.6	20.2	Britz and Hecht (1997)
H. discus hannai	0.4 g weight	Casein	23.3 – 35.6	19.1 – 29.2	17.9 – 27.4	Mai et al. (1995)
H. tuberculata	0.2 g weight	Casein	22.3 -32.3	18.2 – 26.5	17.2 – 24.9	Mai et al. (1995)

BENEFITS

Groups that will benefit from this research and the benefits they will gain are:

1. Abalone growers

Benefits:
1) A decrease in production costs due to enhanced growth rates.
2) Improved health of the abalone through better nutrition and thus an increase in the number of abalone surviving to market size.
3) A reduction in organic wastes produced by the farms due to increased digestibility of diets.

2. Abalone diet manufacturers

Benefits:
1) Improved diet formulation through a greater knowledge of the nutritional requirements of abalone.
2) An increase in sale of manufactured diets as a consequence of the improved growth rates produced by the diets.

FURTHER DEVELOPMENT

Based on the results from this study the author recommends that further abalone manufactured feed development can be made by addressing the following:

- Since the energy from semolina is so poorly digested by both blacklip and greenlip abalone (Objective 1, Table 3) and semolina is one of the main constituents of manufactured diets, additional studies could be done to assess ways of improving semolina's gross energy digestibility for abalone. If semolina's starch is responsible for the poor digestibility of its energy then it may be worth investigating the effects of adding a commercial amylase to diets or of gelatinising semolina before incorporating into diets. If no improvement is observed by the addition of amylase or by gelatinising semolina then it may be that the soluble non-starch polysaccharides present in semolina are responsible for its poor digestibility. In this instance it may be worth assessing a commercial enzymes that hydrolyses arabinoxylans (the main non-starch polysaccharides in wheat).

- Optimal dietary DE content for different production phases.

- Further ingredient assessments.

CONCLUSIONS

Objective 1: To determine the digestibility of nutrients from a range of feedstuffs to be used in artificial diets for blacklip abalone in comparison with existing data on greenlip abalone.

Blacklip abalone's ability to digest the protein and energy from the following 12 ingredients was evaluated: semolina, deffated soyflour, fishmeal, casein, pregelatinised waxy maize starch, whole mung beans, bull kelp, whey, skim milk powder, whole and dehulled *L. angustifolius* and whole *L. luteus* (lupins). Results from this study indicated the following:

- The digestive capacity of blacklip and greenlip abalone differed and therefore they should be fed different diets.
- Blacklip abalone had a greater capacity than greenlip abalone to digest the protein from ingredients that do not contain non-starch polysaccharides, whereas greenlip abalone had a greater capacity than blacklip abalone to digest the protein from ingredients that do contain non-starch polysaccharides.
- Blacklip abalone had a greater capacity to digest cellulose than do greenlip abalone.
- Both abalone species digested the energy from semolina, a major ingredient in commercial abalone feeds, very poorly.
- Of all the ingredients evaluated both abalone digested the nutrients from milk products (casein, whey and skim milk powder) the best.

Objective 2: To determine the optimal protein:energy ratio for growth of blacklip abalone.

The growth of blacklip abalone fed 10 different protein:energy ratio diets (5 different levels of digestible protein at low and high levels of digestible energy) was investigated to determine the ratio at which the rate of growth is optimal. Results from this study indicated the following:

- At equivalent levels of protein, growth of blacklip abalone did not differ when fed diets containing high (11.5 $MJ.kg^{-1}$) or low (9.5 $MJ.kg^{-1}$) levels of digestible energy.
- Optimal growth of blacklip abalone occurred at a digestible protein level of 22.2 % when fed high and low energy diets. For the low energy diet (9.5 $MJ.kg^{-1}$) this equated to a optimal protein:energy ratio for growth of 23.4 g $DP.MJDE^{-1}$ whilst for the high energy diet (11.5 $MJ.kg^{-1}$) it equated to an optimal digestible protein:energy ratio for growth of 19.3 g. $DP.MJDE^{-1}$. It is recommended that for least cost formulation the DP:DE ratio of 23.4 g $DP.MJDE^{-1}$ be used.

- Digestibility coefficients determined for blacklip abalone (Objective 1) were additive and thus can be used reliably to formulate diets to the protein:energy ratio which produces optimal growth.

