Aquaculture Feed Development for Atlantic Salmon (*Salmo salar*)

Edited by

Chris G. Carter

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UNIVERSITY OF TASMANIA

FISHERIES RESEARCH & DEVELOPMENT CORPORATION

Tasmanian Aquaculture & Fisheries Institute
University of Tasmania

FRDC Project No. 1998/322

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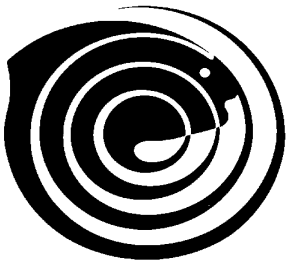

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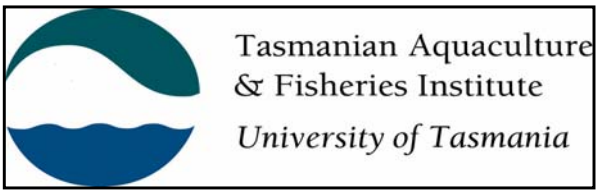

FRDC Project No. 1998/322

First published 2002

First published by
University of Tasmania
Off College Road
Sandy Bay, Tas 7005
Tel: (03) 6226 2761; Fax: (03) 6226 2765

Published by
Fisheries Research and Development Corporation
PO Box 222
Deakin, ACT 2602
Tel: (02) 6232 5222; Fax: (02) 6232 5000

ISBN 1 86295 074 1

Suggested format for referencing:
Carter, C.G., Hauler, R.C. and Foster, C. (Eds.) 2002. Aquaculture Feed Development for Atlantic Salmon (*Salmo salar*). Fisheries Research and Development Corporation Project No. 1998/322. Fisheries Research and Development Corporation: Deakin, Australia.

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2. Non Technical Summary

1998/322 Aquaculture feed development for Atlantic salmon (*Salmo salar*)

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OBJECTIVES:

1. Expand the data base for feed intake, digestibility and utilisation of key nutrients and feed ingredients (principally protein and fat sources) to ensure the optimum balance is used to formulate Atlantic salmon feeds.
2. Determine the lowest level of fish meal that can be used by combining alternative protein sources and to investigate the factors limiting inclusion of the most promising of these combinations.
3. Determine whether at low fish meal inclusion salmon performance is equivalent or better than high fish meal diets.
4. To use the research results to formulate feeds for testing under commercial type conditions.
5. To successfully transfer these results to ingredient producers, feed manufacturers, salmon and trout farmers and the scientific community.

NON TECHNICAL SUMMARY:

The research provided data of strategic importance on the feed requirements of a major farmed Australian fish species, Atlantic salmon, and advanced the study of fish nutrition, particularly our understanding of essential amino acid requirements. This was achieved through collaboration between Skretting Australia, the major manufacturer of salmonid feeds in Australia, and the Nutrition Group at the Tasmanian Aquaculture and Fisheries Institute. The research generated information that will be essential in the evaluation and further development of feeds for Atlantic salmon and is also of relevance for rainbow trout. The focus was on Tasmanian conditions, this is important because they are different to those in which Atlantic salmon are farmed elsewhere in the world.

Atlantic salmon parr were used to assess the apparent digestibility of crude protein (nitrogen), indispensable amino acids and energy of 19 protein sources with potential for use in Atlantic salmon feeds. Protein sources included marine (fish meal), animal (meat, meat and bone, blood, feather, poultry meals) and plant (canola, corn, lupin, soybean, wheat) products. The digestibility data, first limiting amino acid and the indispensable amino acid score indicated how well the ingredients met nutritional requirements for salmonids. As expected, the three fish meals were excellent protein sources with high quality energy, protein and amino acid contents. The rendered animal products were very variable with a wide range of protein and amino acid contents. Recent trends away from the use of rendered animal proteins meant that more importance was placed on plant sources. Soybean is established as a useful protein source in aquafeeds. In addition to soybean products, dehulled plant meals (pea, canola

and lupin) had protein and amino acid contents that indicated their potential as protein sources in salmon feeds. Lysine or methionine was the first limiting amino acid in the plant meals but both are available commercially for use in feeds. Feed intake was measured every week for the four weeks of the trial and compared to feed intake of the fish meal reference diet. Feed intake varied between ingredients but was generally higher after four weeks than after two weeks. This was assumed to reflect a level of acclimation to the ingredient in the diet. Where feed intake remained low after 4 weeks an inclusion level of 15% was shown to be more appropriate.

Lysine is the key essential amino acid and often the first limiting in protein sources. Studies on the quantitative lysine requirements of fish are numerous but the data are highly variable and difficult to use. We conducted a major review of lysine requirements in fish demonstrated very similar utilisation efficiency for lysine across many studies and fish (54.1 mg weight gain.mg^{-1} lysine). This allowed a requirement estimate of 18.5 g lysine.kg^{-1} weight gain to be derived and it is recommended that all future amino acid requirements of fish be expressed relative to growth (wet weight or protein).

The replacement of fish oil with alternative oil sources is an important aspect of Atlantic salmon feed development. An experiment was carried out that test the use of canola oil and a product made from a thraustochytrid, a marine microorganism, in diets for salmon parr. Salmon were fed for over 7 weeks on diets containing only canola oil, canola and fish oil or canola and the thraustochytrid meal. The fish reached smolt size and there were no significant differences in final weight, weight gain, feed intake, feed efficiency, chemical composition or immune status between the diets. However, cumulative mortality was significantly lower in fish fed the diet containing some fish oil than the other two diets following transfer to seawater and two challenges with a bacteria. It was concluded that testing disease resistance is a useful test strategy in nutrition.

Fish meal was replaced by dehulled narrow-leafed lupin with and without the inclusion of supplementary essential amino acids was compared in commercially extruded feeds. A comparison was also made between two methods of oil addition: commercial vacuum oil-coating and manual oil-soaking. There were no statistically significant differences in final weight, weight gain, feed efficiency or survival. Thus, addition of 20% dehulled lupin to a commercial extruded salmon feed produces excellent growth performance under commercial-type conditions.

OUTCOMES ACHIEVED:

- Comprehensive assessment of the nutritional quality of nineteen key protein sources for use in Atlantic salmon feeds.
- Unification of available data on lysine requirements for fish, recommendation of a general approach to expression of lysine requirements for fish in relation to weight gain and recommendation of lysine requirements for Atlantic salmon of all sizes.
- Successful addition of canola and lupin products to Atlantic salmon feeds.
- Demonstration of the effectiveness of a mixed diet feeding regime where by a lower protein feed could be used to replace a higher protein feed at the second feeding time each day.

3. Acknowledgements

Acknowledgements for the financial contribution of Skretting Australia in the provision of a research scholarship as part of the current grant on lysine requirements in Atlantic salmon. Mr. M. Farhangi was in receipt of an Iranian Government Scholarship during completion of the research described in Section 7.7. The research described in Section 7.8 on the potential of thraustochytrids was the result of collaboration between the current grant and FRDC grant (1997 / 329) held by Dr Peter Nicols and Dr Tom Lewis. Further contributions to experiments made by individuals are acknowledged in the relevant sections.

4. Background

This research will advance the study of fish nutrition, provide data of strategic importance for applied research into the feed requirements of major farmed Australian fish species and have broader implications for understanding the biology of fishes. This will be achieved through industrial collaboration and the use of commercially produced feeds and the application of a unique combination of methods that have been developed or used by the Principal Investigator and colleagues. Collaboration is between the major producer of salmonid feeds in Australia and the fish nutritionist at the Key Centre for Aquaculture and Australia's only Department of Aquaculture. The research will generate information that will be essential in the evaluation and further development of feeds for two salmonid species (Atlantic salmon and rainbow trout) of primary importance to Australian aquaculture. The novelty lies in the combination of approaches, the use of experimental feeds produced using commercial facilities, investigation of applied and fundamental aspects of fish growth and the focus on Tasmanian conditions which are different to those in which Atlantic salmon are farmed elsewhere. The expected outcome is a reduction in the cost of feed ingredients through a decrease in the use of protein sources and the development of protocols for the rapid and inexpensive screening of feeds for Atlantic salmon.

5. Need

The cost and potential shortage of marine based products such as fish meals and oils as well as the threat of increases in the price of traditionally used plant meals such as soy bean and the potential problems of using animal meals in feeds for a quality product means that research into maximising the potential of local ingredients is important for ensuring a sustainable feed production in terms of feed costs and the availability of ingredients and the image of the product.

6. Objectives

1. Expand the data base for feed intake, digestibility and utilisation of key nutrients and feed ingredients (principally protein and fat sources) to ensure the optimum balance is used to formulate Atlantic salmon feeds.

2. Determine the lowest level of fish meal that can be used by combining alternative protein sources and to investigate the factors limiting inclusion of the most promising of these combinations.

3. Determine whether at low fish meal inclusion salmon performance is equivalent or better than high fish meal diets.

4. To use the research results to formulate feeds for testing under commercial type conditions.

5. To successfully transfer these results to ingredient producers, feed manufacturers, salmon and trout farmers and the scientific community.

7. Research

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7.5. Hauler, R.C., Carter, C.G. Lysine utilisation by Atlantic salmon parr 3: Influence of dietary protein.
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7.7. Farhangi, M., Carter, C.G. Effect of feeding time and dietary protein level on feed intake, nutrient utilisation and growth of Atlantic salmon fed on dehulled lupin.
7.8. Carter, C.G, Bransden, M.P, Lewis, T.E., Nichols, P.D. The potential of thraustochytrids as a replacement for fish oil for Atlantic salmon.
7.9. Carter, C.G, M.P, Lewis, T.E., Nichols, P.D. Comparison of cholestane and yttrium oxide as digestibility markers for lipid components in Atlantic salmon diets.
7.10. Carter, C.G., Hauler, R.C., Attard, M. Trial of commercially produced Atlantic salmon extruded feeds containing dehulled lupin.

7.1
Feed characteristics and apparent digestibility of ingredients with potential for inclusion in Atlantic salmon feeds

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Abstract

Atlantic salmon parr were used to assess the apparent digestibility of crude protein (nitrogen), indispensable amino acids and energy of 19 protein sources with potential for use in Atlantic salmon feeds. Protein sources included marine (fish meal), animal (meat, meat and bone, blood, feather, poultry meals) and plant (canola, corn, lupin, soybean, wheat) products. The protein content varied between 34% for whole lupin to 88% for blood meal. Apparent digestibility coefficients (ADC) were measured after 2 and 4 weeks of feeding, feeds comprised 30% test ingredient (protein source), 69% reference diet and 1% chromic oxide as the digestibility marker. Crude protein digestibility (ADC N) varied between types of protein meal as well as for the same type of meal. For example, the ADC N for meat meals varied between 70 and 95% and for gluten between 91% (corn) and 99% (wheat). ADC N were generally higher than for ADC kJ although this was not always the case and the size of the difference varied considerably. Taking digestibility values and the first limiting amino acid in to account the indispensable amino acid score (IAAS) provided an indication of how well the ingredients met the amino acid requirements for salmonids. The three fish meals had very high scores of over 1.8; blood (1.6) and poultry (1.2) meals had good scores; meat (0.36 – 1.04) and meat & bone (0.23 – 1.04) meals were very variable. Soybean had the highest score (1.9) followed by field pea (0.94), lupin (0.87) and canola (0.85). Lysine or methionine was the first limiting amino acid in the plant meals. Feed intake was measured every week for the four weeks of the trial and compared to feed intake of the reference diet. Feed intake varied between ingredients but was generally higher after four weeks than after two weeks. This was assumed to reflect a level of acclimation to the ingredient in the diet. The use of a Cumulative Immune Score was tested as an additional method for identifying any potential problems with an ingredient. The index showed differences between ingredients and was tested in further trials.

Keywords: Fish meal replacement; Plant proteins; Digestibility; Ingredient assessment

Introduction

The importance of ingredient digestibility data for feed formulation is well established and there is an increasing amount of information available for salmonids (Anderson *et al.*, 1992; Hajen *et al.*, 1993b; Sugiura *et al.*, 1998; Bureau *et al.*, 1999). However, several of the ingredients that have potential for replacement of fish meal in Australian aquaculture feeds are not readily available in other countries so that there is little information available in the literature. Atlantic salmon provides a good example of this. A considerable amount of research on Atlantic salmon is conducted in Norway and the UK where ingredients such as lupins are not considered important. However, several grain legumes, especially lupins, have considerable potential as Australian grown products (Glenncross, 2001). This raises issues in relation to how applicable

digestibility data is between species and therefore how much use can be made of literature values.

One of the main concerns with digestibility data is the accuracy of the techniques used. There has been much discussion about the appropriate techniques for digestibility determination in fish and aquatic organisms (Sugiura *et al.*, 1998; Refstie *et al.*, 1999; Storebakken *et al.*, 2000; Percival *et al.*, 2001). The collection of faecal samples is important and many studies on salmonids, including the present research, have used modifications of the Guelph-System for collection by settlement (Hajen *et al.*, 1993ab; Sugiura *et al.*, 1998). Studies on larger salmon have tended to use stripping to collect the faeces (Percival *et al.*, 2001). Either approach has some potential problems so that it may be better consider the relative values of digestibility data so that ingredients can be ranked within one experimental approach. The use of marker is critical and chromic oxide has been used for many years in studies on fish (Austreng, 1978). This marker was used in the present study having been validated for use in previous research (Carter, 1998). However, more recent trends are to use yttrium or ytterbium oxides (Sugiura *et al.*, 1998). Finally, the original formula (Cho *et al.*, 1982) for calculating nutrient digestibility from individual ingredients has been questioned (Sugiura *et al.*, 1998). The original formula assumes that the nutrient composition of the test ingredient is the same as that of the reference diet and for this reason the revised formula given in Sugiura *et al*. (1998) is used in the present study. The main aim of the present study was to assess the apparent digestibility of crude protein (nitrogen), indispensable amino acids and energy of 19 protein sources with potential for use in Atlantic salmon feeds. Further aims were to assess these ingredients in terms of feed intake and effect on several measures of immune status.

Materials and methods

Ingredients

Ingredients were selected in consultation with Skretting Australia (Cambridge, Tasmania), the industry partner in the Grant. They were taken to provide a range of animal by-products and plant meals that were readily available in Australia and were either used or had potential for use in commercial salmon feeds. The majority of ingredients were obtained via Skretting Australia (Table 1). Inclusion of ingredients in the list (Table 1) does not necessarily mean they are now or have been used in commercial salmon feeds. Complete details on the ingredients is provided in **Appendix 4.**

Protein quality criteria of ingredients

The indispensable amino acid (IAA) score and index are both based on the relating the AA content of an ingredient to a standard. The IAA requirements for rainbow trout as provided by the NRC (1993) were selected as the standard and the values used were: Arg (15 g/kg); His (7); Iso (9); Leu (14); Lys (18); Met + Cys (10); Phe + Tyr (18); Thr (8); Try (2) and Val (12). The indispensable amino acid score (IAAS) was calculated for each IAA as

$$IAAS = a/a_r$$

where a is the concentration of an amino acid in the ingredient (g/kg) and a_r the dietary requirement (as g/kg feed).

Experimental organisation

Apparent digestibility trials were conducted over several years with Atlantic salmon parr obtained from several Tasmanian hatcheries. Fish were acclimated for at least two weeks before experimentation. Experiments were conducted at the School of Aquaculture, University of Tasmania. Salmon were stocked into 300-L conical bottomed tanks at 10 fish per tank. The tanks were held in a constant environment room and temperature (15.0 ± 0.5^{o}C) and photoperiod (12L:12D) were maintained. The fish were held in a partial recirculation system and water was treated through physical and biofilters with a continuous replacement of approximately 20% per day. Water quality parameters (DO, pH, ammonia, nitrate and nitrite) were monitored every 2-3 days to ensure water quality remained well within limits recommended for Atlantic salmon (Wedemeyer, 1996).

Apparent digestibility

Fish were fed at 2 % BW day^{-1} using belt feeders and equal feeds at 09:00-10:00 and 15:30-16:30 (uneaten feed was removed from the collectors before faecal collection). Apparent digestibility coefficients for crude protein were measured for the reference and test diets in order to calculate ADC for the test ingredients. Faecal samples were collected by settlement in faecal collectors attached to the tanks described above and held in an ice / salt slurry over the collection period. Duplicate groups of salmon were fed a reference diet (RF2, Table 2) and each test diet for 28 days. On days 13 to 15 and days 26 to 28 faecal samples were collected from the settlement trap between 17:00 to 09:00, freeze dried, pooled and used in the analysis of the marker, chromic oxide, and nutrients (see below).

RF2 was mixed with chromic oxide at a ratio of 99:1 and the apparent digestibility coefficients for selected nutrients in RF2 (ADC_{RF2}) calculated using the standard formula

$$ADC_{RF2}\ (\%) = 100 - [100.\ (\%\ I_{diet} / \%I_{faeces}) \times (\%N_{faeces} / \%N_{diet})$$

(Maynard & Loosli, 1969) where I is the inert marker and N the nutrient. Ingredient digestibility was measured by mixing RF2 with the ingredient and chromic oxide at a ratio of 69: 30: 1 (Cho *et al.*, 1982) or 84: 15: 1 as specified and faecal samples taken as described above. The apparent digestibility coefficients for energy, nitrogen and amino acids for each ingredient were calculated as

$$ADC_I\ (\%) = (N_{test} \times ADC_{test} - 0.7 \times N_{ref} \times ADC_{ref}) / (0.3 \times N_I)$$

(Sugiura *et al.*, 1998) where ADC_{ref} and ADC_{test} were the apparent nitrogen digestibility coefficients of the reference and test diets, respectively, and N_{test}, N_{ref} and N_I the nutrient content of the reference diet, test diet and the ingredient, respectively. The equation takes account of differences in the nutrient content and availability between the ingredient and reference diet compared to previously used formula such as the one proposed by Cho (Cho *et al.*, 1982).

Cumulative immune score

Whole blood was used for the measurement of neutrophil activity by the reduction of nitroblue tetrazolium (NBT) to formazan, with values presented as the optical density at 540 nm after reduction (Anderson & Siwicki, 1996). Remaining blood was centrifuged (1000 × *g,* 5 min) to obtain plasma. Plasma was used for measurement of lysozyme activity by determining the rate of lysis of a *Micrococcus lysodeikticus* suspension and using hen egg white lysozyme (Sigma-Aldrich, Castle Hill, NSW, Australia) as standard (Thompson *et al*., 1994). The volume of plasma required to inhibit 50% of a standard trypsin activity was used to quantify antiprotease activity

(Ellis, 1990). The difference in plasma protein concentration (measured according to (Lowry *et al*., 1951)) before and after precipitation with polyethylene glycol was considered to be the plasma immunoglobulin fraction (Siwicki *et al*., 1994). The cumulative immune score (CIS) was calculated as the sum of these assay values when each was expressed relative to the value for the reference diet. When CIS = 1 there was no immuno-modification relative to the reference diet where as CIS of <1 or >1 showed immuno-modification relative to the reference diet.

Results and Discussion

Ingredients

Details including the crude protein, crude lipid, energy and amino acid composition on an 'as is' and digestible basis as well as the first limiting amino acid and IAAS are given for each ingredient in **Appendix 4**.

Feed intake

At 30% inclusion different ingredients had different effects on feed intake (Fig. 1). The major features were the feed intake relative to the RF2 diet and the change between the first and second two-week periods. The feed intake of several ingredients was approximately equal to the RF2 after 2 weeks and had increased by 4 weeks. Fish meal-3, meat meal-3 and corn zein were consumed in excess of 120% RF2 by week 4. Some ingredients were consumed at markedly lower rates than RF2 even after 4 weeks. Thus, consumption rates for blood, canola, soybean-1 were less than 80% and wheat gluten less than 50% of RF2. These data suggest that the latter ingredients may have limitations if their inclusion is linked with a long-term depression in feed intake at 30% dietary inclusion.

Feed intake of blood meal, canola meal–1, corn gluten and soybean–1 were investigated at 15% inclusion and data presented for weeks 1 and 3 to be compared with feed intake for 30% inclusion at the same time (Fig. 2). Decreasing the inclusion level led to a marked increase in feed intake for all of these ingredients that approached or exceeded (corn gluten) 100% of RF2. In addition, there was less change between week 1 and 3 compared with 30% inclusion. This suggested that the use of the ingredients in feeds would need to be managed to take account of differences in feed intake.

Feed intake was not directly influenced by the digestible nutrient value of the test ingredients and there was no relationship between the digestible energy or digestible protein content of the ingredients and relative feed intake. If there had been a direct relationship it is probable that this would have resulted in a negative relationship between digestible energy (or protein) and relative feed intake. The fish would have eaten more of test diets containing test ingredients that supplied lower levels of digestible nutrients. It appeared that feed intake was influenced by ingredient-factors other than macro-nutrient intake.

Digestibility

Table 3 provides a complete summary of the crude protein and energy composition of the ingredients tested and the ADC values for crude protein, total amino acid nitrogen and energy. The digestibility data for the amino acids is given in Table 4. As would be expected the ADC values for crude protein and energy varied between ingredients and were high for purified sources such as the wheat and corn gluten and considerably lower for some of the rendered meals (Table 3). Comparison between the present study and

other studies on salmon species can be made (Table 5). Anchovy meal (Fish meal –1) is the main source of protein in Atlantic salmon feeds and there was excellent consistency in the ADC N obtained from the three studies on different salmon species (Table 5). For other products where there is also likely to be a high degree of similarity in the product such as corn and wheat gluten the digestibility data were similar (Table 5). There is likely to be more variability in the quality of rendered products (Bureau *et al.*, 1999) and this explains part of the difference between and within the studies on salmon.

The crude protein and energy digestibility values for the different ingredients followed the same trend ($n = 7$; $r = 0.96$; $P < 0.001$) and were generally lower for energy than for crude protein. A few crude protein digestibility values were above 100% (wheat gluten and poultry meal) and could indicate slight over estimation of N intake or slight under estimation of faecal nitrogen. Values of above 100% may also suggest that digestibility was not independent of the basal diet and that there was an interaction between the nutrients in RF2 and the test ingredient (Sugiura *et al.*, 1998). It is interesting to note that when nitrogen digestibility was calculated directly from individual amino acids the values were very similar and they followed the same trend as when calculated from crude protein ($n = 11$; $r = 0.66$; $P < 0.01$). A similar observation was made previously with Atlantic salmon (Anderson *et al.*, 1992).

Apparent digestibility values were calculated for individual amino acids including tryptophan (Table 4). For each ingredient the majority of amino acids were similar to each other and similar to the crude protein digestibility. There were a few anomalous data such as very low values (accompanied by high variability) and explained by the very low content of the amino acid in the ingredient but a relatively high faecal content (eg lysine in corn – zein). There were also consistently high amino acid digestibility values of over 100% recorded for the wheat gluten, this may have been a product of the low amounts of faecal material due to the poor feeding response (or nutrient interactions as discussed above). The majority of values were above 90% which might be considered high. This may be partly explained by the use of settlement to collect faecal material and the potential for leaching of nutrients. However, detailed analysis of the approaches taken in other studies must be considered before leaching is taken as the only explanation. Stripping of small salmon is not straightforward and can easily result in faecal material being voided before complete absorption of nitrogenous material via the posterior intestine and contamination, whilst less likely if a correct technique is used, is still possible (Percival *et al.*, 2001). The use of small fish will amplify the effect of these two factors. The issues are complex as illustrated by the similarities and differences in crude protein digestibility values for similar ingredients (Table 5). Two ingredients digestibility values obtained in the present study can be compared with another study (Anderson *et al.*, 1992) that used stripping instead of settlement for similar sized Atlantic salmon to those used in the present experiment. Total nitrogen digestibility for canola meal estimated from amino acid digestibility was 79% for the present study and almost identical to 79% calculated by Anderson et al. (1992). In contrast, the values for extracted soybean were markedly different, around 100% in the present study compared to 70%. The reasons are not clear and will require further research although it clear that considerable variation in digestibility is associated with different products as well as with different batches of the same product.

Cumulative Immune Score

The cumulative immune score represented a first attempt to use indicators of immune status to systematically evaluate ingredients (Fig. 4). It showed that most

ingredients, when included at 30%, did not have a major effect on the immune response of salmon. The index was used to select two ingredients to investigate further (Bransden *et al.*, 2001). Dehulled lupin meal and feather meal were selected as having a lower CIS than most of the other ingredients, considerably lower than fish meal-1 used to make the fish meal only control diet (Bransden *et al.*, 2001). The use of disease challenge as a more focused test of nutritional status is discussed in Section 7.8.

Ingredient Mixtures

In order to develop the use of ingredients the first steps were taken to investigate the use of combinations of ingredients. This was done through the inclusion of 15% solvent extracted soybean (soybean-1) with either 15% corn gluten or dehulled lupin (lupin-1). Feed intake was positively influenced and was higher for the mixed feeds then when any of the ingredients were used separately (Fig. 5). The effect can be seen by comparison between the measured feed intakes and those predicted from the simple arithmetic combination of feed intakes for individual ingredients. The effect was small with lupin but much larger with corn gluten and feed intake compared with predicted intake was 149 and 148% at 2 and 4 weeks, respectively. The explanation could either be due to the lower levels of both plant ingredients or a synergistic effect due to their combination.

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Table 1
Ingredients selected for determination of apparent digestibility coefficients for Atlantic salmon

Ingredient	Product Name	Source
Fish meal – 1	Peruvian Fish Meal	Anchovy / Mackerel, Peru
Fish meal – 2	Petuna FAQ	Petuna Seafoods, TAS
Fish meal – 3	Triabunna Fish Meal	Triabunna, TAS
Blood meal	Blood Meal	Peerless Holdings, VIC
Meat meal – 1	HP Meat Meal	Imported
Meat meal – 2	Provine	Aspen By-products, VIC
Meat meal – 3	Lamb Meal	Fletcher International, NSW
Meat & bone – 1	Meat & Bone	Longford Meatworks, TAS
Meat & bone - 2	Meat & Bone	Peerless Holdings, VIC
Poultry meal	Poultry Meal	Edgell, TAS
Feather meal	Feather Meal	Peerless Holdings, VIC
Canola meal - 1	Extracted Canola Meal	Cargill VIC
Canola – whole	Whole Canola	Gibson's, TAS
Corn – Gluten	Corn Gluten	Imported
Corn – Zein	Zein – Corn	Sigma
Lupin – 1	Dehulled Lupin	Milne Feeds, WA
Lupin – whole	Whole Lupin	Milne Feeds, WA
Soybean – 1	Concentrate	Imported
Soybean – 2	Extracted Soybean Meal	Pivot, TAS
Wheat – Gluten	80% Vital Wheat Gluten	Starch Australasia

Table 2
Ingredient and nutrient composition of the reference diet

Ingredient composition ($g\ kg^{-1}$)	Diet RF2
Fish meal-2	700
Fish oil	150
Wheat flour	138.5
Vitamin & mineral pre-mix	1.5
CMC	10
Proximate composition ($g\ kg^{-1}$ DM)	
Crude protein	459
Gross energy ($kJ\ g^{-1}$ DM)	20.3

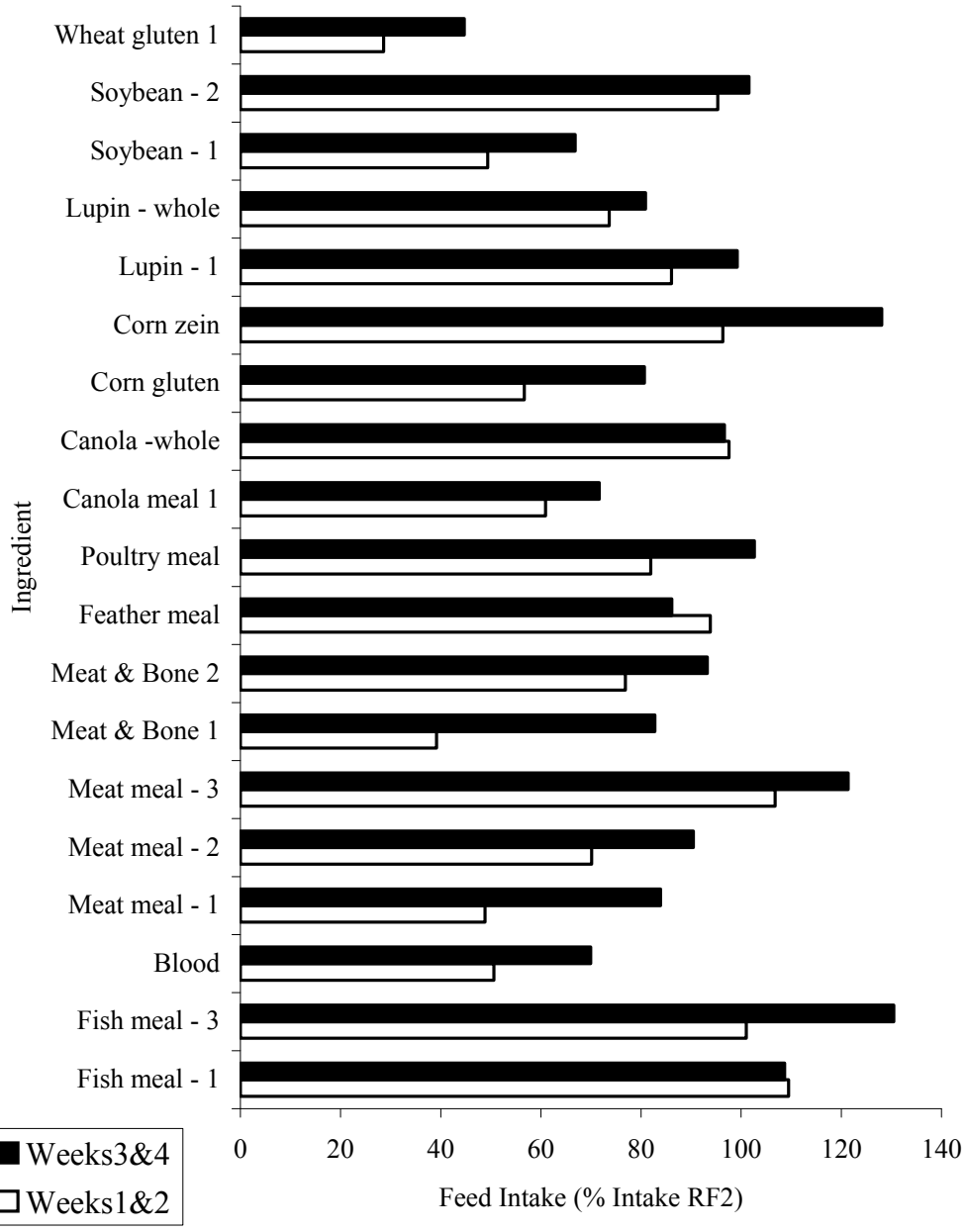

Figure 1
Feed intake as a percentage of RF2 after 2 and 4 weeks of salmon fed test diets containing 30% inclusion of selected ingredients.

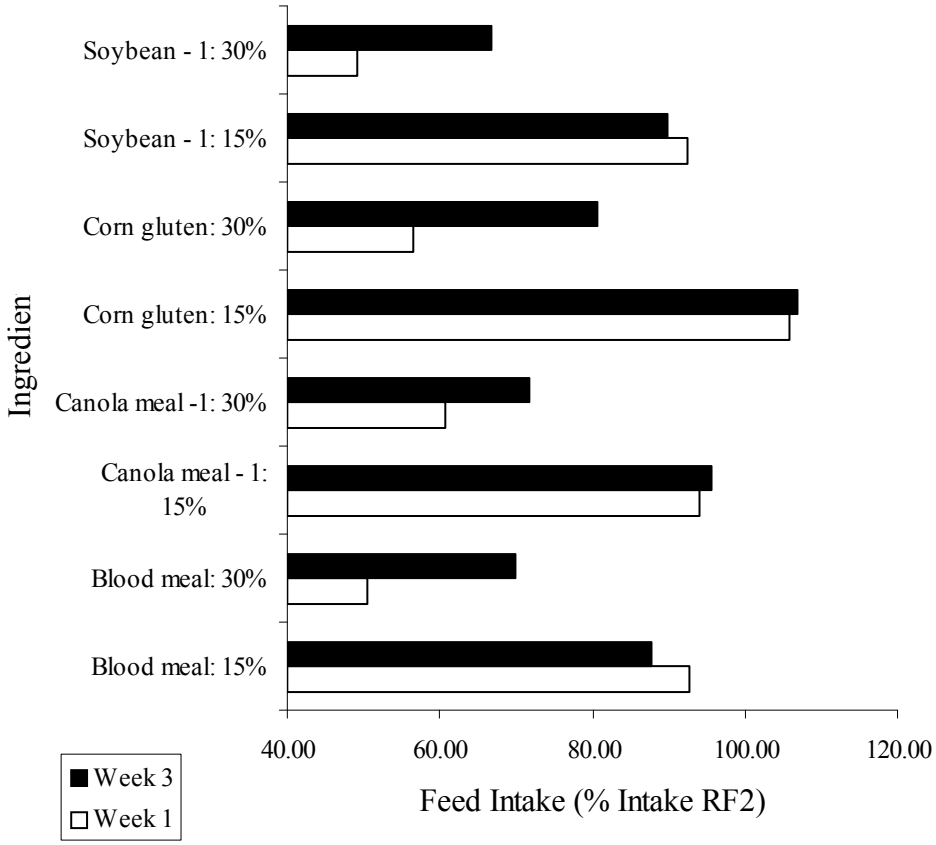

Figure 2
Feed intake as a percentage of RF2 after 1 and 3 weeks of salmon fed test diets containing 15% or 30% inclusion of selected ingredients.

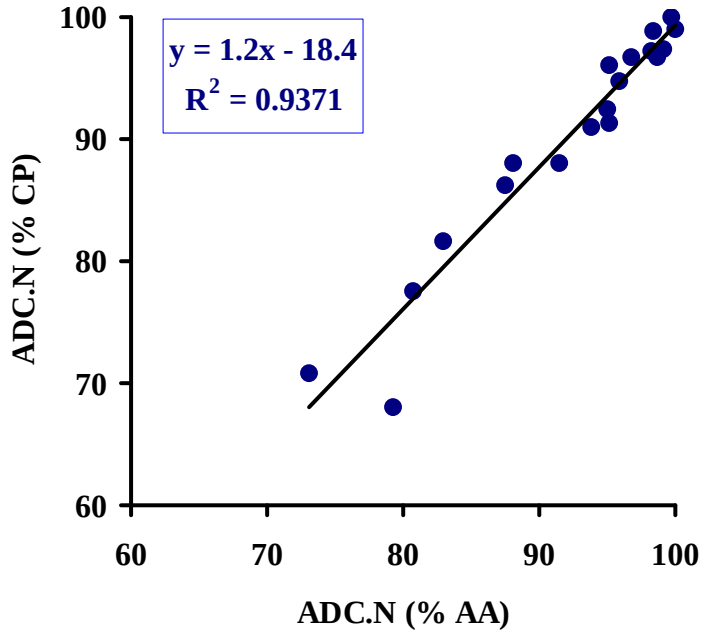

Figure 3
Correlation between the ADC.N calculated from crude protein and from the individual amino acids (taking account of the content and digestibility of all amino acids in each ingredient).

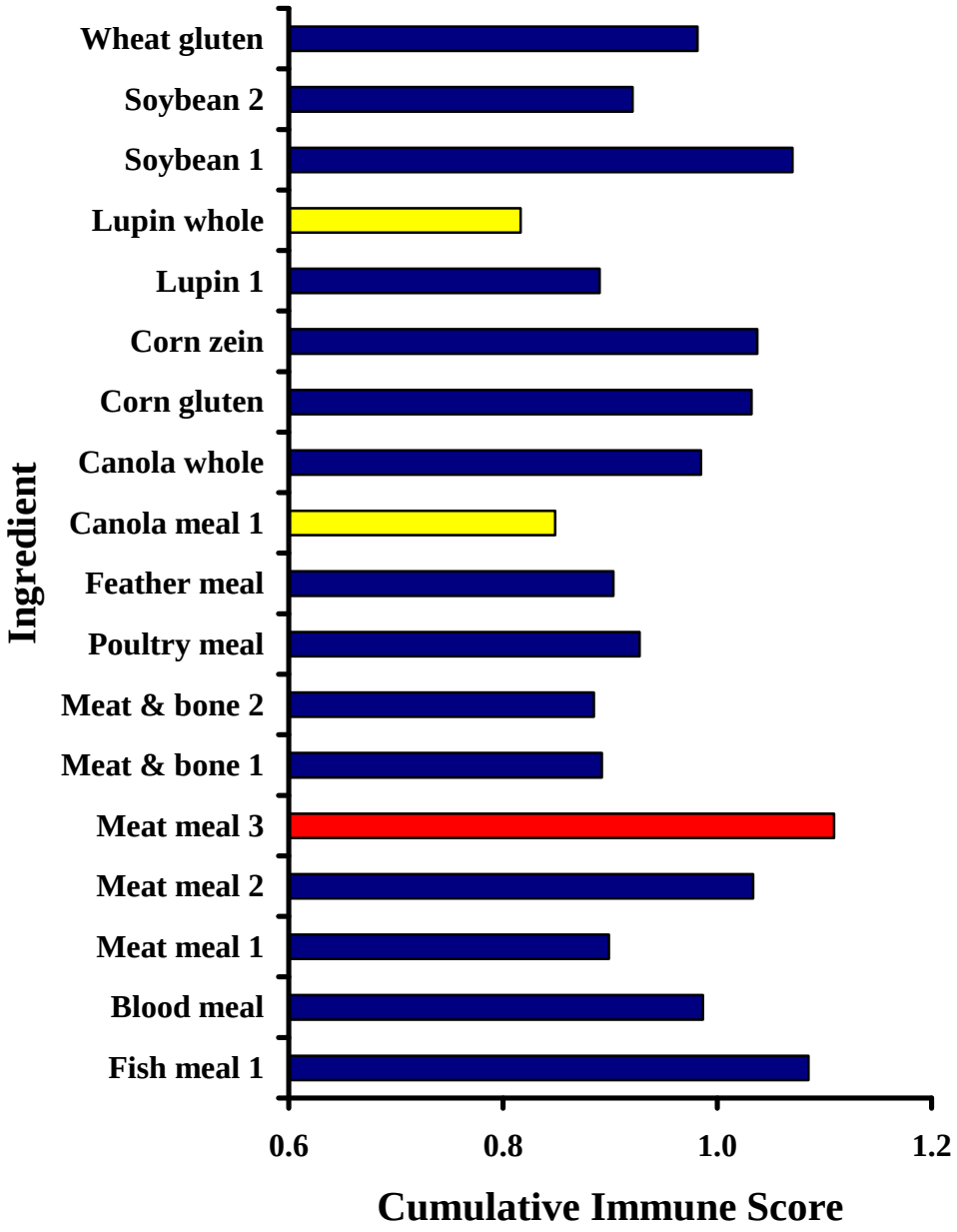

Figure 4
The Cumulative Immune Score for ingredients included in reference feed at 30%. The CIS was based on plasma lysozyme, antiprotease, neutrophil activity and protein relative to the control feed. Lowest CIS is shown by lightest shading and highest CIS is shown by darker shading (other CIS as black).

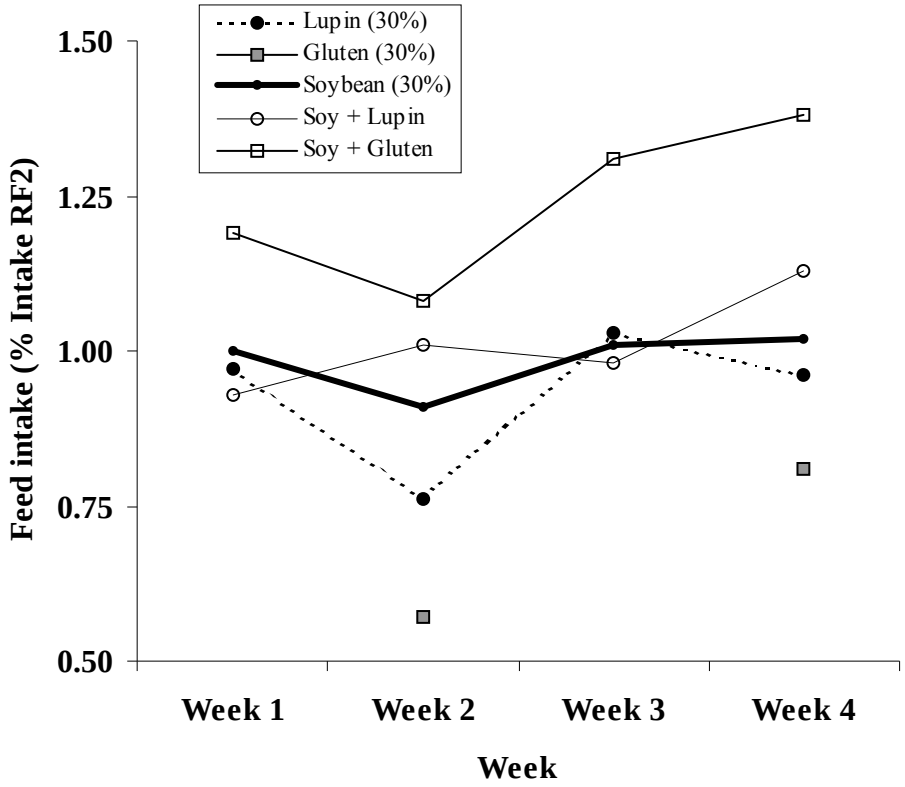

Figure 5
Feed intake as a percentage of RF2 after 1 to 4 weeks of salmon fed test diets containing 30% of single ingredients (dehulled lupin, corn gluten, extracted soybean) or in combination (15% soybean plus 15% lupin; 15% soybean plus 15% corn gluten). Corn gluten data average of weeks 1-2 and 3-4.