REFERENCES

Annison, G. 1990. Antinutritive activity of cereal polysaccharides in monogastric diets. In: Proceedings of the Inaugural Massey Pig and Poultry Symposium, Massey University, 26 – 28th November, 1990. pp.78-85.

Annison, G. 1993. The role of wheat non-starch polysaccharides in broiler nutrition. *Australian Journal of Agricultural Research.* 44: 405-422.

Bautista-Teruel, M. N and O. M. Millamena. 1999. Diet development and evaluation for juvenile abalone, *Haliotis asinina*: protein/energy levels. *Aquaculture*. 178: 117-126.

Bedford, M. R., H. L. Classen and G. L. Campbell. 1991. The effect of pelleting, salt, and pentosanase on the viscosity of intestinal contents and the performance of broilers fed rye. *Poultry Science*. 70: 1571-1577.

Bennett, R. Jr., N. Thanassi and H. I. Nakada. 1971. Hepatopancreas glycosidases of the abalone (*Haliotis rufescens*). *Comparative Biochemistry and Physiology*. 40B: 807-811.

Bikker, P. 1991. Protein and lipid accretion in body components of growing pigs. Dept. of Animal Husbandry, Wageningen Agricultural University. PhD Thesis.

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

Campbell, R. G., M. R. Taverner and D. M. Curic. 1983. The influence of feeding level from 20 to 45 kg live weight on the performance and body composition of female and entire male pigs. *Animal Production*. 36: 193.

Campbell, R. G., M. R. Taverner and D. M. Curic. 1985. Effects of sex and energy intake between 48 and 90 kg live weight on protein deposition in growing pigs. *Animal Production*. 40: 497.

Choct, M. 1997. Feed non-starch polysaccharides: chemical structures and nutritional significance. In: Proceedings from Feed Ingredients Asia 97. Singapore International Convention and Exhibition Centre 18-20th March 1997. p. 1-12.

Choct, M. & G. Annison. 1992. Anti-nutritive activity of wheat arabinoxylans: role of viscosity and gut microflora. *British Poultry Sciences.* 33: 821-834.

Choct, M., R. J. Hughes, J. Wang, M. R. Bedford, A. J. Morgan & G. Annison. 1996. Increased small intestinal fermentation is partly responsible for the anti-nutritive activity of non-starch polysaccharides in chickens. *British Poultry Science.* 37: 609-621.

Close, W. H., F. Berschauer and R. P. Heavens. 1983. The influence of protein:energy value of the ration and level of feed intake on the energy and nitrogen metabolism of the growing pig. 1. Energy metabolism. *British Journal of Nutrition.* 49: 255.

Coote, T. A. 1998. The protein, energy and lysine requirements of greenlip abalone (*Haliotis laevigata*). Ph.D. Dissertation. University of Tasmania, Australia. 118 pp.

Coote, T.A., P.W. Hone, R. J. van Barneveld and G. B. Maguire. 2000. Optimal protein level in a semipurified diet for juvenile greenlip abalone *Haliotis laevigata*. *Aquaculture Nutrition*. 6: 213 – 220.

Dunkin, A. C., J. L. Black and K. J. James. 1986. Nitrogen balance in relation to energy intake in entire male pigs weighing 75 kg. *British Journal of Nutrition*. 55: 201.

Dusel, G., H. Kluge, K. Glaser, O. Simon, G. Harmann, J. v. Lengerken and H. Jeroch. 1997. An investigation into the variability of extract viscosity of wheat – relationship with the content of non-starch-polysaccharide fractions and metabolisable energy for broiler chickens. *Archives of Animal Nutriton*. 50: 121-135.

Elyakova, L. A., N. M. Shevchenko and S. M. Avaeva. 1981. A comparative study of carbohydrase activities in marine invertebrates. *Comparative Biochemistry and Physiology.* 69B: 905-908.

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

Genstat 5. 1998. Lawes Agricultural Trust. Release 4.1.

Hardy, R. E. 1997. Understanding and using apparent digestibility coefficients in fish nutrition. *Aquaculture Magazine.* May/June: 84-85.

Hillebrand, W. F., G. E. F. Lundell, H. A. Bright and J. I. Hoffman. 1953. Applied Inorganic Analysis. Wiley: New York.

Ikeda, K & T. Kusano. 1983. In vitro inhibition of digestive enzymes by indigestible polysaccharides. *Cereal Chemistry*. 60(4): 260-263.