Table 3
Composition and apparent digestibility coefficients for crude protein (N), based on the total nitrogen (% N) and on the individual amino acids (% AA), and for energy (% kJ) of ingredients calculated using equation of Sugiura *et al*., 1998

Ingredient	Time (weeks)	**Inclusion (%)**	Crude protein (g/kg)	ADC.N (% N)	ADC.N (% AA)	Gross Energy (kJ/g)	ADC.kJ (% kJ)
Fish meal – 1	4	30	656 ± 1	91.28 ± 0.29	95.11	18.84 ± 0.01	93.15 ± 0.14
Fish meal - 3	4	30	702 ± 2	98.82 ± 0.97	98.38	19.92 ± 0.04	98.74 ± 2.21
Blood meal	4	30	877 ± 28	92.39 ± 1.96	95.06	21.73 ± 0.05	95.79 ± 0.34
Meat meal – 1	4	30	668 ± 5	94.75 ± 1.53	95.91	19.94 ± 0.08	86.30 ± 3.39
Meat meal – 2	4	30	755 ± 5	87.99 ± 0.26	88.02	23.71 ± 0.01	81.40 ± 1.55
Meat meal - 3	4	30	523 ± 2	70.76 ± 0.00	73.14	15.11 ± 0.06	69.64 ± 1.66
Meat & bone - 1	4	30	527 ± 2	77.55 ± 3.61	80.76	15.80 ± 0.13	75.52 ± 1.48
Meat & bone - 2	4	30	532 ± 1	81.58 ± 1.07	82.96	18.17 ± 0.04	74.63 ± 2.40
Poultry meal	4	30	480 ± 1	90.94 ± 0.41	93.83	26.18 ± 0.24	91.27 ± 0.26
Feather meal	4	30	756 ± 9	86.27 ± 1.57	87.56	23.22 ± 0.23	79.88 ± 0.51
Canola meal - 1	4	30	354 ± 1	67.97 ± 6.94	79.30	17.60 ± 0.26	42.28 ± 1.31
Canola - whole	4	30	222 ± 1	96.13 ± 0.89	95.10	26.13 ± 0.41	80.53 ± 3.99
Corn – Gluten	4	30	603 ± 4	88.05 ± 0.43	91.43	21.25 ± 0.05	83.50 ± 0.71
Corn – Zein	4	30	875 ± 14	96.71 ± 3.67	96.75	23.28 ± 0.01	95.25 ± 1.82
Lupin – 1	4	30	383 ± 1	96.68 ± 4.21	98.70	18.52 ± 0.03	63.08 ± 3.84
Lupin - whole	4	30	338 ± 2	97.44 ± 6.91	99.09	18.16 ± 0.02	65.74 ± 4.27
Soybean – 1	4	30	602 ± 4	97.14	98.28	17.82 ± 0.06	79.42 ± 2.46
Soybean - 2	4	30	463 ± 2	102.24 ± 0.69	99.70	17.81 ± 0.01	89.38 ± 3.24
Wheat – Gluten	4	30	779 ± 7	99.04 ± 0.16	99.94	20.97 ± 0.06	89.66 ± 0.98
Blood meal	4	15	877 ± 28	97.53 ± 1.15	98.99	21.73 ± 0.05	99.88 ± 2.68
Canola meal - 1	4	15	354 ± 1	86.66 ± 8.39	98.81	17.60 ± 0.26	65.89 ± 5.33
Corn – Gluten	4	15	603 ± 4	90.92 ± 1.97	96.20	21.25 ± 0.05	85.65 ± 4.98
Soybean – 1	4	15	602 ± 4	101.73 ± 0.39	99.91	17.82 ± 0.06	92.94 ± 1.26

Table 4

Apparent digestibility coefficients (%) for essential and non-essential amino acids of ingredients calculated using equation of Sugiura *et al*., 1998

Ingredient	Arg	His	Iso	Leu	Lys	Met	Cys	Phe	Tyr	Thr	Try	Val	Ala	Asx	Glx	Gly	Pro	Ser
Fish meal – 1	94.84 (0.27)	95.91 (0.15)	98.04 (0.18)	98.18 (0.19)	98.19 (0.16)	96.65 (0.23)	87.94 (0.39)	95.68 (0.24)	96.49 (0.21)	95.93 (0.25)	98.34 (10.7)	97.48 (0.19)	94.71 (0.31)	91.20 (0.34)	98.08 (0.23)	84.54 (0.59)	92.93 (0.38)	93.24 (0.40)
Fish meal – 3	99.23 (1.92)	98.71 (1.23)	97.99 (0.18)	98.43 (1.46)	99.68 (1.67)	97.42 (1.65)	100.9 (2.00)	97.62 (1.62)	97.24 (1.75)	98.59 (1.92)	101.0 (3.49)	98.56 (1.55)	97.46 (1.91)	99.67 (1.13)	98.91 (1.54)	96.89 (2.05)	94.61 (0.94)	98.06 (1.84)
Blood meal	92.59	92.51	98.53	95.89	97.59	95.78	89.20	95.36	95.93	95.63	63.46	96.03	95.34	95.38	97.13	93.31	92.11	94.20
Meat meal – 1	97.41 (1.24)	94.55 (1.49)	96.90 (1.47)	97.87 (1.16)	98.02 (1.48)	96.75 (2.64)	74.91 (4.31)	95.60 (1.71)	95.15 (1.64)	95.34 (1.80)	91.85 (18.5)	96.79 (1.33)	96.78 (1.38)	88.76 (2.32)	98.16 (1.11)	95.71 (1.40)	96.69 (1.09)	93.94 (1.72)
Meat meal – 2	90.55 (0.51)	88.02 (0.30)	84.41 (0.09)	87.12 (0.14)	90.91 (0.03)	82.35 (0.96)	79.28 (0.44)	86.12 (0.63)	84.94 (0.76)	85.73 (0.34)	82.74 (8.64)	86.12 (0.22)	90.88 (0.16)	82.83 (1.02)	90.29 (0.55)	94.26 (0.14)	88.87 (0.99)	85.72 (0.81)
Meat meal – 3	73.22 (0.76)	79.83 (0.40)	77.68 (1.49)	78.73 (0.17)	79.40 (0.52)	72.52 (2.62)	61.83 (7.18)	77.12 (0.40)	76.42 (0.40)	74.21 (0.50)	77.48 (13.1)	77.02 (1.30)	71.62 (0.02)	71.42 (3.97)	75.76 (0.36)	66.59 (2.37)	67.97 (2.25)	69.49 (2.38)
Meat & bone - 1	78.37 (2.36)	77.45 (2.21)	87.60 (1.93)	86.67 (1.63)	89.22 (1.62)	89.67 (2.59)	53.89 (3.74)	85.56 (1.90)	85.11 (1.96)	83.71 (2.19)	26.96 (7.89)	87.44 (1.63)	80.61 (2.21)	81.94 (2.49)	82.99 (2.12)	75.07 (2.56)	73.96 (2.45)	76.05 (2.95)
Meat & bone - 2	81.33 (0.89)	88.23 (0.79)	90.27 (0.66)	89.25 (0.61)	87.87 (0.83)	93.16 (1.02)	74.47 (0.93)	87.36 (0.73)	89.61 (0.68)	87.03 (0.82)	86.42 (0.01)	88.55 (0.64)	79.64 (0.96)	81.15 (1.06)	86.06 (0.80)	71.51 (1.18)	77.16 (0.89)	84.81 (0.89)
Poultry meal	96.12 (0.22)	92.66 (0.30)	94.90 (0.23)	95.36 (0.19)	98.33 (0.23)	99.67 (0.34)	78.50 (0.30)	94.33 (0.23)	95.38 (0.21)	93.38 (0.26)	105.5 (1.23)	93.66 (0.21)	95.20 (0.27)	86.24 (0.40)	97.53 (0.22)	93.46 (0.33)	92.34 (0.25)	91.82 (0.26)
Feather meal	91.80 (1.14)	71.59 (3.83)	92.42 (0.82)	90.79 (0.92)	81.02 (2.74)	83.63 (3.44)	76.66 (1.42)	90.76 (0.95)	87.06 (1.23)	79.91 (10.5)	74.89 (10.4)	91.27 (0.81)	89.79 (1.68)	75.71 (2.36)	88.53 (1.42)	89.15 (1.81)	88.25 (1.06)	89.58 (0.92)
Canola meal – 1	83.26	77.81	82.06	84.27	81.58	89.89	74.41	81.12	80.81	75.52	69.90	79.72	72.23	72.83	89.55	56.48	74.30	71.82
Canola – whole	99.85 (0.79)	98.55 (3.10)	91.08 (3.97)	90.24 (3.86)	99.03 (0.18)	101.1 (7.13)	96.09 (1.25)	92.67 (5.39)	91.35 (3.51)	91.12 (1.42)	98.60 (0.57)	91.00 (3.72)	98.69 (5.63)	104.0 (5.11)	98.99 (3.40)	108.7 (4.81)	85.54 (5.20)	96.49 (4.60)
Corn – Gluten	90.99	89.02	91.35	92.92	87.84	91.26	87.68	91.89	92.10	89.77	78.21	90.99	92.03	89.41	93.36	79.48	91.79	90.55
Corn – Zein	102.3 (7.15)	96.11 (2.97)	94.61 (3.54)	97.42 (1.93)	33.97 (93.7)	95.18 (5.03)	89.46 (6.21)	95.48 (3.05)	96.51 (2.29)	96.05 (3.37)	140.9 (12.1)	94.24 (2.48)	98.38 (2.47)	94.82 (5.15)	96.98 (2.74)	122.0 (10.6)	98.21 (1.76)	96.29 (3.43)
Lupin – 1	101.0 (2.92)	96.97 (4.20)	97.20 (5.07)	96.28 (4.73)	96.60 (7.08)	108.8 (19.2)	99.77 (5.28)	97.29 (5.12)	99.36 (3.78)	98.72 (7.21)	107.0 (10.2)	97.78 (4.74)	102.8 (11.4)	99.36 (6.13)	99.24 (3.40)	106.7 (18.5)	97.46 (9.99)	99.18 (5.51)
Lupin – whole	102.6 (4.83)	99.08 (5.42)	98.02 (4.58)	98.29 (4.30)	100.9 (6.79)	100.8 (23.7)	102.2 (10.3)	96.72 (4.46)	99.45 (3.97)	100.6 (7.36)	100.5 (7.65)	98.22 (6.82)	102.6 (16.7)	101.6 (7.95)	100.2 (3.66)	111.4 (26.7)	90.62 (15.2)	99.44 (8.11)
Soybean – 1	99.69	97.56	99.86	99.24	101.9	102.4	93.07	91.00	100.2	97.52	101.1	99.11	96.91	96.98	100.3	91.16	95.53	97.26
Soybean – 2	104.8 (1.79)	100.9 (1.95)	99.16 (0.08)	98.50 (0.17)	102.5 (1.11)	106.4 (2.84)	102.3 (0.02)	99.20 (0.63)	100.0 (0.84)	99.69 (1.96)	105.3 (0.81)	99.82 (1.11)	103.2 (1.89)	103.0 (0.19)	100.8 (0.27)	111.7 (4.93)	98.26 (2.85)	101.1 (0.70)
Wheat - Gluten	103.9	95.92	103.5	103.1	116.9	104.7	100.9	101.9	102.5	104.3	108.3	103.7	103.7	107.5	101.1	100.9	100.1	101.6
Soybean 2 + Lupin 1	99.62 (0.95)	96.96 (0.79)	94.78 (0.18)	95.55 (1.26)	98.71 (0.52)	88.11 (10.0)	97.66 (0.81)	94.45 (1.14)	95.68 (0.63)	94.88 (0.29)	102.2 (6.23)	95.75 (0.56)	96.21 (0.25)	98.85 (0.68)	97.74 (0.23)	99.42 (3.06)	88.06 (1.77)	96.11 (0.53)
Soybean 2 + Corn Gluten	95.27 (2.46)	92.25 (0.39)	88.34 (2.16)	91.83 (1.26)	91.69 (2.34)	92.66 (1.00)	88.38 (1.77)	90.26 (1.43)	91.54 (1.29)	88.23 (3.68)	92.69 (2.17)	89.31 (1.98)	91.51 (2.62)	90.71 (1.91)	93.07 (0.58)	90.56 (6.16)	89.68 (2.01)	91.06 (2.26)

Table 5
Comparison between apparent digestibility for crude protein (%) determined for salmon species

Salmon species	Atlantic		Coho	Chinook
	This study	Anderson *et al*. 1992	Sugiura *et al*., 1998	Hajen *et al*., 1993
Collection method	settlement	stripping	settlement	settlement
Ingredient				
Anchovy meal	91.3		91.4	91.7
Blood meal	92.4			29.4
Meat meal	70.8 – 94.8			
Meat and bone meal	77.6 – 81.6			
Poultry meal	90.9		94.2	84.9
Feather meal	86.3		79.7	57.4
Canola meal	68.0	74.1		84.5
Corn gluten	88.1	83.1	91.9	
Lupin meal	96.7			
Soybean concentrate	97.1			86.3
Soybean meal	99.7*	69.9	93.0	77.0
Wheat gluten	99.0		99.6	

* From amino acid digestibility

7.2
The effect of diet change on feed intake of Atlantic salmon in groups and of individuals within groups

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Abstract

Our previous research has shown that the inclusion of very high levels of plant proteins in salmon feeds may lead to decreased feed intake but that this may only be a temporary situation with feed intake returning to normal after a few days (Carter, 1998). In the previous experiment satiation feeding was used. The aim here was to investigate the effect of feeding a lower ration. Pea meal was included in an Atlantic salmon parr diet to replace 50% of the protein supplied by fish meal and resulted in a 50% dietary inclusion of this plant protein. Atlantic salmon parr were stocked into 300-l tanks at 25 fish per tank. Ten tanks were used and after an acclimation period the control diet (FM) was changed to the pea meal diet (PM) in five tanks. A ration of 0.5 mg. g^{-1} initial body weight was hand fed once a day in the morning and uneaten food collected. The fish were fed in this way each day for seven days. The change in diet had no effect on feed intake on any day following the change. The significance of this finding is that the use of a low ration allows the introduction of a new feed without causing changes in group consumption. Changes in group consumption were previously shown to be due to changes in intake of individual fish with different positions in a feeding hierarchy (Carter, 1998) and the use of a restricted ration therefore has the potential to be used to control this.

Keywords: Atlantic salmon; Feed intake; Fish meal replacement; Pea meal

Introduction

Our previous research has shown that the inclusion of very high levels of plant proteins in salmon feeds lead to decreased feed intake but that this was a temporary situation with feed intake returning to normal after a few days (Carter, 1998). In this research satiation feeding was used and feed intake varied substantially between individuals. Some individuals appeared to respond to the new diet more positively than other individuals and ate more at an earlier time. Analysis of feeding hierarchies suggested that fish previously lower in the feeding hierarchy increased their share of the new feed compared to fish higher in the feeding hierarchy (Wybourne & Carter, 1998). Consequently, maintaining feeding to satiation increased individual variation in feed intake. In order to explore this further the following experiment was conducted to investigate the effect of feeding a lower ration. A ration of 0.5 % BW per day was selected, this approximated maintenance ration and was predicted to promote conditions for hierarchical control of feed intake (McCarthy *et al*., 1993). The aim was to determine whether the low ration resulted in less variation in feed intake following the introduction of a new feed containing a plant protein.

Materials and methods

Experimental diets

Two isonitrogenous and isoenergetic feeds were formulated to contain only fish meal (FM) or fish meal and field pea meal (PM) (Table 1). Phenylalanine, individual vitamins and minerals used in the pre-mixes, carboxymethylcellulose (CMC), α-cellulose, bentonite and chromic oxide were supplied by Sigma-Aldrich (Castle Hill, NSW); Rovimix Stay-C by Roche Vitamins Australia (Frenchs Forest, NSW) and pea meal by . The remaining ingredients were supplied by Skretting Australia (Cambridge, Tas.) and were those used in commercial salmon feeds. The feeds were manufactured as 3 mm diameter pellets using a California Pellet Mill (CL-2 Lab Mill), dried and stored at -20^{o}C.

Experiment

The experiment was conducted at the School of Aquaculture, University of Tasmania. Atlantic salmon (*Salmo salar* L.) parr were originally obtained from Springfield Salmon Hatchery (Tas.) and stocked into 300-l tanks held in a constant environment room (temperature, 15.7 ± 0.8^{o}C; photoperiod, 12:12) and part of a partial freshwater recirculation system (Carter & Hauler, 2000). Water was treated through physical and bio-filters with a continuous replacement of approximately 20% per day. Water quality parameters (DO, pH, ammonia, nitrate and nitrite) were monitored to ensure water quality remained well within limits recommended for Atlantic salmon (Wedemeyer, 1996).

Ten groups of 20 fish were maintained in the system for 28 days and fed FM at a ration of 0.5% BW day^{-1}. On day 29 the diet fed to 5 groups was changed to PM, also supplied at a ration of 0.5 % BW day^{-1}. The remaining 5 groups continued to be fed FM and all groups were fed by hand and in the same way each day until day 36 when the experiment ended. Following the change in feed total feed intake (kg DM) was estimated, for all groups and every day, from the amount of feed that was not eaten and collected from the effluent standpipe. Individual feed intake was measured using X-radiography (see below).

At the start and end of the experiment fish were anaesthetised (50 mg l^{-1}, Benzocaine), weight and fork length measured. Fish were individually marked at the start of the experiment (Hart & Pitcher, 1969). Linear growth was assumed and weight calculated for each day in order to calculate feed intake as % BW.

X-radiography

Individual feed intake was measured twice during the FM feeding (days 10 and 26) and twice after the change in feed so that individual feed intake was measured on four occasions for each group. Feed intake of one different group (tank) fed each diet was measured on five consecutive days (days 29-33) and then for all tanks on day 36. Standard procedures using feeds labelled with Ballotini glass beads and for X-radiography equipment described previously were used (Carter *et al.*, 1996; Shelverton & Carter, 1998).

Chemical analysis

Standard methods were used to determine dry matter (freeze dry to constant weight); nitrogen (Kjeldahl using a selenium catalyst); crude fat (Bligh & Dyer, 1959); energy (bomb calorimeter: Gallenkamp Autobomb, calibrated with benzoic acid), ash (AOAC,

1995).

Statistical analysis

Mean values are reported ± standard error of the mean (SEM). Percentage data were arcsine transformed prior to analysis. Normality and homogeneity of variance were confirmed (JMP Version 3.2.1) and comparison between means was by one-way ANOVA. Multiple comparison was by Tukey-Kramer HSD. Significance was accepted at probabilities of 0.05 or less.

Results and Discussion

There were no significant differences in feed intake between groups changed onto the PM diet compared to the groups that continued on the FM diet over the eight days following the change (Fig. 1). The comparatively large decrease in the feed intake of FM on day 3 was not statistically significant. Analysis of individual feeding behaviour showed that only one or two fish had not fed on the day of the X-ray. There were no differences between the two diets in the proportion of non-feeding fish on the days following the diet change. These data were based on one tank per diet on day 0 to 4. Feed intake was measured in all the groups on day 7 and only one fish in the five tanks had not eaten the PM diet on that day. This gave an average of 1 ± 2.2 % non-feeding fish for PM compared to 5 ± 3.3 % on the FM diet where 5 fish between the groups had not eaten any of the diet. In the previous experiment the percentages of non-feeding fish were higher and were 10, 23, 20 and 10 % on days 3, 6, 10 and 12 following the diet change. Thus, in the present experiment the salmon readily accepted the diet containing 50% pea meal and showed no difference to the control fed the fish meal only diet. These differences were reflected in the decreased feed intake of the PM diet in the previous experiment (Fig. 3).

The share of the available food was investigated by calculating the mean share of the meal (MSM, %) from each X-ray (McCarthy *et al.*, 1993). This provided a rank of the feeding intake of each fish on each day that an X-ray was taken. The effect of diet change on the feeding rank of individuals was examined by calculating the Spearman Rank Correlation Coefficient between the average MSM before the date of the change with the two MSM values after the date of the change. For the fish fed FM there was no change in diet. Of the possible 10 correlations (5 tanks per diet and two X-rays) per diet there were two significant correlations for FM (both on day 7) and non for PM. Therefore, the feeding ranks were not stable and suggested that feed intake was primarily determined by scramble competition and not the result of a social regulated "pecking order" (Carter *et al.*, 1994). It is highly likely that the lack of a stable social hierarchy in the groups of salmon was due to a combination of the numbers and density of the fish and the use of a low ration. There was therefore a marked difference between the present experiment and the previous experiment which suggested that an established feeding hierarchy was temporally changed following the change to a new diet.

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Table 1
Ingredient and chemical composition of experimental feeds

	Diet	
	FM	PM
Ingredient composition ($g.kg^{-1}$)		
Fish meal	668.2	341.1
Field pea meal		500.0
Phenylalanine		2.0
Fish oil	97.0	119.1
Dextrin	186.6	12.8
Vitamin mix (ASV4)[1]	3.0	3.0
Rovimix Stay-C[2]	1.0	3.0
Choline chloride	1.2	1.2
Mineral mix (TM4)[3]	0.6	1.0
Bentonite	23.6	
CMC	8.8	8.8
Chromic oxide	10.0	10.0
Chemical composition ($g.kg^{-1}$ DM)		
Dry matter ($g.kg^{-1}$)	890	954
Crude protein	464	463
Crude fat	170	190
Ash	137	84
Gross energy ($MJ.kg^{-1}$ DM)	20.7	21.8

[1]Vitamin mix (ASV4) to supply per kg feed: 2.81 mg thiamin HCl; 10.0 mg riboflavin; 9.15 mg pyridoxine HCl; 25 mg nicotinic acid; 54.35 calcium D-pantothenate; 750 mg myo-inositol; 0.38 mg d-biotin; 2.5 mg folic acid; 0.03 mg cyanocobalamin; 6250 IU retinol acetate; 2800 IU cholecalciferol; 125 mg DL α-tocopherol acetate; 5 mg menadone sodium bisulphate.

[2]Stay-C (L-Ascorbyl-2-polyphosphate) (Roche Vitamins Australia, Frenchs Forest, NSW)

[3]Mineral mix (TM4) to supply per kg feed: 117 mg $CuSO_4$ $5H_2O$; 7.19 mg KI; 1815 mg $FeSO_4$ $7H_2O$; 307 mg $MnSO_4$ H_2O; 659 mg $ZnSO_4$ $7H_2O$; 3.29 mg Na_2SeO_3; 47.7 mg $CoSO_4$ $7H_2O$.

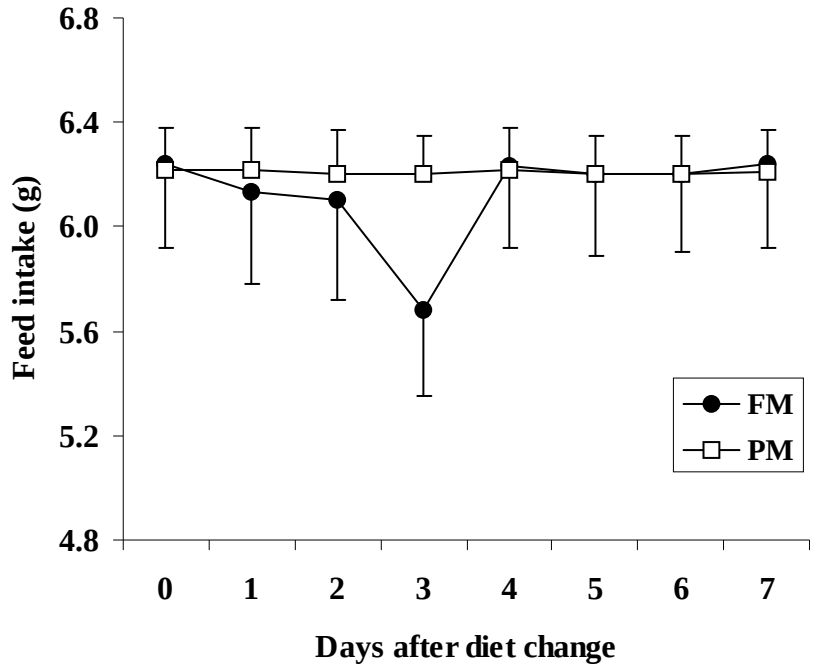

Figure 1
Feed intake (g) of groups of Atlantic salmon fed only a fish meal (FM) diet or after changing from FM to a pea meal (PM) diet. (Mean ± SEM, n = 5).

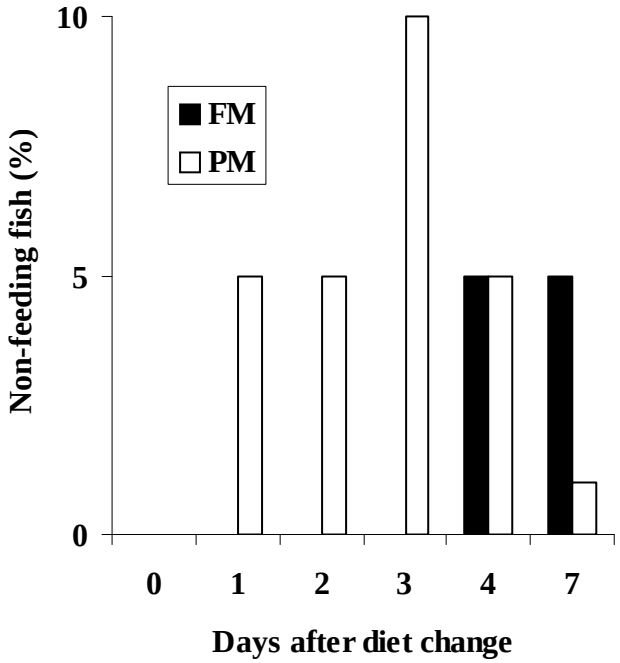

Figure 2
The proportion of non-feeding (%) Atlantic salmon fed only a fish meal (FM) diet or after changing from FM to a pea meal (PM) diet.

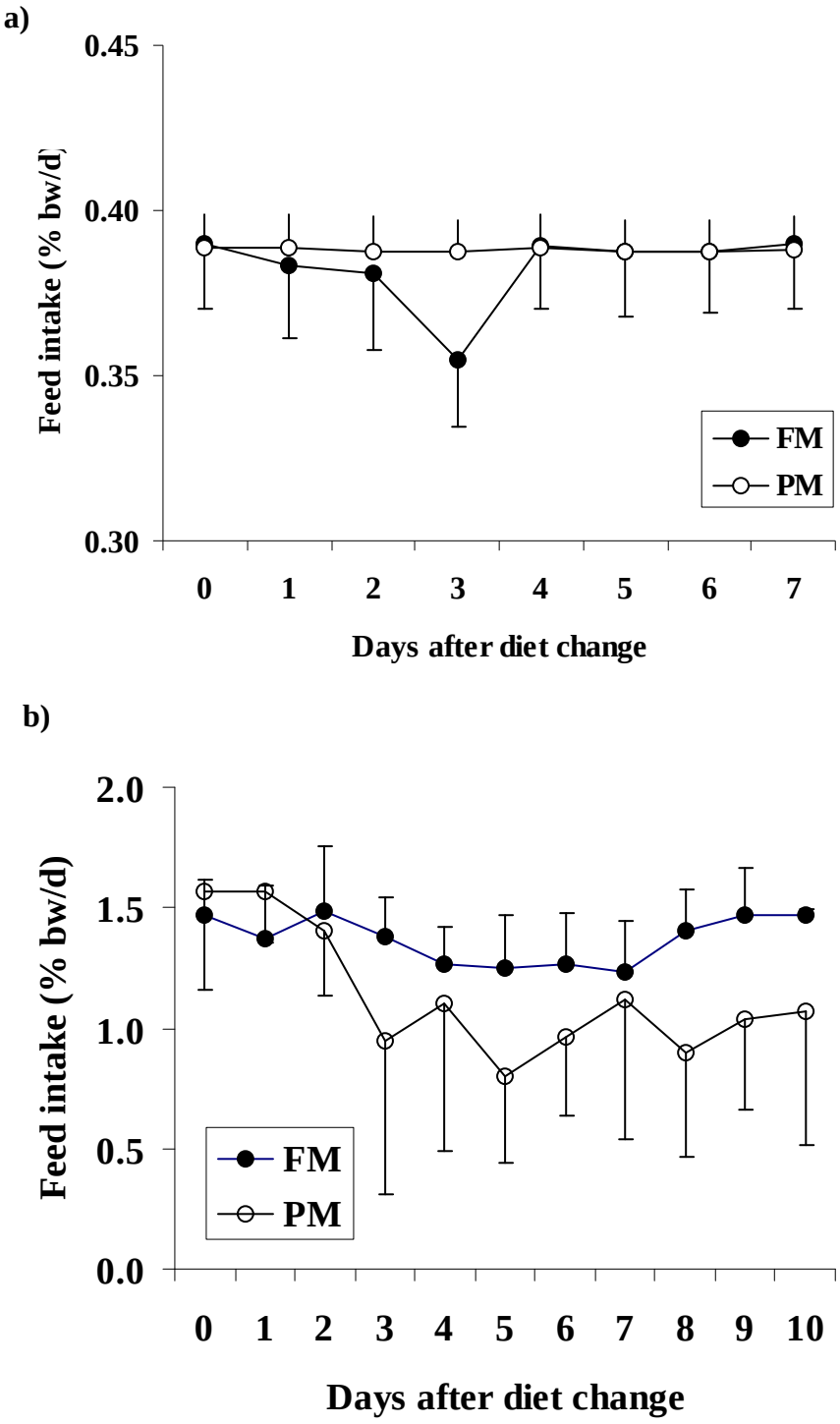

Figure 3
Feed intake (% BW/d) of groups of Atlantic salmon fed only a fish meal (FM) diet or after changing from FM to a pea meal (PM) diet for a) the current experiment in which the fish were supplied a low group ration (0.5% BW/d) or b) where the fish were supplied with a higher group ration (Carter, 1998). (Mean ± SEM, n = 5)

7.3
Lysine utilisation by Atlantic salmon parr 1: Influence of feeding regime

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Abstract

This study investigated if the efficiency of lysine utilisation for liveweight gain, protein gain and lysine gain (LysG) was affected by feeding regime (satiation or controlled ration) in Atlantic salmon (*Salmo salar* L.) parr. Twelve diets (408.3 g digestible protein.kg^{-1} and 20.6 MJ digestible energy.kg^{-1}) using zein, fish meal and crystalline amino acids were formulated to contain a range from 10.15 to 20.79 g dietary digestible lysine.kg^{-1}. Each diet was hand-fed at either satiation or controlled ration (0.85% body weight.d^{-1}) over a 76 day period. Increasing dietary lysine supplementation resulted in a significant ($P < 0.001$) linear increase in feed intake for the satiation fed parr. For both feeding regimes, with increasing DDLys supplementation there was a significant ($P < 0.001$) linear increase in live weight gain and significant increases in protein ($P < 0.001$) and lysine ($P < 0.01$) concentration of live weight gain. Allometric analysis further revealed that controlled fed parr had a significantly higher weight exponent for protein and lysine than satiation fed parr. Linear regressions described relationships between digestible lysine intake (DLysI) and live weight, protein and lysine gain. Efficiency of lysine utilisation for live weight gain above maintenance lysine intake was significantly higher ($P < 0.005$) for the satiation fed parr than the controlled fed parr (47.7 and 34.9 mg LG.mg^{-1} DLysI, respectively). For both feeding regimes, however, there was no significant difference in efficiency of lysine utilisation for protein gain ($P > 0.05$) or lysine gain ($P > 0.5$). For satiation and controlled fed parr, the efficiency of lysine utilisation for lysine gain above maintenance lysine intake was 67% and 71%, respectively, and from extrapolation of relationship between DLysI and lysine gain the lysine requirement for maintenance was estimated to be 1.13 and 1.42 mg.100 g^{-1} body weight.d^{-1}, respectively. This study demonstrated that feed intake does not influence the efficiency of lysine utilisation for protein or lysine gain in Atlantic salmon parr. Furthermore, this study was the first to identify an exogenous affect on the weight-specific lysine composition of fish. The change in weight-specific lysine composition suggests of a robust physiological mechanism maintaining the efficiency of lysine utilisation for lysine gain in Atlantic salmon parr.

Keywords: Amino acid requirements; Lysine requirements; Lysine utilisation

Introduction

For a factorial approach to be a reliable means of estimating the lysine allowance in growing Atlantic salmon (*Salmo salar* L.), the efficiency of lysine utilisation for growth [liveweight gain (LG), protein gain (PG) and lysine gain (LysG)] is required to be constant under various culture conditions. A single study comparing Atlantic salmon

(Hauler & Carter, 2001a) showed the efficiency of lysine utilisation for LysG remained constant with dietary formulation, lysine source and life-stage of production. However, this study also assumed constant lysine concentration of whole-body protein and, hence, did not unequivocally estimate the efficiency of lysine utilisation for Atlantic salmon.

The change in the lysine concentration of whole-body protein gain is an interesting feature of studies investigating lysine utilisation in production animals. The amino acid profile of protein laid down during growth of fish is thought to be reasonably constant (Wilson & Cowey, 1985; Cowey, 1994). However, Rodehutscord *et al.* (2000) demonstrated that the lysine concentration of whole-body protein gain of rainbow trout, *Oncorhynchus mykiss*, was influenced by lysine intake level. Change in the lysine concentration has been attributed to rates of different proteins being laid down at different growth rates (Gahl *et al*., 1994, 1996). Collagen in connective tissue has a lower concentration of lysine than muscle myosin (Bailey, 1989). Lower lysine concentration of whole-body protein gain in slower growing animals suggests that collagen makes a greater contribution to growth than in faster growing animals. However, the hypothesis that growth rate is responsible for the change in lysine concentration of whole-body protein gain in animals is yet to be confirmed.

For a given weight of fish it is generally regarded that the whole-body protein and amino acid composition is controlled within narrow limits (Shearer, 1994). Allometric analysis has been used to examine the weight-specific composition of fish (Weatherley & Gill, 1987; Shearer, 1994). Allometric analysis generates Log composition-Log liveweight plots that describe the composition of animals as linear within a life stage (Laird *et al*., 1968). When weight exponents (slopes) of allometric relationships equal 1, the component and liveweight are increasing at the same rate and when the weight exponent is greater than 1, the component is an increasing portion of the animal. There is little evidence of exogenous factors influencing the weight-specific protein and amino acid composition of fish. The reported changes in protein and amino acid composition of fish under experimentation can usually shown to be weight-specific by allometric analysis (Shearer, 1994).

Studies on lysine utilisation in terrestrial animals and fish have generally suggested that the response (LG, PG or LysG) to lysine intake has been a linear function (Bolton & Miller, 1985; Batterham *et al*., 1990; Adeola, 1995; Susenbeth, 1995; Kim *et al*., 1997; Edwards *et al*., 1999; Rodehutscord *et al*., 2000). Linear function to lysine intake implies that the efficiency of lysine utilisation is constant, and hence, conveniently applied to a factorial approach. Contrary to a linear function, however, studies with rats (Hegar & Frydrych, 1985; Gahl *et al*., 1991,1996) and pigs (Gahl *et al*., 1994) demonstrate a curvilinear pattern in response in which the efficiency of lysine utilisation decreases with lysine intake to less than 50% of the maximum response (diminishing returns). The decline in lysine utilisation limits the ability to estimate lysine allowances because of the trivial response per unit of lysine input (Gahl *et al*., 1996). Success of a factorial approach to estimate lysine allowance of fish depends on confirming constant efficiency of lysine utilisation for live weight, protein or lysine gain.

In the present study, lysine utilisation was investigated in Atlantic salmon parr fed incremental levels of lysine at satiation and controlled percent body weight intake. The difference in feeding regime was designed to elicit differences in growth rate of parr. The primary aim was to compare lysine utilisation for live weight, protein and lysine gain in order to test the hypothesis that efficiency remains constant under different

feeding regimes. Constant lysine utilisation would confirm that a factorial approach would be a useful means of calculating the lysine allowance of growing Atlantic salmon parr. A secondary aim was to compare the lysine concentration of whole-body protein gain of parr. It was proposed that this latter comparison would confirm that growth rate was the contributing factor to the difference in lysine concentration of whole-body protein gain in production animals.

Materials and methods

Experimental diets

A diet was formulated to contain 408.3 g digestible protein (DP).kg^{-1} and 20.6 MJ digestible energy (DE).kg^{-1} with increasing lysine levels (Table 1). Zein, fish meal and extruded wheat were used as sources of protein-bound amino acids. Crystalline amino acids (Sigma Chemical Co. and Musashi, Victoria, Australia) were added to create a dietary amino acid profile (except glycine and lysine) similar to the whole-body of Atlantic salmon parr (Hauler & Carter, 2001) (Table 4.2). The basal diet had a lysine content of 11.7 g dietary lysine (DLys).kg^{-1}. Lysine (L-lysine monohydrochloride, Musashi, Victoria, Australia, 78.5% lysine) was added (substituting against glycine) to the basal diet in 11 increments of 1.05 g DLys.kg^{-1}. Thus, the 12 DLys levels were: 11.70; 12.75; 13.80; 14.85; 15.90; 16.95; 18.00; 19.05; 20.10; 21.15; 22.20; 23.25 g DLys.kg^{-1}. To balance the pH of the feed (to pH 8), ammonium acetate was added at 3 g.kg^{-1} of the amino acid mixture (Berge *et al*., 1998). Vitamin and minerals mixtures were added as previously described (Hauler & Carter, 2001). Ingredients were mixed before being cold pellet pressed to 3 mm using a California Pellet Mill (CL-2 laboratory pellet mill, California Pellet Mill Co., San Francisco, U.S.A.). Pellets were dried at 36°C until they contained less than 100 g.kg^{-1} moisture then stored at 4°C.

Growth experiment

The experiment was conducted at the School of Aquaculture, University of Tasmania with Atlantic salmon (*Salmo salar* L.) that originated from the SALTAS Salmon Hatchery (Tasmania, Australia). Parr were transferred to a recirculation system (Carter & Hauler, 2000), acclimatised for 2 weeks during which time they were supplied a commercial feed (450 g.kg^{-1} protein, 220 g.kg^{-1} fat, 19.4 MJ DE.kg^{-1}). The system was housed in a constant environment room maintained at 12 h light:12 h dark photoperiod and 15.2 ± 0.6°C water temperature. Water quality parameters (pH, DO, ammonia, nitrate and nitrite) were maintained within limits for Atlantic salmon (Wedemeyer, 1996).

At the beginning of the experiment, fish were anaesthetised (50 mg.l^{-1} benzocaine), weighed (mean weight 42.14 g) and twenty-three fish were randomly allocated to each of 24 tanks. Twenty fish were euthanased (100 mg.l^{-1} benzocaine) for assessment of initial whole-body chemical composition (see below). To investigate the influence of feed intake on the dietary lysine requirement estimate, each of the 12 diets were fed at either satiation or a controlled (% BW.d^{-1}) feed intake. For both feeding regimes each of the 12 diets were fed to a single replicate tank. Fish were fed by hand 3 times per day (0900, 1300 and 1700 hours) over an experimental period of 76 days. Judgement of satiation was made by collecting uneaten pellets. Controlled feed intake treatments were fed the equivalent weight specific ration each day (average of 0.85% BW.d^{-1}). The weight specific ration was determined by the performance of the basal diet (11.70 g DLys.kg^{-1}) for which all controlled feed intake replicates were adjusted accordingly. Daily rations of controlled fed fish were divided into 3 equal portions each day. Every

10 days fish were fasted for the day and tanks bulk-weighed. Rations were subsequently re-calculated for the following 10 day period.

At the conclusion of the experiment, all fish were individually weighed after 48 hours of fasting. Five fish per tank were euthanased (100 $mg.l^{-1}$ benzocaine) to measure whole-body chemical composition (see below). Fish were frozen at -20°C, dried individually, homogenised in a cutting mill and then pooled by tank for further chemical analysis.

Apparent digestibility

Apparent digestibility coefficient (ADC) was estimated for all diets. From day 61 of the experiment diets containing 10 $g.kg^{-1}$ chromic oxide were fed as normal. On day 68 faeces were collected overnight (1800 to 0800 hours) in settlement collectors (Cho *et al*., 1982). Faeces were frozen, freeze-dried and stored at -20°C before analysis. Chromic oxide and nutrients were analysed (see below) in diets and faeces and ADC was calculated using:

$$ADC\ (\%) = 100 - [100(\%I_{diet}/\%I_{faeces}) \times (\%N_{faeces}/\%N_{diet})]$$

(Maynard & Loosli, 1969) where I is the inert marker and N is the nutrient.

Chemical analysis

Dry matter of whole-body was determined by lyophilisation and for diets by oven drying at 70°C to constant weight. Whole-body and diets were analysed for nitrogen (Kjeldahl, copper sulfate catalyst), crude fat by petroleum ether extraction (AOAC, 1995), ash by combustion at 550°C for 16 hours (AOAC, 1995) and chromium by acid digestion (Furukawa & Tsukahara, 1966). Amino acid content of the diets was determined by a Waters High Performance Liquid Chromatograph (HPLC) after acid (HCl) hydrolysis (Rayner, 1985). Lysine content of diets, faeces and whole-body was determined by isocratic liquid chromatography (Or-Rashid *et al*., 1999).

Statistical analysis

Least squares linear regression was used to describe relationships between: (1) DLys level and ADC of diet nutrients; (2) dietary digestible lysine (DDLys) and performance of parr; (3) final liveweight of parr and body burden (absolute amount) of protein and lysine (allometric analysis; see below); (4) LG and lysine concentration of whole-body PG in parr; (5) digestible lysine intake (DLysI) and LG, PG and LysG in controlled and satiation feeding regimes. Goodness of fit was described by the coefficient of determination (r^2) which describes the proportion of the total variation around *y* explained by the linear regression, and the standard error of the regression ($s_{y.x}$) which describes the standard deviation of the residuals of the individual values of *y* from the linear regression.

In order to consider weight-specific influence on whole-body composition, allometric analysis was applied as described by Shearer (1994, 1995). The relationship between parr liveweight (final liveweight) and the body burden (absolute amount) of protein or lysine was described by the linear relationship:

$$\text{Log body burden (protein/lysine)} = a + b\ (\text{Log fish weight})$$

where body burden and fish weight was expressed in grams. Linear regressions were performed for means from each of the tank within each feeding regime. The weight exponent (*b* = slope) and elevation of linear regressions were compared between feeding regime as described below.

Dependent variables expressed as a percentage were arcsine-transformed prior to analysis. If linear relationships were significant and variance were equal (variance ratio test, F = 5.87, $P = 0.05$, n = 20) between feeding regimes a *t*-test was used to identify if slopes and elevations were significantly different (Zar, 1984). If it was concluded that slopes and elevations were not significantly different between feeding regimes, data were pooled to quote a common linear regression equation where appropriate. Probabilities of 0.05 or less were considered significant.

Results

Apparent digestibility

The influence of feeding regime on apparent digestibility of dietary nutrients was investigated to identify a possible nutrient modulated modification to lysine utilisation and to establish the DDLys levels. Comparison was made between linear regression describing DLys level and apparent digestibility of dry matter (ADC_{DM}), protein (ADC_N), energy (ADC_{kJ}) and lysine (ADC_{Lys}) (Table 3). For ADC_{DM} and ADC_N a significant linear regression was not established for controlled fed parr. For satiation fed parr, however, a significantly negative linear regression for ADC_{DM} ($P < 0.05$) and ADC_N ($P < 0.01$) indicated a decrease in digestibility with higher feed intake. Significant negative regression was established for ADC_{kJ} for both satiation ($P < 0.001$) and controlled ($P < 0.05$) fed parr, for which the satiation fed parr were significantly lower ($P < 0.02$). Negative slopes for the satiation fed parr suggest that ADC_{DM}, ADC_N and ADC_{kJ} of the experimental diets decreased with increase in feed intake.

In both feeding regimes, ADC_{Lys} increased as DLys level increased. There was no significant difference in slopes ($P > 0.2$) and elevation ($P > 0.5$) of the relationships describing ADC_{Lys} and DLys level between feeding regimes. Consequently, data from both feeding regimes were pooled to calculate a common linear equation:

$ADC_{Lys} = 0.147DLys + 93.882$ ($r^2 = 0.86$, $s_{y.x} = 0.336$, $P < 0.001$)

This equation was used to calculate the 12 DDLys levels, which were 11.19; 12.21; 13.24; 14.26; 15.30; 16.33; 17.37; 18.42; 19.46; 20.51; 21.56; 22.62 g DDLys.kg^{-1}.

Growth performance

Average LG was 48.2 g and 32.7 g for satiation and controlled fed parr, respectively (a weight increase by factors of 2.1 and 1.8, respectively) over the 76-day experiment. For both feeding regimes, highly significant ($P < 0.001$) linear regressions described LG with increasing DDLys level (Fig. 1). For the satiation fed parr the slopes of linear regression was significantly higher ($P < 0.001$) than the controlled fed parr. Linear regressions also described the increase in feed efficiency ratio (FER) with increasing DDLys for both feeding regimes (Table 4). For FER there was no significant difference in slopes ($P > 0.1$) and elevations ($P > 0.2$) of the linear regressions between feeding regimes.

As DDLys increased, whole-body protein increased, while dry matter, lipid and ash decreased in parr under both feeding regimes (Table 5). No significant differences were detected in the slopes of linear regressions for whole-body dry matter ($P > 0.5$), fat ($P > 0.2$) or ash ($P > 0.2$) between the feeding regimes. However the elevation of linear regressions for the satiation fed parr were all significantly higher ($P < 0.001$) suggesting of a greater whole-body content of these nutrients compared to the controlled fed parr. There was no significant difference in slopes ($P > 0.05$) and elevations ($P > 0.2$) between the feeding regimes of linear regressions describing the increase in whole-body protein of the parr with increasing DDLys level. Likewise, for the increase in whole-

body lysine there was no significant difference in slopes ($P > 0.5$) and elevations ($P > 0.5$) between feeding regimes.

Allometric analysis (Table 6) demonstrated the weight exponent (*b*) for protein and lysine was greater than 1 in parr under both feeding regimes. Controlled fed parr had a significantly higher weight exponent for protein ($P < 0.001$) and lysine ($P < 0.05$) than the satiation fed parr.

For both feeding regimes, as DDLys increased, protein and lysine increased, while lipid decreased in the composition of LG (Table 7). Between the two feeding regimes, there was no significant difference in the coefficient for crude protein ($P > 0.2$), lysine ($P > 0.5$) and fat ($P > 0.2$). For controlled fed parr, slope of the relationship between LG and the lysine concentration of whole-body PG was significantly higher ($P < 0.001$) than the satiation fed parr (Figure 2)

Lysine utilisation

The slopes of the relationships between DLysI and LG, PG and LysG represented the efficiency of lysine utilisation above maintenance lysine intake (Fig. 3). The efficiency of lysine utilisation for LG in satiation fed parr was significantly higher ($P < 0.005$) than controlled fed parr (Fig. 3a). Between the feeding regimes there was no significant difference in the efficiency of lysine utilisation for PG ($P > 0.05$) or LysG ($P > 0.5$) and there was no significant difference in the elevations of the linear regressions between DLysI and PG ($P > 0.2$) or LysG ($P > 0.2$). Consequently, a common linear regression for PG and LysG was derived from data pooled from both feeding regimes (Figs. 3b&c).

The present study was not primarily designed to estimate maintenance lysine intake. From extrapolation of the linear relationship between DLysI and LysG (Fig. 3c) maintenance lysine intake was estimated to be 1.13 and 1.42 mg.100 g^{-1} BW.d^{-1} for satiation and controlled fed parr, respectively (Mean weight of fish = weight gain of parr fed 22.62 g DDLys.kg^{-1}/2). Expressed as metabolic body weight, maintenance lysine intake was 5.91 and 7.06 mg.$(kg^{0.75})^{-1}$.d^{-1}, respectively.

Discussion

The present study demonstrated that feeding regime did not influence the efficiency of lysine utilisation for PG and LysG in Atlantic salmon parr. The efficiency of lysine utilisation for LysG confirmed previous estimates for Atlantic salmon (Hauler & Carter, 2001) and rainbow trout (Rodehutscord *et al*., 2000) and suggests a factorial approach to be a useful means of calculating the lysine allowance of growing salmonids. The present study was first to evaluate the lysine concentration of whole-body PG in animals maintained at different growth rates and feeding regime. Growth rate does not appear to induce the change in lysine composition of whole-body protein as suggested in previous studies on lysine utilisation.

To quote a single efficiency of lysine utilisation, it must be assumed that a linear function describes LG, PG or LysG to marginal lysine intake (lysine intake below or at maximum response). Many researchers have debated whether utilisation of the first-limiting dietary amino acid is best described by a linear or curvilinear function (Fisher, 1972; Heger & Frydrych, 1985, 1989; Fuller & Garthwaite, 1993; Baker *et al*., 1996). Curvilinear functions described lysine utilisation to be slowly decreasing from less than 50% of the maximum response (diminishing returns) (Heger & Frydrych, 1985; Gahl *et*

al., 1991, 1994, 1996). Baker *et al*. (1996) has questioned the use of curvilinear functions to describe lysine utilisation. It was alternatively suggested that even in the studies that have applied curvilinear functions, careful investigation shows the response to marginal lysine intake could just as adequately be described a linear function. The use of a linear function in the present study was consistent with the approach used with rats (Bolton & Miller, 1985) pigs (Batterham *et al*., 1990; Adeola, 1995; Susenbeth, 1995) poultry (Kim *et al*., 1997; Edwards *et al*., 1999) and rainbow trout (Rodehutscord *et al*., 2000). In both feeding regimes, lysine intake was not sufficient to reach maximum response, and was considered marginal. In Section 7.5, the equivalent size parr held under the same environmental conditions (except photoperiod) were demonstrated to require a DLysI of 17.54 mg DLysI.d^{-1} to reach the maximum in PG. This would suggest that the highest lysine intake of the satiation fed parr in the present study was approximately 90% of that need to reach maximum response.

An interesting feature of the present study was the linear response in LG (Fig. 1) to DDLys levels exceeding the current quantitative dietary lysine requirement of Atlantic salmon parr of 20 g.kg^{-1} (Anderson *et al*., 1993). This represents further evidence that quantitative requirements of fish are not accurately represented by a dietary concentration. Based on the current understanding that fish require a lysine intake per unit of LG (Hauler & Carter, 2001), dietary lysine requirements are influenced by feed efficiency ratio (FER). It was on this basis that a significant correlation could be established between the dietary lysine requirements and FER (Hauler & Carter, 2001). Consequently, as both satiation and controlled fed parr achieved a high FER (ie 1.21 g.g^{-1} for 22.62 g DDLys.kg^{-1}; Table 4), the response of LG was linear up to DDLys levels exceeding the current quantitative dietary lysine requirement of Atlantic salmon parr.

The present study does, however, confirm that efficiency of lysine utilisation for LG to be influenced by feeding regime and, hence, an unreliable efficiency to estimate the lysine allowance of fish. The efficiency of lysine utilisation for LG of fish was estimated to be 54.1 mg LG.mg^{-1} lysine intake (Hauler & Carter, 2001). In this study, however, it was acknowledged that lysine utilisation for LG was limited by not knowing the whole-body composition of the fish. Efficiencies of lysine utilisation for LG satiation and controlled fed parr were 47.7 and 34.9 mg LG.mg^{-1} lysine intake, respectively, which correspond to a lysine requirements for LG of 20.9 and 28.6 g lysine.kg^{-1} LG, respectively. In this case, both lysine requirements for LG are considerably greater than 18.5 g lysine.kg^{-1} LG previously estimated for fish (Hauler & Carter, 2001).

The difference in efficiency of lysine utilisation for LG between satiation and controlled fed parr can be attributed to the controlled fed parr having a greater lysine composition of LG. The influence lysine composition on the efficiency for LG is illustrated in Figure 4. At intake 1, there was no difference in the lysine concentration of LG of parr (Table 7, diet 11.19 g DDLys.kg^{-1}) and, hence, the efficiency of lysine utilisation for LG was equivalent. However at intake 2, the lysine composition of LG in controlled fed parr was greater (1.12%, diet 22.62 g DDLys.kg^{-1}) than satiation fed parr receiving the same lysine intake (0.84 %, diet 16.33 g DDLys.kg^{-1}). As a consequence of a greater lysine composition of LG, the efficiency of lysine utilisation for LG was lower in controlled fed parr.

In the present study, the constraints of marginal lysine intake and controlled feed intake (ration) represented an exogenous influence on the weight-specific composition of parr. Allometric analysis has been used to examine the weight-specific composition of fish (Weatherley & Gill, 1987; Shearer, 1994). Applying allometric analysis to published data for fish Shearer (1994), demonstrated that change in whole-body protein composition associated with different ration or graded levels of amino acid (tryptophan) was alternatively be explained to be weight-specific. In the present study, however, weight exponents for protein and lysine were both greater than 1 in both feeding regimes indicating these components were making up an increasing portion of the parr (Table 7). For protein, weight exponents greater than 1 for Atlantic salmon parr may have been anticipated considering that that protein concentration naturally increases over this life-stage (Shearer *et al*., 1994). Accordingly, for the study of Shearer *et al*. (1994) it was demonstrated the weight exponent for protein to be 1.06 in Atlantic salmon parr (Shearer, 1994). For the controlled fed parr, however, the significantly greater weight exponent for protein and lysine than the satiation fed parr suggests that the combination of marginal lysine intake and controlled feed intake have an exogenous effect on the weight-specific composition. Previously, a weight exponent for lysine has not been reported for fish. However, it is proposed that the weight exponent for lysine of 1.95 in controlled fed parr represents a significant deviation from 'normal' weight-specific lysine composition, and consequently, is evidence that the physiological mechanism maintaining the efficiency of lysine utilisation for LysG to be robust under nutritional constraints.

The different composition of parr in each feeding regime may also suggest a need to evaluate the influence of growth rate on the lysine concentration of whole-body PG in production animals. There is increasing evidence from studies on lysine utilisation in rainbow trout (Rodehutscord *et al*., 2000) and terrestrial animals (Batterham *et al*., 1990; Gahl *et al*., 1994, 1996; Adeola, 1995; Edwards *et al*., 1999) that the lysine concentration of PG does not remain constant. Lysine concentration of the whole-body protein is influenced by the contribution that different proteins make to the total protein gain. Lower lysine concentration in slower growing animals suggests that collagen makes a greater contribution to growth than in faster growing animals (Gahl *et al*., 1994, 1996). Contrary to this, the present study demonstrates lysine concentration of whole-body PG of parr was equivalent despite the difference in growth rate in each feeding regime. In this case, controlled fed parr had a significantly greater lysine concentration of whole-body PG when considered as a function of LG (Figure 2). Results from this study would suggest that the lysine concentration of whole-body PG to be closely regulated by DDLys level rather than growth rate. Further anecdotal evidence that lysine concentration may not be related to growth rate can be interpreted from the amino acid requirement study for rainbow trout (Rodehutscord *et al*., 1997). Low lysine concentration of the whole-body protein of rainbow trout was shown not to necessarily correspond with the lowest level of amino acid supplementation in these dose-response studies. It is unlikely, therefore, that lysine concentration of whole-body PG is regulated solely by growth rate.

The change in lysine concentration of whole-body PG means the efficiency of lysine utilisation for PG must be applied to a factorial approach with caution. Assuming the physiological mechanism of parr maintains constant lysine utilisation for LysG, the change in lysine concentration of whole-body PG will influence the efficiency of lysine utilisation for PG. It is proposed that change in lysine concentration of PG accounts for the positive protein balance estimated from the ordinate (zero lysine intake) in the

relationship describing lysine utilisation for PG (Fig. 3b). It is recommended, therefore, that efficiency of lysine utilisation for PG be applied to a factorial approach baring in mind that this efficiency will be influenced by changes in lysine concentration of whole-body PG.

The efficiency of lysine utilisation for LysG above maintenance lysine intake estimated in the present study is proposed to represent the maximum for salmonids under adequate nutrition. The efficiency of lysine utilisation for LysG of 67 and 72% for the satiation and control fed parr, respectively, confirm the efficiency of 71% previously estimated for Atlantic salmon (Hauler & Carter, 2001a,b). These estimates for Atlantic salmon are also equivalent to rainbow trout fed a high dietary crude protein level (Rodehutscord *et al*., 2000). In this latter study with rainbow trout, the efficiency of lysine utilisation for LysG was 71% when fed high dietary protein (550 $g.kg^{-1}$) in which wheat gluten was the main protein source. However, the efficiency of lysine utilisation for LysG was only 55% when fed a low dietary protein (350 $g.kg^{-1}$) in which wheat gluten and free amino acids (supplying 65% N) were the combined protein source. While it was concluded by Rodehutscord *et al*. (2000) that lysine utilisation was improved with increased dietary crude protein level, it remained unclear whether the results reflected the inability of rainbow trout to efficiency assimilate crystalline amino acid (350$g.kg^{-1}$). Considering that in the present study crystalline amino acids contributed 27% of dietary N, it appears modest levels of crystalline amino acids are able to support an efficiency of lysine utilisation equivalent to whole protein. The decrease in lysine utilisation observed by Rodehutscord *et al*. (2000) was therefore most probably an effect of inadequate supply of amino acids to support maximum lysine utilisation. It is proposed that when protein (amino acid) supply is adequate, the efficiency of lysine utilisation at marginal intake for salmonids can be expected to be within the range observed in this study.

The present study confirms the low lysine requirement for maintenance identified for rainbow trout (Rodehutscord *et al*., 1997). Lysine requirement for maintenance was estimated by extrapolation of the relationship between DLysI and LysG (Fig. 3c). These maintenance estimates must be discussed with caution considering that lysine intakes close to maintenance are utilised with greater efficiency (Gahl *et al*., 1996). The estimates of lysine requirement for maintenance of 1.13 and 1.42 $mg.100\ g^{-1}\ BW.d^{-1}$ from satiation and controlled fed parr, respectively, compare favourably to the estimate of 1.93 $mg.100\ g^{-1}\ BW.d^{-1}$ for rainbow trout (Rodehutscord *et al*., 1997). Interestingly, Rodehutscord *et al*. (1997) demonstrated lysine requirement for maintenance was as low as 4% of the total requirement for maximum response in rainbow trout. Despite that lysine intake was marginal, and hence, the maximum response was not achieved in the present study, lysine requirement for maintenance of the satiation fed parr was 5.15% of the total requirement for maximum response. This confirms that the low lysine requirement for maintenance to be common for salmonids.

In conclusion, feed intake does not influence the efficiency of lysine utilisation for PG and LysG in Atlantic salmon parr, and the change in weight-specific lysine composition of observed in this study suggests of a robust mechanism controlling the efficiency of lysine utilisation in this species. Constant efficiency of lysine utilisation means that a factorial approach is an appropriate means of estimating the lysine requirement for growing parr. In application, however, a factorial approach requires knowledge of the composition throughout the production cycle. As establishing the lysine composition throughout Atlantic salmon production is likely to be prohibitive, a

factorial approach based on the efficiency of lysine utilisation for PG would present itself as a more feasible option. In a factorial approach based on lysine utilisation for PG, means any significant change in protein composition of LG of Atlantic salmon results in an equivalent change in the lysine allowance for LG. For example, Shearer *et al*. (1994) demonstrated the protein composition of parr increases from 120 to 180 $g.kg^{-1}$ from first feed to 125 g liveweight. Based on the efficiency of lysine utilisation for PG in this study, 1 kg liveweight gain in first feeding parr requires 15 g of lysine, whereas 1kg of liveweight gain in 125g parr require 22.5 g of lysine (estimates did not include maintenance lysine requirement). This example demonstrates that implementation of a factorial approach will not only offer the means to achieve the greatest efficiency but also the greatest growth potential of cultured fish species.

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Table 1
Ingredient and nutrient composition of the experimental diet

Ingredient composition ($g.kg^{-1}$)	
Zein	176.00
Fish meal	192.00
Amino acid mix[a]	102.40
Glycine/Lysine	14.97
Extruded wheat	200.00
Fish oil	202.00
Ammonium acetate	3.08
Vitamins[b]	4.22
Minerals[c]	0.97
Potassium phosphate[d]	14.87
Sipernat®50S	40.00
α-Cellulose	49.49
Chemical composition ($g.kg^{-1}$ DM)	
Dry matter ($g.kg^{-1}$)	970.7
Nitrogen	72.6
Crude protein[e]	424.7
Crude fat	241.9
Ash	84.9
Gross energy ($MJ.kg^{-1}$)	22.7

[a] Amino acids added ($g.kg^{-1}$ diet): Arg, 20.8; His, 4.4; Ile, 5.5; Met, 6.8; Cys, 1.6; Tyr, 0.4; Thr, 9.7; Trp, 2.9; Val, 8.7; Ala, 1.9; Asx, 20.3; Gly, 16.6; Ser, 2.8.
[b] Vitamin mixture (mg or $IU.kg^{-1}$ diet): Retinol, 2500IU; Cholecalciferol, 2400IU; α-Tocopherol, 200; Menadione, 40; Thiamine, 10; Riboflavin, 20; Calcium pantothenate, 40; Biotin, 1; Folic acid, 5; Cyanocobalamin, 0.02; Nicotinic acid, 150; Pyridoxine, 10; Choline chloride, 1200; Inositol, 400; Ascorbic acid (Rovimix stay C 35%), 750.
[c] Mineral mixture ($mg.kg^{-1}$ diet): Mn, 35 ($MnSO_4.H_20$); Fe, 80 ($FeSO_4.7H_2O$); Cu, 12 ($CuSO_4$ anhydrous); Zn, 90 ($ZnSO_4.7H_2O$); Co, 7 ($CoSO_4.6H_20$); I, 2 (KI); Se, 0.2 (Na_2SeO_3).
[d] Monobasic molecule (KH_2PO_4).
[e] Crude protein = N × 5.85 (Gnaiger and Bitterlich, 1984).

Table 2
Amino acid composition ($g.kg^{-1}$) of experimental diets

Amino acid	$g.kg^{-1}$
Arginine	32.85
Histidine	13.05
Isoleucine	20.90
Leucine	50.25
Lysine	-
Methionine	14.50
Cystine [a]	4.66
Phenylalanine	20.40
Tyrosine	14.80
Threonine	22.00
Tryptophan [a]	4.09
Valine	25.70
Alanine	29.25
Aspartic acid	44.85
Glutamic acid	72.50
Glycine	27.10
Proline	26.35
Serine	19.80

[a] Calculated from that added in crystalline form and measured composition of fish meal, zein and wheat

Table 3
Apparent digestibility coefficients (ADC, %) for dry matter (DM), protein (N), energy (kJ) and lysine (Lys)

Dietary lysine	ADC_{DM}		ADC_N		ADC_{kJ}		ADC_{Lys}	
$g.kg^{-1}$	Controlled	Satiation	Controlled	Satiation	Controlled	Satiation	Controlled	Satiation
11.70	81.88	80.80	96.24	96.08	90.92	90.20	95.29	95.34
12.75	81.72	82.36	96.52	96.24	90.47	90.97	95.78	96.02
13.80	81.00	81.46	96.32	96.39	90.97	90.54	96.04	96.13
14.85	81.81	81.37	95.90	96.13	90.76	90.54	96.41	96.08
15.90	80.83	81.45	96.03	96.16	90.18	90.50	96.16	95.96
16.95	81.84	80.39	96.23	95.98	90.76	90.12	96.67	96.51
18.00	81.13	80.92	96.11	95.77	90.26	89.96	96.14	96.31
19.05	81.08	79.92	96.14	95.23	90.71	89.24	96.66	96.58
20.10	81.85	79.67	96.17	95.78	90.42	89.37	96.90	96.63
21.15	81.24	80.69	95.88	95.87	90.28	89.95	96.80	97.35
22.20	81.83	80.82	96.21	95.58	90.42	89.52	96.95	97.23
23.25	81.43	79.70	95.79	95.33	90.30	88.77	97.31	97.43
Regression[a]								
Slope (b)[b]		-0.146		-0.075	-0.042	-0.142*	0.145	0.163
Intercept (a)		83.354		97.199	91.278	92.458	95.48	95.40
$\mathbf{r^2}$		0.48		0.63	0.34	0.70	0.85	0.88
$s_{y.x}$		0.422		0.307	0.217	0.333	0.330	0.344
P[c]	NS	*	NS	**	*	***	***	***

[a] Linear regression, $y = a + b * x$ in which: y = independent variable, x = dependent variable, $a = y$ intercept and b = slope of regression.
[b] Denotes significant difference between feeding regime within nutrient digestibility, * < 0.05.
[c] Denotes significant relationship between DLys and nutrient digestibility, NS - not significant, * < 0.05, ** < 0.01, *** < 0.001.

Table 4

Performance of Atlantic salmon parr (per fish basis) after a 76 day experimental period

Dietary digestible lysine	Initial weight (g)		Final weight (g)		Feed intake (g DM)		FER (g.g DM^{-1})		Mortality	
g.kg^{-1}	Controlled	Satiation	Controlled	Satiation	Controlled	Satiation	Controlled	Satiation	Controlled	Satiation
11.19	42.27	41.94	68.85	70.09	30.31	32.99	0.61	0.85	3	
12.21	41.86	41.63	67.62	71.83	29.90	34.00	0.80	0.89	1	
13.24	42.67	42.61	71.31	79.34	29.75	39.64	0.96	0.87		1
14.26	42.17	42.27	74.09	77.91	30.42	35.76	0.99	0.99	1	
15.30	42.48	42.33	74.37	78.70	30.27	37.11	1.05	0.97		
16.33	41.56	42.43	74.30	90.19	29.47	45.62	1.11	1.04		
17.37	42.57	42.67	75.45	97.04	30.45	50.78	1.02	1.03	1	1
18.42	42.18	41.70	75.88	96.90	29.86	48.55	1.13	1.13		
19.46	41.83	41.71	77.66	103.85	30.00	53.16	1.19	1.17		
20.51	42.42	42.25	77.90	99.64	30.27	49.76	1.17	1.11		
21.56	42.30	42.41	79.82	109.38	30.30	55.05	1.24	1.17		1
22.62	41.67	41.49	80.9	109.20	30.90	54.60	1.21	1.21	1	1
Regression[a]										
Slope (b)[b]			1.049	3.706***		2.100	0.044	0.032		
Intercept (a)			57.139	27.817		9.323	0.291	0.495		
r^2			0.93	0.95		0.88	0.80	0.93		
$s_{y.x}$			1.036	2.286		2.836	0.082	0.032		
P[c]	NS	NS	***	***	NS	***	***	***		

Feed intake = total feed consumed over 76 days.

FER: feed efficiency ratio = total weight gain (g)/total feed consumed (g DM).

Moralities: total in each treatment replicate.

[a] Linear regression, $y = a + b * x$ in which: y = independent variable, x = dependent variable, $a = y$ intercept and b = slope of regression.

[b] Denotes significant difference between feeding regime within growth performance, *** < 0.001.

[c] Denotes significant relationship between DDLys and growth performance, NS - not significant, *** < 0.001.

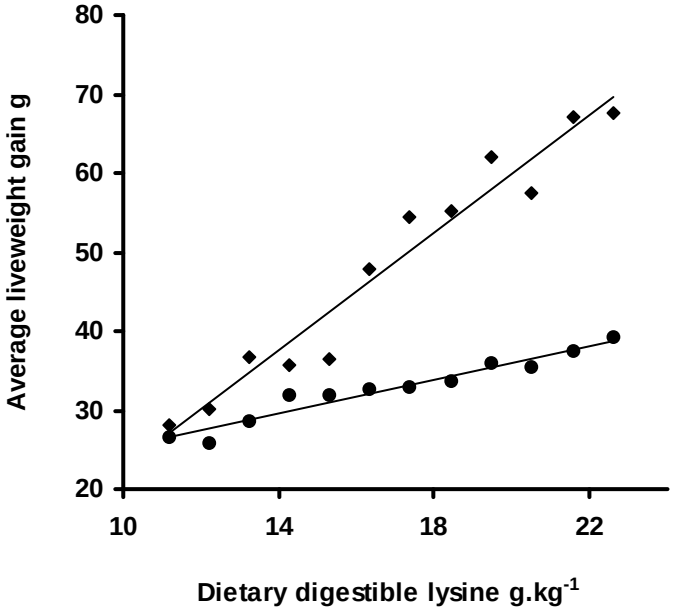

Figure 1
Average liveweight gain (LG, g) of Atlantic salmon parr to dietary digestible lysine level (DDLys, $g.kg^{-1}$) fed for 76 days at either satiation (υ, LG = 3.72DDLys – 14.58, $r^2 = 0.95$, $s_{y.x} = 3.233$, $P < 0.001$) or controlled (λ, LG (g) = 1.07DDLys + 14.64, $r^2 = 0.94$, $s_{y.x} = 1.006$, $P < 0.001$) feed intake. Slopes of regression equations between Log-LG (transformed) and DDLys were significantly different ($P < 0.001$) between feeding regimes.

Table 5

Whole-body composition (% wet weight) of Atlantic salmon parr

Dietary digestible lysine	Dry matter		Crude protein[a]		Lysine		Fat		Ash	
g.kg^{-1}	Controlled	Satiation	Controlled	Satiation	Controlled	Satiation	Controlled	Satiation	Controlled	Satiation
11.19	31.92	32.38	13.62	13.67	0.95	0.96	14.08	14.20	2.42	2.51
12.21	30.93	32.34	13.89	13.97	0.94	0.95	12.65	14.26	2.39	2.54
13.24	31.24	31.86	14.28	13.72	0.99	0.92	12.94	12.74	2.42	2.49
14.26	30.70	31.69	14.16	14.26	1.05	1.04	11.94	12.08	2.39	2.54
15.30	30.21	31.33	14.43	14.57	0.91	0.98	11.07	12.25	2.42	2.51
16.33	30.58	32.20	14.54	14.74	0.97	0.96	11.69	13.25	2.34	2.41
17.37	30.50	31.97	14.52	14.83	0.99	1.13	11.34	12.36	2.33	2.48
18.42	30.93	31.14	14.54	14.42	1.11	1.02	12.47	12.63	2.32	2.38
19.46	29.39	31.64	14.81	14.58	1.10	1.08	10.25	12.84	2.43	2.36
20.51	29.54	31.12	14.90	15.30	1.08	0.97	10.70	11.11	2.24	2.41
21.56	29.64	30.73	14.94	14.98	1.09	1.14	10.07	11.07	2.31	2.37
22.62	30.42	30.70	15.34	14.91	1.10	1.11	10.87	11.85	2.31	2.33
Regressio										
Slope (b)	-0.150	-0.131	0.122	0.119	0.015	0.015	-0.264	-0.208	-0.011	-0.018
Intercept (a)	33.030	33.810	12.44	12.49	0.774	0.764	16.139	16.065	2.551	2.740
r^2	0.58	0.70	0.93	0.75	0.62	0.55	0.69	0.59	0.50	0.77
$s_{y.x}$	0.298	0.200	0.104	0.207	0.124	0.146	0.462	0.451	0.079	0.067
P[c]	**	***	***	***	**	**	***	**	**	***

Initial group (mean): 30.19% DM; 14.52% crude protein[a]; 1.08% lysine, 11.88% fat; 2.45% ash.

[a] Crude protein = N × 5.85 (Gnaiger and Bitterlich, 1984)

[b] Linear regression, $y = a + b * x$ in which: y = independent variable, x = dependent variable, $a = y$ intercept and b = slope of regression.

[c] Denotes significant relationship between DDLys and whole-body composition, ** < 0.01, *** < 0.001.

Table 6

Linear regression[a] between Log fish weight and Log body burden protein or Log body burden lysine of parr fed satiation and controlled feeding regime

Feeding regime	*a*	*b*	r^2	$s_{y.x}$	*P*[b]
	Protein				
Controlled	- 1.900	1.567	0.99	0.005	***
Satiation	- 1.193	1.181	0.99	0.009	***
P[c]		***			
	Lysine				
Controlled	-3.786	1.958	0.85	0.019	***
Satiation	-2.684	1.355	0.95	0.021	***
P[d]		*			

[a] Linear regression, $y = a + b * x$ in which: y = Log body burden (protein/lysine), x = Log fish weight, $a = y$ intercept and b = weight exponent (slope).
[b] Denotes significant relationship between Log fish weight and Log body burden (protein/lysine) of parr, *** < 0.001.
[c] Denotes significant difference between feeding regime within weight exponent (*b*), *** < 0.001.
[d] Denotes significant difference between feeding regime within weight exponent (*b*), * < 0.05.

Table 7
Composition (%) of liveweight gain

Dietary digestible lysine	Crude protein[a]		Lysine		Fat	
g.kg^{-1}	Controlled	Satiation	Controlled	Satiation	Controlled	Satiation
11.19	12.19	12.40	0.76	0.77	17.58	17.66
12.21	12.87	13.21	0.72	0.77	13.90	17.54
13.24	13.92	12.79	0.85	0.72	14.51	13.75
14.26	13.68	13.95	1.00	0.98	12.04	12.31
15.30	14.31	14.63	0.70	0.86	9.99	12.67
16.33	14.56	14.94	0.83	0.84	11.47	14.45
17.37	14.52	15.07	0.86	1.16	10.65	12.73
18.42	14.57	14.34	1.15	0.96	13.21	13.20
19.46	15.15	14.62	1.13	1.07	8.35	13.49
20.51	15.35	15.87	1.07	0.94	9.29	10.54
21.56	15.41	15.27	1.10	1.18	8.04	10.57
22.62	16.21	15.15	1.12	1.13	9.80	11.82
Regressionn						
Slope (b)	0.287	0.245	0.037	0.035	-0.620	-0.482
Intercept (a)	9.560	10.222	0.319	0.367	22.023	21.538
r^2	0.92	0.74	0.66	0.65	0.68	0.63
$s_{y.x}$	0.275	0.488	0.301	0.279	0.588	0.562
P[c]	***	***	**	**	**	**

[a] Crude protein = N × 5.85 (Gnaiger and Bitterlich, 1984).
[b] Linear regression, $y = a + b * x$ in which: y = independent variable, x = dependent variable, $a = y$ intercept and b = slope of regression.
[c] Denotes significant relationship between DDLys and composition of liveweight gain, ** < 0.01, *** < 0.001.

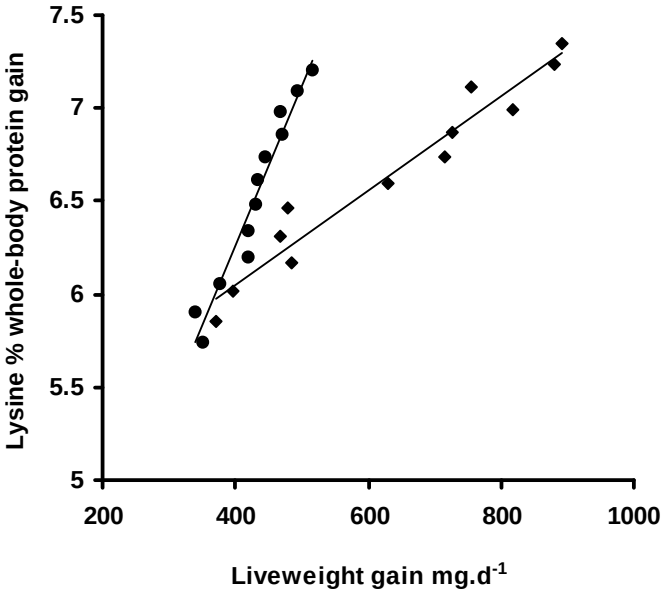

Figure 2
Lysine concentration of whole-body protein gain (Lys%WBPG, %) to liveweight gain (LG, $mg.d^{-1}$) fed at either satiation (υ, Lys%WBPG = 0.0025LG + 5.03, $r^2 = 0.95$, $s_{y.x} = 0.112$, $P < 0.001$) or controlled (λ,Lys%WBPG = 0.0086LG + 2.84, $r^2 = 0.95$, $s_{y.x} = 0.112$, $P < 0.001$) feed intake. Slopes of regression equations were significantly different ($P < 0.001$) between feeding regimes.

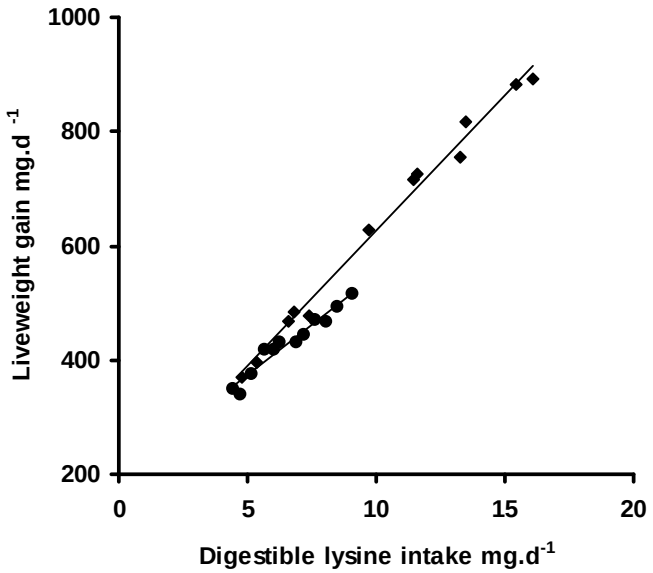

Figure 3a
Liveweight gain (LG, mg.d^{-1}) to digestible lysine intake (DLysI, mg.d^{-1}) fed at either satiation (υ, LG = 47.67DLysI + 150.06, r^2 = 0.99, $s_{y.x}$ = 19.023, $P < 0.001$) or controlled (λ, LG = 34.90DLysI + 199.11, r^2 = 0.94, $s_{y.x}$ = 13.011, $P < 0.001$) feed intake. Slopes of regression equations were significantly different ($P < 0.005$) between feeding regimes.

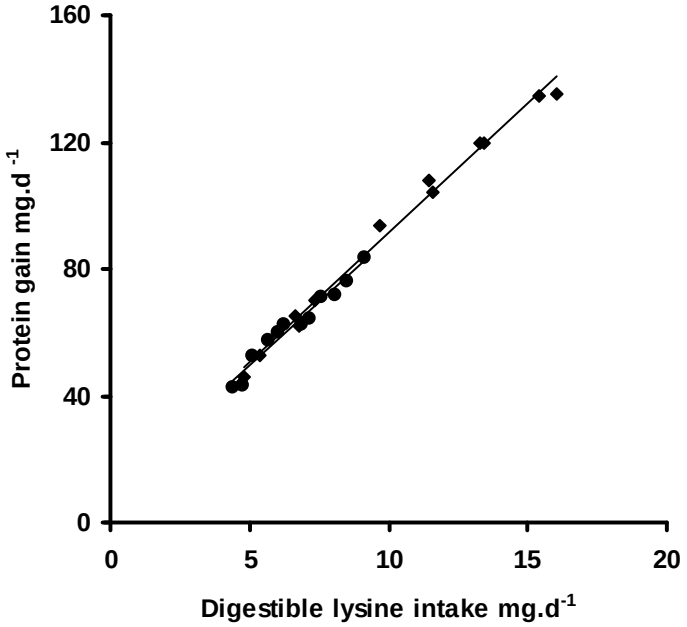

Figure 3b

Protein gain (PG, $mg.d^{-1}$) to digestible lysine intake (DLysI, $mg.d^{-1}$) fed at either satiation (υ, PG = 8.14DLysI + 9.82, $r^2 = 0.99$, $s_{y.x} = 3.165$, $P < 0.001$) or controlled (λ, PG = 8.02DLysI + 9.36, $r^2 = 0.96$, $s_{y.x} = 2.453$, $P < 0.001$) feed intake. There was no significant difference between slopes ($P > 0.05$) and elevations ($P > 0.2$) for both feeding regimes. Regression equation of pooled data: PG = 8.23DLysI + 8.47 (n = 24, $r^2 = 0.99$, $s_{y.x} = 2.823$, $P < 0.001$).

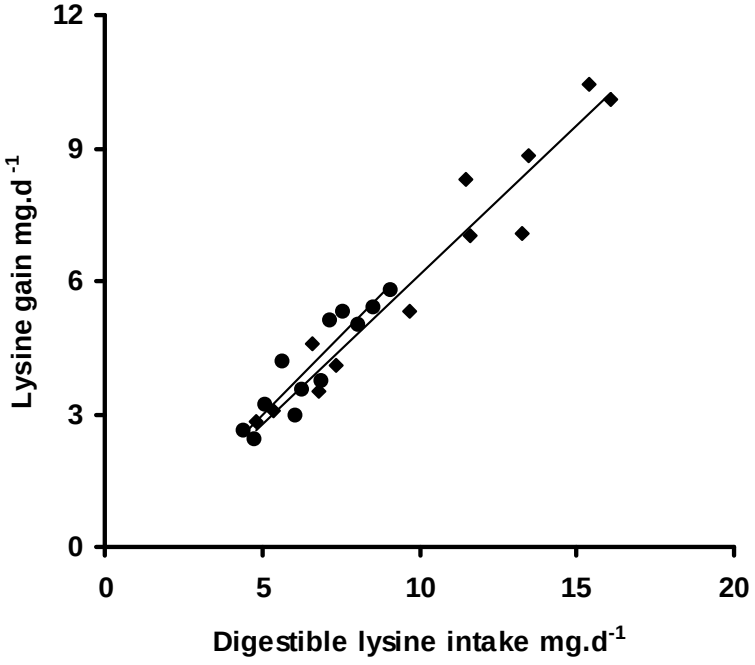

Figure 3c
Lysine gain (LysG, mg.d^{-1}) to digestible lysine intake (DLysI, mg.d^{-1}) fed at either satiation (υ, LysG = 0.67DLysI – 0.57, $r^2 = 0.94$, $s_{y.x} = 0.667$, $P < 0.001$) or controlled (λ, LysG = 0.72DLysI – 0.63, $r^2 = 0.85$, $s_{y.x} = 0.459$, $P < 0.001$) feed intake. There was no significant difference between slopes ($P > 0.5$) and elevations ($P > 0.2$) for both feeding regimes. Regression equation of pooled data: LysG = 0.66DLysI – 0.32 (n = 24, $r^2 = 0.94$, $s_{y.x} = 0.573$, $P < 0.001$).

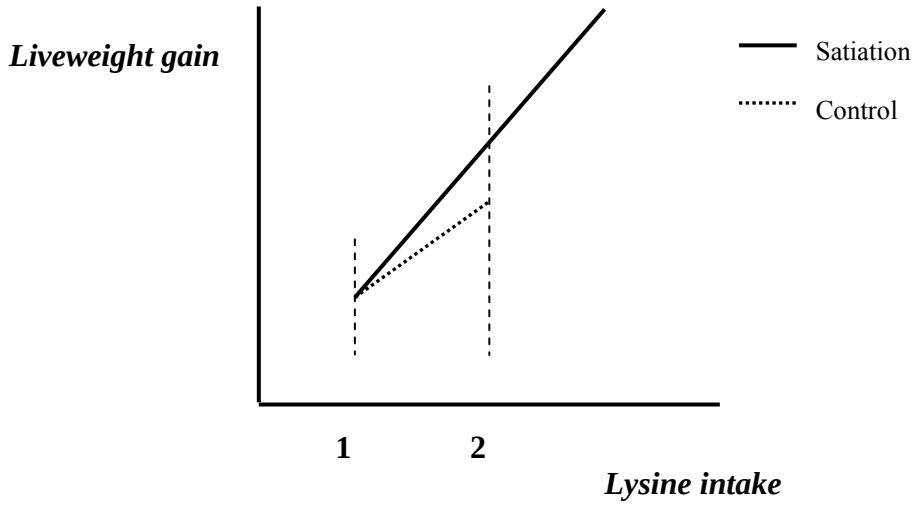

Figure 4
Illustration of LG to lysine intake of Atlantic salmon parr fed increasing digestible dietary lysine at satiation and control feed intake.

7.4
Lysine utilisation by Atlantic salmon (*Salmo salar* L.) parr 2: Comparison of two diet formulations

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Abstract

This study investigated if the efficiency of lysine utilisation for liveweight gain (LG), protein gain (PG) and lysine gain (LysG) was affected by diet formulation in Atlantic salmon (*Salmo salar* L.) parr. To test the hypothesis that the efficiency of lysine utilisation remains constant in different diet formulations, data from a previous experiment (Section 7.3) in which parr were fed a diet with 19.8 g digestible protein (DP).MJ digestible energy (DE)$^{-1}$ (ie 19.8 DP.DE^{-1}) was compared to a study in which parr were fed a diet with 25.0 DP.DE^{-1}. Twelve diets (461.0 g DP.kg^{-1} and 18.4 MJ DE.kg^{-1}) using zein, fish meal and crystalline amino acids were formulated to contain a range from 10.15 to 20.79 g digestible dietary lysine (DDLys).kg^{-1}. Each diet was hand-fed a controlled ration (kJ DE. kg^{-1} body weight (BW).d^{-1}) over a 50 day period. With increasing DDLys supplementation there was a significant ($P < 0.001$) linear increase in live weight gain. Allometric analysis demonstrated the weight exponent for protein and lysine to be 1.58 and 2.47, respectively, in parr fed 25.0 DP.DE^{-1}, but this was not significantly difference to parr fed 19.8 DP.DE^{-1}. Linear regression described relationships between digestible lysine intake (DLysI) and live weight, protein and lysine gain for parr fed 25.0 DP.DE^{-1}. There was no significant difference in the efficiency of lysine utilisation for LG ($P > 0.2$), PG ($P > 0.2$) or LysG ($P > 0.1$) above maintenance lysine intake between diets. The elevation of the relationship between DLysI and live weight gain was, however, significantly higher ($P < 0.001$) for parr fed 25.0 DP.DE^{-1}. For the present study, efficiency of lysine utilisation for lysine gain above maintenance lysine intake was estimated to be 77%. Extrapolating the relationship between DLysI and lysine gain the lysine requirement for maintenance was estimated to be 7.7 mg.(kg$^{0.75}$)$^{-1}$.d^{-1}. This study demonstrates that providing protein and energy intake sufficiently meets the requirements for lean gain (protein and lysine gain), diet formulation does not influence the efficiency of lysine utilisation. Furthermore, the efficiency of lysine utilisation for lysine gain above maintenance lysine intake in parr was shown to be equivalent to terrestrial animals. Compared to terrestrial animals, lysine requirement for maintenance expressed as metabolic body weight was approximately 10-18 fold lower for fish but was equivalent (approximately 10%) when expressed as a portion of the total requirement for maximum gain. This confirms the observation that fishes and terrestrial animals are similar in their use of protein as a nutrition source.

Keywords: Amino acid requirements; Lysine requirements; Lysine utilisation

Introduction

For a factorial approach to be a flexible means of estimating the lysine allowance in growing Atlantic salmon (*Salmo salar* L.), the efficiency of lysine utilisation for growth

[liveweight gain (LG), protein gain (PG) and lysine gain (LysG)] is required to remain constant with changes in diet formulation. At the time this experiment was conducted, lysine utilisation had not been compared in different diets for fish. A single study comparing life-stages of Atlantic salmon (Hauler & Carter, 2001a) showed the efficiency of lysine utilisation for LysG remained constant using different diets but did not unequivocally estimate the true efficiency because of assumptions made about body-composition (lysine content). Since then, however, it was demonstrated in Section 7.3 that the efficiency of lysine utilisation was equivalent in Atlantic salmon and rainbow trout, *Oncorhynchus mykiss* (Rodehutscord *et al*., 2000) fed different diets. This comparison further supported the hypothesis that diet formulation does not influence lysine utilisation in Atlantic salmon.

Currently there is little evidence that fish composition is influenced by exogenous factors. Applying allometric analysis to published data, Shearer (1994) demonstrated the differences in protein and amino acid composition of fish to be weight-specific. Allometric analysis generates Log composition-Log liveweight plots that describe the composition of animals as linear within a life stage (Laird *et al*., 1968). When weight exponents (slopes) of allometric relationships equal 1, the composition and liveweight are increasing at the same rate and when the weight exponent is greater than 1, the composition is an increasing portion of the animal. Contrary to the composition of fish being weight-specific, allometric analysis demonstrated the combination of marginal lysine intake and controlled feed intake (ration) had an exogenous affect on the weight exponent for protein and lysine composition in Atlantic salmon parr (see Section 7.3). This exogenous influence of combined marginal lysine intake and controlled feed intake on weight-specific composition is yet to be confirmed in fish.

It is becoming increasingly apparent from studies on lysine utilisation of the similarity in protein nutrition between fish and terrestrial animals. Equivalent lysine utilisation for LG demonstrated for Atlantic salmon, rat and chick (Hauler & Carter, 2001) confirmed the observation that fishes and terrestrial animals are similar in their use of protein as a nutritional source (Bowen, 1987). To date, protein efficiency ration (PER; weight of growth achieved per weight of protein ingested) and protein productivity value (PPV; proportion of ingested protein retained in growth) have been the common means of comparing protein nutrition of fish and terrestrial animals (Bowen, 1987; Kaushik, 1995). However, as PER and PPV are affected by diet formulation and rates of protein intake it cannot be unequivocally stated that utilisation of dietary protein is equivalent (Bowen, 1987; Kaushik, 1995). Furthermore, as 'protein' is not a quantitative term, it does not provide the vital information about the efficiency at which individual amino acids are utilised for maintenance and growth. Only recently has amino acid utilisation for maintenance and growth have been investigated in fish (Mambrini & Kaushik, 1995; Rodehutscord *et al*., 1997, 2000). With estimates of the lysine requirement for maintenance and utilisation for growth generated in these studies with Atlantic salmon (present Section and Section 7.3), a more detailed comparison of lysine utilisation by fish and terrestrial animals is warranted to confirm the reported similarity in protein nutrition.

In the present study, lysine utilisation was investigated in Atlantic salmon parr fed a diet with 25.0 g digestible protein (DP).MJ digestible energy $(DE)^{-1}$ (ie 25.0 $DP.DE^{-1}$) at a controlled ration (kJ DE. kg^{-1} body weight (BW).d^{-1}). Data were compared with data from Section 7.3 in which parr were fed at an equivalent controlled ration in a 19.8 $DP.DE^{-1}$ diet. The primary aim was to compare lysine utilisation for live weight, protein

and lysine gain in order to test the hypothesis that efficiency remains constant in different diet formulations for parr. Considering the optimal digestible protein to digestible energy ratio for parr is approximately 21 DP.DE^{-1} (Helland *et al*., 1991), the diets compared encompasses the range of formulations fed to this life-stage. Allometric analysis was also investigated to confirm that the combination of marginal lysine intake and controlled ration represented an exogenous influence on weight-specific protein and lysine composition of parr identified previously in Section 7.3. Based on the current estimates of lysine utilisation for lysine gain and lysine requirement for maintenance, a comparison of lysine utilisation by fish and terrestrial animals is made to confirm the similarity in protein nutrition.

Materials and methods

Experimental diets

A diet was formulated to contain 461.0 g DP.kg^{-1} and 18.4 MJ DE.kg^{-1} (Table 1). Zein, fish meal and extruded wheat were used as sources of protein-bound amino acids. Crystalline amino acids (Sigma Chemical Co. and Musashi, Victoria, Australia) were added to create a dietary amino acid profile (except glycine and lysine) similar to the whole-body of Atlantic salmon parr (Hauler & Carter, 2001a) (Table 2). Each basal diet had a lysine content of 10.80 g dietary lysine (DLys).kg^{-1}. Lysine (L-lysine monohydrochloride, Musashi, Victoria, Australia, 78.5% lysine) was added (substituting against glycine) to the basal diet in 11 increments of 0.96 g DLys.kg^{-1}. Thus, the 12 DLys levels were: 10.80; 11.76; 12.72; 13.68; 14.64; 15.60; 16.56; 17.52; 18.48; 19.44; 20.40; 21.36 g DLys.kg^{-1}. To balance the pH of the feed (to pH 8), ammonium acetate was added at 3g.kg^{-1} of the amino acid mixture (Berge *et al*., 1998). Vitamin and minerals mixtures were added as previously described (Hauler & Carter, 2001a). Ingredients were mixed before being cold pellet pressed to 3 mm using a California Pellet Mill (CL-2 laboratory pellet mill, California Pellet Mill Co., San Francisco, U.S.A.). Pellets were dried at 36°C until they contained less than 100 g.kg^{-1} moisture then stored at 4°C.

Growth experiment

The experiment was conducted at the School of Aquaculture, University of Tasmania with Atlantic salmon (*Salmo salar* L.) that originated from the SALTAS Salmon Hatchery (Tasmania, Australia). Parr were transferred to a recirculation system (Carter & Hauler, 2000), acclimatised for 2 weeks during which time they were supplied a commercial feed (500 g.kg^{-1} protein, 140 g.kg^{-1} fat, 17.8 MJ DE.kg^{-1}). The system was housed in a constant environment room maintained at 12 h light:12 h dark photoperiod and 14.9 ± 0.6°C water temperature. Water quality parameters (pH, DO, ammonia, nitrate and nitrite) were maintained within limits for Atlantic salmon (Wedemeyer, 1996).

At the beginning of the experiment, fish were anaesthetised (50 mg.l^{-1} benzocaine), weighed (mean weight 43.72 g) and twenty-three fish were randomly allocated to each of 12 tanks. Twenty fish were euthanased (100 mg.l^{-1} benzocaine) for assessment of initial whole-body chemical composition (see below). Each tank of fish was fed one of the 12 diets by hand 3 times per day (0900, 1300 and 1700 hours) over an experimental period of 50 days. Parr were fed a daily ration (0.94 %BW.d^{-1}) that was equivalent to the weight-specific digestible energy intake (173 kJ DE. kg^{-1} BW.d^{-1}) to controlled fed parr in Section 7.3. Daily rations of fish were divided into 3 equal portions each day. Every 10 days fish were fasted for the day and each tank of fish bulk-weighed. Rations

were subsequently recalculated for the following 10 day period. No mortality was recorded during the trial.

At the conclusion of the experiment, all fish were individually weighed after 48 hours of fasting. Five fish per tank were euthanased (100 $mg.l^{-1}$ benzocaine) to measure whole-body chemical composition (see below). Fish were frozen at -20°C, dried individually, homogenised in a cutting mill and then pooled within each tank for further chemical analysis.

Apparent digestibility

Apparent digestibility coefficient (ADC) was estimated for all diets. From day 31 of the experiment diets containing 10 $g.kg^{-1}$ chromic oxide were fed as normal. On day 38 faeces were collected overnight (1800 to 0800 hours) in settlement collectors (Cho *et al*., 1982). Faeces were frozen, freeze-dried and stored at -20°C before analysis. Chromic oxide and nutrients were analysed (see below) in diets and faeces and ADC was calculated using:

$$ADC\ (\%) = 100 - [100(\%I_{diet}/\%I_{faeces}) \times (\%N_{faeces}/\%N_{diet})]$$

(Maynard and Loosli, 1969) where I is the inert marker and N is the nutrient.

Chemical analysis

Dry matter of whole body was determined by lyophilisation and for diets by oven drying at 70°C to constant weight. Whole-body and diets were analysed for nitrogen (Kjeldahl, copper sulfate catalyst), crude fat by petroleum ether extraction (AOAC, 1995), ash by combustion at 550°C for 16 hours (AOAC, 1995) and chromium by acid digestion (Furukawa & Tsukahara, 1966). Amino acid content of the diets was determined by a Waters High Performance Liquid Chromatograph (HPLC) after acid (HCl) hydrolysis (Rayner, 1985). Lysine content of diets, faeces and whole-body was determined by isocratic liquid chromatography (Or-Rashid *et al*., 1999).