Mai, K., J. P. Mercer and J. Donlon. 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.

Mercer. J. P., K. S. Mai and J. Donlon. 1993. Comparative studies on the nutrition of two species of abalone, *Haliotis tuberculata* Linnaeus and *Haliotis discus hannai* Ino I. Effects of algal diets on growth and biochemical composition. *Invertebrate Reproduction and Development.* 23(2-3): 75-88.

Ogino, C. and N. Kato. 1964. Studies on the nutrition of abalone – II. Protein requirements for growth of abalone, *Haliotis discus*. *Bulletin of the Japanese Society of Scientific Fisheries.* 30(6): 523 – 526.

Oshima, K. 1931. Digestive enzymes appeared in abalone viscera. *Journal of Agricultural Chemistry*. 7: 328-331.

Ragan, M.A. and K. W. Glombitza. 1986. Phlorotannins, brown algal polyphenols, *Progressive Phycological Research*. 4: 129-241.

Reed, J.D. 1995. Nutritional toxicology of tannins and related polyphenols in forage legumes. *Journal of Animal Science.* 73: 1516-1528.

Royal Australian Chemical Institute. 1999. Cereal Chemistry Division - Official Methods. Dumas (combustion) total nitrogen determination. Method No: 02-03.

SAS Institute Inc. 1988. SAS/STAT® Users guide, Release 6.03 Edition. SAS Institute Inc, Cary, North Carolina, USA. 1028 p.

Schneider, W., G. Gaus, A. Michel, A. Susenbeth and K. H. Menke. 1982. Effect of level of feeding and body weight on partition of energy in growing pigs. In: A. Ekern and F. Sundstol (eds.). Energy metabolism of farm animals. EAAP publ. no. 29. p. 96. The Agric. Univ. of Norway.

Schweiger, R. G. 1978. Carbohydrate Sulfates. A symposium sponsored by the ACS Division of Carbohydrate Chemistry at the 174th Meeting of the American Chemical Society, Chicago, Illinois, August 30-31, 1977. ACS Symposium Series 77. pp. 214-243.

Shepherd, S.A. 1973. Studies on southern Australian abalone (genus *Haliotis*). I. Ecology of five sympatric species. *Aust. J. mar. Freshwat. Res.* 24: 217-257.

Shepherd, S.A. 1975. Distribution, habitat and feeding habits of abalone. *Australian Fisheries.* 34: 12-15.

Steinberg, P.D. 1989. Biogeographical variation in brown algal polyphenolics and other secondary metabolites, comparison between temperate Australasia and North America. *Oecologia*. 78: 373-382.

Stewart, W. D. P. 1974. Algal physiology and biochemistry. W. D. P. Stewart (ed.). Blackwell Scientific Publications, Osney Mead, Oxford. 989 p.

Sugahara, M., D. H. Baker, B. G. Harman and A. H. Jensen. 1969. Effect of excess levels of dietary crude protein on carcass development in swine. *Journal of Animal Science.* 29: 598 – 601.

Taylor, B. 1992. Abalone Nutrition: Optimum protein levels in artificial diets for *Haliotis kamtschatkana. Journal of Shellfish Research.* 11: 556.

Uki, N., A. Kemuyama and T. Watanabe. 1985. Development of semipurified test diets for abalone. *Bulletin of the Japanese Society of Scientific Fisheries.* 51(11): 1825-1833.

Uki, N., A. Kemuyama and T. Watanabe. 1986. Optimum protein level in diets for abalone. *Bulletin of the Japanese Society of Scientific Fisheries.* 52(6): 1005–1012.

van Barneveld R. J. and R. J. Hughes. 1994. The nutritive value of lupins for pigs and poultry. In: Proceedings of the First Australian Lupin Technical Symposium, Perth, Western Australia. Sponsored by the Western Australian Grain Pool. pp. 49-57.

APPENDIX 1: INTELLECTUAL PROPERTY

The intellectual property developed in this project is shared between the South Australian Research and Development Institute and the Fisheries Research and Development Corporation as defined in section of C7 of the project application.

APPENDIX 2: STAFF

Principal Investigator: Meegan Vandepeer

Co-investigators: Dr Ann Fleming and Dr Robert van Barneveld

Technical Support: Annette Doonan