Statistical analysis

Least squares linear regression was used to describe relationships between: (1) DLys level and apparent lysine digestibility (ADC_{Lys}); (2) dietary digestible lysine (DDLys) and performance of parr; (3) final liveweight of parr and body burden (absolute amount) of protein and lysine (allometric analysis; see below); (4) digestible lysine intake (DLysI) and LG, PG and LysG. Goodness of fit was described by the coefficient of determination (r^2) which describes the proportion of the total variation around *y* explained by the linear regression, and the standard error of the regression ($s_{y.x}$) which describes the standard deviation of the residuals of the individual values of *y* from the linear regression.

In order to consider weight-specific influence on whole-body composition, allometric analysis was applied as described by Shearer (1994, 1995). The relationship between parr liveweight (final liveweight) and the body burden (absolute amount) of protein or lysine was described by the linear relationship:

$$\text{Log body burden (protein/lysine)} = a + b\ (\text{Log fish weight})$$

where body burden and fish weight was expressed in grams. Linear regressions were performed for means from each of the tank within each feeding regime. The weight exponent (*b* = slope) and elevation of linear regressions were compared between feeding regime as described below. Comparison between diets was limited to allometric analysis and linear regressions between DLysI and LG, PG and LysG. Dependent variables expressed as a percentage were arcsine-transformed prior to analysis. If linear

regressions were significant and variance equal (variance ratio test, F = 5.87, $P = 0.05$, n = 20) between diets, a *t*-test was used to identify if slopes and elevations were significantly different (Zar, 1984). If it was concluded that slopes and elevations were not significantly different between diets, data were pooled to quote a common linear regression equation. Probabilities of 0.05 or less were considered significant.

Results

The results presented herein refer mostly to the present study in which parr were fed the 25.0 $DP.DE^{-1}$ diet. Comparison made between 19.8 and 25.0 $DP.DE^{-1}$ diets was limited to allometric analysis (as described in Table 6, Section 7.3; see below) and DLysI and LG, PG and LysG (as described in Figure 1 a,b & c; see below). Refer to Section 7.3 for apparent digestibility of the diet, growth performance and lysine requirement for maintenance of parr fed the 19.8 $DP.DE^{-1}$ diet.

Apparent digestibility

Apparent digestibility of dietary nutrients was investigated to ascertain the daily DP and DE intake and to establish the DDLys levels of the 25.0 $DP.DE^{-1}$ diet (Table 3). There was no relationship between DLys level and apparent digestibility of dry matter (ADC_{DM}), protein (ADC_N), energy (ADC_{kJ}). Consequently, data were pooled to quote an average ADC_{DM}, for the 25.0 $DP.DE^{-1}$ diet. Based on average ADC_N and ADC_{kJ}, parr fed the 25.0 $DP.DE^{-1}$ diet were receiving a daily intake of 4.33 g $DP.kg^{-1}$ $BW.d^{-1}$ and 171.0 kJ DE. kg^{-1} $BW.d^{-1}$ whereas parr fed the 19.8 $DP.DE^{-1}$ diet were receiving a daily intake of 3.47 g $DP.kg^{-1}$ $BW.d^{-1}$ and 175.1 kJ DE. kg^{-1} $BW.d^{-1}$.

For the 25.0 $DP.DE^{-1}$ diet, ADC_{Lys} increased as DLys increased, resulting in a significant ($P < 0.001$) regression being established (Table 3). This equation was used to calculate the 12 DDLys levels, which were 10.15, 11.09, 12.03, 12.98, 13.94, 14.90, 15.87, 16.84, 17.82, 18.80, 19.79, 20.79 g $DDLys.kg^{-1}$.

Growth performance

Average LG of the parr was 22.9 g, an increase by a factor of 1.5 over the 50-day experiment (Table 4). A significant ($P < 0.001$) linear relationship described the increase in LG and feed efficiency ratio (FER) as DDLys level increased. The change in whole-body composition with increasing DDLys levels was also described by linear regressions (Table 5). As DDLys increased, crude protein increased ($P < 0.001$), while dry matter ($P < 0.05$), lipid ($P < 0.001$) and ash ($P < 0.05$) decreased.

Allometric analysis (Table 6) demonstrated the weight exponent (*b*) for protein and lysine was greater than 1 in parr fed both diets. There was no significant difference in the weight exponent for protein ($P > 0.1$) and lysine ($P > 0.2$) in the parr fed both diets. However for the parr fed the 25.0 $DP.DE^{-1}$ diet, elevations of the allometric relationships were significantly higher for both protein ($P < 0.001$) and lysine ($P < 0.001$) than the parr fed the 19.8 $DP.DE^{-1}$ diet (significant difference in elevation was not indicated in Table 6).

Lysine utilisation

The slopes of the relationships between DLysI and LG, PG and LysG represented the efficiency of lysine utilisation above maintenance lysine intake (Fig. 1). The efficiency of lysine utilisation for LG was not significantly different ($P > 0.2$) between diets, however the elevation of the regression equation was significantly higher ($P <$

0.001) for the 25.0 DP.DE^{-1} diet (Fig. 1a). Between the diets there was no significant difference in the efficiency of lysine utilisation for PG ($P > 0.2$) or LysG ($P > 0.2$) and there was no significant difference in elevation ($P > 0.2$) of the linear regressions between DLysI and PG ($P > 0.5$) or LysG ($P > 0.1$). Consequently, a common efficiency of lysine utilisation for PG and LysG was derived from data pooled from both diets (Figs. 1b & c).

The present study was not primarily designed to estimate lysine requirement for maintenance. However, by extrapolation of the linear relationship between digestible lysine intake and LysG (Fig. 1c) on parr fed the 25.0 DP.DE^{-1} diet, lysine requirement for maintenance was estimated to be 1.58 mg.100g^{-1} BW.d^{-1} (Mean weight of fish = weight gain of parr fed 21.36 g.kg^{-1} DDL/2). Expressed as metabolic body weight, lysine requirement for maintenance was 7.7 mg.(kg$^{0.75}$)$^{-1}$.d^{-1}.

Discussion

The present study demonstrated that diet formulation did not influence the efficiency of lysine utilisation for PG and LysG of Atlantic salmon parr. Allometric analysis confirmed that the combination of marginal lysine intake and controlled feed intake had an exogenous effect on the weight-specific protein and lysine composition of parr. This study is also the first to compare lysine utilisation for LysG and lysine requirement for maintenance in fish and terrestrial animals. Based on utilisation of lysine, it was concluded that fish and terrestrial animals are very similar in their use of protein as a nutrition source.

The constraints of marginal lysine intake and controlled ration were confirmed to have an exogenous effect on the weight-specific protein and lysine composition of Atlantic salmon parr. For fish, there is currently little evidence of exogenous factors affecting the weight-specific protein composition (Shearer, 1994). Contrary to this, however, allometric analysis in Section 7.3 demonstrated that when parr were fed at marginal lysine intake (see Section 7.3) the weight exponent for both protein and lysine were higher under a controlled feed intake (ration) compared to satiation feeding. On this basis it was concluded that the combined constraints of marginal lysine intake and controlled feed intake represented an exogenous effect on the weight-specific composition of parr. In the present study, parr were fed 25.0 DP.DE^{-1} at an equivalent controlled ration (kJ DE. kg^{-1} BW.d^{-1}) to parr fed 19.8 DP.DE^{-1} in Chapter 4. For parr fed 25.0 DP.DE^{-1} weight exponents for protein and lysine were 1.58 and 2.47, respectively, and were not significantly difference to parr fed 19.8 DP.DE^{-1} (Table 6), indicating an equivalent influence on weight-specific composition. Shearer (1994) demonstrated in the published studies with salmonids the weight exponent for protein range between 0.99 and 1.07, suggesting the weight exponent for protein observed for parr fed 25.0 DP.DE^{-1} to be a considerable deviation from normal weight specific protein composition of salmonids. Unfortunately, a weight exponent for lysine has not been reported for fish. However, it was proposed in Chapter 7.3 that the weight exponent for lysine of the order seen the present study represents a considerable deviation from the 'normal' weight-specific lysine composition. This deviation from the normal weight-specific lysine composition is considered evidence that the physiological mechanism maintaining the efficiency of lysine utilisation for LysG to be robust under nutritional constraints. It is recommended that future study on lysine utilisation, allometric analysis be applied to confirm the exogenous effect on weight-specific protein and lysine composition in fish.

The influence of whole-body composition on the efficiency of lysine utilisation for LG has previously been discussed for Atlantic salmon parr (Chapter 7.3). In the present study, the efficiency of lysine utilisation for LG was not significantly different between diets (Figure 1a). However, the linear regression describing lysine utilisation for LG was significantly higher for parr fed 25.0 $DP.DE^{-1}$, suggesting in this case, of a greater absolute growth per mg lysine intake. This greater absolute growth can be attributed to parr fed 25.0 $DP.DE^{-1}$ having a lower lysine concentration of LG. As the efficiency of lysine utilisation for LysG was equivalent for both diets (Figure 2c), parr with the lower lysine concentration of LG had a greater absolute growth. The average lysine concentration of LG in parr fed 25.0 $DP.DE^{-1}$ was 0.89% (not shown in the results of the present study), whereas average lysine concentration of LG in parr fed 19.8 $DP.DE^{-1}$ was 0.94% (estimated from the equation in Table 7 in Chapter 7.3). The 5% difference in lysine concentration of LG accounts almost in total for the increase in lysine utilisation for LG. The present study reiterates that the efficiency of lysine utilisation for LG is influenced by changes in composition of LG, and hence, is an unreliable efficiency to estimate the lysine allowance of fish.

Influence of diet formulation on lysine utilisation

The constant efficiency of lysine utilisation for PG and LysG observed for parr suggests a factorial approach to be an appropriate means of estimating the lysine allowance (requirement) for fish with changes in diet formulation. Contrary to the present study, Rodehutscord *et al*. (2000) demonstrated that diet formulation (protein level) had an influence on the efficiency of lysine utilisation for LysG in rainbow trout. The low efficiency of lysine utilisation observed in rainbow trout fed low dietary protein (350 g $protein.kg^{-1}$), however, was proposed in Chapter 7.3 to be due to an inadequate supply of amino acid to support maximum lysine utilisation; which will be addressed in Chapter 7.5. Alternatively, the high protein diet (550 g $protein.kg^{-1}$; 27.5 $DP.DE^{-1}$) in the same study provides confirmatory evidence that diet formulation does not influence lysine utilisation in salmonids. The high protein diet fed to rainbow trout was a different formulation than had been fed to Atlantic salmon parr (present chapter). On the basis that the efficiency of lysine utilisation for LysG for rainbow trout (71%) and parr fed 19.8 $DP.DE^{-1}$ (67%) and parr fed 25.0 $DP.DE^{-1}$ (77%) was not considerably different, diet formulation does not appear to influence lysine utilisation in salmonids.

Confirming the results from salmonids, studies with terrestrial animals have generally concluded that diet formulation does not influence lysine utilisation. The diet influences on lysine utilisation commonly considered in terrestrial animals have been excesses of amino acids (protein) and energy intake. The influence of dietary amino acid excesses on lysine utilisation has been a particular focus in poultry nutrition (Fisher *et al*., 1960; Morris *et al*., 1987; D'Mello, 1988; Abebe & Morris, 1990; Morris *et al*., 1999). Although results have been contradictory, when first-limiting lysine has been demonstrated to be utilised less efficiently with excessive dietary amino acids supply (D'Mello, 1988, Morris, 1999). Decreased lysine utilisation in these studies was attributed to metabolic interactions with other amino acids (D'Mello, 1994; Morris *et al*., 1999) or additional energy expenditure associated with the catabolism of excess dietary protein (D'Mello, 1988; Henry *et al*., 1992). Contrary to the observations in poultry, however, amino acid excess did not influence utilisation of lysine when first-limiting in the diet of pigs (Langer & Fuller, 1996). From this latter study it was generally regarded that providing the requirement for protein (lysine) deposition is met, amino acid excesses do not influence lysine utilisation.

For pigs it has been proposed when total dietary energy limits protein deposition, dietary protein (amino acids) are catabolised 'preferentially' to supply energy (Whittemore, 1983; Moughan, 1991). Consequently, when lysine is the first-limiting dietary amino acid, preferential catabolism arises in a decrease in the efficiency of lysine utilisation (Mohn *et al*., 2000). The biological basis for preferential catabolism is considered to be a physiological requirement to maintain a minimum level of body lipid (Whittemore, 1983). The influence of preferential catabolism on the efficiency of lysine utilisation has been confirmed in pigs (Mohn *et al*., 2000), however, only occurs under severe energy intake restrictions.

In salmonids and terrestrial animals, modest changes in diet formulation do not appear to influence the efficiency of lysine utilisation. Providing the requirement for PG and LysG are met, it is proposed that the efficiency of lysine utilisation is not considerably different when fed different diets. This information can be applied a factorial approach to calculate the lysine allowance (requirement) of growing Atlantic salmon.

Lysine utilisation by fish and terrestrial animals

It is becoming increasing apparent that fish and terrestrial animals are similar in their utilisation for lysine for growth. Similar efficiency of lysine utilisation above maintenance lysine intake in fish and terrestrial animals was first identified by Hauler and Carter (2001b; Chapter 7.3). To date, further studies on lysine utilisation in fish are scarce (Rodehutscord *et al*., 1997; 2000). With estimates of lysine requirement for maintenance and utilisation for LysG generated for Atlantic salmon (present study and Chapter 4), a more detailed comparison of lysine utilisation by fish and terrestrial animals is warranted to confirm the reported similarity in protein nutrition (Bowen, 1987). In the comparison presented herein, lysine requirements for maintenance were a combination of reported values (as indicated in Table 8) and estimates calculated from lysine utilisation studies that were not necessarily designed to consider maintenance. Those lysine utilisation studies that required extrapolation of linear relationships to estimate maintenance included those of Batterham *et al*. (1990), Adeola (1995), Kim *et al*. (1997), Rodehutscord *et al*. (2000) and the studies with Atlantic salmon (present study and Chapter 4).

Lysine utilisation for LysG

The efficiency of lysine utilisation for LysG above maintenance lysine intake is reasonably consistent for fish and terrestrial animals (Table 7). The degree of variation in lysine utilisation appears to prevail within and between animals. Granted that a constant efficiency of lysine utilisation cannot be assumed for all animals or strains of species, there are several reasons for variation between these individual studies. Of the many reasons, the efficiency of lysine utilisation depends on an evaluation of the digestibility or availability of dietary lysine. In chemically defined, crystalline amino acid diets, digestibility and availability of lysine is unlikely to have a significant influence. In some protein sources, however, lysine may not be in a form unavailable for protein metabolism (Batterham *et al*., 1990; Adeola, 1995). As whole-proteins were used to supply at least some of the lysine in all studies except Edward *et al*. (1999), a degree of variation in Table 5.7 may be attributed to lysine availability. The lysine intake range studied may also have a considerable influence on the efficiency of lysine utilisation. Assuming the pattern of LysG to lysine intake is sigmoidal as described for pigs (Gahl *et al*., 1994), lysine intake studied on this curve may be a predisposing factor for efficiency estimates by linear regression. Natural variation in LysG and the ability to accurately estimate whole-body lysine in animals would also contribute significantly to

variation in estimates. Considering individual lysine utilisation studies are influenced by a number of factors, variation in the efficiency of lysine utilisation within and between animals is considered unavoidable.

With this in mind, it is generalised that for both fish and terrestrial animals that up to 30% of the absorbed lysine above maintenance lysine intake is consistency catabolised in normal physiology. The efficiency of absorbed lysine above maintenance lysine intake would not be expected to be totally efficient because of the inevitable catabolism of amino acid in animals during animal growth (Heger & Frydrych, 1985,1989). The mechanism for inevitable catabolism of amino acids has been proposed to occur due to a 'consequence of the operation of mechanisms controlling the degradation of amino acids in the body' (Hegar & Frydrych, 1989). Alternatively, Millward and Rivers (1988) and Millward (1989) explained the inevitable catabolism as 'anabolic drive' for which the oxidation of amino acids represents a regulatory influence on growth and maintenance. Based on the consistent efficiency of lysine utilisation, it would appear that fish and terrestrial animals share the mechanism for inevitable lysine catabolism. It is therefore proposed that any major difference in lysine utilisation between fish and terrestrial animals could only derive from the lysine requirement for maintenance.

Lysine requirement for maintenance

Expressed as metabolic body weight, the lysine requirement for maintenance in salmonids is undoubtedly lower than terrestrial animals (Table 8). For salmonids, lysine requirement for maintenance ranges between 3.1 and 7.7 $mg.(kg^{0.75})^{-1}.d^{-1}$, with an average of 6.3 $mg.(kg^{0.75})^{-1}.d^{-1}$. In comparison, lysine requirements for maintenance vary considerably in terrestrial animals, although a distinction with regard to methodology must be made. As nitrogen balance methodology tends to overestimate protein retention in amino acid utilisation studies, maintenance is likely to be underestimated (Langer & Fuller, 1996; Susenbeth, 1995). Accordingly, lysine requirement for maintenance in nitrogen balance studies with pigs (Fuller *et al.*, 1989) and chick (Leveille & Fisher, 1959) are the lowest for these animals (Table 5.8). Disregarding nitrogen balance studies, lysine requirement for maintenance expressed as metabolic body weight in fish is approximately 10-36 times lower than for terrestrial animals. Excluding the study of Adeola (1995) in which LysG (response) of pigs was variable and data required considerable extrapolation to estimate maintenance, the range is then reduced to 10-18 times. Contrary to this comparison, Mambrini and Kaushik (1995) demonstrated the sulphur amino acid requirement for maintenance expressed as metabolic body weight in rainbow trout was two-fold higher than pigs, indicating a different basal metabolism in fish was greater for fish.

Expressed as a proportion of the total requirement for maximum gain, there is less than a two-fold difference in the lysine requirement for maintenance for fish and terrestrial animals (Table 8). The average lysine requirement for maintenance expressed as a proportion of the total requirement is 8.6% for fish, 17.6% for pigs and 9.7% for chicks. Again, excluding the study of Adeola (1995) the requirement for pigs is 12%. Considering that terrestrial animals have a higher weigh-specific lysine requirement for maintenance, similarity when expressed as a proportion of the total requirement for maximum gain arises from greater weight-specific feed intake than fish. It is also worthy to note that as a consequence of many of the animals being fed at marginal lysine intakes in studies detailed in Table 8, the lysine requirement for maintenance expressed as a proportion of the would be somewhat overestimated. Despite this, values for terrestrial animals are consistent with the figure of 10% estimated for rapidly

growing pigs in a simulated model of the physiological processes of lysine metabolism (Moughan, 1989,1991).

Considering lysine utilisation for LysG and lysine requirement for maintenance there appears to be little difference in the protein nutrition of rapidly growing fish and terrestrial animals. For both fish and terrestrial animals, intakes above maintenance requirements are utilised with a similar efficiency due to the mechanism of inevitable catabolism shared by all animals. Terrestrial animals, however, have a higher weight-specific lysine required for maintenance but is off-set by a higher weight-specific feed intake to the extent that when growing at a rapid rate are equivalent in their utilisation of lysine as fish. These results support the findings of Bowen (1987) of similar protein nutrition in fish and terrestrial animals. Protein utilisation, however, would depend on the utilisation of all ten essential amino acids for growth and maintenance. It is conceivable that difference in protein retention may arise between fish and terrestrial animals from an animal-specific requirement for one particular amino acid. Contrary to the findings of the present study, Mambrini and Kaushik (1995) demonstrated rainbow trout had a greater sulphur amino acid requirement for maintenance expressed as metabolic body weight than pigs. This suggests there is some basis for a difference in protein nutrition between fish and terrestrial animals arising from animal-specific requirements for particular amino acids.

For fish production, the combination of inevitable lysine catabolism and lysine requirement for maintenance can account for a considerable proportion of the inefficiency of protein retention. In rapidly growing fish, an inevitable loss of up to 30% lysine and lysine requirement of 9% means maximum lysine retention can only be as high as 61%. Assuming lysine to be the first-limiting dietary amino acid in the dietary protein of fish, PPV (proportion of ingested protein retained in growth) could only be as high as 61%. Generally, PPV observed for rapidly growing fish range between 30 to 45% (Cowey, 1994; Kaushik, 1995). Presumably, the lower observed PPV than predicted from lysine utilisation would be due to protein supply exceeding the individual amino acid requirements for growth and maintenance.

Alternatively, the lower PPV commonly observed in fish may, at least in part, result from amino acids other than lysine being utilised less efficiently for growth and/or having greater requirement for maintenance. In comparative studies, the efficiency of threonine utilisation has been demonstrated to be lower than lysine in pigs (Adeola, 1995) and rats (Gahl *et al*., 1997). If the efficiency of threonine utilisation of 60% estimated for pigs by (Adeola, 1995) is any indication, the PPV of less than 60% observed in fish culture would be partly contributed to the greater inevitable loss of particular essential amino acids. Maintenance requirement of some amino acids are also considerably greater than lysine expressed as a proportion of the total requirement for maximum gain. Rodehutscord *et al*. (1997) demonstrated for rainbow trout that the tryptophan and leucine requirements for maintenance were 30 and 34% of the total requirement for maximum gain, respectively. If tryptophan or leucine have a similar inevitable loss that has been observed for lysine (ie up to 30%), combined with the maintenance requirement, this accounts in total for the PPV less than 40% observed for rapidly growing fish. Based on these examples it can be appreciated that potential to improve PPV in fish culture depends on establishing the efficiency at which these particular amino acids are utilised for growth and maintenance.

Acknowledgments

We gratefully acknowledge the assistance of S. Edwards, School of Biomedical Science in the analysis of lysine.

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Table 1
Ingredient and nutrient composition of the experimental diet

Ingredient composition ($g.kg^{-1}$)	
Zein	219.00
Fish meal	173.00
Amino acid mix[a]	128.00
Glycine/Lysine	13.58
Extruded wheat	200.00
Fish oil	124.00
Ammonium acetate	3.84
Vitamins[b]	4.22
Minerals[c]	0.97
Potassium phosphate[d]	15.46
Sipernat®50S	40.00
α-Cellulose	77.93
Chemical composition ($g.kg^{-1}$ DM)	
Dry matter ($g.kg^{-1}$)	951.1
Nitrogen	82.3
Crude protein[e]	481.4
Crude fat	163.0
Ash	84.6
Gross energy ($MJ.kg^{-1}$)	21.2

[a] Amino acids added ($g.kg^{-1}$ diet): Arg, 25.9; His, 5.9; Ile, 6.9; Met, 8.4; Cys, 2.1; Tyr, 0.5; Thr, 11.9; Trp, 3.6; Val, 11.0; Ala, 2.4; Asx, 25.1; Gly, 20.9; Ser, 3.6.
[b] Vitamin mixture (mg or $IU.kg^{-1}$ diet): Retinol, 2500IU; Cholecalciferol, 2400IU; α-Tocopherol, 200; Menadione, 40; Thiamine, 10; Riboflavin, 20; Calcium pantothenate, 40; Biotin, 1; Folic acid, 5; Cyanocobalamin, 0.02; Nicotinic acid, 150; Pyridoxine, 10; Choline chloride, 1200; Inositol, 400; Ascorbic acid (Rovimix stay C 35%), 750.
[c] Mineral mixture ($mg.kg^{-1}$ diet): Mn, 35 ($MnSO_4.H_20$); Fe, 80 ($FeSO_4.7H_2O$); Cu, 12 ($CuSO_4$ anhydrous); Zn, 90 ($ZnSO_4.7H_2O$); Co, 7 ($CoSO_4.6H_20$); I, 2 (KI); Se, 0.2 (Na_2SeO_3).
[d] monobasic molecule (KH_2PO_4).
[e] Crude protein = N × 5.85 (Gnaiger and Bitterlich, 1984).

Table 2
Amino acid composition ($g.kg^{-1}$) of experimental diets

Amino acid	$g.kg^{-1}$
Arginine	37.45
Histidine	14.80
Isoleucine	23.80
Leucine	58.60
Lysine	-
Methionine	15.80
Cystine [a]	5.30
Phenylalanine	23.30
Tyrosine	16.25
Threonine	24.10
Tryptophan [a]	4.65
Valine	29.30
Alanine	33.45
Aspartic acid	50.50
Glutamic acid	81.60
Glycine	33.30
Proline	30.70
Serine	22.40

[a] Calculated from that added in crystalline form and measured composition of fish meal, zein and wheat

Table 3
Apparent digestibility coefficients (ADC, %) for dry matter (DM), crude protein (N), energy (kJ) and lysine (Lys)

Dietary lysine $g.kg^{-1}$	ADC_{DM}	ADC_N	ADC_{kJ}	ADC_{Lys}
10.80	77.82	95.09	87.08	94.19
11.76	77.57	96.08	86.38	94.19
12.72	78.93	96.10	87.29	94.68
13.68	78.48	96.33	86.61	94.75
14.64	78.19	96.03	86.96	95.02
15.60	77.09	95.55	86.51	95.58
16.56	77.91	95.97	86.79	95.75
17.52	78.39	95.65	87.71	95.85
18.48	78.20	96.05	87.18	96.68
19.44	78.68	94.85	87.30	97.17
20.40	78.28	95.69	86.92	96.61
21.36	79.21	95.91	87.41	97.44
Mean	78.23	95.78	87.01	
Regression[a]				
Slope (b)				0.317
Intercept (a)				90.559
$\mathbf{r^2}$				0.95
$s_{y.x}$				0.243
P[b]	NS	NS	NS	***

[a] Linear regression, $y = a + b * x$ in which: y = independent variable, x = dependent variable, $a = y$ intercept and b = slope of regression.
[b] Denotes significant relationship between DLys and nutrient digestibility, NS - not significant, *** < 0.001.

Table 4
Performance of Atlantic salmon parr (per fish basis) after a 50 day experimental period

Dietary digestible lysine ($g.kg^{-1}$)	Initial weight (g)	Final weight (g)	Average liveweight gain (g)	Feed intake (g DM)	FER ($g.g\ DM^{-1}$)
10.15	44.14	62.68	18.54	20.68	0.90
11.09	44.15	63.44	19.29	20.73	0.93
12.03	43.43	63.31	19.88	20.67	0.96
12.98	43.86	66.61	22.75	21.05	1.08
13.94	43.93	65.57	21.63	20.92	1.03
14.90	43.82	67.58	23.76	21.16	1.12
15.87	43.52	67.07	23.55	20.93	1.12
16.84	43.30	67.37	24.07	20.94	1.15
17.82	43.82	67.80	23.98	20.96	1.14
18.80	43.59	68.20	24.61	20.93	1.18
19.79	43.39	69.88	26.49	21.33	1.24
20.79	43.70	69.59	25.88	21.41	1.21
Regression[a]					
Slope (b)		0.646	0.693	0.053	0.030
Intercept (a)		56.626	12.183	20.155	0.622
$\mathbf{r^2}$		0.89	0.90	0.62	0.91
$s_{y.x}$		0.787	0.797	0.144	0.034
P[b]	NS	***	***	**	***

Feed intake = feed consumed over 50 days.
FER: feed efficiency ratio = total weight gain (g)/total feed intake (g DM).
[a] Linear regression, $y = a + b * x$ in which: y = independent variable, x = dependent variable, $a = y$ intercept and b = slope of regression.
[b] Denotes significant relationship between DDLys and growth performance, NS - not significant, ** < 0.01.

Table 5
Whole-body composition (% wet weight) of Atlantic salmon parr

Dietary digestible lysine ($g.kg^{-1}$)	Dry matter	Crude protein[a]	Lysine	Fat	Ash
10.15	28.04	13.65	0.93	9.75	2.43
11.09	26.47	13.49	0.91	8.77	2.33
12.03	26.36	13.49	0.94	8.36	2.43
12.98	25.81	13.88	0.99	7.42	2.51
13.94	26.40	13.88	0.99	7.77	2.40
14.90	26.10	14.03	1.00	8.15	2.43
15.87	26.05	14.05	1.05	7.93	2.36
16.84	25.42	13.96	1.01	7.37	2.36
17.82	25.49	14.25	1.00	6.57	2.37
18.80	25.68	14.52	1.07	6.98	2.38
19.79	25.17	14.41	1.09	6.18	2.34
20.79	25.42	14.45	1.07	4.51	2.26
Regression[b]					
Slope (b)	-0.180	0.083	0.066	-0.349	-0.011
Intercept (a)	28.808	12.698	6.171	12.855	2.552
$\mathbf{r^2}$	0.67	0.77	0.74	0.82	0.35
$s_{y.x}$	0.439	0.157	0.137	0.577	0.051
P[c]	**	***	***	***	*

Initial group (mean): 26.32% DM; 14.20% crude protein[a]; 1.04% lysine, 8.53% fat; 2.25% ash.

[a] crude protein = N × 5.85 (Gnaiger and Bitterlich, 1984).

[b] Linear regression, $y = a + b * x$ in which: y = independent variable, x = dependent variable, $a = y$ intercept and b = slope of regression.

[c] Denotes significant relationship between DDLys and whole-body composition, * < 0.05, ** < 0.01, *** < 0.001.

Table 6
Linear regression[a] between Log fish weight and Log body burden protein or Log body burden lysine of parr fed 25 and 19.8 DP.DE^{-1}

Diet formulation DP.DE^{-1}	*a*	*b*	r^2	$s_{y.x}$	*P*[b]
	Protein				
25.0	-1.917	1.583	0.97	0.0046	***
19.8	- 1.900	1.567	0.99	0.0045	***
P[c]		NS			
	Lysine				
25.0	-4.683	2.473	0.94	0.0097	***
19.8	-3.786	1.958	0.85	0.0194	***
P[d]		NS			

[a] Linear regression, $y = a + b * x$ in which: y = Log body burden (protein/lysine), x = Log fish weight, $a = y$ intercept and b = weight exponent (slope).
[b] Denotes significant relationship between Log fish weight and Log body burden (protein/lysine) of parr, *** < 0.001.
[c] Denotes significant difference between diet formulation within weight exponent (*b*), NS - not significant.
[d] Denotes significant difference between diet formulation within weight exponent (*b*), NS - not significant.

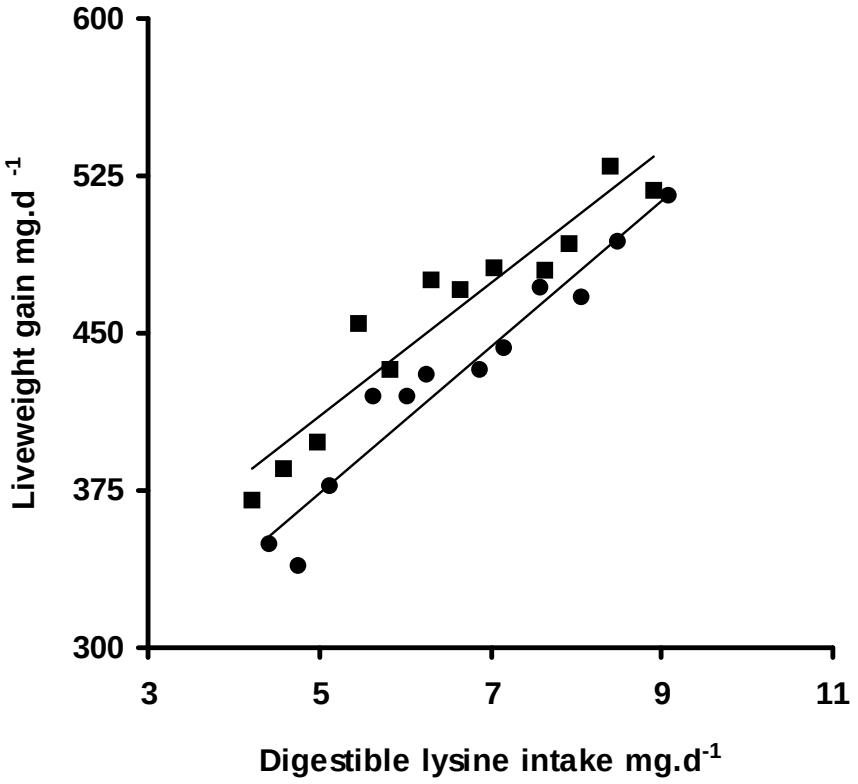

Figure 1a
Liveweight gain (LG, mg.d^{-1}) to digestible lysine intake (DLysI, mg.d^{-1}) fed 25.0 DP.DE^{-1} diet (ν, LG = 31.54DLysI + 252.58, $r^2 = 0.90$, $s_{y.x} = 15.928$, $P < 0.001$) and 19.8 DP.DE^{-1} diet (λ,LG = 34.90DLysI + 199.11, $r^2 = 0.94$, $s_{y.x} = 13.011$, $P < 0.001$). Slopes of regression equations were not significantly different (P > 0.2) between diets. The elevation of the 25.0 DP.DE^{-1} diet was significantly higher ($P < 0.001$).

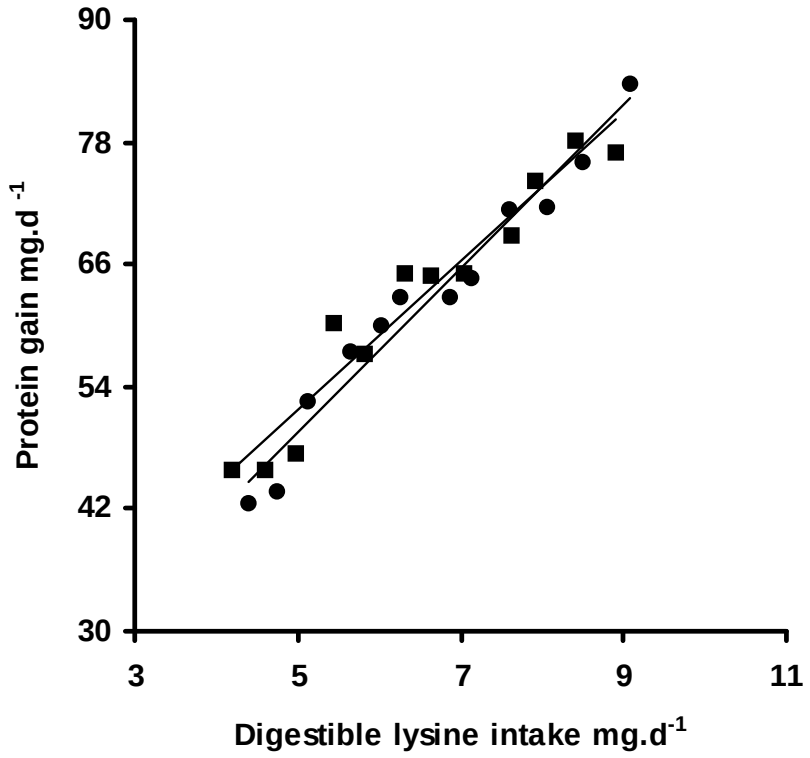

Figure 1b
(b) Protein gain (PG, $mg.d^{-1}$) to lysine intake (DLysI, $mg.d^{-1}$) fed 25.0 $DP.DE^{-1}$ diet (ν, PG = 7.31DLysI + 15.05, r^2 = 0.94, $s_{y.x}$ = 2.891, $P < 0.001$) and 19.8 $DP.DE^{-1}$ diet (λ, PG = 8.02DLysI + 9.35, r^2 = 0.96, $s_{y.x}$ = 2.453, $P < 0.001$)). There was no significant difference between the slopes (P > 0.2) and elevations (P > 0.2) for the regression equations of both diets. Regression equation of pooled data: PG = 7.65DLysI + 12.35 (n = 24, r^2 = 0.95, $s_{y.x}$ = 2.723, $P <$ 0.001).

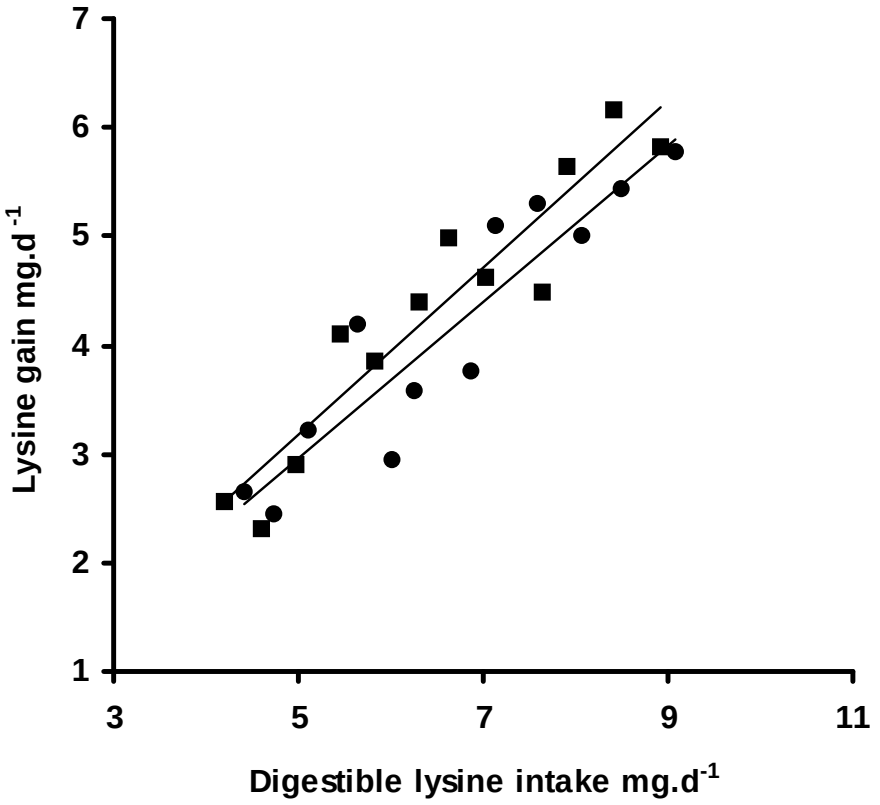

Figure 1c
(c) Lysine gain (LysG, mg.d^{-1}) to lysine intake (DLysI, mg.d^{-1}) fed 25.0 DP.DE^{-1} diet (ν, LysG = 0.77DLysI – 0.69, r^2 = 0.89, $s_{y.x}$ = 0.459, P < 0.001) and 19.8 DP.DE^{-1} diet (λ, LysG = 0.72DLysI – 0.63, r^2 = 0.85, $s_{y.x}$ = 0.414, P < 0.001)). There was no significant difference between the slopes (P > 0.5) and elevations (P > 0.1) for the regression equations of both diets. Regression equation of pooled data: LysG = 0.74DLysI – 0.63 (n = 24, r^2 = 0.85, $s_{y.x}$ = 0.455, P < 0.001).

Table 7
The efficiency of lysine utilisation for lysine gain in experiments with terrestrial animals and fish

Animal	Efficiency (%)	Reference
Rat	83	Bolton and Miller, 1985
Pig	77[a]	Batterham *et al*., 1990
	72	Adeola, 1995
Chick	76 & 79	Edwards *et al*., 1999
Rainbow Trout	71[b]	Rodehutscord *et al*., 2000
Atlantic Salmon	67 & 72	Section 7.3
	77	Present Section

[a] Originally estimated to be 86% (Batterham *et al*., 1990) but requoted to be 77% excluding the treatment with the lowest lysine intake that displayed problems with growth (E.S. Batterham pers. comm. in Langer and Fuller, 1996)
[b] Reported for the high crude protein diet only.

Table 8
Maintenance lysine intake expressed as metabolic body weight and % of total requirement for maximum gain in fish and terrestrial animals

Animal	Maintenance lysine requirement		Methodology[b]	Reference
	Metabolic body weight[a] $mg.(kg^{0.75})^{-1}.d^{-1}$	Total Requirement %		
Rat	64[c]	na	CS	Owens *et al*., 1985
Pig	38[c]	na	NB	Fuller *et al*., 1989
	80	12.0	CS	Batterham *et al*., 1990
	233	23.3	CS	Adeola, 1995
Chick	29[c]		NB	Leveille and Fisher, 1959
	99[c]	na	CS	Owens *et al*., 1985
	61	7.0	CS	Kim *et al*., 1997
	89[c]	10.5	CS	Edwards *et al*., 1999
	114[c]	11.6	CS	Edwards *et al*., 1999
Rainbow Trout	7.7	4.0[c]	CS	Rodehutscord *et al*., 1997
	3.1	13.9	CS	Rodehutscord *et al*., 2000
Atlantic salmon	5.9	5.3	CS	Section 7.3
	7.1	9.6	CS	Section 7.3
	7.7	10.0	CS	Present Section

[a] Body weight assuming weight = (initial weight + final weight)/2 of treatment with greatest weight change.
[b] na, not available; CS, comparative slaughter; NB, nitrogen balance.
[c] Estimates as quoted in particular studies.

7.5
Lysine utilisation by Atlantic salmon parr 3: Influence of dietary protein

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Abstract

This study investigated the effect of dietary protein on the efficiency of lysine utilisation for protein gain in Atlantic salmon (*Salmo salar* L.) parr. The aim was to identify the relative dietary protein concentration critical to the efficiency of lysine utilisation for protein gain. This, the critical relative protein concentration, was used to evaluate the dietary lysine requirements that achieve maximum lysine utilisation in fish. Wheat gluten, fish meal and crystalline amino acids were used to formulate two isoenergetic diets (19.9 MJ digestible energy (DE).kg^{-1}) containing low and high protein level (326.6 and 449.6 g digestible protein (DP).kg^{-1}) but the equivalent 12 lysine levels ranging from 16.0 to 30.9 g dietary digestible lysine (DDLys).kg^{-1}. Parr fed the same lysine level in each protein level received the equivalent ration (% body weight.d^{-1}) over a 56 day period. The lysine intakes in this study were designed to include those from marginal to exceeding maximum protein gain. A rectilinear (broken-line) model determined the breakpoint in growth performance to lysine supplementation in each protein level. Liveweight gain, total feed intake and feed efficiency ratio responded linearly to lysine supplementation in each protein level: the breakpoints in growth parameters in the low protein level were 25.5, 25.3 and 25.5 g DDLys.kg^{-1}, respectively, and in the high protein level were 26.5, 25.8 and 26.6 g DDLys.kg^{-1}, respectively. Protein gain (mg.d^{-1}) to digestible lysine intake (DLysI, mg.d^{-1}) was more accurately described by the four-parameter saturated kinetics model (4SKM) in the low protein diet and the rectilinear model in the high protein diet. From the first derivative plot of the 4SKM model a lysine concentration of 6.0% dietary protein was shown to be critical to the efficiency of lysine utilisation for protein gain in Atlantic salmon. Hence, dietary lysine concentrations greater than 6.0% dietary protein maintain a sub-optimal efficiency of lysine utilisation. The majority of dietary lysine requirement recommendations for fish are less than 6.0% dietary protein. Being lower than the critical relative protein concentration, most dietary lysine requirement recommendations for fish would therefore achieve maximum lysine utilisation. Alternatively, as protein intake has not had a regulatory role in these studies, inter and intra-species variation observed in lysine requirements cannot be expected to improve when expressed as a percentage of dietary protein.

Introduction

Rodehutscord *et al*. (2000) highlighted that the current dietary lysine requirements for fish will not necessarily achieve maximal lysine utilisation when applied to feed formulation. Critical to the efficiency of lysine utilisation is the relative concentration of dietary protein (amino acids). It is proposed that dose-response lysine requirement studies that used low dietary protein level have potentially compromised lysine utilisation by reaching a critical relative concentration to the second-limiting amino acid. In the current environment of fish production where dietary protein concentration

is being reduced and feed efficiency is developing as a priority, it would be useful to identify the critical relative protein concentration that optimises lysine utilisation.

Commonly, dose-response lysine requirement studies have employed a low basal lysine diet and gradually supplemented crystalline lysine to which the response of fish measured. In dose-response studies that use a low dietary protein level, there are potentially three distinct stages of interaction with the second-limiting amino acid (see Figure 3 for further detail). These stages are based on a theoretical interaction between lysine and sulphur amino acid (methionine + cystine) in pigs as described by Susenbeth (1995). Initially there is no interaction as other amino acids are in relative excess and the efficiency of lysine utilisation maximised. Eventually, at critical point the efficiency of lysine utilisation is diminished, but still existent (diminishing returns), as the second-limiting amino acid becomes co-limiting. Beyond diminishing returns the second-limiting amino acid replaces lysine as first-limiting and lysine utilisation ceases. In the present study it was proposed the relative protein concentration corresponding to the beginning of diminishing returns to be critical to lysine utilisation.

Curvilinear models have been used to describe the diminishing returns in lysine utilisation in rats (Hegar & Frydrych, 1985; Gahl *et al*., 1991, 1996) and pigs (Gahl *et al*., 1994). In these studies the models applied have included both the four-parameter logistics model (Finke *et al*., 1987) and third degree polynomials. To evaluate diminishing returns first derivative plots of these models have been essential [(*dr/dI*, derivative of the response (r) with respect to lysine intake (I)]. Plots of the first derivative illustrate instantaneous efficiency, which is the efficiency in the response when adding an additional increment of lysine to the diet (Gahl *et al*., 1991). The point where instantaneous efficiency is greatest is the point of maximum efficiency in lysine utilisation. In the same manner, the greatest instantaneous efficiency can be used to evaluate the relative protein concentration critical to lysine utilisation.

In the present study, lysine utilisation for protein gain (PG) was investigated in Atlantic salmon parr fed incremental levels of lysine in diets that contained either 326.6 or 449.6 g digestible protein (DP).kg^{-1}. Lysine intake at each dietary protein level ranged from marginal to exceeding maximum for protein gain. The primary aim was to identify the critical relative protein concentration for maximum lysine utilisation in the low protein diet. The critical relative protein concentration identified in this study was then used to evaluate which current dietary lysine requirement estimated for fish are likely to achieve maximum lysine utilisation.

It is acknowledged, however, that the critical relative protein concentration for lysine utilisation defined in this study does not apply to all situations in formulation and fish culture. Firstly, it is the relative concentration of the second-limiting amino acid that determines the critical protein concentration. Thus, diets with a different amino acid profile will have a different critical protein concentration. Secondly, the dietary amino acid profile required for maintenance differs considerably from the profile for protein gain (growth) in fish (Rodehutscord *et al*., 1997). Therefore, the relative amino acid profile critical to lysine utilisation depends on what fraction of the total requirement is due to maintenance (Gahl *et al*., 1996). Because the fraction for maintenance changes with different circumstances (rate of protein gain, fish size, etc), the relative dietary protein concentration critical to lysine utilisation in fish is not definitive. Nevertheless, investigating the relative protein concentration critical to the efficiency of lysine

utilisation is warranted, as currently it is unknown what recommendations for formulation achieve maximum utilisation.

Materials and methods

Experimental diets

Two isoenergetic (20.4 MJ digestible energy (DE).kg^{-1}) diets were formulated to contain 326.6 and 449.6 g DP.kg^{-1} with increasing lysine levels (Table 1). Vital wheat gluten, fish and extruded wheat were used as sources of protein-bound amino acids. Crystalline amino acids (Sigma Chemical Co. and Musashi, Victoria, Australia) were added to create a profile (except glycine and lysine) similar to the whole-body of Atlantic salmon parr (Hauler & Carter, 2001) (Table 2). Each basal diet had a lysine content of 16.5 g dietary lysine (DLys).kg^{-1}. Lysine (L-Lysine monohydrochloride, Sewon 99%, BASF, 78.5% lysine) was added (substituting against glycine isonitrogenously) to each basal diet in 9 increments of 1.2 g DLys.kg^{-1} and 2 final increments of 2.1 g DLys.kg^{-1}. Thus, the 12 DLys levels at each protein level were; 16.5, 17.7, 18.9, 20.1, 21.3, 22.5, 23.7, 24.9, 26.1, 27.3, 29.4, 31.5 g DLys.kg^{-1}. To balance the pH of the feed (to pH 8), ammonium acetate was added at 3 g.kg^{-1} of the amino acid mixture (Berge *et al*., 1998). Vitamin and minerals mixtures were added as previously described (Hauler & Carter, 2001). Ingredients were mixed before being cold pellet pressed to 3 mm using a California Pellet Mill (CL-2 laboratory pellet mill, California Pellet Mill Co., San Francisco, U.S.A.). Pellets were dried at 36°C until they contained less than 100 g.kg^{-1} moisture then stored at 4°C.

Growth experiment

Atlantic salmon (*Salmo salar* L.) parr originating from the SALTAS Salmon Hatchery (Tasmania, Australia) were transferred to a recirculation system (Carter & Hauler, 2000), acclimatised for 2 weeks during which time they were supplied a commercial feed (500 g.kg^{-1} protein, 14 g.kg^{-1} fat and 17.8 MJ DE.kg^{-1}). The system was housed in a constant environment room maintained at 16 h light:8 h dark photoperiod and 15.2 ± 0.6°C water temperature. Water quality parameters (pH, DO, ammonia, nitrate and nitrite) were maintained within limits for Atlantic salmon (Wedemeyer, 1996).

At the beginning of the experiment, fish were anaesthetised (50 mg.l^{-1} benzocaine), weighed (mean weight 37.87 g) and 21 fish randomly allocated to each of 24 tanks. Twelve fish were euthanased (100 mg.l^{-1} benzocaine) for assessment of initial whole-body chemical composition (see below). Each diet was fed to a single tank. A paired feeding design was implemented in which an equivalent ration (% BW.d^{-1}) was fed at each dietary lysine level in each protein level. Rations were adjusted to achieve a level of intake judged to be close to satiation for each pair of diets and uneaten pellets were collected. Fish were fed by hand 3 times each day (0900, 1300 and 1700 hours) in which daily rations were divided into 3 approximately equal portions. Diets were fed over a period of 56 days. Every 14 days fish were fasted for the day and each tank of fish bulk-weighed.

At the conclusion of the experiment, all fish were individually weighed after 48 hours of fasting. Five fish per tank were euthanased (100 mg.l^{-1} benzocaine) to measure whole-body chemical composition (see below). Fish were frozen at -20°C, dried individually, homogenised in a cutting mill and then pooled by tank for further chemical analysis.

Apparent digestibility

Apparent digestibility coefficient (ADC) was estimated for all diets. On days 48 and 49 faeces were collected overnight (1800 to 0800 hours) in settlement collectors (Cho *et al*., 1982). Faeces were frozen, freeze-dried, pooled from both collection days and stored at -20°C before analysis. Yttrium and nutrients were analysed (see below) in diets and faeces and ADC was calculated using:

$$ADC\ (\%) = 100 - [100(\%I_{diet}/\%I_{faeces}) \times (\%N_{faeces}/\%N_{diet})]$$

(Maynard and Loosli, 1969) where I is the inert marker and N is the nutrient.

Chemical analysis

Dry matter of whole-body was determined by lyophilisation and for diets by oven drying at 70°C to constant weight. Whole-body and diets were analysed for nitrogen (Kjeldahl, copper sulfate catalyst), crude fat by petroleum ether extraction (AOAC, 1995), ash by combustion at 550°C for 16 hours (AOAC, 1995) and Yttrium by ICP spectrophotometry after acid digestion (Refstie *et al*., 1997). Amino acid content of the diets was determined by a Waters High Performance Liquid Chromatograph (HPLC) after acid (HCl) hydrolysis (Rayner, 1985). Lysine content of diets, faeces and whole-body was determined by isocratic liquid chromatography (Or-Rashid *et al*., 1999).

Statistical analysis

Least squares linear regression was used to describe relationships between (1) DLys level and apparent lysine digestibility (ADC_{Lys}) and (2) digestible lysine intake (DLysI) and PG of parr fed each protein level. Goodness of fit was described by the coefficient of determination (r^2) which describes the proportion of the total variation around y explained by the linear regression, and the standard error of the regression ($s_{y.x}$) which describes the standard deviation of the residuals of the individual values of y from the linear regression. ADC_{Lys} was arcsine-transformed prior to analysis. If linear regressions were significant and variance equal (variance ratio test, $F = 5.87$, $P = 0.05$, $n = 20$ and $F = 6.41$, $P = 0.05$, $n = 13$) between dietary protein levels, a t-test was used to identify if slopes and elevations were significantly different (Zar, 1984). Where a significant linear regression was not established between DLys level and ADC of dietary nutrients, data was pooled and a t-test used to identify if means were significantly different between each protein level after confirmation of normality and homogeneity. Probabilities of 0.05 or less were considered significant.

The following approaches were used for curve fitting procedures:

(i) Rectilinear (broken-line):

As adapted by Rodehutscord and Pack (1999) from Robbins *et al*. (1979):

$$y = f + 0.5g(x - h - |x - h|)$$

where, y = dependent variable (response criteria), x = independent variable, f = ordinate, h = abscissa of the breakpoint and g = slope of the line for $x < h$.

(ii) Exponential:

As published by Robbins *et al*. (1979):

$$y = u + v(1 - e^{bx})$$

where, y = dependent variable (response criteria), x = independent variable, $u = y$ at $x = 0$, v = maximum response ($u + v$ = asymptote) and b = parameter describing the steepness of the curve.

(iii) Four-Parameter Saturation Kinetics Model (4SKM)
As published by Mercer (1982):

$$y = d(K_{0.5})^n + y_{max}(x)^n / (K_{0.5})^n + (x)^n$$

where, y = dependent variable (response criteria), x = independent variable, d = intercept on the y axis, y_{max} = maximum theoretical response, $K_{0.5}$ = lysine level or intake for ½ of (y_{max} + d) and n = apparent kinetic order. The 4SKM equation was written for use in a curve-fitting program as described by Shearer (2000).

Model parameters were estimated using SIGMAPLOT (2000 version 6.0, Abacus Concepts, Berkeley, CA, USA) which solves the equations iteratively using the Maruardt-Levenberg algorithm.

Results

Apparent digestibility

The relationship between DLys level and apparent digestibility of dry matter (ADC_{DM}), protein (ADC_N), energy (ADC_{kJ}) and ADC_{Lys} was investigated (Table 3). In each protein level the relationship between DLys and ADC_{DM}, ADC_N and ADC_{kJ} was not significant ($P > 0.05$) and consequently, data were pooled. ADC_{DM}, ADC_N and ADC_{kJ} of the high protein diet was significantly greater ($P < 0.001$) than the low protein diet. The digestible protein and energy composition of the low protein diet was 326.6 g $DP.kg^{-1}$ and 20.0 MJ $DE.kg^{-1}$, respectively, and of the high protein diet was 449.6 g $DP.kg^{-1}$ and 19.7 MJ $DE.kg^{-1}$, respectively.

In each protein level, ADC_{Lys} increased as DLys increased. Between each protein level there was no significant difference in the slopes ($P > 0.5$) of the relationship describing DLys and ADC_{Lys}. However, elevation of the relationships for the low protein diet was significantly higher ($P < 0.001$) than the high protein diet. Consequently, further analysis was based on digestible dietary lysine (DDLys) levels specific to each protein level. DDLys levels quoted in Tables 4, 5 and 6 were the average of each protein level.

Growth performance

Average liveweight gain (LG) was 37.6 g and 41.1 g for the parr fed low and high protein level, respectively (an increase by a factor of 2.0 and 2.1, respectively) over the 56 day experiment. The rectilinear model was used to compare the growth performance of parr to DDLys at each protein level. Parameters for the rectilinear model are summarised in Tables 4 and 5. Average liveweight gain (ALG), total feed intake (TFI), feed efficiency ratio (FER) and protein of whole-body increased with increasing DDLys at each protein level. Dry matter, fat and ash of whole-body decreased with increasing DDLys at each protein level. The DDLys level required to reach the breakpoint in ALG, TFI and FER in the low protein diet was only slightly lower (no less than 95%) than the high protein diet. The DDLys level required to reach the breakpoint in protein in whole-body in the low protein diet was 80.8% of the high protein diet.

Lysine utilisation

The rectilinear model described the relationship between digestible lysine intake (DLysI) and PG (lysine utilisation for PG) at each protein level (Fig. 1a). Maximum PG was 111.8 and 140.6 $mg.d^{-1}$ for the low and high protein diet, respectively. The breakpoint of PG in the low protein diet was reached at a lysine intake of 84.6% of that

in the high protein diet. In the low protein diet the slope of rectilinear model to the breakpoint was 77.4% of that in the high protein diet. In each protein level a linear model was applied to the lysine intake lower than the breakpoint defined by the rectilinear model (Figure 1b). The slope of the low protein diet was significantly lower ($P < 0.01$) than the high protein diet.

In the low protein diet lysine utilisation was also described by an exponential (Fig. 2a) and a 4SKM (Fig. 2c) model. Based on r^2 and $s_{y.x}$, the 4SKM model more accurately described the data than the exponential function. First derivative plots of the exponential (Fig. 2b) and 4SKM (Fig. 2d) models illustrated the instantaneous efficiency of lysine utilisation in the low protein diet. The derivative plot of the exponential model suggests the efficiency of lysine utilisation decreased from the first lysine intake in the low protein diet (7.9 mg DLysI.d^{-1}). In comparison the 4SKM model, after an initial increase, decreased at lysine intakes beyond 10.3 mg DLysI.d^{-1}. In the 4SKM model the diet corresponding to the decrease in instantaneous efficiency of lysine utilisation was 19.53 g DDLys.kg^{-1} (4th DDLys level of the low protein diet). Based on the results from the 4SKM model, 19.53 g DDLys.kg^{-1} in the low protein diet was considered the critical relative protein concentration to lysine utilisation. In this diet formulation the relative protein concentration was 6.0% (ie 19.53 g DDLys/326.6 g DP = 5.97%).

Discussion

The present study compared lysine utilisation in a low (326.6 g DP.kg^{-1}) or high (449.6 g DP.kg^{-1}) protein diet in Atlantic salmon parr. The response in lysine utilisation in the low protein diet confirmed the theoretical interaction between lysine and the second-limiting amino acid as proposed by Susenbeth (1995). A dietary lysine concentration of 6.0% dietary protein was shown to be critical to the efficiency of lysine utilisation. Considering that most lysine requirement estimates for fish are less that 6.0% dietary protein, these recommendations are likely to achieve maximum lysine utilisation.

Influence of dietary protein on lysine utilisation

The influence of dietary protein level on lysine utilisation was consistent with a similar study with rainbow trout, *Oncorhynchus mykiss* (Rodehutscord *et al*., 2000). Rodehutscord *et al*. (2000) compared the efficiency of lysine utilisation for lysine gain (LysG) in two dietary protein levels (350 and 550 g protein.kg^{-1}). By fitting a linear model to the response in each protein level they concluded that the efficiency of lysine utilisation for LysG was 23% (0.71 v 0.55) lower in the low protein diet. Following the same approach, both the rectilinear and linear models were applied to each protein level in the present study (Fig. 1a&b). Comparing the slopes of the rectilinear and linear models, the efficiency of lysine utilisation for PG were 23 and 22% lower, respectively, in the low protein diet. Both studies with salmonids, therefore, demonstrate the equivalent influence of dietary protein on the efficiency of lysine utilisation. However, it is proposed this interpretation of the influence of protein level on lysine utilisation has been too simplistic.

The response of PG to lysine intake in each protein level was consistent with the theoretical interaction between lysine and the second-limiting amino acid proposed by Susenbeth (1995). Based on diet formulations used in the present study, Figure 3 illustrates the influence that relative sulphur amino acids (methionine + cystine)

concentration had on lysine utilisation for PG. As the second-limiting amino acid was not actually known, the figures quoted for the interaction herein serves as an example only.

In the low protein diet in the present study, initially (Range A) there was no interaction as other amino acids are in relative excess and the efficiency of lysine utilisation maximised. Eventually, a critical point (Range B) was reached where the efficiency of lysine utilisation diminished, but was still existent (diminishing returns), as the second-limiting amino acid became co-limiting. Beyond diminishing returns (Range C) the second-limiting amino acid replaced lysine as first-limiting and lysine utilisation ceases. Over the same range in the high protein diet, the relative concentration of the second-limiting amino acid was in relative excess and maximum lysine utilisation was maintained (represented by the dotted line in Figure 3).

Based on the interaction observed in the low protein diet, concluding that lysine utilisation was consistently low would be inaccurate. Alternatively, both the present study and that of Rodehutscord *et al*. (2000) displayed diminishing returns in lysine utilisation in the low protein diet. The difference in slopes of linear models, therefore, was attributed to the decline in protein gain to the higher lysine intakes of the low protein level (Range B). This can be confirmed in figure 3 in Rodehutscord *et al*. (2000), which on close inspection shows that a decrease in the efficiency of lysine utilisation only occurred at highest lysine level of the low protein diet.

It was intriguing to find lysine utilisation of parr fed the high protein diet to be constant at lysine intakes up to maximum protein gain (Figure 1a). In terrestrial animals, the response in lysine utilisation under conditions of relative protein (essential amino acid) excess has often been contradictory. For both rats (Hegar & Frydrych, 1985; Gahl *et al*., 1991,1996) and pigs (Gahl *et al*., 1994) a decrease in utilisation was evident at lysine intakes < 50% of the requirement for maximum protein gain (diminishing returns). The decrease in lysine utilisation under conditions of protein excess was contributed to the difference in activities of enzymes involved in both protein synthesis and degradation and subsequent competition for substrate (Gahl *et al*., 1991). However, contrary to diminishing returns in lysine utilisation under protein excess, other studies with pigs (Batterham *et al*., 1990; Susenbeth, 1995) have demonstrated a constant lysine utilisation up to maximum response. Interesting, the efficiency of lysine utilisation for PG of parr fed the high protein level (7.54 mg protein deposition (PD).mg^{-1} DLysI; Figure 6.1 a) was equivalent to the efficiency 7.51 mg PD.mg^{-1} lysine intake (LysI) observed for pigs (Susenbeth, 1995). The efficiency of utilisation for PG of parr fed the high protein level also agrees favourably to the average in Section 7.3 (8.28 mg PD.mg^{-1} DLysI) and Section 7.4 (7.65 mg PD.mg^{-1} DLysI). It is proposed that based on these comparisons, parr fed the high protein diet attained the maximum lysine utilisation for animals under adequate nutrition. Accordingly, further reference to the maximum lysine utilisation herein refers to this efficiency.

Critical relative protein concentration to lysine utilisation

In the present study, the beginning of diminishing returns in the low protein diet was considered indicative of the critical relative protein concentration in lysine utilisation. As the mathematical model chosen to describe diminishing returns can affect the final assessment (Gahl *et al*., 1996), more than one curvilinear model was considered herein.

Despite diminishing returns being displayed in the low protein diet, the rectilinear model more accurately described the data than curvilinear models when assessed by r^2 and $s_{y.x}$. However, the assumption of constant utilisation by fitting a rectilinear model was not a realistic representation of the interaction of lysine and the second-limiting amino acid. The use of curvilinear models, therefore, was based on an *a priori* decision considering the expected biological response (Gahl *et al*., 1994) or biological significance (Rodehutscord & Pack, 1999).

By comparing curvilinear models using r^2 and $s_{y.x}$, the more sophisticated 4SKM model described lysine utilisation in the low protein diet more accurately than the exponential model (Fig. 2a&c). From similar observations when applying these models to dietary amino acid requirements, Rodehutscord and Pack (1999) highlighted that the 4SKM model describes the curve around the inflection point as nearly linear whereas an exponential model describes diminishing returns from the very beginning of the curve. The exponential model, therefore, is useful when only a certain part of the response range can be studied. In the present study, diminishing return from the beginning of the curve in the low protein diet (Fig. 2b) was not entirely consistent with the expected interaction between lysine and the second-limiting amino acid. Initially the second-limiting amino acid was in relative excess and the efficiency of lysine utilisation maximised (Range A). Therefore, the ability of the 4SKM model to describe the curve around the inflection point as linear attributed to the more accurate fit. If the critical relative protein concentration had been lower than the lysine levels observed, an exponential function may have more appropriately described diminishing returns in lysine utilisation.

The 4SKM model did, however, suggest the response in lysine utilisation in the low protein diet was sigmoidal (Fig. 2c) in which lysine retention (utilisation) was greater at low lysine intake. In both rats (Hegar & Frydrych, 1985; Gahl *et al*., 1991, 1996) and pigs (Gahl *et al*., 1994) the efficiency of lysine utilisation has shown to be greater at lysine intake close to maintenance. For the present study, however, the sigmoidal response suggests the efficiency of lysine utilisation in the low protein diet was greater than the maximum observed in the high protein diet. A metabolic reason for improved efficiency in the low protein diet cannot be suggested. Alternatively, the difference in PG at low lysine intake in each protein level is considered to represent experimental variation. In this case, the lower instantaneous efficiency of lysine utilisation of the 4SKM model through Range A (Fig. 2d) was also considered an artifact and not representative of the true response. The diet corresponding to the inflection point of the 4SKM model (ie 19.53 g DDLys.kg^{-1}) was critical to lysine utilisation. Hence, the relative protein concentration critical to lysine utilisation was 6.0% dietary protein.

The relative protein concentration critical to lysine utilisation in the present study was greater than previously observed for rainbow trout. Re-evaluating the study of Rodehutscord *et al*. (2000), the efficiency of lysine utilisation was compromised at 5.0% dietary protein in rainbow trout (ie calculated as 17.3 g DDLys/345.2 g DP × 100 = 5.01%). The difference in critical relative protein concentration observed in these studies may be attributable to different dietary amino acid profiles and/or that maintenance requirement for particular amino acids were greater in the present study. The considerably greater estimate in the present study does reiterate the limitation of estimating the relative protein concentration critical to the efficiency of lysine utilisation. Nevertheless, based on what would appear to be an elevated estimate, some

important conclusions can be drawn concerning the relative protein and amino acid concentrations critical to the efficiency of lysine utilisation in fish nutrition.

Implications for the critical relative protein and amino acid concentration in fish nutrition

Critical relative protein concentration

From the present study, it is proposed that those dose-response dietary lysine requirements greater than 6.0% dietary protein will achieve a sub-optimal efficiency of lysine utilisation in fish. Rodehutscord *et al*. (2000) discovered on re-calculating dose-response lysine requirement experiments conducted in their laboratory (Pfeffer *et al*., 1992; Rodehutscord *et al*., 1997), that lysine utilisation in rainbow trout was higher when fed 470 g protein.kg^{-1} protein with no free amino acids (Pfeffer *et al*., 1992) compared to when fed 340 g protein.kg^{-1} protein with more than 50% free amino acids (Rodehutscord *et al*., 1997). It was speculated purified dietary ingredients (crystalline amino acids) were utilised less efficiently by fish and as a consequence, lysine concentrations in practical diets (protein-bound amino acid diets) could be lower than some dose-response studies suggest. However, the lysine requirement estimates in these same studies for rainbow trout were 4.18 and 8.76% dietary protein, respectively. In this case, the low efficiency of lysine utilisation in the latter study can be attributed to exceeding the relative protein concentration critical to the efficiency of lysine utilisation. Furthermore, in studies that have estimated a lysine requirement less than 6.0% dietary protein, crystalline amino acids have demonstrated the ability to maintain an equivalent utilisation to protein-bound amino acids (Hauler & Carter, 2001). Requirement recommendations from studies using purified ingredients are therefore considered applicable to practical formulation, so long as lysine utilisation has not been compromised by relative protein intake.

The critical relative protein concentration identified in this study would suggest that only a few lysine requirements for fish would maintain a sub-optimal efficiency of lysine utilisation. Of the current 33 dose-response dietary lysine requirement studies with fish, only 3 have exceeded 6.0% dietary protein: 8.76% (Rodehutscord et al., 1997) and 6.10% (Ketola, 1983) for rainbow trout and 6.23% for Indian carp, *Catla catla* (Fagbenro *et al*., 1998). It is not surprising, therefore, that lysine utilisation has been equivalent (at a maximum) in the lysine requirement studies for fish (Hauler & Carter, 2001). As twenty-three dose-response lysine requirement studies for fish have recommended a requirement of 5.0% dietary protein or less, it could be alternatively argued that protein supply has been excessive in most studies. Because the lysine requirements are lower that the critical protein concentration for lysine utilisation, it is unlikely that protein intake has had a regulatory role in these studies. It is only under regulatory protein intake that amino acids should be expressed as a percentage of dietary protein (Almquist, 1972). Consequently, intra and inter-species variation in lysine requirements of fish will not be improved when expressed as a percentage of dietary protein.

Critical relative amino acid profile

In the present study, the relative amino acid profile critical to lysine utilisation confirms that fishes whole-body amino acid profile to be misrepresentative as the ideal dietary profile. It has been demonstrated that fishes whole-body amino acid profile strongly correlates with quantitative dose-response amino acid requirements (Wilson & Poe, 1985; Arai & Ogata, 1991; Mambrini & Kaushik, 1995). On this basis, it may be thought the whole-body amino acid profile to be indicative of the ideal dietary profile.

However, expressed relative to lysine, the amino acid profile in the whole-body of fish differs considerably from the profile that required for protein gain and maintenance in fish (Rodehutscord *et al*., 1997). The relative dietary amino acid concentrations critical to lysine utilisation in the present study exceeded the profile of Atlantic salmon parr whole-body (Table 6), indicating that the amino acid profile based on whole-body will not maintain maximum efficiency of lysine utilisation at all levels of protein gain.

Furthermore, the relative amino acid concentrations critical to lysine utilisation in the present study exceeded the requirements for 65 % of maximum protein gain recommended by Rodehutscord *et al*. (1997) in rainbow trout (Table 6). At the critical point in the present study, protein gain was approximately 60% of maximum (of the high protein diet), indicating the recommendations for 65% of maximum protein gain in rainbow trout may not necessarily achieve maximum efficiency of lysine utilisation. In fact, it is unlikely that these recommendations would not achieve maximum efficiency considering that they were derived from a dose-response study in which lysine utilisation was compromised by low dietary protein (Rodehutscord *et al*., 1997). This example demonstrates importance of investigating amino acid utilisation under excessive protein supply to ensure recommended dietary amino acid profiles achieve maximum utilisation.

In conclusion, the present study has demonstrated that protein supply has a considerable influence on the efficiency of lysine utilisation in Atlantic salmon parr. For most of the recommended current lysine requirements for fish, lysine would be utilised with maximum efficiency. However, as dietary protein content decrease and optimising protein efficiency becomes more of a priority in fish culture, utilisation must become a feature of amino acid requirement values for practical feed formulation.

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Table1
Ingredient and nutrient composition of the experimental diets

	Diet	
	Low Protein	High Protein
Ingredient composition (g.kg^{-1})		
Wheat gluten	105.00	190.00
Fish meal	235.00	225.00
Amino acid mix	80.34[a]	161.37[b]
Glycine/Lysine	14.97	14.97
Extruded wheat	200.00	200.00
Fish oil	238.00	148.00
Ammonium acetate	2.86	5.31
Vitamins[c]	4.23	4.23
Minerals[d]	0.97	0.97
Potassium phosphate[e]	9.15	9.15
Sipernat®50S	40.00	40.00
α-Cellulose	68.48	-
Yttrium oxide	1.00	1.00
Chemical composition (g.kg^{-1} DM)		
Dry matter (g.kg^{-1})	984.4	989.3
Nitrogen	58.4	79.3
Crude protein[f]	341.6	463.9
Crude fat	277.9	190.3
Ash	92.4	86.6
Gross energy (MJ.kg^{-1})	23.1	21.8

[a] Amino acids added (g.kg^{-1} diet): Arg, 10.6; His, 1.9; Ile, 4.4; Leu, 6.7; Met, 4.2; Phe, 2.4; Tyr, 2.7; Thr, 6.5; Trp, 1.4; Val, 5.3; Ala, 8.8; Asx, 14.8; Gly, 7.6; Ser, 3.1.
[b] Amino acids added (g.kg^{-1} diet): Arg, 20.6; His, 5.1; Ile, 9.1; Lue, 14.2; Met, 8.1; Phe, 5.7; Tyr, 5.7; Thr, 12.4; Trp, 2.6; Val, 11.0; Ala, 17.1; Asx, 28.0; Gly, 15.7; Ser, 6.8.
[c] Vitamin mixture (mg or IU.kg^{-1} diet): Retinol, 2500IU; Cholecalciferol, 2400IU; α-Tocopherol, 200IU; Menadione, 40; Thiamine, 10; Riboflavin, 20; Calcium pantothenate, 40; Biotin, 1; Folic acid, 5; Cyanocobalamin, 0.02; Nicotinic acid, 150; Pyridoxine, 10; Choline chloride, 1200; Inositol, 400; Ascorbic acid (Rovimix stay C 35%), 750.
[d] Mineral mixture (mg.kg^{-1} diet): Mn, 35 ($MnSO_4.H_20$); Fe, 80 ($FeSO_4.7H_2O$); Cu, 12 ($CuSO_4$ anhydrous); Zn, 90 ($ZnSO_4.7H_2O$); Co, 7 ($CoSO_4.6H_20$); I, 2 (KI); Se, 0.2 (Na_2SeO_3).
[e] monobasic molecule (KH_2PO_4).
[f] Crude protein = N × 5.85 (Gnaiger and Bitterlich, 1984).

Table 2
Amino acid composition ($g.kg^{-1}$) of the experimental diets

	Diet	
Amino acid	Low Protein	High Protein
Arginine	22.27	41.86
Histidine	10.67	13.30
Isoleucine	16.99	24.46
Leucine	30.24	41.86
Lysine	-	-
Methionine	10.36	14.55
Cystine[a]	3.60	5.19
Phenylalanine	15.43	23.83
Tyrosine	12.53	16.51
Threonine	17.92	20.71
Tryptophan[a]	3.16	4.56
Valine	17.61	23.83
Alanine	18.75	24.82
Aspartic acid	32.31	44.99
Glutamic acid	63.91	82.39
Glycine	24.85	34.19
Proline	19.06	23.03
Serine	13.69	15.97

[a] Calculated from that added in crystalline form and measured composition of fish meal, zein and wheat

Table 3
Apparent digestibility coefficients (ADC, %) for dry matter (DM), crude protein (N), energy (kJ) and lysine (Lys)

Dietary Lysine	ADC_{DM}		ADC_N		ADC_{kJ}		ADC_{Lys}	
$g.kg^{-1}$	Low	High	Low	High	Low	High	Low	High
16.50	77.06	82.64	95.62	96.95	86.87	90.49	96.82	96.54
17.70	76.57	81.94	95.74	96.92	86.60	90.25	96.98	96.56
18.90	78.72	82.66	95.58	96.95	87.99	90.75	97.20	97.00
20.10	77.00	82.59	95.70	96.82	86.74	90.70	97.31	97.13
21.30	77.93	82.38	95.64	96.77	87.14	90.42	97.27	97.40
22.50	76.43	82.72	95.74	97.27	86.16	90.65	97.61	97.27
23.70	76.36	81.70	95.51	96.80	86.50	90.03	97.46	97.58
24.90	78.55	81.59	95.83	97.15	87.36	89.88	97.86	97.59
26.10	76.74	82.53	95.26	96.95	86.38	90.48	97.80	97.67
27.30	76.33	82.41	95.48	96.78	85.95	90.37	98.05	97.74
29.40	77.79	81.61	95.44	96.86	86.62	89.79	98.02	97.76
31.50	76.77	82.78	95.68	96.82	86.01	90.35	98.21	98.08
Mean[a]	77.19	82.30***	95.60	96.92***	86.69	90.35***		
Regression[b]								
Slope (*b*)							0.092	0.097
Intercept (*a*)							95.40	95.10
r^2							0.95	0.92
$s_{y.x}$							0.19	0.24
P[c]	NS	NS	NS	NS	NS	NS	***	***

[a] Denotes significant difference between each protein level within nutrient digestibility, *** < 0.001.
[b] Linear regression using model $y = a + b * x$ in which: y = independent variable, x = dependent variable, $a = y$ intercept and b = slope of regression.
[c] Denotes significant relationship between DLys and nutrient digestibility, NS - not significant, *** < 0.001.

Table 4
Performance of Atlantic salmon parr (per fish basis) after a 56 day experimental period

Dietary digestible lysine	Initial weight (g)		Final weight (g)		Average liveweight gain (g)		Total feed intake (g DM)		FER (g.g DM^{-1})		Mortality	
g.kg^{-1}	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High
15.97	38.14	38.54	68.68	68.03	30.54	29.49	27.65	28.11	1.10	1.05		
17.15	37.89	37.59	71.60	69.90	33.71	32.31	29.79	30.04	1.13	1.08		
18.34	38.50	38.05	72.40	72.03	33.90	33.98	30.38	30.86	1.12	1.10		
19.53	37.68	37.71	71.83	72.37	34.15	34.65	30.66	31.35	1.11	1.11		
20.72	37.62	37.67	70.29	76.82	32.67	39.16	30.38	32.45	1.08	1.21		
21.91	38.17	37.99	75.85	78.65	37.68	40.66	32.47	33.39	1.16	1.22		
23.11	37.39	37.68	76.87	80.13	39.48	42.45	33.40	34.50	1.18	1.23		
24.30	37.37	37.27	79.00	82.21	41.63	44.94	34.58	35.76	1.20	1.26		
25.50	38.19	38.56	80.75	86.04	42.57	47.48	34.88	36.27	1.22	1.31		
26.71	38.02	38.05	80.21	87.19	42.18	49.14	35.17	36.81	1.20	1.33	1	
28.82	37.29	38.61	78.30	88.07	41.01	49.45	34.56	36.57	1.19	1.35		1
30.94	37.19	37.84	78.88	86.78	41.69	48.94	35.09	36.84	1.19	1.33		1
Regression[a]												
f			79.400	87.425	41.827	49.177	34.925	36.740	1.197	1.337		
g			1.119	1.837	1.175	1.859	0.725	0.827	0.011	0.029		
h			25.549	26.793	25.529	26.531	25.336	25.779	25.525	26.649		
$\mathbf{r^2}$			0.88	0.99	0.91	0.99	0.95	0.99	0.74	0.98		
$s_{y.x}$			1.61	0.82	1.46	0.63	0.58	0.29	0.03	0.02		
P[b]	NS	NS	***	***	***	***	***	***	**	***		

Total feed intake = total feed consumed over 56 days.
FER: feed efficiency ratio = total weight gain (g)/total feed intake (g DM).
Mortality: total in each treatment replicate.
[a] Regression analysis using rectilinear model $y = f + 0.5g(x - h - |x - h|)$ in which: y = dependent variable, x = independent variable, f = ordinate, g = slope of the line for $x < h$ and h = abscissa of the breakpoint.
[b] Denotes significant relationship between DDLys and growth performance, NS - not significant, ** < 0.01, *** < 0.001.

Table 5
Whole-body composition (% wet weight) of Atlantic salmon parr

Dietary digestible lysine	Dry matter		Crude protein[a]		Fat		Ash	
g.kg^{-1}	Low	High	Low	High	Low	High	Low	High
15.97	32.16	30.95	13.86	13.80	14.15	12.67	2.25	2.27
17.15	32.03	31.12	13.96	14.01	13.96	12.61	2.21	2.19
18.34	32.08	31.35	14.39	14.53	13.16	12.83	2.23	2.19
19.53	31.49	30.72	14.74	14.75	12.70	11.76	2.15	2.22
20.72	31.50	30.43	14.79	14.64	12.92	12.01	2.18	2.18
21.91	31.66	30.42	14.81	14.63	12.73	11.35	2.11	2.14
23.11	31.06	30.54	14.81	15.11	12.15	11.15	2.10	2.08
24.30	31.17	30.17	14.83	15.26	12.55	10.53	2.09	2.08
25.50	31.07	30.29	14.77	15.04	12.50	11.21	2.09	2.04
26.71	31.21	29.95	14.74	15.41	12.34	10.87	2.13	2.07
28.82	31.05	30.06	14.81	15.44	12.04	10.68	2.10	2.04
30.94	31.24	29.61	14.98	15.43	12.18	10.12	2.08	2.07
Regression[b]								
f	31.148	29.835	14.818	15.330	12.356	10.557	2.098	2.056
g	-0.139	-0.108	0.259	0.159	-0.313	-0.222	-0.021	-0.021
h	23.610	28.325	20.006	24.748	21.624	26.270	23.249	25.481
$\mathbf{r}^2$	0.88	0.83	0.96	0.90	0.87	0.86	0.89	0.91
$s_{y,x}$	0.16	0.23	0.08	0.19	0.27	0.37	0.02	0.03
P[c]	***	***	***	***	***	***	***	***

Initial group (mean): 30.09% DM; 14.67% crude protein[a], 10.99% fat; 2.22% ash.
[a] Crude protein = N × 5.85 (Gnaiger and Bitterlich, 1984).
[b] Regression analysis using rectilinear model $y = f + 0.5g(x - h - |x - h|)$ in which: y = dependent variable, x = independent variable, f = ordinate, g = slope of the line for $x < h$ and h = abscissa of the breakpoint.
[c] Denotes significant relationship between DDLys and whole-body composition, *** < 0.001.

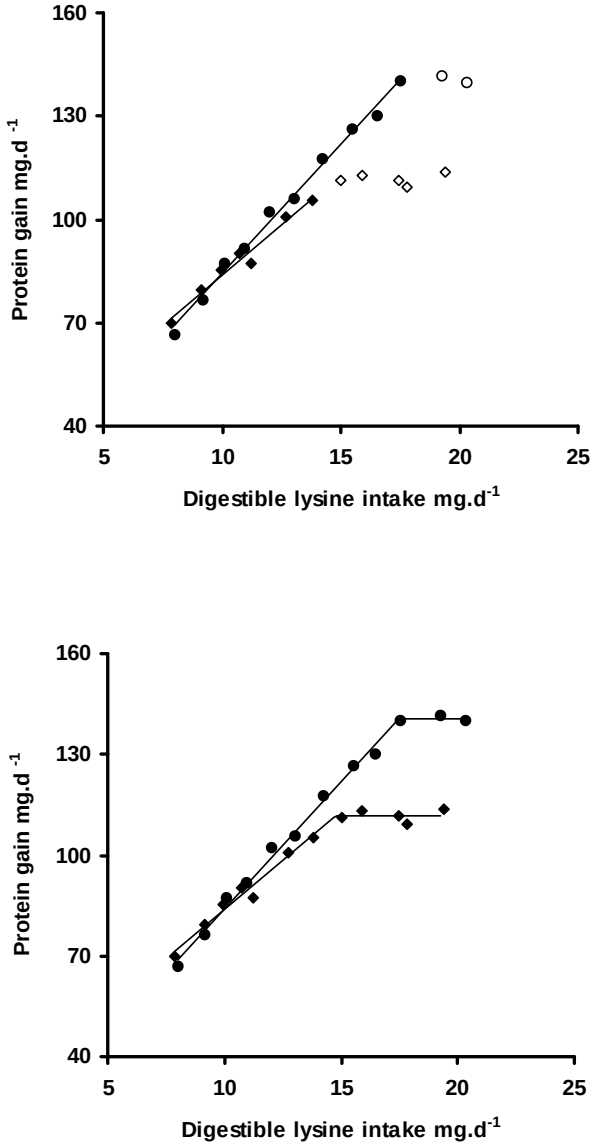

Figure 1
(a) Regression analysis using rectilinear model: Protein gain (PG, mg.d^{-1}) to digestible lysine intake (DLysI, mg.d^{-1}) fed high (λ, PG = 140.65 + 0.5*7.54(DLysI – 17.49 - | DLysI – 17.49|), r^2 = 0.99, $s_{y.x}$ = 2.08, $P < 0.001$) or low (υ, PG = 111.78 + 0.5*5.84(DLysI – 14.80 - | DLysI – 14.80|), r^2 = 0.98, $s_{y.x}$ = 2.08, $P < 0.001$) dietary protein.

(b) Comparison of linear model applied to lysine intakes lower than the breakpoint of the rectilinear model: Protein gain (PG, mg.d^{-1}) to digestible lysine intake (DLysI, mg.d^{-1}) fed high (λ, PG = 7.50DLysI + 9.18, r^2 = 0.99, $s_{y.x}$ = 2.05, $P < 0.001$) or low (υ, PG = 5.85DLysI + 25.26, r^2 = 0.97, $s_{y.x}$ = 2.16, $P < 0.001$) dietary protein. Slopes of regression equations were significantly different ($P < 0.01$) between dietary crude protein levels. Open symbols indicate data excluded from linear functions.

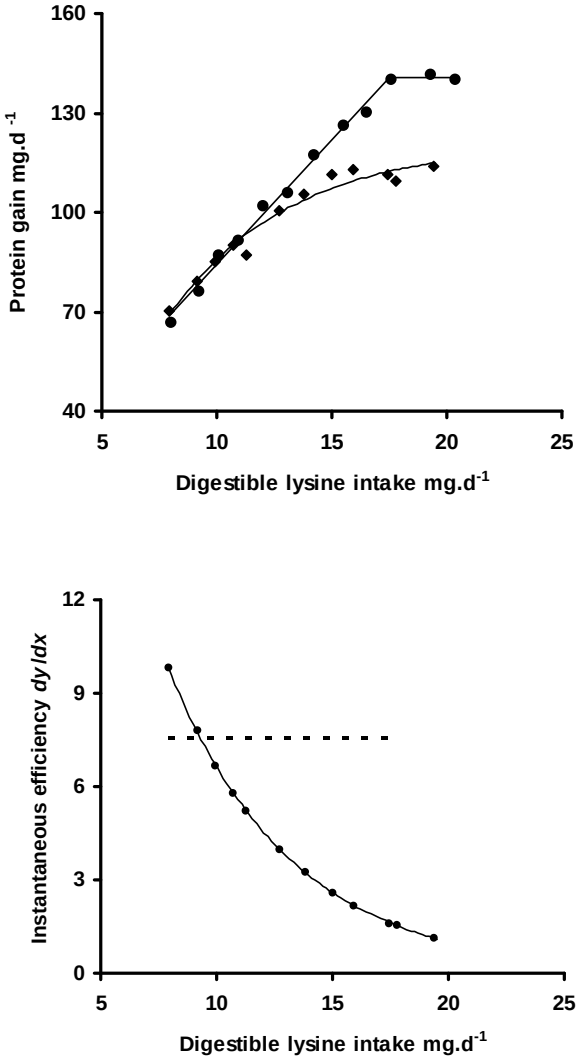

Figure 2 (a & b)

Lysine utilisation for protein gain by Atlantic salmon parr in relation to curvilinear models fit to low protein diet:

(a) Regression analysis using rectilinear model for high protein diet and exponential model for low protein diet: Protein gain (PG, $mg.d^{-1}$) to digestible lysine intake (DLysI, $mg.d^{-1}$) fed high (λ, PG = 140.65 + 0.5*7.54(DLysI – 17.49 - | DLysI – 17.49|), $r^2 = 0.99$, $s_{y.x} = 2.08$, $P < 0.001$) or low (υ, PG = -110.24 + 231.18$(1 - e^{0.188DLysI})$, $r^2 = 0.97$, $s_{y.x} = 3.08$, $P < 0.001$) dietary protein.

(b) Instantaneous efficiency of lysine utilisation to maximum protein gain from first-derivative plots of the rectilinear model for the high protein diet (- -) and exponential model for the low protein diet (—). Points along the first-derivative of the exponential model correspond to dietary lysine levels in the low protein diet.

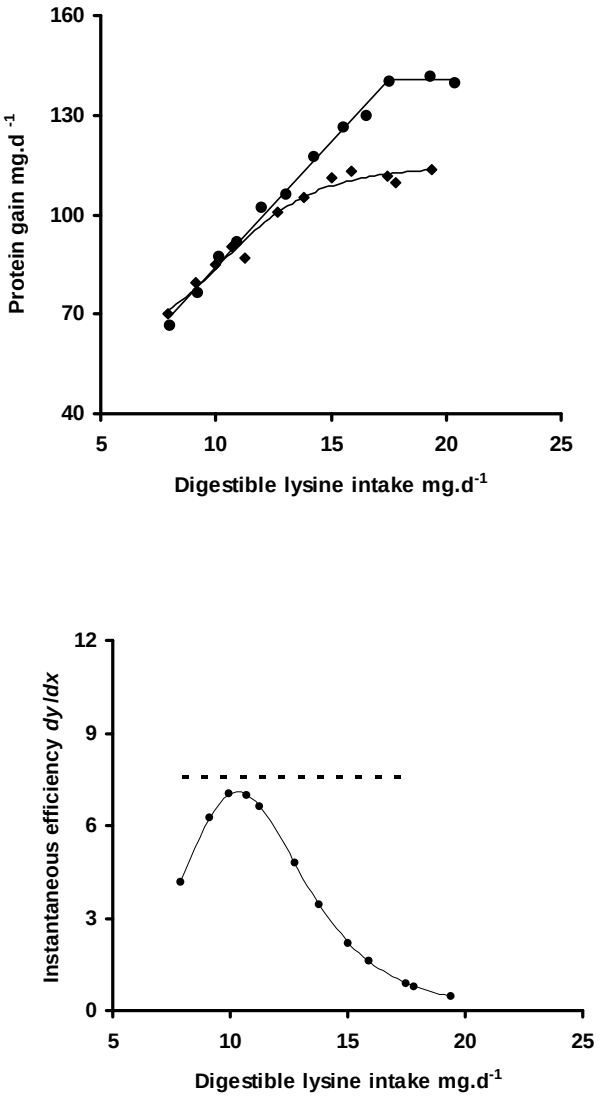

Figure 2 (c & d)

(c) Regression analysis using rectilinear model for high protein diet and 4SKM model for low protein diet: Protein gain (PG, $mg.d^{-1}$) to digestible lysine intake (DLysI, $mg.d^{-1}$) fed high (λ, PG = 140.65 + 0.5*7.54(DLysI – 17.49 - | DLysI – 17.49|), $r^2 = 0.99$, $s_{y.x} = 2.08$, $P < 0.001$) or low (υ, PG = $65.11(10.91)^{6.04} + 114.99(DLysI)^{6.04} / (10.91)^{6.04} + (DLysI)^{6.04}$, $r^2 = 0.98$, $s_{y.x} = 2.77$, $P < 0.001$) dietary protein.

(d) Instantaneous efficiency of lysine utilisation to maximum protein gain from first-derivative plots of the rectilinear model for the high protein diet (- -) and 4SKM model for the low protein diet (—). Points along the first-derivative of the 4SKM model correspond to dietary lysine levels in the low protein diet.

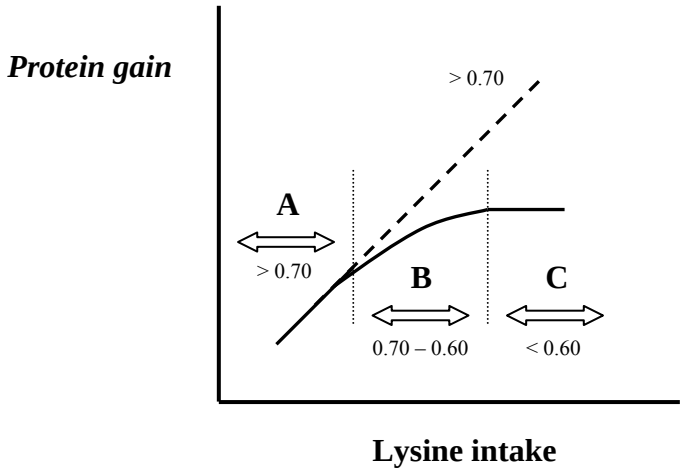

Figure 3
Schematic illustration describing the interaction between lysine and the second-limiting amino acid (methionine + cystine) and the efficiency of lysine utilisation (as previously illustrated by Susenbeth, 1995). It has been assumed that the lysine to methionine + cystine ratio of 0.70 is critical to the efficiency of lysine utilisation for protein gain in the present study.

Range A. No interaction: Ratio of lysine to the second-limiting amino acid was > 0.70 and efficiency of lysine utilisation was at maximum.
Range B. Critical interaction: Ratio of lysine to the second-limiting amino acid was critical (0.70 and 0.60) and efficiency of lysine utilisation was diminished, but still existent (diminishing returns).
Range C. Lysine excess: Ratio of lysine to the second-limiting amino acid was < 0.70, the second-limiting amino acid became first-limiting and lysine utilisation ceases.

Table 6
Amino acid profiles (relative to lysine) in experimental diets, whole-body of Atlantic salmon and recommendations (Rodehutscord *et al*., 1997) for different levels of protein gain and maintenance in rainbow trout

Amino acid	Diet 4 Low Protein	Atlantic salmon whole-body [1]	Rainbow trout % of maximum protein gain 95	65	maintenance
Arginine	114	84	42	52	83
Histidine	55	28	21	22	27
Isoleucine	87	48	50	57	78
Leucine	154	80	49	83	189
Methionine	53	33	29	33	46
Methionine + cystine	71	44			
Phenylalanine + tyrosine	143	79			
Threonine	92	52	37	50	92
Tryptophan	16	10	7	12	25
Valine	90	56	57	74	128

[1] whole-body profile of Atlantic salmon (Hauler & Carter, 2001a)

7.6
Effects of dietary protein source on growth, immune function, blood chemistry and disease resistance of Atlantic salmon parr*

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* Adapted from Bransden*, M.P., Carter, C.G. & Nowak, B.F. 2001. Effects of dietary protein source on growth, immune function, blood chemistry and disease resistance of Atlantic salmon (*Salmo salar* L.) parr. Animal Science 73: 105-114

Abstract

Many studies with fin fish have demonstrated the potential to use alternative dietary protein sources to fish meal based on growth responses, though these trials mostly neglect to determine if such protein sources affect immune function. This study investigated the effect of fish meal replacement with dehulled lupin mea or hydrolyzed poultry feather meal. Atlantic salmon (*Salmo salar*) parr were supplied isonitrogenous and isoenergetic feeds with 40% of the dietary protein provided by lupin or feather meal, or 40% of the dietary protein provided equally by lupin and feather meal. A feed mainly containing fish meal protein acted as a control. Growth, immune function, blood chemistry and disease resistance were assessed after 56 days. Significant differences ($P < 0.05$) in weight gain were detected between Atlantic salmon fed the control and feather meal feeds, whilst those salmon fed lupin or a mixture did not differ from any other. Productive protein values were significantly lower ($P < 0.01$) for poultry meal-fed salmon compared to those fed the control or mixture. Immune function (as assessed by lysozyme, antiprotease, neutrophil oxygen radical production and plasma total immunoglobulin) and blood chemistry (as assessed by plasma total protein and glucose) were not significantly ($P > 0.05$) affected by any feed. Mortality rates of Atlantic salmon challenged with *Vibrio anguillarum* were not influenced by diet. These data suggest Atlantic salmon could be supplied feeds with the fish meal component reduced to supply approximately 60% of the total protein, with the remaining 40% supplied by dehulled lupin meal or a combined dehulled lupin and hydrolyzed poultry feather meal without any adverse effects on growth, immune function or blood chemistry.

Keywords: Atlantic salmon; Feathermeal; Immune function; Lupin;

Introduction

Atlantic salmon, *Salmo salar* L. are the most economically valuable of the salmonid family worldwide, with global production exceeding 600 000 metric ton per annum (FAO, 1997). Salmonids are carnivorous fishes, and similarly to all other species of fish so far examined, have an absolute dietary requirement for ten amino acids (NRC, 1993). Fish meal, which provides all of these essential amino acids (EAA), and which has a similar EAA profile to the carcass amino acid composition of salmonids including Atlantic salmon (Pike *et al.*, 1990), is subsequently the protein of choice in salmonid feeds. However, an increasing (although highly variable) cost of fish meal due to increased demand from various feed-lot sectors has resulted in the ongoing search for alternative protein sources in aquafeeds. To date, the majority of research on fish meal replacement with alternative proteins in Atlantic salmon feeds has focused on the use of

proteins derived from plant sources (Anderson *et al.*, 1992; Carter *et al.*, 1994; Olli *et al.*, 1995; Carter & Hauler, 2000). The use of other protein sources, such as meat and bone meals and poultry by-products from terrestrial slaughterhouses, has not been fully investigated in Atlantic salmon. However studies with other salmonids, such as rainbow trout, *Oncorhynchus mykiss* (Pfeffer *et al.*, 1994; Steffens, 1994; Bureau *et al.*, 1999, 2000) and chinook salmon, *O. tshawytscha* (Fowler, 1990), have evaluated the potential of some of these terrestrial animal meals.

Whilst fish growth will usually be the main criteria in identifying alternative protein sources with potential for use in aquafeeds, the affect these proteins have on immune function and disease resistance should also be investigated, yet is seldom addressed. Preliminary research by Wedemeyer & Ross (1973) demonstrated rainbow trout fed an isonitrogenous and isoenergetic feed differing in its protein source (corn gluten or cottonseed meal) had no effect on its susceptibly to infection from corynebacterial kidney disease. More recently however, Rumsey *et al.* (1994, 1995) found that rainbow trout supplied isonitrogenous feeds, but differing in their protein source (fish meal or soybean meal) were subject to changes to their immune response. In this instance, feeding soybean increased serological and nonspecific defense mechanisms. Growth however, was depressed in trout fed the soybean. Krogdahl *et al.* (2000) also investigated the effect of immune function and disease resistance in Atlantic salmon supplied feeds containing various soybean products. Similarly to Rumsey *et al.* (1994, 1995) immune parameters including lysozyme activity and total immunoglobulin increased with the inclusion of soybean, whilst disease resistance varied depending on the soybean product incorporated. Finally, Neji *et al.* (1993) demonstrated that rainbow trout supplied feeds with all of the protein provided by plant sources (corn gluten and soybean meals) were more susceptible to *Aeromonas salmonicida* infection compared to trout fed animal protein sources (poultry by-products and blood meal). With these trials in mind, and with the understanding that aquafeed manufacturers would be hesitant to incorporate alternative protein sources that may negatively impact upon immune function (although still providing adequate growth), the present trial was undertaken. The aim was to identify partial substitutes to fish meal that promoted both growth and immune function in Atlantic salmon relative to fish meal-fed controls.

Proteins sources utilized in the present trial, dehulled lupin meal (*Lupinus angustifolius*) and hydrolyzed poultry feather meal, were selected on the basis that they were domestically available, a key criteria in identifying useful alternative proteins for the Australian salmonid industry. Furthermore, both lupin (De la Higuera *et al.*, 1988; Morales *et al.*, 1994; Burel *et al.*, 1998; Carter and Hauler, 2000) and feather meal (Fowler, 1990; Pfeffer *et al.*, 1994; Steffens, 1994; Bureau *et al.*, 2000) have shown varying degrees of success (in relation to growth) when fed as partial fish meal substitutes in salmonid feeds in the past. Dehulled lupin or poultry feather meal were used to partially substitute (40%) the fish meal protein, whilst a combination of dehulled lupin and hydrolyzed poultry feather meal, with each supplying 20% of the protein, was also included. To ensure differences that may be observed could only be attributed to protein source and not protein or energy content of the whole feed, diets were formulated to be isonitrogenous and isoenergetic on a digestible basis. Growth was assessed after 56 days. Further, lysozyme activity, antiprotease activity, neutrophil oxygen radical production, and plasma concentrations of total immunoglobulin (Ig), total protein and glucose were measured to evaluate immune function and blood chemistry. Finally, a challenge against *Vibrio anguillarum* was used as this bacterium has the potential to cause significant mortality in Atlantic salmon populations (Roberts

& Shepherd, 1997), and disease resistance trials provide the best indication of fish health in relation to diet (Waagbø, 1994).

Material and methods

Feeds and growth trial

Feeds were prepared to provide 380 g digestible crude protein/kg and 15.7 MJ digestible energy/kg (Table 1) using ingredient *in vivo* digestibility values from a previous trial with Atlantic salmon (C.G. Carter *et al*. unpublished results). A control (CON) feed was formulated with fish meal supplying over 95% of the dietary protein. Dehulled lupin (*Lupinus angustifolius*, LPN) and hydrolyzed poultry feather meal (FTH) were selected as alternative protein sources, and incorporated into feeds to replace 40% of the fish meal protein. A fourth feed was formulated whereby 40% of the fish meal protein was replaced with 20% dehulled lupin meal, and 20% hydrolyzed poultry feather meal (MIX). Feeds were pelleted through a cold pellet press (CL-2 laboratory pellet mill, California Pellet Mill Co., San Francisco, U.S.A.).

Growth trials were conducted at the School of Aquaculture, University of Tasmania (Australia). The fresh water semi-recirculation system consisted of twelve 300 l circular tanks. A constant supply of fresh water meant an exchange rate of approximately 15% per day with recirculation water treated through physical and bio-filters. Water temperature was maintained at 15 ± 1°C, whilst photoperiod was natural (approximately 10 h light:14 h dark). Dissolved oxygen, ammonia, nitrate, nitrite and pH were monitored and remained within limits recommended for Atlantic salmon (Wedemeyer, 1996).

Atlantic salmon parr (mean body weight 35.9 g) were randomly allocated between each of the twelve tanks until a total of twenty-five fish were in each tank. A further five fish were euthanased with a lethal concentration of benzocaine (ethyl-p-aminobenzoate, Sigma Aldrich, Castle Hill, NSW, Australia, 100 mg/l) and frozen (-20°C) for initial carcass chemical composition. Triplicate tanks of fish were fed by hand one of the four feeds once per day (9.00 hours). Based on earlier comparable studies, a daily ration of 1.25% of tank biomass was used to ensure all feed would be consumed, and to avoid differences in feed intake often associated with satiation feeding of plant substituted feeds (De la Higuera *et al.*, 1988; Refstie *et al.*, 1998). Fish were weighed after being anaesthetized (benzocaine, 30 mg/l) at 14 d intervals following a starvation period of 36 h. The growth trial was conducted for a total of 56 d, at which time all fish were removed and weighed.

Sample collection and analysis

At the end of the growth trial, after final weighing, five fish per tank were euthanased (benzocaine, 100 mg/l) and bled via the caudal vein using a heparinised syringe and needle for measurement of immune function and blood chemistry. Whole blood was used for the measurement of neutrophil activity by the reduction of nitroblue tetrazolium (NBT) to formazan, with values presented as the optical density at 540 nm after reduction (Anderson & Siwicki, 1996) Remaining blood was centrifuged (1000 × *g*, 5 min) to reveal plasma. Plasma was used for measurement of lysozyme activity by determining the rate of lysis of a *Micrococcus lysodeikticus* suspension and using hen egg white lysozyme (Sigma Chemical Co., Castle Hill, N.S.W., Australia) as standard as described by Thompson *et al.* (1994). The volume of plasma required to inhibit 50%

of a standard trypsin activity was used to quantify antiprotease activity (Ellis, 1990). The difference in plasma protein concentration (measured according to Lowry *et al.* (1951)) before and after precipitation with polyethylene glycol was considered to be the plasma immunoglobulin fraction (Siwicki *et al.*, 1994). Finally, plasma glucose was measured using a commercial diagnostic kit (Sigma Chemical Co., Castle Hill, N.S.W., Australia). A further three fish per tank were euthanased, frozen (-20°C) and kept for final carcass chemical composition. Feeds and carcasses were analyzed for dry matter, crude protein (AOAC, 1990) using a conversion factor of N × 5.85 (Gnaiger & Bitterlich, 1984), crude fat (Bligh & Dyer, 1959) and ash (AOAC, 1995). Gross energy of feeds was measured by bomb calorimetry.

Disease resistance trial

Eleven Atlantic salmon from each of the growth trial tanks were stocked into one of twelve 120 l rectangular fiberglass tanks supplied with flow-through fresh water. The tanks were housed in an insulated room with controlled photoperiod (10 h light:14 h dark) but under ambient temperature (range 10-14°C). Salmon were allowed to acclimate for 3 days before the challenge commenced. The challenge bacterium, *Vibrio anguillarum* (serovar O1) was passaged through several Atlantic salmon prior to the disease resistance experiment to increase pathogen virulence. Twenty-four hours before *V. anguillarum* was administered, a fresh culture of the passaged bacterium was prepared (Oxoid Nutrient Broth No. 2, Oxoid Ltd, Basingstoke, England, plus 10% NaCl). On the day of the challenge, the culture was washed in sterile phosphate buffered saline (PBS, pH 7.2) with intermediate centrifugation (3000 × *g*, 10 min). Cells were then counted in a haemocytometer under phase contrast microscopy and adjusted (with PBS) to 3 x 10^5 cells/ml. Cell densities were confirmed by serially diluting the culture and distributing suspensions onto dry horse blood agar plates (Tasmanian Laboratory Services, Launceston, Tas., Australia) using a calibrated pipette. Colonies were then counted after 48 h. One-hundred µl of the 3 × 10^5 cells/ml suspension was injected into the intraperitoneal cavity of each Atlantic salmon after they had been anaesthetized (benzocaine, 30 mg/l). Tanks were assessed for mortalities at 17.00 hours on each day post-injection, with death by the challenge organism confirmed by culturing kidney swabs (after sterile dissection) on blood agar and checking colonial morphology. The challenge was terminated after 14 days at which time mortalities had ceased.

Statistical analysis

Significant differences were determined by performing one way analysis of variance (ANOVA) followed by a Tukey-Kramer multiple comparison of means. Statistical significance was accepted at a probability value of 5% or less. Data from the growth trial was analyzed using the mean value from a tank as the experimental unit, whilst individual fish were used as the experimental unit (as is the accepted convention in fish immunology and related studies (Jarp and Tverdal, 1997)) for analysis of nonspecific and specific immune function and blood chemistry. Mortality rates during challenges were compared using the log-rank test (Peto *et al.*, 1977). Statistical analyses were performed using J.M.P. version 3.2.1 (SAS Institute Inc.). Data are presented as the means of each group and standard error together with the significance levels of the main effects.

Results

Growth and carcass chemical composition

After 56 days, groups of Atlantic salmon fed the CON feed had significantly ($\underline{P} < 0.05$) elevated weight gain compared to those fed the FTH feed, whilst weight gain of LPN- and MIX-fed salmon were intermediate and not different from any other feed (Table 2). Productive protein value (PPV) differed between feeds, with those salmon fed the CON and MIX feeds having significantly ($\underline{P} < 0.01$) greater PPV's than those fed the FTH feed, whilst salmon on the LPN feed were intermediate. During the growth trial, survival was not influenced by diet with all groups equal or very close to 100%.

Dietary treatment had a significant effect on carcass crude protein and ash, though there were no differences in dry matter or total lipid content (Table 3). Carcass crude protein was significantly ($\underline{P} < 0.01$) reduced in salmon fed FTH compared to all other feeds. Ash content was highest in salmon fed LPN and CON which was significantly greater ($\underline{P} < 0.05$) than their FTH-fed counterparts, whilst MIX-fed salmon did not differ from any other.

Immune function, blood chemistry and disease resistance

No significant differences ($\underline{P} > 0.05$) in lysozyme activity, antiprotease activity, neutrophil oxygen radical production, or plasma concentrations of total Ig, total protein or glucose were recorded between dietary treatments (Table 4).

Mortalities from *V. anguillarum*-challenged Atlantic salmon first occurred on day 6 post-challenge in all groups excluding those fed the CON feed (Figure 1). Mortalities ceased firstly in the LPN-fed salmon after day 9, whilst the final mortalities occurred for the CON- and MIX-fed salmon on day 11. At this time, cumulative mortality rates of LPN- and FTH-fed salmon were approximately 16%, whilst MIX- and CON-fed salmon had mortality rates of approximately 22%. Analysis by the log-rank test revealed no significant difference in mortality rates.

Discussion

Growth and efficiencies

Despite growth rates of Atlantic salmon fed the CON feed out-performing the remaining fish meal-substituted feeds, a lack of any statistical significance (at 5% probability) demonstrated dehulled lupin or a combination of dehulled lupin and poultry feather meal could be incorporated into Atlantic salmon feeds, whilst poultry feather meal alone appears inadequate as a replacement to fish meal at 40% of the protein. PPV's were found not to differ for the LPN and MIX feeds relative to the CON feed, and suggested lupin or the combined lupin and poultry feather meal satisfactorily promoted protein growth. PPV of approximately 40% for the control feed is in close agreement with other closely related studies on salmonids where fish meal is the principal dietary protein (Rumsey *et al.*, 1993; Perera *et al.*, 1995; Carter & Hauler, 2000), whilst protein productivity in the LPN-fed salmon was approximately 10% higher (De la Higuera *et al.*, 1988; Carter and Hauler, 2000) or lower (Carter & Hauler, 1998) when lupin meal was supplied to other salmonids at 30-40% of the dietary protein. Given that all feeds were balanced for digestible crude protein and energy, the significant reduction in PPV in the salmon fed the FTH feed suggested the protein

quality in the poultry feather meal was insufficient relative to the control. A similar reduction in protein productivity has been recorded in rainbow trout when 50% or more of the fish meal protein was replaced with combined poultry by-product and poultry feather meal (Steffens, 1994), with this difference attributed to an inadequate amino acid profile of these ingredients. Bureau *et al.* (2000) also suggested that growth rates of rainbow trout were inversely proportional to the dietary level of feather meal when it replaced fish meal, by virtue of feather meal's limited lysine content. Finally, when Pfeffer *et al.* (1994) supplemented feeds for rainbow trout containing hydrolyzed feather meal with lysine both energy and protein retention were considerably improved. In the present trial however, the small but significant difference in weight gain between salmon fed the FTH and CON feeds was not attributable to dietary amino acids. All feeds were found to have very similar digestible amino acid profiles (C.G. Carter *et al*., unpublished results), which exceeded the requirements calculated for salmonids (NRC, 1993). A reduction in the carcass ash content of FTH-fed salmon suggested poor bone mineralization. In salmonids this has been attributed to dietary deficiencies of phosphorus, also a causative agent of growth retardation (Lall, 1991). However, literature values show that typical phosphorus concentrations and their digestibility coefficients in salmonids are higher in poultry feather meal (Sugiura *et al.*, 1998) compared with lupin meal (Petterson *et al.*, 1997; Burel *et al.*, 1998). Subsequently, the exact reason for the slight reduction in growth of FTH-fed salmon relative to the control remains unknown, and warrants further investigation. Interestingly, reducing the poultry feather meal to 20% of the protein, and including lupin meal overcame the mechanisms responsible for this lower weight gain.

Lupin meal was shown to be a good protein source for partial replacement of fish meal. Under similar experimental conditions, previous research has shown that growth performance in Atlantic salmon was not affected when 33 (Carter & Hauler, 2000) or 40% (Carter & Hauler, 1998) of the fish meal protein was replaced with lupin meal. Similarly, (De la Higuera *et al.*, 1988) demonstrated that rainbow trout could be supplied a feed containing up to 30% of lupin meal without causing reduced growth compared to a fish meal based control. The potential to include lupin meal beyond 40% of the protein in Atlantic salmon feeds however is probably limited by the high levels of carbohydrate in this ingredient, which would ultimately result in a decreased digestible energy content and therefore growth (Morales *et al.*, 1994).

Immune function, blood chemistry and disease resistance

Immune parameters measured in the present trial are important components of a fish's defense system. Antiproteases are critical in inactivating extra-cellular proteases from invading pathogens, whilst lysozyme and neutrophil oxygen radical production are active parasiticidal agents (Secombes, 1994). The production of immunoglobulin too, has a critical role in forming complexes with pathogens to bring about opsonisation or cellular lysis (Alexander & Ingram, 1992). Whilst these parameters have been previously shown to be modulated by dietary change in various teleosts (e.g. Blazer, 1992; Waagbø *et al.*, 1993; Rumsey *et al.*, 1994; Thompson *et al.*, 1996), in the present trial no modification relative to the control feed occurred. Thompson *et al.* (1996) however, proposed that measuring defense mechanisms prior to challenge only represents resting levels. Measuring immune function shortly after challenge may highlight dietary modifications that were not evident until stimulation (via challenge) of the immune system had occurred. This is further highlighted by mammalian research which has demonstrated that nutrients are preferentially directed towards the immune system, rather than growth, during times of infection (Beisel, 1977). This includes the

distribution of amino acids towards the liver for synthesis of acute phase proteins such as antiprotease (Wannemacher, 1977), and suggests sub-optimal nutrient intake prior to infection may result in a diminished immune response. It seems a logical step therefore to evaluate immune function after the dietary protein source (and subsequently the amino acid profile) has been altered. Whilst studies on the use of soybean meal (because of its antigenic properties) in feeds for salmonids and its subsequent effects on immune function have been widely undertaken (e.g. Neji *et al.*, 1993; Rumsey *et al.*, 1994, 1995; Krogdahl *et al.*, 2000), few have investigated other proteins. Notably, Steffens (1994) examined some basic hematological parameters in rainbow trout fed practical feeds with 50 or 100% (plus additional lysine, or lysine and methionine) of the fish meal replaced by a combined poultry by-product and poultry feather meals. Similarly to the present trial, none of the measured parameters (haematocrit, hemoglobin and erythrocyte numbers) were adversely affected. To our knowledge, the affect lupin meal may have on immune function has not previously been examined.

Challenge with *V. anguillarum* in the present trial failed to result in mortality rates greater than 22%. Unfortunately these low rates of mortality suggest the concentration of bacteria administered to the salmon was probably not sufficient enough to clearly demonstrate inadequacies (if any) in the feeds. Nevertheless, data presented suggest the alternative protein sources tested were not detrimental to disease resistance.

In conclusion, the supply of marine products such as fish meal is not a finite resource, and the aquaculture industry must be vigilant in identifying alternative protein sources to fish meal. Whilst growth and efficiencies of aquatic species fed novel protein sources will most probably determine their use in aquafeeds, the affect it may have on immune function must not be overlooked. The present study is one of very few to investigate both growth and immune parameters, and we have successfully shown that dehulled lupin in particular is an excellent source of protein for Atlantic salmon, stimulating growth and immune function as well as fish meal fed controls.

Acknowledgements

The authors wish to thank Dr J. Carson from DPIWE, Tasmania, for the *V. anguillarum* strain and technical expertise, and the technical assistance of B. Verbeeten, R. Morrison and M. Foale. The provision of ingredients from Pivot Aquaculture, Peerless Holdings and Milne Feeds is gratefully appreciated. This work was funded by the Cooperative Research Centre for Aquaculture and the Fisheries Research and Development Corporation.

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Table 1
Ingredient and chemical composition of the experimental feeds

	Feed			
	CON	LPN	FTH	MIX
Ingredient composition (g/kg)				
Fish meal	591.0	331.5	331.5	331.0
Poultry feather meal	0.0	0.0	229.0	114.6
Lupin (dehulled)	0.0	443.0	0.0	222.0
Wheat flour	100.0	100.0	100.0	100.0
Wheat gluten	40.0	40.0	40.0	40.0
Vitamin & mineral premix†	3.0	3.0	3.0	3.0
Stay-C 35%	0.2	0.2	0.2	0.2
Fish oil	87.0	82.2	113.7	98.0
Dextrose	50.0	0.0	74.0	0.0
Diatomaceous earth	58.8	0.0	38.6	21.2
Bentonite	70.0	0.1	70.0	70.0
Chemical composition (g/kg DM)				
Dry matter (g/kg)	950.9	957.3	960.7	962.6
Crude protein	442.8	428.8	438.2	436.4
Crude fat	171.8	169.9	184.7	183.5
Ash	205.2	59.4	152.5	137.6
Gross energy (MJ/kg DM)	18.6	20.8	20.0	19.9
Digestible CP	389.0	377.1	377.1	377.1
Digestible energy (MJ/kg DM)	15.7	15.7	15.7	15.7
Digestible CP:Digestible energy (g CP/MJ)	24.8	24.1	24.1	24.1

† Supplied to exceed dietary requirements for salmonids (NRC, 1993).

DM, dry matter; CP, Crude protein.

Table 2
Effect of experimental feeds on growth, feed efficiency, productive protein value and survival of Atlantic salmon parr (means of three replicates)

		FEED				Significance
		CON	LPN	FTH	mix	
Final wet weight (g)	Mean	71.0[a]	67.4[ab]	67.1[b]	67.9[ab]	*
	SEM	1.2	0.3	1.0	0.2	
Weight gain (g)	Mean	34.9[a]	31.7[ab]	30.8[b]	32.2[ab]	*
	SEM	1.1	0.2	0.7	0.5	
FER (g/g DM) †	Mean	1.13	1.07	1.07	1.10	ns
	SEM	0.02	0.00	0.01	0.02	
PPV (%) ‡	Mean	39.5[a]	36.9[ab]	34.4[b]	37.8[a]	**
	SEM	1.0	0.5	0.3	0.7	
Survival (%) §	Mean	100.0	100.0	98.6	100.0	ns
	SEM	0.0	0.0	1.3	0.0	

[a,b] mean values across each row not sharing a common superscript were significantly different.

† FER, feed efficiency ratio = total weight gain (g) / total feed consumption (g DM).

‡ PPV, productive protein value = 100.[fish protein gain (g CP) / total protein consumption (g CP)].

§ Percentage survival from growth trial.

DM, dry matter.

Table 3
Effect of experimental feeds on carcass chemical composition of Atlantic salmon parr (means of three replicates) †

		FEED				Significance
		CON	LPN	FTH	MIX	
Dry material (g/kg)	Mean	292.7	293.6	296.2	293.5	ns
	SEM	3.0	1.3	0.7	0.3	
Crude protein (g/kg WW)	Mean	158.6[a]	156.0[a]	152.5[b]	156.4[a]	**
	SEM	0.4	0.9	0.8	0.1	
Total lipid (g/kg WW)	Mean	104.2	105.7	113.3	105.5	ns
	SEM	3.1	1.8	3.1	0.6	
Ash (g/kg WW)	Mean	22.1[a]	22.3[a]	20.9[b]	21.7[ab]	*
	SEM	0.1	0.4	0.1	0.3	

[a,b] mean values across each row not sharing a common superscript were significantly different.

† Carcass chemical composition (g/kg WW) of initial group (mean ± s.e., n=5): Dry matter, 283.3 ± 1.7 g/kg; crude protein, 162.4 ± 0.5; total lipid, 93.2 ± 2.1; ash, 21.6 ± 0.2.

WW, wet weight

Table 4
Effect of experimental feeds on immune function and blood chemistry of Atlantic salmon parr (means of fifteen replicates). No significant differences (P > 0.05) were recorded.

		FEED			
		CON	LPN	FTH	MIX
Lysozyme (μg/ml)	Mean	4.61	3.27	3.86	4.88
	SEM	0.48	0.34	0.43	0.68
Antiprotease (μl plasma for 50% inhibition)	Mean	0.50	0.43	0.52	0.53
	SEM	0.04	0.03	0.04	0.05
Neutrophil (OD)	Mean	0.197	0.196	0.203	0.208
	SEM	0.009	0.012	0.015	0.015
Total immunoglobulin (mg/ml)	Mean	20.5	14.6	19.2	17.1
	SEM	5.0	2.2	3.9	4.3
Total protein (mg/ml)	Mean	40.3	38.5	39.4	40.5
	SEM	0.6	0.5	0.5	1.0
Glucose (mmol/L)	Mean	3.10	3.38	3.52	3.40
	SEM	0.10	0.18	0.22	0.18

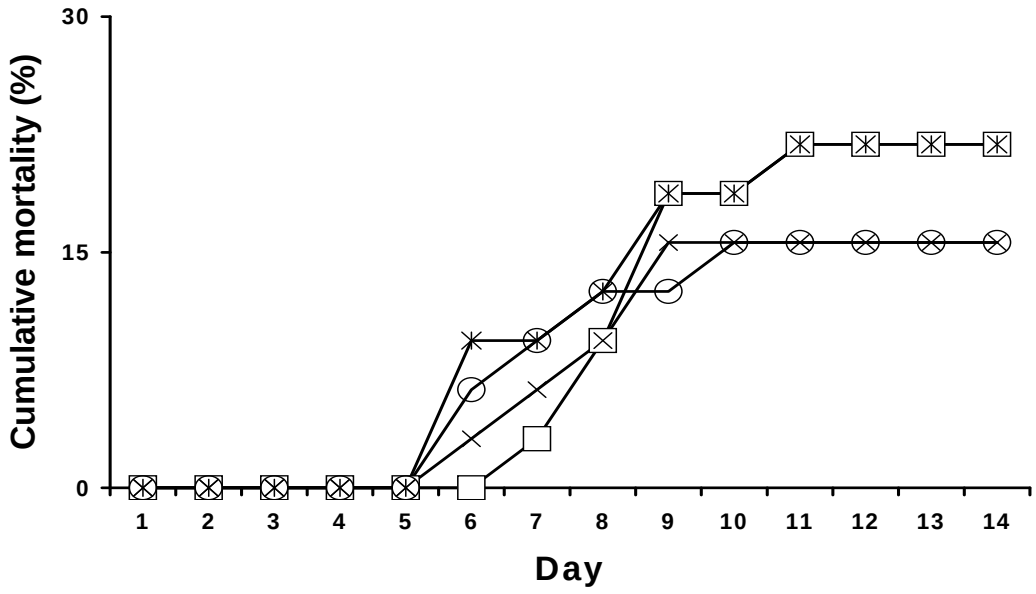

Figure 1
Cumulative mortality (%) observed in Atlantic salmon fed the CON (□), LPN (×), FTH (○) or MIX (P) feeds for 56 days and then challenged with *Vibrio anguillarum* (Serovar O1). No significant differences (P > 0.05) were recorded by log rank analysis.

7.7
Effect of feeding time and dietary protein level on feed intake, nutrient utilization and growth of Atlantic salmon fed on dehulled lupin

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Abstract

This experiment aimed to determine the effects of feeding time on growth performance using a mixed feeding regime in which fish were fed diets with higher (45%) and lower (40%) crude protein at different times on the same day. A further aim was to investigate the replacement of fish meal with lupin (both diets in the mixed feeding regime contained 30% lupin). Regardless of protein source, the 40% crude protein diets had significantly lower growth compared to 45% crude protein diets. The main finding in this experiment was the similar growth performance of fish that were fed on a mixed feeding regime (both 45% and 40% crude protein diets in one day) as compared with those groups of fish that received 45% crude protein diets (with or without lupin) at both feeds. Growth efficiency, measured by FCR, EER and LER did not show significant differences between the feeding regimes either. However, PER was significantly higher for the mixed feeding regime and showed that there was potential for lowering the daily protein intake.

Keywords: Atlantic salmon; Dehulled lupin; Feeding time; Fish meal replacement;

Introduction

Much research has been conducted to establish the best time for feeding various species of fish by measuring feed intake and more recently by considering differences in nutrient utilisation in relation to feeding at different times (Bolliet *et al*., 2001; Madrid *et al*., 2001). Under most conditions when food is freely available to Atlantic salmon they feed diurnally, exhibit peaks in feed intake around dawn and dusk and often have higher intake earlier in the day (Paspatis & Boujard, 1996; Madrid *et al*., 2001). Within day variation in appetite and feed intake are suggested to indicate changes in metabolism, nutrient utilisation and nutrient retention (Bolliet *et al*., 2001). For example, feed intake, growth and growth efficiency were higher for rainbow trout fed one meal in the morning compared to one meal fed in the afternoon (Boujard *et al*., 1995). Efficiency of nutrient utilization in various fish varies at different times of the day (Noeske-Hallin *et al*., 1985; Boujard & Leatherland, 1992; Boujard *et al*., 1995; Spieler, 2001). Protein synthesis and the efficiency of protein turnover were shown to differ between trout fed in the morning or evening (Bolliet *et al*., 2000).

In the present experiment it was proposed that it might be possible to improve overall nutrient utilization by feeding diets with differing nutrient composition at different times of the day in order to exploit differences in the timing of nutrient utilisation. Mixed feeding schedules have been used successfully with carp species and these concentrated on changing fish and plant meal based feeds on different days rather than on using more than one diet on the same day (De Silva, 1985; Nandeesha *et al*., 1995). The aims of this study were to assess the growth perfomance of Atlantic salmon fed feeds with higher and lower protein contents, feeds containing dehulled lupin or

only fish meal, and fed two different feeds each day. Four feeds were formulated to contain either 40 or 45% crude protein supplied by two different combinations of protein sources (fish meal and corn gluten with or without 30% dehulled lupin). A mixed feeding regime of feeding 40 and 45% crude protein diets (both with 30% dehulled lupin) on the same day but either in the morning or afternoon, was also evaluated. The feed preference between 45% or 40% crude protein was investigated by feeding the two diets simultaneously and analysing the ratio of two markers in the uneaten feed (Refstie *et al*., 1997). Finally, the morphological response of the digestive tract (both proximal and distal intestine) to 30% inclusion level of dehulled lupin was compared to fish meal.

Material and methods

Experimental animals and culture conditions

Juvenile Atlantic salmon were supplied by a local hatchery (Springfield, Tasmania). Fish were kept in 2000-l tanks and fed commercial salmon feed until required. Experiments were conducted in an indoor air-conditioned recirculation system (Carter & Hauler, 2000). Fish were weighed and randomly stocked into 300-l fiberglass tanks in duplicate groups of 20 fish (twelve tanks). Tanks were covered with black plastic mesh to minimize disturbance and prevent escape, and supplied with continuously aerated fresh water (9 l/min) at a temperature of 14°C ± 0.5. Temperature, dissolved oxygen, total ammonia, nitrite and pH were measured three times weekly, and did not exceed values recommended for Atlantic salmon (Tarazona & Munoz, 1995). A constant photoperiod of 12L/12 D was used. Prior to the experiment, fish were fed with the commercial feed for a ten day adaptation period, fasted for two days and re-weighed (38.99 ± 0.13 g) prior to starting the experiment. Experimental diets were fed to satiation twice a day one hour after lights-on and one hour before lights-off. The sequence of feeding the different groups (tanks) was changed each day to reduce any possible effect of feeding time (within feeding period) on nutrient utilization for each tank. Feed was distributed by hand to observe feeding behaviour and to record daily feed intake for each tank.

Experimental diets

Four experimental diets including two fish meal based diets containing 40% CP (FM40) and 45% CP (FM45) and two dehulled lupin based diets also containing 40% CP (DL40) and 45% CP (DL45) were formulated (Table 1) to exceed essential amino acid requirements (Table 2). Dehulled lupin was added at 30% in both DL40 and DL45. Commercial dehulled lupin (*Lupinus angustifolius*) (Milne Feeds Company, Western Australia) was finely ground (about 1mm particle size) to prepare a homogenized flour. Ytterbium oxide (Yb_2O_3) was used as a marker in the FM based diets. Ytterbium and yttrium oxides (Y_2O_3) were used as markers in DL40 and DL45, respectively. Both markers were included at the level of 100 mg/kg in all diets. All ingredients were mixed, and after adding 10% water, cold pelleted (pellet diameter of 3 mm). The moisture content of the diets was reduced using a drier (30ºC for 24 h). The diets were stored in thick plastic containers in dark conditions at -18°C until required.

Feeding regime

The feeding regimes are shown in Table 3. Four duplicated treatment groups of fish were fed in the morning and in the afternoon with FM40, FM45, DL40 or DL45. The fifth treatment was fed with DL40 in the morning and DL45 in the afternoon (DL4045) and the sixth treatment (DL4540) was the reverse: DL45 was fed in the morning and DL40 was fed in the afternoon.

Experimental procedure and sampling

Before starting the experiment, random samples of the diets and fish were collected for whole-body chemical analysis. The experiment lasted for 50 days. Fish were killed at the end of the experiment for whole-body chemical analysis. A further three fish per tank were killed and samples of their proximal and distal intestine taken, flushed with cold (4°C) phosphate buffered saline (pH 7.2) to remove the faeces and then fixed in 10% buffered formalin at room temperature for histological assay. Fixed samples of both proximal and distal intestine were dehydrated in an ethanol solution series of 50% to 95%. Tissues were embedded in paraffin and after sectioning (5 μm), stained with haematoxylin and eosin for light microscopic examination.

Apparent digestibility coefficients

A few days before finishing the experiment, faecal samples were collected by settlement (Carter & Hauler, 2000) for measuring crude protein (nitrogen) digestibility in diets DL40, FM40, DL45 and FM45. The apparent crude protein digestibility for experimental diets was also calculated using the standard formula (Maynard & Loosly, 1962):

$$ADC\ (\%) = 100*[1-(\%M_{diet}/\ \%M_{faeces})*(\%N_{faeces}/\%N_{diet})$$

where M is the inert marker and N is the nutrient. Digestibility was not calculated for DL4045 and DL4540 diets.

Feed preference

A feed preference study was carried out on treatments 5 and 6 after finishing the growth experiment, using the method described by (Refstie *et al*., 1997). To do this, fish were starved for three days to ensure that there was no marker left in the digestive tract from their previous feedings regime. Equal mixtures of DL45 (with Y_2O_3) and DL40 (with Yb_2O_3) were fed to both groups 5 and 6 for three days at normal feeding times. After each feeding, tanks were flushed to remove uneaten pellets and faeces. Prior to the next feeding, faecal samples were collected and pooled to quantify the preferred diet through the quantification of a related marker.

Analytical methods

All feed samples were milled before analysis, which was carried out in duplicate. Dry matter was determined using a freeze drier. Total nitrogen was measured by Kjeldahl (CP as N×6.25). Ytterbium and yttrium oxides were quantified using an ICP spectrometer after digestion of faecal samples in HCL: HNO_3 2:1 (v/v), as described by (Refstie *et al*., 1997).

Statistical analysis

Mean values are reported ± (S.E.M.) of each treatment. All statistical tests were performed using the SPSS Statistical Analysis Software Program (version 10 for Windows, 2001). Kolmogorov-Smirnov and Levene's tests were applied to test normality and homogeneity of variance, respectively. One-way analysis of variance was conducted to assess the effect of dietary treatments on different responses and, when appropriate, the differences between means were tested using Tukey's multiple range test. Paired t-tests were conducted to compare feed intake between morning and afternoon feeding times and between feeds in the feed preference study. Probability values of less than 0.05 were considered as significant.

Results

Final weight and weight gain was significantly lower for the 40% CP diets (FM40 and DL40) but similar for the other treatments and this was mainly explained by significantly lower feed intake of FM40 and DL40 (Table 4). Thus, there were few significant differences in FCR although they tended to be slightly higher for the 40% CP diets. Overall the 40% treatments resulted in the poorer growth and nutrient retention, condition factor, FCR, EER and LER all suggested slower growing less fat fish compared to the 45% CP treatments. In contrast, the mixed feeding regimes resulted in similar growth but produced less fatty fish. Digestible protein intake showed a significant relationship to specific growth rate (Fig. 1) that confirmed nutrient intake was the main factor explaining growth differences between the treatments (rather than differences in digestibility or nutrient utilisation efficiency).

Although feed intake was generally higher in the afternoon, there were no significant differences between feed intake in the morning and in the afternoon (with the exception of FM40 group) when this parameter was compared for each experimental diet. Feed intake however, was significantly higher in the afternoon when the values for all experimental diets were pooled (Table 5).The feed preference study was conducted for DL4540 and DL4045 and showed there was no preference for either diet (Table 6).

Apparent crude protein digestibility was generally higher in those groups of fish that received dehulled lupin in their diet (DL40 vs FM40) and (DL45 vs FM45). The differences, however were not significant. Regardless of the protein source in the diets, apparent crude protein digestibility in 45% CP diets was higher than 40% CP diets, however this differences were not significant. Apparent crude protein digestibility of DL45 diet was significantly higher than the same value for FM40 diet (Fig. 2). There was no evidence of abnormalities in the proximal and distal intestine.

Discussion

The main finding in this experiment was the similar growth performance of fish that were fed DL4540 and DL4045 regimes as compared with those groups of fish that received 45% crude protein diets (FM45 and DL45) at both feeds. Growth efficiency, measured by FCR, EER and LER did not show significant differences between the feeding regimes either. Interestingly, PER was significantly higher for the mixed feeding regime and showed that there was potential for controlling dietary protein to lower daily protein intake without affecting growth. This may have related to differences in nutrient utilisation at different times (see below) but probably suggests an optimum dietary crude protein between the 40 and 45% levels used in the present experiment. This conclusion is supported by there being no statistical difference between the two mixed feeding regimes. However, there was a suggestion in all growth performance parameters that feeding higher protein in the afternoon was more effective than in the morning. This trend should be explored further. Nitrogen excretion was not measured in this experiment but the results suggest lower nitrogen excretion on the mixed feeding regime. This might be a useful mechanism for managing overnight water quality. Apparent crude protein digestibility was not measured directly but there were no significant differences between 40 and 45% crude protein diets. Consequently, it was assumed differences in PER were not related to protein digestibility.

The effect on growth performance of reducing the crude protein content of an Atlantic salmon diet from 45% to 40%, while fish were fed to satiation with diets that

fulfilled the amino acid requirements (on a % diet basis), was of further interest. Regardless of the protein source, those groups of fish that received 40% crude protein diets had significantly lower growth compared to 45% crude protein diets. Despite lower growth PER was significantly higher than for 45% crude protein diets. In other words, the lower dietary protein content resulted in more efficient use for wet weight growth. This is in agreement with findings on carp that showed higher PER for lower protein diets (Nandeesha *et al*., 1995). Use of the lower protein diets would decrease growth, but there are situations where an increase in protein retention efficiency would be more useful can be envisaged and might include the need to manage water quality. This would be achieved by reducing nitrogen excretion due to both the reduction in absolute nitrogen intake-excretion as well as the added advantage that an increased proportion would be retained.

Feed preference

To ensure that the higher feed intake in the afternoon was entirely related to the feeding time and not to preference for a specific diet, a feed preference test was carried out after the growth trial. Results showed that there was no feed preference when both DL 40 and DL45 diets were offered simultaneously, in equal amounts and twice a day to fish that had already been fed both DL4540 and DL4540. This highlights the importance of feeding to satiation and recording feed intake on a daily basis. Fish are sensitive to nutritional intake but it is unlikely the salmon were balancing their intake to achieve a 42.5% crude protein intake. However, this would be worthy of further research.

Feeding time and nutrient utilisation

A significant effect of feeding time on growth performance and nutrient utilization in some fish but not others is described in the literature (Reddy *et al*., 1994 ; Boujard *et al*., 1995; Bolliet *et al*., 2000, 2001). Effects of feeding time have been attributed to differences in both feed intake and growth efficiency (Bolliet *et al*., 2000). The importance of accurate measurement of feed intake is noted and thought to explain some of the discrepancies between different studies (Bolliet *et al*., 2001). Other than experimental error, the reasons for the effect of feeding time on growth performance are not yet clearly understood (Boujard & Leatherland, 1992; Sanchez-Vazquez & Tabata, 1998; Bolliet et al., 2001). Greenback flounder fed a range of rations and to satiation either in the morning or evening clearly showed greater growth in evening fed fish was due to both increased feed intake and more efficient use of the feed (Verbeeten et al., 1999). Nitrogen excretion was higher in the morning and showed both a ration and time of day effect suggesting the importance of protein metabolism in determining differences in growth efficiency. Protein synthesis was higher for morning fed trout and this resulted in better growth performance than trout fed in the evening (Bolliet et al., 2000). It is therefore possible that a similar mechanism was operating in the salmon in the present experiment and resulted in the tendency for more efficient protein

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Table 1
Ingredient and chemical composition of experimental diets

	Diets			
	FM40	DL40	FM45	DL45
Ingredients (g / kg)				
Fish meal	569.7	347.8	519.7	327.0
Dehulled lupin	0	300.0	0	300.0
Corn gluten	46.8	94.0	184.7	200.0
Fish oil	175.0	175.0	150.0	151.8
Alpha-cellulose	103.7	23.7	93.7	0
Dextrose	83.6	38.3	30.7	0
CMC	10.0	10.0	10.0	10.0
Vitamin	10.0	10.0	10.0	10.0
Stay C	0.5	0.5	0.5	0.5
Choline chloride	0.7	0.7	0.7	0.7
Chemical composition (% DM)				
Dry matter (%)	91.58	91.86	93.42	92.85
Crude protein	39.86	39.63	45.39	44.53
Crude lipid	31.39	30.97	27.11	27.09
Ash	9.28	6.02	8.77	6.27
Gross energy (MJ/kg)	22.12	22.53	22.28	22.13
DP/DE (g DP/MJ DE)	20.23	20.53	22.62	22.71

Table 2
Amino acid composition of experimental diets and fish requirement (g/kg)

	40 FM	DL40	FM45	DL45	Requirement
Arg	29.47	33.95	29.82	35.11	18.4
His	13.62	12.42	14.12	13.21	6.2
Iso	18.47	18.11	20.54	20.24	10.6
Leu	33.27	35.13	45.17	45.17	17.6
Lys	35.8	27.09	33.80	26.65	22.0
Met	12.13	9.23	13.17	10.38	7.4
Phe	17.25	17.86	21.12	21.32	9.6
Val	21.06	19.30	22.64	21.13	12.3

Table 3
Feeding regimes used during growth study (DL dehulled lupin)

Treatment	Feed time		Name	DL inclusion (%)
	AM	PM		
1	40% CP	40% CP	FM40	-
2	45% CP	45% CP	FM45	-
3	40% CP	40% CP	DL40	30
4	45% CP	45% CP	DL45	30
5	45% CP	40% CP	DL4540	30
6	40% CP	45% CP	DL4045	30

Table 4
Growth response, feed intake and nutrient utilization of Atlantic salmon fed with different experimental diets

	Diets						
Parameter	FM40	DL40	FM45	DL45	DL 4540	DL4045	P
Initial weight	38.53	39.12	38.64	39.23	39.06	39.35	
	0.06	0.41	0.17	0.41	0.35	0.29	
Final weight (g)	148.55^{a}	153.00^{a}	167.86^{b}	173.80^{b}	169.35^{b}	174.54^{b}	<0.001
	1.49	1.48	1.48	1.48	2.23	2.22	
Weight gain (g)	110.02^{a}	113.89^{a}	129.21^{b}	134.57^{b}	130.29^{b}	135.19^{b}	<0.001
	1.54	1.07	1.30	1.90	2.58	1.93	
FI (g/d/fish)	1.89^{a}	1.99^{a}	2.18^{b}	2.31^{b}	2.23^{b}	2.27^{b}	<0.001
	0.04	0.02	0.02	0.01	0.03	0.02	
Condition factor	1.44	1.43	1.48	1.48	1.37	1.40	NS
	0.03	0.00	0.07	0.01	0.02	0.04	
FCR (g /g)	0.87^{ab}	0.88^{b}	0.85^{ab}	0.86^{ab}	0.86^{ab}	0.84^{a}	<0.05
	0.01	0.00	0.00	0.01	0.00	0.005	
PER (g/g)	2.88^{b}	2.86^{b}	2.59^{a}	2.61^{a}	2.74^{ab}	2.82^{b}	<0.002
	0.03	0.005	0.01	0.04	0.02	0.05	
EER (g/MJ)	51.49	50.42	52.75	52.44	51.67	53.22	NS
	0.15	0.05	0.19	0.77	0.44	0.95	
LER (g/g)	3.66^{a}	3.66^{a}	4.33^{c}	4.28^{c}	3.96^{b}	4.10^{bc}	<0.001
	0.04	0.005	0.01	0.06	0.03	0.07	

Each value is the mean (± SEM) of two replicates.
Means within the same row with unlike superscript letters were significantly different (Tukey's multiple range test).
CF: Condition factor = 100×[(whole live body weight (g))/(fork length (cm^3)].
FCR: Feed conversion ratio = g feed intake /g live weight gain
PER: Protein efficiency ratio = g weight gain /g crude protein intake
EER: Energy efficiency ratio = g live weight gain / MJ energy intake
LER: Lipid efficiency ratio = g weight gain / g lipid intake

Table 5

Comparison of feed intake (% total daily intake) in the morning and in the afternoon for six feeding regimes

	Diets						
Feed intake	FM40	DL40	FM45	DL45	DL4540	DL4045	All diets
AM	48.72[a]	47.97	48.00	48.52	48.15	49.38	48.46[a]
PM	51.28[b]	52.03	52.00	51.48	51.85	50.62	51.54[b]
SEM	0.12	0.36	0.67	0.96	0.88	2.5	0.47
P	<0.05	ns	ns	ns	ns	ns	<0.001

Each value is the mean (± SEM) of two replicates.
Means within the same column with unlike superscript letters were significantly different (Paired t-test).

Table 6

Feed preference (%) test of fish received mixed diets

Treatment	Diet		SEM	P
	DL40	DL45		
DL4045	49.62	50.38	2.97	ns
DL4540	50.56	49.35	2.07	ns
All diets	50.09	49.91	1.58	ns

Each value is the mean (± SEM) of two replicates
Means within the same row with unlike superscript letters were significantly different (Paired t-test)

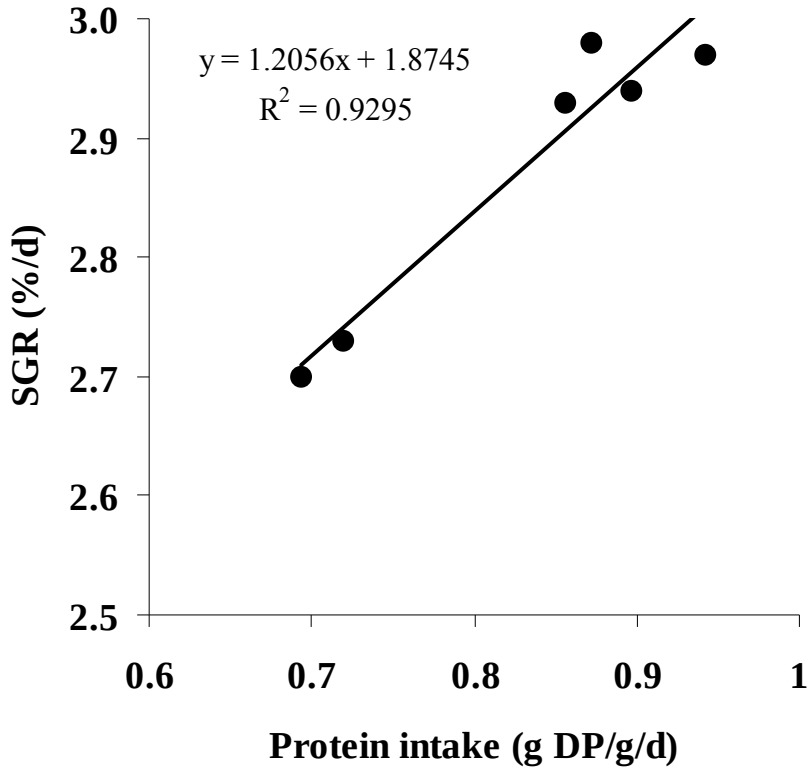

Figure 1.
The relationship between digestible protein intake (mg DP / g / d) and specific growth rate (% / d) for the six feeding regimes. SGR (%/day) = $(\log_e W_2 - \log_e W_1)/t \times 100$.

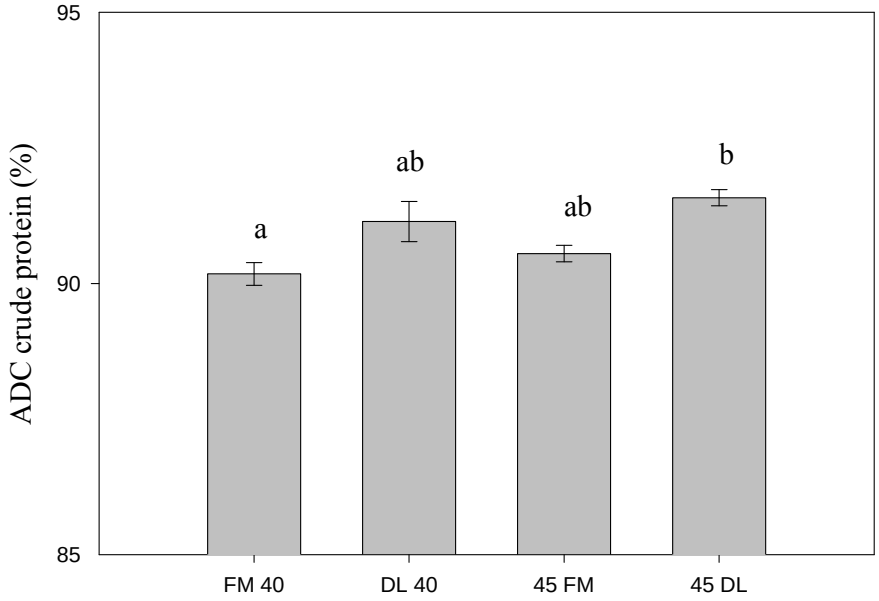

Figure 6.2. Apparent crude protein digestibility in different experimental diets (%).

Figure 2.
Apparent digestibility coefficients (%) for crude protein for different diets

7.8
The potential of thraustochytrids as a replacement for fish oil for Atlantic salmon

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Abstract

The replacement of fish oil with a product made from thraustochytrids, a marine microorganism, and canola oil was investigated in diets for Atlantic salmon parr. Salmon (37 g) were fed for 51 days on diets containing only canola oil (CO), canola and fish oil (CFO) or canola and the thraustochytrid meal (CTH). There were no significant differences in final weight (106.1 ± 1.1 g), weight gain (69.6 ± 1.1 g), feed consumption (16.5 ± 0.2 mg dry matter.g^{-1}.d^{-1}), feed efficiency ratio (1.15 ± 0.03 g.g^{-1}) or productive protein value (51.2 ± 1.7%) between the diets. Nor were there any significant differences in whole-body chemical composition (dry matter, crude protein, total lipid) or organ somatic indices (liver, heart, eye). There were significant differences in apparent digestibility coefficients for total lipid ($P < 0.01$) and phosphorus ($P < 0.05$) but these were small and not thought to be of biological significance. There were no differences in apparent digestibility coefficients for energy or crude protein. Fatty acid digestibility values were very high (>99%) for all fatty acids and differences between the diets were very small. There were no significant differences in measures of immune function (plasma concentrations of neutrophil, lysozyme, antiprotease). However, cumulative mortality was significantly ($P < 0.05$) lower in fish fed CFO (68% mortality) than the other two diets (92% mortality) following transfer to seawater and two challenges with *Vibrio anguillarum*. Compared to differences in lipid distribution and the ability to withstand disease challenge growth performance measures were not as sensitive as indicators of dietary induced changes in salmon.

Keywords: Alternative oil sources; Atlantic salmon; canola oil; fatty acids; fish oil; thraustochytrids

Introduction

As intensive aquaculture continues to expand so does the requirement for high quality protein and oil sources (Barlow, 1989; Hardy, 1996; Naylor *et al*., 2000). Fish meal and oil are major and increasingly expensive components of commercial aquaculture feeds and their replacement has been the focus of considerable research for many species for many years. Atlantic salmon are a major aquaculture species but until recently there has been comparatively little information on the potential of alternative ingredients (Hardy, 1982; Carter *et al*., 1994; Olli *et al*., 1994, 1995). Vegetable sources offer the most potential for the replacement of fish meal protein and wheat and corn gluten, and products from soybean, rapeseed (canola) and lupin show potential for Atlantic salmon (Carter & Hauler, 2000; Storebakken *et al*., 2000; Refstie *et al*., 2001). The replacement of fish oil has been viewed as a greater problem due to the balance of

essential fatty acids in vegetable proteins. However, this has been proposed to have advantages in diets for salmon parr which have to undergo transfer to seawater (Bell *et al*., 1997). Microbial ingredients have received less attention and have mainly been investigated as protein sources (Perera *et al*., 1995a,b) but some have potential as alternative oil sources in aquaculture (Lewis *et al*., 1999, 2001). Thrastochytrids are marine microheterotrophs that have both a high lipid content and extremely high concentrations of DHA (Lewis *et al*., 1999). Thrastochytrids may also have more potential for industrial scale production of essential fatty acids than autotrophs because their culture is less complex (Lewis *et al*., 1999).

This study aimed to investigate the use of a vegetable oil (Canola) on the growth performance of Atlantic salmon parr held at a higher temperature in freshwater than used for previous research (Bell *et al*., 1997; Tocher *et al*., 2000) but that was more representative of local production (Carter & Hauler, 2000; Bransden *et al*., 2001). Furthermore, the canola oil diet was marginally deficient in omega-3 essential fatty acids (NRC, 1993) and had imbalances in key fatty acid ratios (Bell, 2000). This was in order to test the potential of fatty acid supplementation using either fish oil or a prototype fermentation product derived from a Tasmanian thraustochytrid. The potential of canola oil was assessed using growth performance criteria after the freshwater phase but longer term effects were investigated by consideration of immune status and the response to disease challenge following transfer to seawater. In this way the relationship between fatty acid profile and performance over a combination of critical events was investigated (Bransden *et al*., 2001).

Materials and methods

Experimental diets

Three diets were formulated to compare canola oil (CO) as the only source of oil with supplementation by either the thraustochytrid meal (CTH) or fish oil (CFO). The thraustochytrid meal (Table 1) was a freeze dried product made from strain ACEM 6063 and produced experimentally (Lewis *et al*., 2001). The meal contained protein as well as oil so the casein and soybean meal contents were reduced to account for this and the diets formulated to be isonitrogenous and isoenergetic (Table 2). Chilean fish meal was defatted twice using a 2:1 mixture of hexane and ethanol (400 ml 100 g^{-1} fish meal). Soybean meal (ADM Bioproducts, Beenleigh, Qld., Australia); lactic casein (New Zealand Dairy Board, Wellington, New Zealand); wheat gluten (Starch Australasia, Tamworth, NSW, Australia), pre-gelatinised starch (Goodman Fielder, Summer Hill, NSW, Australia) were also used. Fish oil from jack mackeral (Skretting Australia, Cambridge, Tas., Australia) and a domestic source of pure Canola oil were used. Stay-C and Rovimix E50 were supplied by Roche Vitamins Australia (Frenchs Forest, NSW, Australia), vitamins A and D by ICN (Seven Hills, NSW, Australia) and the remaining ingredients were supplied by Sigma-Aldrich (Castle Hill, NSW, Australia). The diets were manufactured as 3 mm diameter pellets using a California Pellet Mill (CL-2 Lab Mill). Diets were then dried and stored at -20^{o}C.

Growth experiment

The experiment was conducted at the School of Aquaculture, University of Tasmania. Atlantic salmon (*Salmo salar* L.) parr (36.5 ± 5.7 g) were obtained from Springfield Salmon Hatchery (Tas., Australia) and stocked into 300-l tanks at 30 fish per tank. These fish were acclimated for 2 weeks. The tanks were held in a constant environment room (temperature, 15.7 ± 0.8^{o}C; photoperiod, 12:12) and part of a partial freshwater recirculation system (Carter & Hauler, 2000). Water was treated through

physical and biofilters with a continuous replacement of approximately 20% d^{-1}. Water quality parameters (DO, pH, ammonia, nitrate and nitrite) were monitored to ensure water quality remained well within limits recommended for Atlantic salmon (Wedemeyer, 1996).

At the start of the experiment fish were anaesthetised (50 mg l^{-1}, Benzocaine) and weight and fork length measured. A group of fifteen fish was used for assessment of initial chemical composition of whole-body and tissues: white muscle, liver, heart and eye (see below). Twenty-five fish were returned to each of nine tanks and distributed to ensure there were no significant differences between initial group mean weight for triplicate groups to be fed one of the 3 diets. The fish were re-weighed every 14 days and ration adjusted accordingly. A ration of 1.25 $mg.g^{-1}$ initial body weight was supplied twice a day in the morning and afternoon and dispensed over 1 h by automatic belt feeders. The fish were fed in this way each day. Total feed consumption (kg DM) was estimated every fourth day approximately (on 13 days over the experiment) from the amount of feed that was not eaten and was collected from the effluent standpipe. A mean value for feeding efficiency (feed consumed / feed supplied) was calculated for each tank and applied to the total feed fed over the experiment.

The experiment continued for 51 days when the fish had more than doubled in weight. At the end of the experiment the fish were not fed for a day and all individual fish weights were then measured and tank means calculated. Fish were killed by transection of the spinal cord after immersion in anaesthetic and five removed from each tank to determine whole-body chemical composition. A further five fish were removed, dissected to determine liver, heart and eye weights and samples of white muscle, heart, liver and eyes taken for fatty acid analysis. Tissue samples were frozen in liquid nitrogen.

Apparent digestibility

Apparent digestibility coefficients were measured during the experiment using all tanks. Faecal samples were collected by settlement (Cho *et al*., 1982) in settlement collectors attached to the tanks described above (Carter & Hauler, 2000). Groups of salmon were fed the experimental diets containing digestibility markers cholestane and yttrium oxide (10 g. kg^{-1}) for 7 days. On days 5,6 and 7 faecal samples were removed from the settlement collector between 1700 to 0900 h, freeze-dried and used in the analysis of the digestibility markers and nutrients (see below). The apparent digestibility coefficients (ADC) were calculated using the standard formula ADC (%) = 100 - [100. (% I_{diet} / %I_{faeces}) x (%N_{faeces} / %N_{diet}) (Maynard & Loosli, 1969), where I is the inert marker and N the nutrient.

Chemical analysis

Standard methods were used to determine dry matter (freeze dry to constant weight); crude protein (N x 6.25, Kjeldahl using a selenium catalyst); total lipid (Bligh & Dyer, 1959) and energy (bomb calorimeter: Gallenkamp Autobomb, calibrated with benzoic acid), ash (AOAC, 1995). Yttrium was analysed using inductively coupled plasma emission spectrometry (ICP-OES) following digestion with nitric acid at 180°C for 48 h. Cholestane was determined during the fatty acid analysis (see below).

Total lipid extracts were obtained from all samples using a modified one-phase (chloroform:methanol:water) extraction (Bligh & Dyer, 1959). A portion of the total lipid extract recovered from the lower chloroform phase was used for fatty acid

analyses. This portion was transmethylated by reaction for 1 h at 80 °C with methanol:hydrochloric acid:chloroform (10:1:1 v/v/v, 3 ml). After cooling and addition of water (1 ml), the resultant fatty acid methyl esters (FAME) were extracted with hexane:chloroform (4:1 v/v, 3 x 2 ml). For analysis by gas chromatography (GC), samples were diluted with chloroform containing a known concentration of either 19:0 or 23:0 FAME as the internal injection standard. FAME analyses were performed with a Hewlett Packard 5890 GC equipped with a 50 m x 0.32 mm internal diameter cross-linked methyl silicone fused-silica capillary column (HP5) and flame ionization detector (Nichols, 1995). Peak areas were recorded and quantified using the software package Millenium 32 v3.05.01 (Waters Corporation, USA). Confirmation of FAME identity was achieved using a GCQ (Thermoquest, USA) GC-mass spectrometer (GC-MS). Component identification was by comparison of retention time and MS data with that obtained for authentic and laboratory standards.

Lipid class data was obtained by analysing a portion of the total lipid extract using thin layer chromatography – flame ionisation detection (TLC-FID) (Iatroscan Mk V TH-10, Iatron Laboratories, Japan). Aliquots (1 µl) from a known volume of each extract were applied onto duplicate Iatroscan chromarods using disposable micro-pipettes. Chromarods were developed using a hexane-diethyl ether-acetic acid (60:17:0.2 v/v/v) solvent system in a sealed glass tank containing pre-extracted filter paper. After development, the chromarods were oven-dried at 60°C for 10 min and analysed immediately. Peak areas were recorded and quantified using the chromatography software DAPA (Scientific Software, Kalamunda, WA, Australia).

Immune function and blood chemistry

Whole blood was used for the measurement of neutrophil activity by the reduction of nitroblue tetrazolium (NBT) to formazan, with values presented as the optical density at 540 nm after reduction (Anderson & Siwicki, 1996). Osmotic erythrocyte fragility (OEF) was measured by modifications of methods described previously (Siddall *et al*., 1995; Hansen, 1998). Briefly, a series of phosphate buffered saline (PBS) solutions were prepared with salt concentrations from 0.15 to 0.90% in 0.05% increments except between 0.4-0.6% where increments were 0.025%. Ten µl of whole blood was placed into wells of round-bottomed microtiter plates, and 190 µl of the PBS series added. Plates were incubated at room temperature for 1 h, centrifuged (1000 × *g*, 10 min) and supernatants removed and transferred to flat-bottomed microtiter plates. Supernatant absorbance was read at 540 nm. Optical densities (OD_{obs}) were converted to proportional haemolysis values (*H*) using the equation described by Siddall *et al*., (1995): $Hi = OD_{obs} - OD_{blank} / OD_{0.15}$ where $OD_{0.15}$ is the maximal optical density for that fish at 0.15% PBS, and OD_{blank} is the optical density of 10µl of haemoglobin-free plasma plus 190 µl of the 0.90% PBS. Proportional haemolysis was plotted against PBS concentration, and each curve fitted with the function: $H = (1 + e^{-\alpha + \beta [PBS]})^{-1}$. The logistic positional and rate parameters (α and β, respectively) were used for statistical comparisons of OEF, whilst the value where 50% haemolysis occurred (C_{50}) was used for descriptive purposes only.

Remaining blood was centrifuged (1000 × *g,* 5 min) to obtain plasma. Plasma was used for measurement of lysozyme activity by determining the rate of lysis of a *Micrococcus lysodeikticus* suspension and using hen egg white lysozyme (Sigma-Aldrich, Castle Hill, NSW, Australia) as standard (Thompson, 1994). The volume of plasma required to inhibit 50% of a standard trypsin activity was used to quantify antiprotease activity (Ellis, 1990). The difference in plasma protein concentration

(measured according to Lowry *et al*., 1951) before and after precipitation with polyethylene glycol was considered to be the plasma immunoglobulin fraction (Siwicki *et al*., 1994). Plasma glucose was measured using a commercial diagnostic kit (Sigma-Aldrich, Castle Hill, NSW, Australia).

Disease resistance

Ten salmon from each tank not bled for measurement of immune function were anaesthetized and tagged with alcian blue using a Panjet (Hart & Pitcher, 1969) so the experimental dietary treatment could be identified. All tagged salmon from all groups were then transferred to a 'post-challenge' system, consisting of a 1500-l tank containing recirculating freshwater under aeration at 15°C, water was replaced at 25% day^{-1}. The system was used in the same way when salinity was increased (see below). Salmon were allowed to acclimate for 5 days prior to disease challenge.

The challenge bacterium, *V. anguillarum* (serovar O1) was passaged through several Atlantic salmon prior to the disease resistance experiment to increase pathogen virulence. Twenty-four hours before *V. anguillarum* was administered, a fresh culture of the passaged bacterium was prepared (Oxoid Nutrient Broth No. 2, Oxoid Ltd, Basingstoke, UK, plus 10% NaCl). On the day of the challenge, the culture was washed in sterile PBS (pH 7.2) with intermediate centrifugation (3000 × *g*, 10 min). All fish were removed from the post-challenge system, and placed in a 'challenge' vessel containing 15‰ saline water. The suspension of *V. anguillarum* was added to the vessel so as to provide a final cell density of 3 x 10^5 $cells.ml^{-1}$. Cells densities were confirmed by counting in a haemocytometer under phase contrast microscopy, and by serially diluting the culture from the reservoir and distributing suspensions onto dry horse blood agar plates (Tasmanian Laboratory Services, Launceston, Tas., Australia) using a calibrated pipette. Salmon were held in the vessel for 1 h with constant aeration. All salmon were then removed and returned to the post-challenge system that had been filled with 15‰ saline water.

Fish were monitored daily for mortality. The presence of *V. anguillarum* was confirmed using sterile swabs of the kidney, culture on blood agar and assessment of the colonial morphology. By day 23, little mortality had occurred so a secondary challenge was performed under similar conditions to those described above: all salmon were transferred to the challenge vessel at a salinity of 35‰. A *V. anguillarum* culture was added to provide a cell density of 2.8 × 10^6 $cells.ml^{-1}$. Salmon were returned to the post-challenge system (35‰) and daily monitoring of mortality continued. The challenge experiment was ended at day 32 by which time mortalities had ceased.

Statistical analysis

Mean values are reported ± standard error of the mean (SEM). Percentage data were arcsine transformed prior to analysis. Normality and homogeneity of variance were confirmed and comparison between means was by one-way ANOVA. Multiple comparison was by Tukey-Kramer HSD. Mortality rates during the challenge were compared using the log-rank test (Peto *et al*., 1977). Statistical analyses were performed using J.M.P. version 3.2.1 (SAS Institute Inc.). Significance was accepted at probabilities of 0.05 or less.

Results

After 51 days in freshwater there were no significant differences between the dietary treatments in growth performance as measured by final weight, weight gain or survival (Table 4). Total feed consumption and weight specific feed consumption were not different and this meant that diet had no effect on FER or PPV (Table 4). Diet had no effect on whole body chemical composition (Table 5) or on organ somatic indices (Table 6). However, diet did have an effect on the distribution of lipid in tissues and the tissue pr ofiles tended to reflect the dietary fatty acid profiles (Table 6). Thus, OA, LA and LNA were higher for the canola oil diet, DHA higher for the thraustochytrid diet and EPA was higher for the diet with fish oil (Table 6).

In the liver the PL fraction accounted for more of the lipid than TAG. The PL fraction was significantly and markedly higher for CTH than for the other diets, being almost four times larger than for CO. The proportion of FA as OA and LNA were significantly higher in CO, EPA was highest in CFO and DHA higher in CTH than CO. There were no significant differences in major n-6 FA. In the heart TAG were significantly higher in CO than CTH but there were no significant differences between PL. Heart TAG only accounted for a small part of the FA (between 8-15% of the FA from TAG plus PL). Consequently, differences in FA would mainly be related to changes in the FA composition of the PL. As in the liver, OA and LNA were highest for CO and EPA highest for CFO. In contrast to the liver, LA was significantly different for all diets and highest in CO, but there were no differences in DHA or AA. In white muscle, TAG and PL were approximately equal and there were no differences between diets. OA, LA, AA and LNA were highest in CO, EPA highest for CFO and DHA highest for CTH. In the eye, OA, LA and LNA were higher for CO compared with CFO.

The n-3/n-6 ratio was significantly higher for CFO for all tissues although the magnitude of the ratio differed between tissues and was highest in muscle (Table 6). The n-3/n-6 ratio for CO and CTH was higher than the ratio in the diets and suggested an accumulation of n-3 FA in preference to n-6 FA in the liver, muscle and eye. The n-3/n-6 ratio was similar in the diet (1.7), liver (1.6), heart (1.5) and eye (1.6) for CFO, but approximately double this in the white muscle (3.4).

There was no significant difference in the apparent digestibility coefficients for crude protein or energy, whereas phosphorous was higher for CTH than for CFO (Table 7). ADC for total lipid and TAG were affected by diet and significantly lower for CTH (Table 7). PL was more poorly digested although there were no significant dietary differences. Digestibility of fatty acids was high at over 99% and, apart from hexadecanoic acid (16:0), there were few major differences between the diets (Table 7).

No significant differences ($P > 0.05$) were detected between diets for any of the immune function or blood chemistry parameters measured (Table 8). Similarly, no significant differences were detected between osmotic erythrocyte fragility curves between the different diets (Fig. 1). Mean concentrations of NaCl where 50% haemolysis occurred were 0.44 (± 0.01), 0.47 (± 0.01) and 0.44 (± 0.01) % for diets CFO, CO and CTH, respectively.

Significant differences were detected in cumulative mortality between different diets (Fig. 2). Mortality first occurred 5 days after the primary challenge in those

salmon fed the CO feed and subsequently on day 6 in both CO and CTH-fed salmon. There was no further mortality after day 7. No mortality occurred on CFO. Following the secondary challenge mortality occurred in all groups between days 24 and 28, after which time no further deaths occurred. Final cumulative mortality rates were 68%, 92% and 92% for salmon given diets CFO, CO and CTH, respectively.

Discussion

The diets were designed to test the addition of either fish oil or the thraustochytrid product to a diet containing canola oil as the only other oil source. Canola oil was used to make a diet (CO) that was marginal in terms of meeting the essential n-3 fatty acid requirements. Thraustochytrid was added to CO to increase the essential n-3 fatty acid content and the addition of 10% thraustochytrid doubled the essential n-3 fatty acid content due to its high content of DHA. However, the total n-3 content was still lower than added by fish oil. The CTH diet had a n-3/n-6 ratio intermediate between CO and CFO. The growth-experiment showed that using only canola oil was adequate for 40 g salmon parr when grown to 105 g smolt size. Changes in dietary fatty acid profiles, due to the addition of fish oil or thraustochytrid meal, had no effect on growth and growth efficiency or on other measures of performance such as survival, measures of immune response or nutrient digestibility. The experiment represented a significant part of the salmon freshwater stage where by the salmon almost tripled their wet weight and achieved smolt status. The different distribution of fatty acids in the diets affected the salmon fatty acid composition in all tissues examined and it is suggested that this explained the different response to the combined stressors of sea water and disease challenge. Salmon fed the diet containing the thraustochytrid showed similar mortality to those fed the canola oil diet despite there being differences in content of some fatty acids. Consequently, assessment of the possible importance of differences in fatty acid composition focused on values that were similar for CO and CTH but both different from CFO.

The major differences in key indices of dietary fatty acid status were in n-3 and n-6 fatty acids. CFO had lower linoleic acid but higher arachidonic acid concentrations, as well as a higher proportion of n-3 fatty acids and therefore a higher n-3/n-6 ratio than the other diets. Although, salmonids grow well in fresh- and sea-water when fed vegetable oils, the concentrations and ratios of key fatty acids have to be exceeded for this to occur (Bell, 2000). However, the consequences of poor dietary fatty acid status may not be evident under normal relatively stress-free experimental conditions (Bell *et al*., 1996). Differences in dietary fatty acid status in the present trial did not result in significant differences in immune function or blood chemistry in Atlantic salmon after seven weeks in freshwater. When Thompson *et al*. (1996) supplied two feeds containing either sunflower or fish oil and with n-3/n-6 ratios of 0.3 or 5.2, respectively, they also failed to find any alteration to non specific defense mechanisms including lysozyme, antiprotease, macrophage respiratory burst and phagocytosis. Similarly, there was no effect on serum protein, lysozyme activity or kidney phagocyte respiratory burst when different oils (sunflower, linseed or two fish oils) with n-3/n-6 ratios of between 0.3 to 10.6 were fed to Atlantic salmon (Bell *et al*., 1996). Rainbow trout (*Oncorhynchus mykiss*) with a 'history' of adequate nutritional status did not show signs of immunomodulation when fed diets which would normally compromise younger fish (Kiron *et al*., 1995). In the present trial, immune parameters were measured at the conclusion of a growth trial and values probably represented resting values (Thompson *et al*., 1996) and did not highlight differences between the dietary

treatments. Consequently, the salmon were followed through transfer to seawater and subjected to disease challenge procedures. In contrast to the growth and immune parameters, the high level of stress imposed jointly by seawater transfer and / or disease challenge resulted in significant differences in mortality between the dietary treatments. The fish oil supplement significantly increased survival compared with both the control canola diet and the thraustochytrid supplement. Although better survival suggested better fatty acid status of salmon fed fish oil, it was difficult to identify key dietary differences that may have explained this.

Differences in dietary concentrations of arachidonic acid, eicosapentanoic acid and the n-3/n-6 ratio may have influenced smolt physiology and health status via their complex effects on the synthesis of various eicosanoids from arachidonic acid (Bell *et al*., 1996). However, tissue concentrations of arachidonic acid were not different between the diets and it also appeared that the arachidonic acid / eicosapentanoic acid ratio was nearer optimal for fish fed CTH rather than CFO (Bell *et al*., 2000). Part of the explanation may have been that the absolute levels of dietary arachidonic acid were considerably lower in CO and CTH than in CFO and lower than in similar studies (Bell *et al*., 1997; Dosanjh *et al*., 1998). Sunflower and linseed oil based diets (Bell *et al*., 1996) had different dietary arachidonic acid contents that were similar to CTH and CO, respectively. These differences resulted in significant differences in the production of leukotrienes (LTB_4 and LTB_5) in kidney macrophage-enriched preparations and thought suggestive of major differences in immune function and inflammatory activity (Bell *et al*., 1996). Neither the arachidonic acid / eicosapentanoic acid ratio nor the dietary content of arachidonic acid appear to provide an explanation for the difference in mortality. The dietary n-3/n-6 ratio separated the CFO diet from the other two, and the difference was reflected in all the tissues so that higher survival was associated with higher n-3/n-6. Although CTH had a similar n-3/n-6 ratio to a successful parr diet containing rapeseed oil (Tocher *et al*., 2000) the salmon from the later trial were not exposed to disease challenge. These experiments were carried out under different circumstances and the explanation for the patterns of mortality requires further study.

Several studies have demonstrated the potential for replacement of substantial amounts of fish oil with plant oils in diets for salmon during their freshwater parr stage (Bell *et al*., 1997; Dosanjh *et al*., 1998; Tocher *et al*., 2000). For example, the performance of two feeds, predominantly fish meal and fish oil (with some plant meal) or predominantly plant meals and oil (with low fish meal), were compared in Atlantic salmon parr (Bell *et al*., 1997). There were no differences in growth performance over the winter freshwater phase with parr growing from around 20 g to a final weight of 35 g (Bell *et al*., 1997). Diets containing added oil (accounting for 68% of total dietary lipid) from either fish oil, rapeseed oil or linseed oil produced equivalent winter growth performance in growing 30 g parr to exceed 40 g after 19 weeks (Tocher *et al*., 2000). The higher temperatures used in the present study allowed much faster growth and 40 g parr reached over 100 g after less than eight weeks. It is of interest that the CO diet performed well despite a marginal essential n-3 fatty acid content. This is likely to be explained by the high residual levels in the fish (2.2% wet weight total lipid) even though the fish were fed on a low oil diet prior to the experiment.

It has been proposed that fish oil replacement during the parr stage may be beneficial for smoltification due to a reduction in a high dietary content of n-3 PUFA from fish oil (Bell *et al*., 1997). The study by Bell *et al*. (1997) was significant because

it showed desaturation activity (in isolated hepatocytes) increased towards the time of seawater transfer and that this activity was higher on the plant oil diet. Desaturation is inhibited by high n-3 PUFA levels and desaturation along n-3 pathways occurs in preference to n-6 pathways (Bell, 2000). Thus, high n-3 PUFA levels from the fish oil diet were thought to inhibit desaturation of both n-3 and n-6 fatty acids. This is of importance because arachidonic acid acts as the main precursor for prostaglandins important in smoltification. Bell et al. (1997) found liver phospholipid arachidonic acid / eicosapentanoic acid ratios were over 1, 50 percent higher, on a plant oil diet than on a fish oil diet. The higher ratio was reflected by higher synthesis of $PGF_{3\alpha}$, a prostaglandin derived from arachidonic acid. In the present study the arachidonic acid / eicosapentanoic acid ratios in the liver (total lipid) were over 1.3 for the CO and CTH diets compared with 0.3 for the CFO diet. Although there were no significant differences in the relative concentration of arachidonic acid in liver, eye or heart tissue between dietary treatment white muscle concentrations were significantly lower for CFO. This may have been a consequence of differences in desaturation activity, especially in view of the considerably higher arachidonic acid content in CFO.

In the present experiment salmon were transferred to seawater and then fed a commercial fish oil based feed. Similarly, some studies changed from plant oil diets to commercial diets containing fish oil for the seawater period (Bell *et al*., 1997; Tocher *et al*., 2000), whereas other studies fed plant oils to seawater salmon (Bell *et al*., 1996; Dosanjh *et al*., 1998; Torstensen *et al*., 2000). Neither Bell *et al*. (1997) nor Tocher *et al*. (2000) observed any difference in the growth of salmon after transfer to seawater and fed a normal commercial feed when previously fed diets containing more than 90% plant oil (Bell *et al*., 1997; Tocher *et al*., 2000). Canola oil successfully replaced 47% of the fish oil in 28% lipid diets fed to seawater Atlantic salmon parr growing from 145-180 g to around 400 g (Dosanjh *et al*., 1998). Similarly there were no major differences in the growth of Atlantic salmon (100 to >300 g) fed diets containing either fish oil or soybean oil as the only added oil sources (Grisdale-Helland *et al*., 2002). Although diets containing high amounts of plant oil are successful in terms of growth performance, they result in significant differences in fatty acid composition compared with fish oil diets. These differences have different consequences at different stages in the salmon life / production cycle especially in relation to the fishes ability to withstand challenging conditions.

In conclusion, thraustochytrid had no detrimental effects on the performance of salmon although, at the current inclusion of 10%, failed to confer the same effect as fish oil under challenging conditions. Consequently, it would be informative to test a higher inclusion level under similar procedures to the present experiment.

Acknowledgements

The authors gratefully acknowledge technical assistance from M. Attard, M. Aigerinos, B. Mooney and D.Holdsworth and the Fisheries Research and Development Corporation (97/329) for further financial support.

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Table 1
Chemical composition of the thraustochytrid meal

Nutrient	$g.kg^{-1}$
Dry matter	979
Crude protein	207
Total fat	589
Ash	85
GE ($MJ.kg^{-1}$)	20.7
Essential amino acids	
Arginine	14.6
Histidine	5.3
Isoleucine	9.1
Leucine	16.9
Lysine	13.0
Methionine	5.5
Phenylalanine	10.4
Threonine	10.9
Tryptophan	2.6
Valine	20.9
Essential fatty acids	
18:2n-6	0.14
20:4n-6	0.53
18:3n-3	0.23
20:5n-3	2.63
22:6n-3	92.21

Table 2
Ingredient and chemical composition of experimental diets

	Diet		
	CO	CTH	CFO
Ingredient composition ($g.kg^{-1}$)			
Defatted fish meal	250.0	250.0	250.0
Soybean meal	56.2	36.5	56.2
Casein	143.2	132.9	143.2
Wheat gluten	100.0	100.0	100.0
Canola oil	169.6	110.5	78.2
Fish oil			91.4
Thraustochytrid meal		100.0	
Pre-gel starch	150.0	150.0	136.2
Vitamin mix (ASV4)[1]	3.0	3.0	3.0
Stay C[2]	3.0	3.0	3.0
Choline chloride	2.0	2.0	2.0
Mineral mix (TM4)[3]	5.0	5.0	5.0
Ca $(H_2PO_4)_2$	21.8	23.0	21.8
α-Cellulose	36.2	24.2	50.0
Bentonite	49.0	49.0	49.0
CMC	9.0	9.0	9.0
Cholestane	1.0	1.0	1.0
Yttrium Oxide[4]	1.0	1.0	1.0
Chemical composition ($g.kg^{-1}$ DM)			
Dry matter ($g.kg^{-1}$)	940 ± 2	960 ± 1	901 ± 2
Crude protein	398 ± 3	391 ± 3	410 ± 2
Total lipid	206 ± 4	207 ± 12	194 ± 3
Ash	121 ± 1	130 ± 1	124 ± 3
Gross energy ($MJ.kg^{-1}$ DM)	20.9 ± 3.9	20.8 ± 0.6	20.7 ± 0.6

[1]Vitamin mix (ASV4) to supply per kg feed: 2.81 mg thiamin HCl; 10.0 mg riboflavin; 9.15 mg pyridoxine HCl; 25 mg nicotinic acid; 54.35 calcium D-pantothenate; 750 mg myo-inositol; 0.38 mg d-biotin; 2.5 mg folic acid; 0.03 mg cyanocobalamin; 6250 IU retinol acetate; 2800 IU cholecalciferol; 100 IU DL α-tocopherol acetate; 5 mg menadone sodium bisulphate.

[2]L-Ascorbyl-2-polyphosphate (Stay-C, Roche Vitamins Australia, Frenchs Forest, NSW, Australia)

[3]Mineral mix (TM4) to supply per kg feed: 117 mg $CuSO_4$ $5H_2O$; 7.19 mg KI; 1815 mg $FeSO_4$ $7H_2O$; 307 mg $MnSO_4$ H_2O; 659 mg $ZnSO_4$ $7H_2O$; 3.29 mg Na_2SeO_3; 47.7 mg $CoSO_4$ $7H_2O$.

[4]Yttrium oxide was replaced with bentonite outside of the digestibility experiment

Table 3
Lipid class and fatty acid composition of experimental diets

	CO	CTH	CFO
Lipid class composition[1] ($g.kg^{-1}$ DM)			
TAG	199	196	186
PL	4.0	8.3	4.0
Free fatty acid	1.0	1.1	2.0
Sterol	0.4	0.6	1.0
Fatty acid composition (% fatty acids)			
14:0	0.50	5.55	4.06
16:0	6.64	12.52	11.90
18:0	2.13	1.58	2.78
Total saturates[2]	10.22	21.28	21.62
16:1n-7	0.44	1.37	4.29
18:1n-7	3.13	2.86	3.15
18:1n-9	54.82	39.42	34.25
20:1n-9	1.20	0.73	1.04
Total monosaturates[3]	60.15	44.28	43.39
18:2n-6	22.19	16.27	11.84
20:4n-6	0.04	0.09	0.61
Total n-6[4]	22.39	18.44	12.71
18:3n-3	6.09	4.38	3.81
18:4n-3	0.45	0.14	1.05
20:5n-3	0.51	0.81	9.58
22:5n-3	0.03	0.20	1.01
22:6n-3	0.65	9.93	4.90
Total n-3[5]	7.83	15.61	21.61
n-3/n-6	0.35	0.85	1.70
20:4n-6/20:5n-3	0.05	0.11	0.06
22:6n-3/20:5n-3	1.28	12.26	0.51

[1] calculated from % composition and total lipid.
[2] includes 15:0, 17:0, 20:0.
[3] includes 16:1n-9, 20:1n-7.
[4] includes 18:3n-6, 20:3n-6, 22:5n-6.
[5] includes 20:4n-3.

Table 4
The performance of Atlantic salmon fed experimental diets containing different oil sources (no significant differences)

Parameter		Diet		
		CO	CTH	CFO
Initial weight	(g)	36.4 ± 0.1	36.7 ± 0.5	36.5 ± 0.2
Final weight	(g)	107.6 ± 2.8	105.8 ± 0.4	105.0 ± 2.4
Weight gain	(g)	71.1 ± 2.8	69.2 ± 0.7	68.5 ± 2.4
Total feed consumption	(g DM)	1499 ± 41	1562 ± 41	1423 ± 10
FC	(mg $DM.g^{-1}.d^{-1}$)	16.3 ± 0.3	17.1 ± 0.4	16.2 ± 0.4
FER	($g . g^{-1}$)	1.19 ± 0.03	1.10 ± 0.04	1.16 ± 0.05
PPV	(%)	49.1 ± 4.6	52.6 ± 2.3	51.9 ± 2.0
Overall survival	(%)	100	98.7 ± 1.3	98.7 ± 1.4

Each value is the mean (± SEM) of three replicates
FC. feed consumption = total feed consumption (g DM) / ∑ individual mid-weight (g) / 51 days
FER. Feed efficiency ratio = (total weight gain (g) / total feed consumption (g DM))
PPV. productive protein value = 100.(fish protein gain (g CP) / total protein consumption (g CP))

Table 5
Chemical composition of Atlantic salmon fed experimental diets containing different oil sources (No significant differences)

Parameter		Diet		
		CO	CTH	CFO
Dry matter	(%)	26.9 ± 1.5	29.4 ± 0.5	27.7 ± 0.9
Crude protein	(%)	16.0 ± 0.9	17.3 ± 0.1	16.6 ± 0.5
Total lipid	(%)	2.4 ± 0.3	2.9 ± 0.2	2.8 ± 0.3
Ash	(%)	1.9 ± 0.1	2.2 ± 0.1	1.9 ± 0.1

Each value is the mean (± SEM) of three replicates (3 fish pooled per replicate)
Initial group (mean ± sd; n = 6): 25.9 ± 1.0 % DM; 15.1 ± 0.1 % crude protein; 2.2 ± 0.1 % total lipid; ± % ash.

Table 6
Organ somatic indices and tissue content of triacylglycerol (TAG), phospholipid (PL) and selected fatty acids (% total fatty acid) of Atlantic salmon fed experimental diets containing different oil sources

Parameter	Diet			P
	CO	CTH	CFO	
Liver (% wet weight)	1.64 ± 0.08	1.46 ± 0.05	1.40 ± 0.12	ns
TAG ($mg.g^{-1}$)	2.3 ± 1.4	5.3 ± 1.6	8.6 ± 2.6	ns
PL ($mg.g^{-1}$)	6.5^{b} ± 1.1	24.0^{a} ± 3.9	13.2^{b} ± 3.1	<0.05
18:1n-9	49.50^{a} ± 1.30	42.06^{b} ± 0.13	40.35^{b} ± 1.28	<0.005
18:2n-6	8.83 ± 0.19	8.73 ± 0.52	7.93 ± 0.43	ns
20:4n-6	1.60 ± 0.30	1.77 ± 0.16	0.97 ± 0.17	ns
18:3n-3	5.50^{a} ± 0.15	4.67^{b} ± 0.01	4.48^{b} ± 0.14	<0.005
20:5n-3	1.17^{b} ± 0.19	1.30^{b} ± 0.15	3.37^{a} ± 0.15	<0.0001
22:6n-3	3.26^{b} ± 0.69	7.40^{a} ± 0.55	6.10^{ab} ± 0.95	<0.05
n-3/n-6	1.02^{b} ± 0.06	1.10^{b} ± 0.03	1.55^{a} ± 0.11	<0.005
Heart (% wet weight)	0.18 ± 0.01	0.16 ± 0.01	0.18 ± 0.01	ns
TAG ($mg.g^{-1}$)	0.8^{a} ± 0.1	0.3^{b} ± 0.1	0.4^{ab} ± 0.1	<0.05
PL ($mg.g^{-1}$)	4.4 ± 0.3	3.7 ± 0.1	4.4 ± 0.6	ns
18:1n-9	43.17^{a} ± 0.57	33.66^{b} ± 0.44	30.45^{c} ± 0.25	<0.0001
18:2n-6	10.27^{a} ± 0.14	7.33^{b} ± 0.27	5.57^{c} ± 0.41	<0.0001
20:4n-6	0.83 ± 0.09	0.87 ± 0.09	0.80 ± 0.06	ns
18:3n-3	4.80^{a} ± 0.06	3.74^{b} ± 0.05	3.38^{c} ± 0.03	<0.0001
20:5n-3	0.70^{b} ± 0.10	0.63^{b} ± 0.14	1.80^{a} ± 0.20	<0.005
22:6n-3	2.23 ± 0.28	4.36 ± 0.87	3.93 ± 0.48	ns
n-3/n-6	0.78^{b} ± 0.02	0.95^{b} ± 0.07	1.46^{a} ± 0.01	<0.0001
White muscle				
TAG ($mg.g^{-1}$)	2.0 ± 0.4	2.3 ± 0.7	2.9 ± 1.6	ns
PL ($mg.g^{-1}$)	3.7 ± 1.3	2.5 ± 0.7	3.4 ± 0.6	ns
18:1n-9	34.65^{a} ± 0.36	26.91^{b} ± 1.89	24.03^{b} ± 1.41	<0.005
18:2n-6	11.73^{a} ± 0.26	8.80^{ab} ± 0.74	7.23^{b} ± 0.53	<0.005
20:4n-6	1.30^{a} ± 0.06	1.10^{ab} ± 0.10	0.93^{b} ± 0.03	<0.05
18:3n-3	3.85^{a} ± 0.04	2.99^{b} ± 0.21	2.67^{b} ± 0.16	<0.005
20:5n-3	3.17^{b} ± 0.12	2.60^{b} ± 0.06	6.80^{a} ± 0.30	<0.0001
22:6n-3	12.33^{b} ± 0.32	19.43^{a} ± 1.83	17.00^{ab} ± 1.53	<0.05
n-3/n-6	1.59^{b} ± 0.04	2.05^{b} ± 0.19	3.37^{a} ± 0.33	<0.005
Eye (% wet weight)	0.50 ± 0.01	0.50 ± 0.01	0.49 ± 0.02	ns
TAG ($mg.g^{-1}$)	27.5 ± 3.7	17.9 ± 3.0	23.4 ± 4.1	ns
PL ($mg.g^{-1}$)	7.6^{b} ± 2.5	11.9^{b} ± 3.0	23.7^{a} ± 2.5	<0.05
18:1n-9	41.58^{a} ± 1.31	35.58^{b} ± 1.07	32.04^{b} ± 0.32	<0.005
18:2n-6	9.63^{a} ± 0.81	7.33^{ab} ± 0.82	5.97^{b} ± 0.19	<0.01
20:4n-6	0.43 ± 0.03	0.33 ± 0.03	0.27 ± 0.09	ns
18:3n-3	4.62^{a} ± 0.15	3.95^{b} ± 0.12	3.56^{b} ± 0.04	<0.005
20:5n-3	1.93 ± 0.33	1.80 ± 0.42	2.37 ± 0.12	ns
22:6n-3	3.73 ± 0.93	4.73 ± 0.90	3.77 ± 0.13	ns
n-3/n-6	0.91^{c} ± 0.01	1.21^{b} ± 0.03	1.56^{a} ± 0.01	<0.0001

Each value is the mean (± SEM) of three replicates (3 fish pooled per replicate)
Initial group (mean ± sd; n = 9) liver = 1.03 ± 0.16%BW; heart = 0.19 ± 0.04 %BW; eye = 0.79 ± 0.12 %BW

Table 7
Apparent digestibility coefficients (ADC) for crude protein (N), energy (kJ) and phosphorous (P) and different lipid and fatty acid fractions for diets containing different oil sources

Parameter	Diet			P
	CO	CTH	CFO	
ADC_N (%)	$93.2^{a} \pm 0.2$	$92.4^{b} \pm 0.1$	$93.2^{a} \pm 0.5$	ns
ADC_{kJ} (%)	85.9 ± 0.2	84.1 ± 1.1	84.0 ± 1.4	ns
ADC_P (%)	$38.1^{ab} \pm 1.9$	$42.7^{a} \pm 0.2$	$34.2^{b} \pm 1.2$	< 0.05
ADC_{TL} (%)	$95.9^{a} \pm 0.3$	$94.1^{b} \pm 0.2$	$95.5^{ab} \pm 0.6$	<0.05
ADC_{TAG} (%)	$99.9^{a} \pm 0.0$	$99.6^{b} \pm 0.0$	$99.9^{a} \pm 0.0$	<0.0001
ADC_{PL} (%)	79.3 ± 1.9	73.3 ± 1.9	67.1 ± 7.6	ns
$ADC_{16:0}$ (%)	$99.5^{a} \pm 0.1$	$97.6^{c} \pm 0.1$	$99.0^{b} \pm 0.1$	<0.0001
$ADC_{18:1n-9}$ (%)	$99.7^{ab} \pm 0.1$	$99.8^{a} \pm 0.1$	$99.6^{b} \pm 0.1$	<0.05
$ADC_{18:2n-6}$ (%)	$99.8^{ab} \pm 0.1$	$99.9^{a} \pm 0.0$	$99.7^{b} \pm 0.1$	<0.05
$ADC_{18:3n-3}$ (%)	99.7 ± 0.1	99.8 ± 0.1	99.6 ± 0.1	ns
$ADC_{20:5n-3}$ (%)	99.4 ± 0.3	99.7 ± 0.1	99.9 ± 0.0	ns
$ADC_{22:6n-3}$ (%)	99.6 ± 0.1	99.5 ± 0.1	99.7 ± 0.1	ns

Each value is the mean (± SEM) of three replicates
Means with the same letter are not significantly different (Tukey-Kramer HSD multiple comparison)

Table 8
Immune response of Atlantic salmon fed diets containing different oil sources (no significant differences)

Parameter	Diet		
	CO	CTH	CFO
Neutrophil ($mg.ml^{-1}$)	0.465 ±0.026	0.519 ±0.021	0.485 ±0.080
Lysozyme ($\mu g.ml^{-1}$)	3.45 ± 0.29	3.36 ± 0.43	3.24 ± 0.41
Antiprotease (µl plasma for 50% inhibition)	1.14 ± 0.08	1.40 ± 0.09	1.29 ± 0.16
Total protein ($mg.ml^{-1}$)	43.9 ± 4.2	45.0 ± 4.7	54.6 ± 1.2
Total immunoglobulin ($mg.ml^{-1}$)	14.1 ± 2.9	20.5 ± 4.3	21.3 ± 4.6
Glucose ($\mu mol.ml^{-1}$)	7.87 ± 1.27	6.97 ± 1.11	8.41 ± 1.16

Each value is the mean (± SEM) of three replicates
Initial samples (mean ± SE, n=4): neutrophil 0.405 ± 0.038 $mg.ml^{-1}$; lysozyme 3.43 ± 0.57 $\mu g.ml^{-1}$; antiprotease 1.10 ± 0.09 µl serum for 50% inhibition; total protein 44.2 ± 3.8 $mg.ml^{-1}$; total immunoglobulin 19.0 ± 3.1 $mg.ml^{-1}$; glucose 6.82 ± 0.9 $\mu mol.ml^{-1}$.

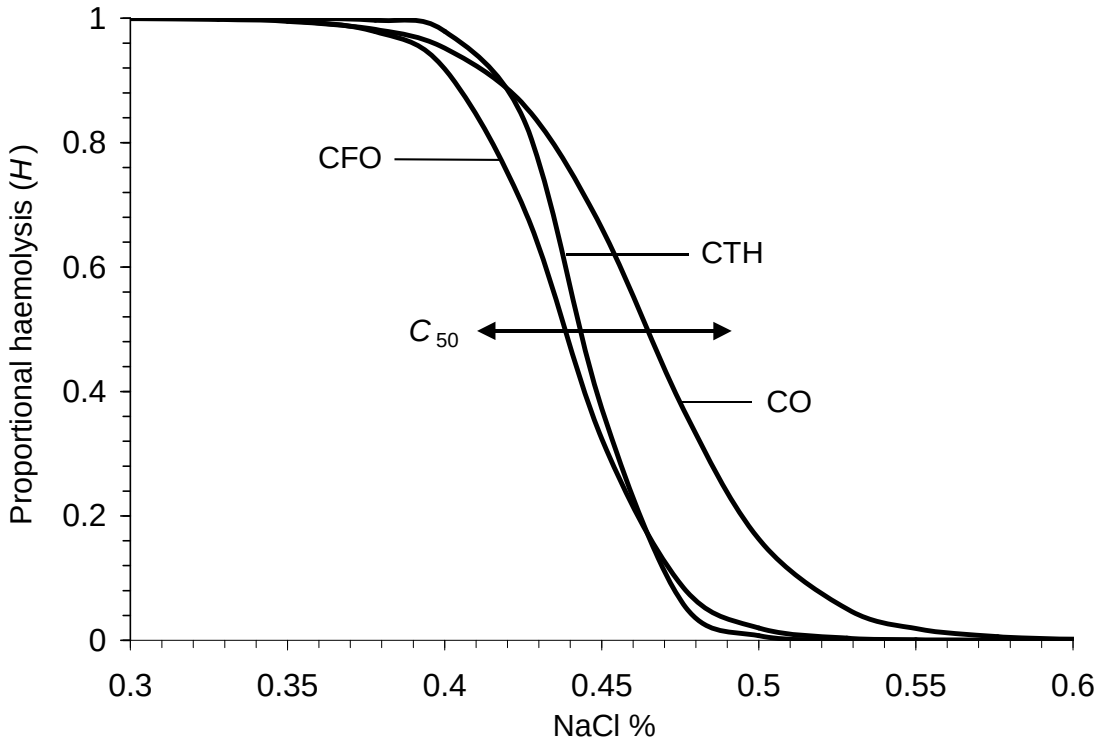

Figure 1

Osmotic erythrocyte fragility curve of Atlantic salmon fed diets containing different oil sources. Curves are marked for each diet (CFO, CO, CTH), with the point where 50% haemolysis occurs marked (C_{50}). No significant differences ($P > 0.05$) were found between diets.

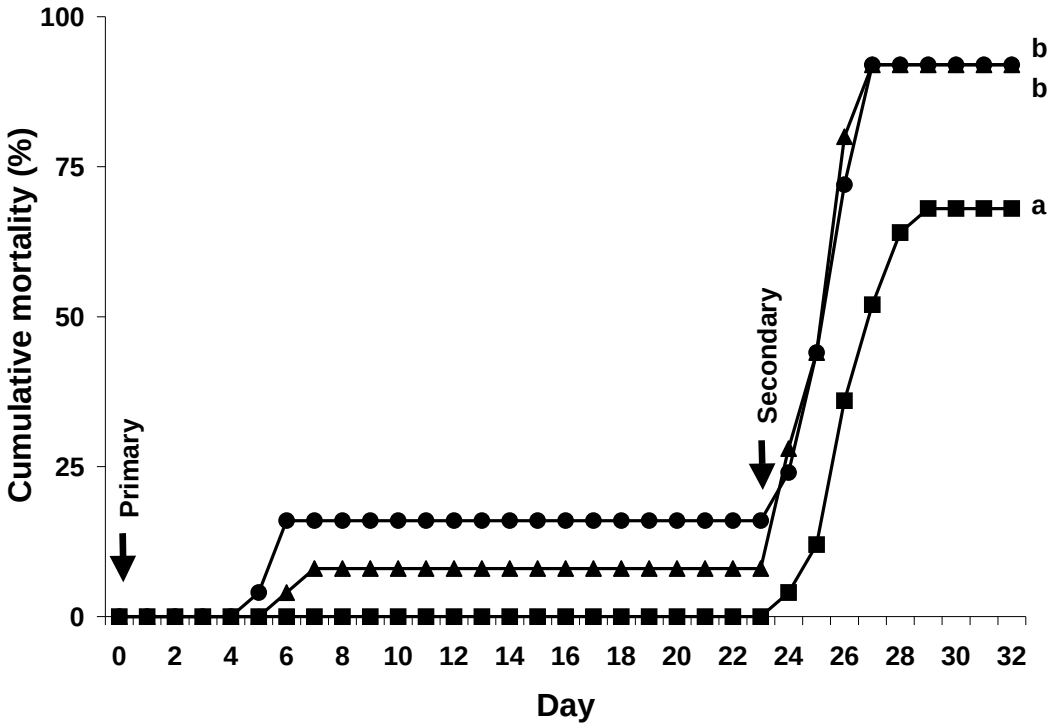

Figure 2

Cumulative mortality (%) observed in Atlantic salmon fed diets containing different oil sources CFO (ν), CO (λ) or CTH (σ) for 51 days and then challenged with *Vibrio anguillarum* (Serovar 01). Arrows indicate points of primary (15‰ salt water, 3×10^5 cells.ml^{-1}) and secondary (35‰ salt water, 2.8×10^6 cells.ml^{-1}) challenges. Superscripts denote significant differences ($P < 0.05$) between diets.

7.9
Comparison of cholestane and yttrium oxide as digestibility markers for lipid components in Atlantic salmon diets

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Abstract

The study aimed to test the similarity between apparent digestibility coefficients (ADC) calculated using either yttrium oxide or cholestane. Atlantic salmon were fed three diets containing a different mix of oil sources: diets contained only canola oil, canola oil and fish oil or canola oil and thraustochytrid meal (a marine microorganism with potential as an alternative oil source). Both markers were concentrated by the same amount in the faeces compared to the diets. ADC values using cholestane tended to be higher than Yttrium, and there was a significant marker effect for crude protein, energy, phosphorous, total lipid and triacylglycerol. Although statistically significant the marker effect was due to numerically small differences in ADC values and probably of limited biological significance. ADC values for the majority of fatty acids did not show marker effects. Consequently, it was concluded that the broad similarity in the digestibility of lipid components between markers validated the use of cholestane for calculating lipid digestibility in Atlantic salmon.

Keywords: Digestibility; Inert marker; Cholestane; Yttrium oxide; Atlantic salmon, Thraustochytrid, Fish oil replacement

Introduction

As intensive aquaculture continues to expand so does the requirement for high quality protein and oil sources (Hardy, 1996; Naylor *et al.*, 2000). Fish oil is a major and increasingly expensive component of commercial aquaculture feeds and its replacement has been the focus of recent research on Atlantic salmon (Bell *et al.*, 1997). Plant oils have been investigated (Bell *et al.*, 1989; Bell *et al.*, 1993; Tocher *et al.*, 2000) and although microbial ingredients have received less attention some have potential as alternative oil sources in aquaculture feeds (Lewis *et al.*, 1999). For example, thraustochytrids are marine microheterotrophs that have both a high lipid content and extremely high concentrations of docosahexaenoic acid (Lewis *et al.*, 1999).

In order to fully explore the potential of alternative oil sources information on digestibility of different components is needed. A variety of inert markers are available to calculate digestibility (Austreng, 1978; Sigurgisladottir *et al.*, 1992; Kabir *et al.*, 1998; Austreng *et al.*, 2000). Chromic oxide has been used in the majority of studies in aquaculture but its suitability as an inert marker is now in question (Ringø, 1993; Austreng *et al.*, 2000). Cholestane has advantages in relation to measuring the digestibility of lipid components, particularly because it is recovered with the lipid components from diets and faeces (Sigurgisladottir *et al.*, 1992). In a previous study on

Atlantic salmon, cholestane was compared with chromic oxide and there were no differences in apparent digestibility of fatty acids using either markers (Sigurgisladottir *et al.*, 1992). The present study compared cholestane with yttrium oxide, a more acceptable digestibility marker than chromic oxide (Austreng *et al.*, 2000), across three combinations of replacement oil sources. The aim was to validate, or otherwise, the conclusion of Sigurgisladottir et al. (1992) that cholestane was a suitable lipid digestibility marker.

Materials and methods

Experimental diets

Three diets were formulated to compare canola oil (CO) as the only source of oil with supplementation by either a thraustochytrid meal (CTH) or fish oil (CFO). The thraustochytrid meal (Lewis *et al.*, 2001) contained protein as well as oil so the casein and soybean meal contents were reduced to account for this and the diets formulated to be isonitrogenous and isoenergetic (Table 1), but to have different fatty acid compositions (Table 2). Chilean fish meal was defatted twice using a 2:1 mixture of hexane and ethanol (400 ml 100 g^{-1} fish meal). Soybean meal (ADM Bioproducts, Beenleigh, Qld., Australia); lactic casein (New Zealand Dairy Board, Wellington, New Zealand); wheat gluten (Starch Australasia, Tamworth, NSW, Australia), pre-gelatinised starch (Goodman Fielder, Summer Hill, NSW, Australia) were also used. Fish oil from jack mackerel (Skretting Australia, Cambridge, Tas., Australia) and a domestic source of pure Canola oil were used. Stay-C and Rovimix E50 were supplied by Roche Vitamins Australia (Frenchs Forest, NSW, Australia), vitamins A and D by ICN (Seven Hills, NSW, Australia) and the remaining ingredients by Sigma-Aldrich (Castle Hill, NSW, Australia). Cholestane and yttrium oxide were added as digestibility markers. The diets were manufactured as 3 mm diameter pellets using a California Pellet Mill (CL-2 Lab Mill). Diets were then dried and stored at -20^{o}C.

Apparent digestibility

The experiment was conducted at the School of Aquaculture, University of Tasmania. Atlantic salmon (Salmo salar L.) parr (36.5 ± 5.7 g) were obtained from Springfield Salmon Hatchery (Tas., Australia) and stocked into 300-l conical bottom tanks at 30 fish per tank. These fish were acclimated for 2 weeks. The tanks were held in a constant environment room (temperature, 15.7 ± 0.8^{o}C; photoperiod, 12:12) and a partial freshwater recirculation system (Carter & Hauler, 2000). Water was treated through physical- and bio-filters with a continuous replacement of approximately 20% day^{-1}. Water quality parameters (DO, pH, ammonia, nitrate and nitrite) were monitored to ensure water quality remained well within limits recommended for Atlantic salmon (Wedemeyer, 1996).

At the start of the experiment fish were anaesthetised (50 mg l^{-1}, Benzocaine) and weight and fork length measured. Twenty-five fish were returned to each of nine tanks and distributed to ensure there were no significant differences between initial group mean weight for triplicate groups to be fed one of the 3 experimental diets. The fish were re-weighed every 14 days and the ration adjusted accordingly. A ration of 1.25 mg. g^{-1} initial body weight was supplied twice a day in the morning and afternoon and dispensed over 1 h by automatic belt feeders. The fish were fed in this way each day. Faecal samples were collected in settlement collectors attached to the tanks described above (Cho *et al.*, 1982; Carter & Hauler, 2000). Faeces settled into removable containers that were held in ice over the collection period. Following a period of 30 days feeding the three experimental diets, the salmon were fed experimental diets

containing both digestibility markers, Yttrium oxide (10 g. kg^{-1}) and cholestane (10 g. kg^{-1}), for 7 days. On days 5, 6 and 7, faecal samples were removed from settlement collectors after collection from 1700 to 0900 h, freeze-dried and used in the analysis of yttrium, cholestane and nutrients (see below). The apparent digestibility coefficients (ADC) were calculated using the standard formula ADC (%) = 100 - [100. (% I_{diet} / $\%I_{faeces}$) x ($\%N_{faeces}$ / $\%N_{diet}$) (Maynard & Loosli, 1969), where I is the inert marker and N the nutrient.

Chemical analysis

Standard methods were used to determine dry matter (freeze dry to constant weight); crude protein (N x 6.25. Kjeldahl using a selenium catalyst); total lipid (Bligh & Dyer, 1959), energy (bomb calorimeter: Gallenkamp Autobomb, calibrated with benzoic acid), and ash (AOAC, 1995). Yttrium was analysed using inductively coupled plasma emission spectrometry (ICP-OES) following digestion with nitric acid at 180°C for 48 h.

Analysis of lipids

Total lipids were obtained from all samples using a modified one-phase (chloroform:methanol:water) extraction (Bligh & Dyer, 1959). A portion of the total lipids recovered from the lower chloroform phase was used for fatty acid analyses. This portion was transmethylated by reaction for 1 h at 80°C with methanol:hydrochloric acid:chloroform (10:1:1 v/v/v, 3 ml). After cooling and addition of water (1 ml), the resultant fatty acid methyl esters (FAME) were extracted with hexane:chloroform (4:1 v:v, 3 x 2 ml). For analysis by gas chromatography (GC), samples were diluted with chloroform containing a known concentration of either 19:0 or 23:0 FAME as the internal injection standard. FAME analyses were performed with a Hewlett Packard 5890 GC equipped with a 7673 autosampler, a 50 m x 0.32 mm internal diameter cross-linked methyl silicone fused-silica capillary column (HP5) and flame ionization detector. Peak areas, including cholestane, were recorded and quantified using the software package Millenium 32 v3.05.01 (Waters Corporation, USA). Confirmation of FAME identity was achieved using a GCQ (Thermoquest, USA) GC-mass spectrometer (GC-MS). Component identification was by comparison of retention time and MS data with that obtained for authentic and laboratory standards.

Lipid class data was obtained by analysing a portion of the total lipids using thin layer chromatography – flame ionisation detection (TLC-FID) (Iatroscan Mk V TH-10, Iatron Laboratories, Japan). Aliquots (1 µl) from a known volume of sample extract were applied onto duplicate Iatroscan chromarods using disposable micro-pipettes. Chromarods were developed using a hexane-diethyl ether-acetic acid (60:17:0.2 v/v/v) solvent system in a sealed glass tank containing pre-extracted filter paper. After development, the chromarods were oven-dried at 60°C for 10 min and analysed immediately. Peak areas were recorded and quantified using the chromatography software DAPA (Scientific Software, Kalamunda, WA, Australia). Data for triacylglycerols (TAG), phospholipids (PL), free fatty acids and cholesterol are presented.

Statistical analysis

Mean values are reported ± standard error of the mean (SEM). Percentage data were arcsine transformed prior to analysis. Normality and homogeneity of variance were confirmed and comparison between means was by two-way ANOVA. Statistical

analyses were performed using J.M.P. version 3.2.1 (SAS Institute Inc.). Significance was accepted at probabilities of 0.05 or less.

Results

Both cholestane (34.3 ± 2.0 mg kg^{-1}) and yttrium oxide (34.3 ± 0.2 mg kg^{-1}) had the same concentration in the faeces (paired t-test). However, there was a significant marker effect on the apparent digestibility coefficients for the non-lipid components but there were no dietary effects (Table 3). ADC data obtained using cholestane were significantly higher than ADC data using yttrium. The lipid classes showed a more varied response (Table 3). The ADC for total lipid and TAG showed diet and marker effects where-as ADC for PL showed no effects. ADC for total lipid using cholestane were higher than using yttrium, and the diets were ranked CTH < CFO < CO for both markers (Table 3).

Digestibility of selected fatty acids was higher than 99% (Table 4). The ADC for palmitic (16:0) and arachidonic (20:4 n-6) acids showed marker, diet and interaction effects where-as eicosapentanoic (20:5 n-3) and docosahexaenoic (22:6 n-3) acids showed no effects. The remaining fatty acids showed only diet effects with higher digestibility for the CTH diet. In effect, the differences in ADC (of less than 0.7%) were unlikely to have been of biological significance.

Discussion

The accurate measurement of apparent digestibility of ingredients and whole feeds is of considerable importance in the development of aquafeeds. Measurement in fish is complicated at several levels of the process including methods of collecting faeces (Hajen *et al.*, 1993; Storebakken *et al.*, 1998a; Percival *et al.*, 2001; Vandenberg & De la Noue, 2001) and the choice of inert marker (Sigurgisladottir *et al.*, 1992; Kabir *et al.*, 1998; Austreng *et al.*, 2000; Vandenberg & De la Noue, 2001).

Chromic oxide has been used as an inert digestibility marker for many years and, although it has its supporters, there are a number of studies that suggest it should be used under strictly defined conditions (Austreng *et al.*, 2000). Chromic oxide may also affect intestinal bacterial flora and faecal fatty acid composition (Ringø, 1993). Austreng et al. (2000) made a detailed analysis of several alternative trivalent metal oxides as inert digestibility markers in Atlantic salmon and confirmed the suitability of yttrium oxide. The use of yttrium oxide (along with ytterbium oxide) is becoming standard in the measurement of digestibility of fish diets (Storebakken *et al.*, 1998b; Sugiura *et al.*, 1998; Torstensen *et al.*, 2000) and was consequently selected for use in the present study. Cholestane may have advantages as an inert marker for fatty acid digestibility in fish due to it being a hydrocarbon and therefore recovered in the lipid fraction (Sigurgisladottir *et al.*, 1992). Atlantic salmon showed minimal absorption of cholestane, and there were only small differences between digestibility of fatty acids calculated using either chromic oxide or cholestane (Sigurgisladottir *et al.*, 1992). The issues with the use of chromic oxide made it necessary to compare the use of cholestane with a more appropriate inert marker in order to confirm, or otherwise, the earlier conclusion that cholestane was a suitable inert marker for lipid digestibility because it produced similar results to chromic oxide.

Re-analysis of mean data from Atlantic salmon (Sigurgisladottir *et al.*, 1992) showed that ADC values for a range of fatty acids were significantly higher for chromic

oxide (83.5 ± 5.1%) than for cholestane (80.8 ± 5.8%) (paired t-test, $P < 0.01$). This suggests that there was a relatively lower concentration of cholestane than chromic oxide in the faeces. In the present study there was also a marker effect for the three non-lipid components as well as the major lipid classes. Concentration of the markers in the faeces appeared to be the same and small differences in the dietary cholestane and yttrium oxide concentrations were thought to be the cause of this difference. Although statistically significant the marker effect was due to numerically small differences in ADC values and probably of limited biological significance. For example, pooled mean values for ADC values with yttrium and cholestane, respectively, were 97.0 and 97.6% for total lipid and 92.9 and 94.3% for crude protein (crude protein showed the most significant marker effect). Only two out of the seven major fatty acids showed a marker effect. The broadly similar digestibility values for lipid components suggested both cholestane and yttrium oxide were equally suitable as inert digestibility markers in Atlantic salmon.

In the present study faeces were collected by settlement using a Guelph-type system. Direct comparisons have shown such systems provide higher digestibility values than stripping or dissection (Hajen *et al.*, 1993; Vandenberg & De la Noue, 2001). For example, crude protein digestibility was between 109 and 124% higher when Guelph-type settlement was compared to stripping (Hajen *et al.*, 1993; Vandenberg & De la Noue, 2001). Crude protein digestibility for the current fish meal based diets were similar (within 5%) to other studies on salmonids using indirect collection methods for measuring digestibility (Satoh, 1992; Gomes *et al.*, 1993; Burel *et al.*, 2000). Similarly, lipid digestibility was 103 to 109% higher when Guelph-type settlement was compared to stripping (Spyridakis *et al.*, 1989; Vandenberg & De la Noue, 2001). Lipid digestibility in salmonids from fish oil based feeds range around 92 ± 4% (Arnesen *et al.*, 1995; Bolliet *et al.*, 2000; Storebakken *et al.*, 2000) although there are examples of lower values closer to 80% (Refstie *et al.*, 1998). Polyunsaturated fatty acids (fed as triacylglycerols) had values in Atlantic salmon of 90 to 97% (stripped) (Sigurgisladottir *et al.*, 1992) and 80 to 90% (dissection) (Røsjø *et al.*, 2000), and 87 to 90% in Arctic charr (dissection) (Olsen *et al.*, 1998). Lower digestibility of saturated fatty acids compared to PUFAs has been found with salmonids (Sigurgisladottir *et al.*, 1992; Røsjø *et al.*, 2000), but was not shown in the present study. The majority of studies measuring digestibility of lipid and fatty acids by Atlantic salmon used stripping or dissection. This makes comparison with the present study difficult, although the higher values were predicted. Digestibility of the TAG class was high and approached 100% as did the major fatty acids (the majority of which were in TAGs).

In conclusion, the present study supported the use of either marker with lipid components from a variety of sources. The preference would be for cholestane because it is recovered with the other lipid components.

Acknowledgements

The authors gratefully acknowledge M. Attard, D. Holdsworth and B. Mooney for technical assistance, and the Fisheries Research and Development Corporation (1998/322 and 1997/329) for financial support.

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Table 1

Ingredient and chemical composition of experimental diets (CO, canola oil; CTH, canola oil supplemented with thraustochytrid; CFO, canola oil supplemented with fish oil)

	Diet		
	CO	CTH	CFO
Ingredient composition ($g.kg^{-1}$)			
Defatted fish meal	250.0	250.0	250.0
Soybean meal	56.2	36.5	56.2
Casein	143.2	132.9	143.2
Wheat gluten	100.0	100.0	100.0
Canola oil	169.6	110.5	78.2
Fish oil			91.4
Thraustochytrid meal		100.0	
Pre-gel starch	150.0	150.0	136.2
Vitamin mix (ASV4)[1]	3.0	3.0	3.0
Stay C[2]	3.0	3.0	3.0
Choline chloride	2.0	2.0	2.0
Mineral mix (TM4)[3]	5.0	5.0	5.0
Ca $(H_2PO_4)_2$	21.8	23.0	21.8
α-Cellulose	36.2	24.2	50.0
Bentonite	49.0	49.0	49.0
CMC	9.0	9.0	9.0
Cholestane	1.0	1.0	1.0
Yttrium oxide	1.0	1.0	1.0
Chemical composition ($g.kg^{-1}$ DM)			
Dry matter ($g.kg^{-1}$)	940 ± 2	960 ± 1	901 ± 2
Crude protein	398 ± 3	391 ± 3	410 ± 2
Total lipid	206 ± 4	207 ± 12	194 ± 3
Ash	121 ± 1	130 ± 1	124 ± 3
Gross energy ($MJ.kg^{-1}$ DM)	20.9 ± 3.9	20.8 ± 0.6	20.7 ± 0.6

[1]Vitamin mix (ASV4) to supply per kg feed: 2.81 mg thiamin HCl; 10.0 mg riboflavin; 9.15 mg pyridoxine HCl; 25 mg nicotinic acid; 54.35 calcium D-pantothenate; 750 mg myo-inositol; 0.38 mg d-biotin; 2.5 mg folic acid; 0.03 mg cyanocobalamin; 6250 IU retinol acetate; 2800 IU cholecalciferol; 100 IU DL α-tocopherol acetate; 5 mg menadone sodium bisulphate.

[2]L-Ascorbyl-2-polyphosphate (Stay-C, Roche Vitamins Australia, Frenchs Forest, NSW, Australia)

[3]Mineral mix (TM4) to supply per kg feed: 117 mg $CuSO_4$ $5H_2O$; 7.19 mg KI; 1815 mg $FeSO_4$ $7H_2O$; 307 mg $MnSO_4$ H_2O; 659 mg $ZnSO_4$ $7H_2O$; 3.29 mg Na_2SeO_3; 47.7 mg $CoSO_4$ $7H_2O$.

Table 2
Lipid class and fatty acid composition of experimental diets (CO, canola oil; CTH, canola oil supplemented with thraustochytrid; CFO, canola oil supplemented with fish oil)

	Diet		
	CO	CTH	CFO
Lipid class composition[1] ($g.kg^{-1}$ DM)			
TAG	199	196	186
PL	4.0	8.3	4.0
Free fatty acid	1.0	1.1	2.0
Cholesterol	0.4	0.6	1.0
Fatty acid composition (% fatty acids)			
14:0	0.50	5.55	4.06
16:0	6.64	12.52	11.90
18:0	2.13	1.58	2.78
Total saturates[2]	10.22	21.28	21.62
16:1n-7	0.44	1.37	4.29
18:1n-7	3.13	2.86	3.15
18:1n-9	54.82	39.42	34.25
20:1n-9	1.20	0.73	1.04
Total monosaturates[3]	60.15	44.28	43.39
18:2n-6	22.19	16.27	11.84
20:4n-6	0.04	0.09	0.61
Total n-6[4]	22.39	18.44	12.71
18:3n-3	6.09	4.38	3.81
18:4n-3	0.45	0.14	1.05
20:5n-3	0.51	0.81	9.58
22:5n-3	0.03	0.20	1.01
22:6n-3	0.65	9.93	4.90
Total n-3[5]	7.83	15.61	21.61
n-3/n-6	0.35	0.85	1.70
20:4n-6/20:5n-3	0.05	0.11	0.06
22:6n-3/20:5n-3	1.28	12.26	0.51

[1] calculated from % composition and total lipid.
[2] includes 15:0, 17:0, 20:0.
[3] includes 16:1n-9, 20:1n-7.
[4] includes 18:3n-6, 20:3n-6, 22:5n-6.
[5] includes 20:4n-3.

Table 3
Apparent digestibility coefficients (ADC) for crude protein (N) and energy (kJ), and different lipid class (TL, total lipid; TAG, triacylglycerol; PL, phospholipid) for diets containing different oil sources (CO, canola oil; CTH, canola oil supplemented with thraustochytrid; CFO, canola oil supplemented with fish oil)

Parameter	Diet	Marker			
		Y_2O_3	Cholestane	Factor	P
Non-lipid component					
ADC_N (%)	CO	93.2 ± 0.2	95.1 ± 0.6	Marker	0.0032
	CTH	92.4 ± 0.1	94.0 ± 0.4	Diet	ns
	CFO	93.2 ± 0.5	93.9 ± 0.7	Interaction	ns
ADC_{kJ} (%)	CO	85.9 ± 0.2	89.7 ± 1.2	Marker	0.015
	CTH	84.1 ± 1.1	87.5 ± 1.6	Diet	ns
	CFO	84.0 ± 1.4	85.7 ± 1.6	Interaction	ns
Lipid class					
ADC_{TL} (%)	CO	95.9 ± 0.3	98.5 ± 0.1	Marker	0.0089
	CTH	94.1 ± 0.2	96.6 ± 0.1	Diet	0.0001
	CFO	95.5 ± 0.6	97.7 ± 0.2	Interaction	ns
ADC_{TAG} (%)	CO	99.9 ± 0.0	100 ± 0.0	Marker	0.0167
	CTH	99.6 ± 0.0	100 ± 0.0	Diet	0.0001
	CFO	99.9 ± 0.0	99.7 ± 0.0	Interaction	0.0141
ADC_{PL} (%)	CO	79.3 ± 1.9	82.2 ± 2.2	Marker	ns
	CTH	73.3 ± 1.9	74.8 ± 6.6	Diet	ns
	CFO	67.1 ± 7.6	79.6 ± 4.5	Interaction	ns

Each value is the mean (± SEM) of three replicates

Table 4
Apparent digestibility coefficients (ADC) for selected fatty acid for diets containing different oil sources (CO, canola oil; CTH, canola oil supplemented with thraustochytrid; CFO, canola oil supplemented with fish oil)

Parameter	Diet	Marker			
		Y_2O_3	Cholestane	Factor	P
$ADC_{16:0}$ (%)	CO	99.5 ± 0.1	99.7 ± 0.1	Marker	0.0016
	CTH	97.6 ± 0.1	98.2 ± 0.1	Diet	0.0001
	CFO	99.0 ± 0.1	99.1 ± 0.1	Interaction	0.0364
$ADC_{18:1n-9}$ (%)	CO	99.7 ± 0.1	99.8 ± 0.1	Marker	ns
	CTH	99.8 ± 0.1	99.9 ± 0.1	Diet	0.0004
	CFO	99.6 ± 0.1	99.6 ± 0.1	Interaction	ns
$ADC_{18:2n-6}$ (%)	CO	99.8 ± 0.1	99.9 ± 0.0	Marker	ns
	CTH	99.9 ± 0.0	99.9 ± 0.0	Diet	0.0005
	CFO	99.7 ± 0.1	99.7 ± 0.0	Interaction	ns
$ADC_{18:3n-3}$ (%)	CO	99.7 ± 0.1	99.7 ± 0.0	Marker	ns
	CTH	99.8 ± 0.1	99.8 ± 0.0	Diet	0.0009
	CFO	99.6 ± 0.1	99.6 ± 0.0	Interaction	ns
$ADC_{20:4n-6}$ (%)	CO	99.4 ± 0.1	99.2 ± 0.1	Marker	0.0184
	CTH	99.9 ± 0.1	99.7 ± 0.1	Diet	0.0001
	CFO	99.9 ± 0.1	99.9 ± 0.1	Interaction	0.0002
$ADC_{20:5n-3}$ (%)	CO	99.4 ± 0.3	99.6 ± 0.1	Marker	ns
	CTH	99.7 ± 0.1	99.8 ± 0.1	Diet	ns
	CFO	99.9 ± 0.0	99.9 ± 0.1	Interaction	ns
$ADC_{22:6n-3}$ (%)	CO	99.6 ± 0.1	99.7 ± 0.1	Marker	ns
	CTH	99.5 ± 0.1	99.6 ± 0.1	Diet	ns
	CFO	99.7 ± 0.1	99.7 ± 0.1	Interaction	ns

Each value is the mean (± SEM) of three replicates

7.10
Trial of commercially produced Atlantic salmon extruded feeds containing dehulled lupin

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Abstract

Fish meal was replaced by dehulled narrow-leafed lupin in commercially extruded diets for Atlantic salmon parr. A comparison was made between two methods of oil addition: commercial vacuum oil-coating (diet, COM) and manual oil-soaking (diet, T1). A second comparison was made between T1 and addition of supplementary essential amino acids (diet, T2). Feed intake and growth performance were measured every two weeks over a 10 week period. Over this time there were no statistically significant differences in final weight, weight gain, feed efficiency ratio or survival. Feed intake was significantly higher on COM than on T2. Differences between feed intake of replicate groups explained much of the difference in growth rates. Total feed intake was significantly correlated with total growth ($r = +0.77$; $n = 9$; $P < 0.05$) and the three COM fed groups had the three highest feed intakes.

Keywords: Atlantic salmon; Extruded feeds; Fish meal replacement; Lupin

Introduction

The main aim of this experiment was to minimise the use of fish meal through its replacement by plant meal in an extruded Atlantic salmon (*Salmo salar* L.) feed. Narrow-leafed lupin (*Lupinus angustifolius*) was selected because it is readily available as a dehulled meal in Australia and we had demonstrated good nutrient digestibility in an earlier part of this project. Very little information has been published on the use of lupins in Atlantic salmon feeds (Glenncross, 2001) and majority of research has been undertaken as part of FRDC projects on salmon feeds (Carter, 1998). Narrow-leafed lupin meal was successfully used to replace 25% of fish meal protein in an extruded feed for Atlantic salmon parr growing from 46 to over 110 g (Carter & Hauler, 2000). At this level of inclusion, 21.8% of the diet, the lupin produced similar growth to the soybean and field pea meals. However, at a higher inclusion level of 29.2% there was some reduction in growth performance (Carter & Hauler, 2000).

In comparison to the previous trial (Carter & Hauler, 2000), which used an experimental extruder, the current trial was designed to consider the performance of a commercially produced extruded feed containing commercially available dehulled narrow-leafed lupin meal. Comparisons were made between: a commercially oil-coated dehulled lupin diet; the same dehulled lupin diet that was manually oil-coated prior to feeding; the same manually oil-coated duhulled lupin diet but containing additional lysine and methionine to increase the content of these two essential amino acids. Conditions of relatively high density were used to provide a more realistic assessment of performance under commercial conditions.

Materials and methods

Three diets prepared using the Wenger twin-screw extruder at Skretting Australia (Cambridge, Tas.) were compared (Table 1). All three diets used 200 $g.kg^{-1}$ dehulled narrow-leafed lupin to partially replace fish meal. All diets were manufactured in a normal commercial process in which dry ingredients (ie fish meal, dehulled lupin, wheat, etc) were pelletized as 4 mm pellets. Comparisons were made between three diets: COM, oil-coating was made under vacuum during normal commercial production; T1, oil-coating was performed manually by bathing the diets in warm oil; T2, the original diet-mash used in the extruder but with additional crystaline L-lysine and DL-methionine and followed by manual oil-coating. During manual oil-coating the pellets were soaked in warmed oil at 60°C for approximately 60 min, this process resulted in lower total lipid of T1 and T2 compared with COM (Table 1).

The experiment was conducted at the School of Aquaculture, University of Tasmania. Atlantic salmon parr were obtained from Springfield Salmon Hatchery (Tasmania) and maintained in the experimental system until required. The experimental system consisted of 300-l tanks in a recirculating freshwater system with a 2000-l reservoir and temperature control unit, held undercover and exposed to natural photoperiod (Bransden *et al.*, 2001). Water was treated through physical and bio-filters with a 50% replacement twice a week. The fish were held at a constant temperature ($15.0 \pm 1.0^{o}C$). Water quality parameters (DO, pH, ammonia, nitrate and nitrite) were monitored to ensure water quality remained well within limits recommended for Atlantic salmon (Wedemeyer, 1996). The experiment was conducted during Spring between August and November.

At the start of the experiment the stock fish (36.9 ± 8.1 g: mean ± sd, n = 720) were anaesthetised (50 $mg.l^{-1}$, Benzocaine), individually weighed and randomly allocated to nine tanks in groups of ten until each tank contained eighty fish. The initial mean stocking density was 9.9 ± 0.15 $kg.m^{-3}$. Triplicate groups were fed each diet. Each group of fish was fed by a clockwork belt-feeder at nominal rations of 1.5 to 1.8 % BW day^{-1} divided over two feeds daily. The fish were re-weighed (in bulk) every two weeks and the rations adjusted accordingly. Actual feed intake was measured by collecting uneaten pellets and adjusting the feed intake from the weighed ration accordingly. The main experiment ended after ten weeks when the fish had more than doubled in weight. Fish were fed to excess for a further two weeks and re-weighed at 12 weeks. Detailed measurement of feed intake was not made over this final period.

Statistical analysis

Mean values are reported ± standard error of the mean (SEM). Percentage data were arcsine transformed prior to analysis. Statistical comparison between means was made using ANOVA. Significance was accepted at probabilities of 5 % or less.

Results & Discussion

The salmon generally grew well in the present study and had doubled their initial weight by 7 to 8 weeks with high survival of over 98% for all treatments (Table 2). Detailed analysis of growth performance was conducted after 10 weeks and there were no statistically significant differences in final weight, weight gain or feed efficiency ratio (Table 2). The trial was continued for 12 weeks but no statistically significant

differences became apparent although the COM treatment fish had trebled their initial weight by this time (Fig. 1).

The SGR of the COM treatment was 1.47 %/day (Fig. 3) which was similar to the SGR in the only other study with lupins on Atlantic salmon using extruded feeds (Table 3). Inclusion of 22 and 29% lupin in extruded diets resulted in SGR of 1.43 % d^{-1} for both diets. In this study the salmon maintained similar growth rates on the higher lupin diet by increasing feed consumption from 1.4 to 1.7 % BW d^{-1} (Carter & Hauler, 2000) which decreased FER from 97 to 78% (Table 3). In the present study the FER was higher (at 120%) and partly explained by the lower feed intake of 1.12 ± 0.01 % BW d^{-1} (calculated as total feed intake g DM per fish per gram mid-weight per day). A diet with a markedly higher lupin content of 44% was fed to Atlantic salmon under similar conditions to the present experiment (Bransden *et al.*, 2001). Although the SGR, of 1.1 % d^{-1}, was lower than in the studies discussed above there was no difference between the lupin and fish meal control diet in this experiment (Bransden *et al.*, 2001). Data on maximum inclusion levels of dehulled lupin is not available for Atlantic salmon but research on rainbow trout demonstrated that an inclusion of 40% did not cause a significant difference in growth performance compared with the fish meal control but that 50% inclusion did (Farhangi & Carter, 2001). It is likely that the inclusion level of 44% in the salmon diet was around the maximum possible and may have been above it. However, the use of a restricted ration (1.25% BW d^{-1}) resulted in efficient utilisation of the diet as indicated by the FER of over 1 (Table 3).

The COM diet out performed the two other diets where oil was added manually by soaking and not using commercial vacuum-coating technology. Poorer growth appeared to be largely explained by the lower feed intake of salmon fed the T1 and T1 diets. There was a strong correlation between total feed intake and total growth when all groups were considered individually (Fig. 3). The addition of supplementary essential amino acids did not appear to significantly improve the performance of T2 compared with T1. However, there was a suggestion of a higher mean FER for T2. Also of interest though was a tendency for feed intake of T2 to be lower (Fig. 3). It would be of interest to investigate further the use of supplementary essential amino acids in diets containing lupin.

High stocking density was used in the present experiment so as to make it more similar to commercial hatchery operations. The initial density was nearly 10 kg m^{-3} and this increased by three times to around 30 kg m^{-3} at the end of the 12 weeks (Fig. 4).

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Table 1
Ingredient and chemical composition of extruded diets. Ingredients were added to a basal commercial mash (557-560 $g.kg^{-1}$).

	Diet		
	COM	T1	T2
Ingredient composition ($g.kg^{-1}$)			
Dehulled lupin meal	200	200	200
Added fish oil	220	220	220
L-lysine	-	-	2.0
DL-methionine	-	-	1.0
Chemical composition ($g.kg^{-1}$ as is)			
Dry matter*	915	940	880
Crude protein*	401	429	396
Total lipid*	250	240	221
Ash	69	75	70
Gross energy ($MJ.kg^{-1}$ DM)	24.2	25.1	24.4
Amino Acids* (% Protein)			
Arg	8.80	8.81	8.72
His	3.46	3.69	3.24
Met	2.43	2.38	2.33
Ile	4.68	4.78	4.78
Leu	9.00	9.21	9.16
Lys	8.60	8.83	9.28
Phe	5.84	6.05	5.65
Tyr	3.86	4.06	3.74
Thr	4.54	4.48	4.41
Val	6.23	6.16	6.21
Ala	5.00	4.95	5.01
Gly	10.69	9.90	9.94
Pro	5.35	5.53	5.63
Ser	4.59	4.44	4.45
Tau	0.40	0.37	0.31
Asp	9.63	9.47	9.85
Glu	6.91	6.88	7.28

* determined by Australian Government Analytical Laboratories

Table 2
The performance of Atlantic salmon fed three extruded diets for 10 weeks

Parameter	Unit	Diet			P
		COM	T1	T2	
Initial weight	(g fish^{-1})	37.2	37.1	36.6	ns
		0.4	0.4	0.1	
Final weight	(g fish^{-1})	104.1	95.5	95.0	ns
		2.7	1.6	6.2	
Weight gain	(g fish^{-1})	67.0	58.4	58.4	ns
		2.4	1.6	6.3	
Total initial weight	(kg)	2.97	2.97	2.93	ns
		0.04	0.03	0.01	
Total final weight	(kg)	8.19	7.55	7.51	ns
		0.25	0.17	0.56	
Total weight gain	(kg)	5.22	4.58	4.58	ns
		0.21	0.18	0.57	
Total feed consumption	(kg DM)	4.35^{a}	3.91^{ab}	3.61^{b}	0.036
		0.13	0.10	0.20	
FER	(g . g^{-1} DM)	1.20	1.17	1.26	ns
		0.02	0.02	0.11	
Overall survival	(%)	98.3	98.8	98.8	ns
		0.2	0.01	0.1	

Each value is the mean (± SEM) of three replicates
Means with same letter are not significantly different (Tukey-Kramer HSD multiple comparison)
FC. feed consumption = total feed consumption (g) / ∑ individual mid-weight (g) / 70 days
FER. Feed efficiency ratio = (total weight gain (g) / total feed consumption (g DM))
* Based on 90% DM for all feeds

Table 3
Comparison of Atlantic salmon performance fed diets containing lupin

Lupin type, Feed type, Inclusion	Temp. (°C)	Initial Weight (g)	SGR (% d^{-1})	FER (g g^{-1})	Reference
N-L, EX, 20%	15.0	37	1.47	1.20	This study
N-L, EX, 22%	15.7	46	1.43	0.97	Carter & Hauler, 2000
29%			1.43	0.78	
N-L, PP, 30%	14	39	2.97	1.16	Farhangi & Carter, unpub.
N-L, PP, 44%	15	36	1.18	1.07	Bransden *et al*., 2001

N-L, narrow-leafed lupin; EX, extruded feed; PP, pellet-press feed

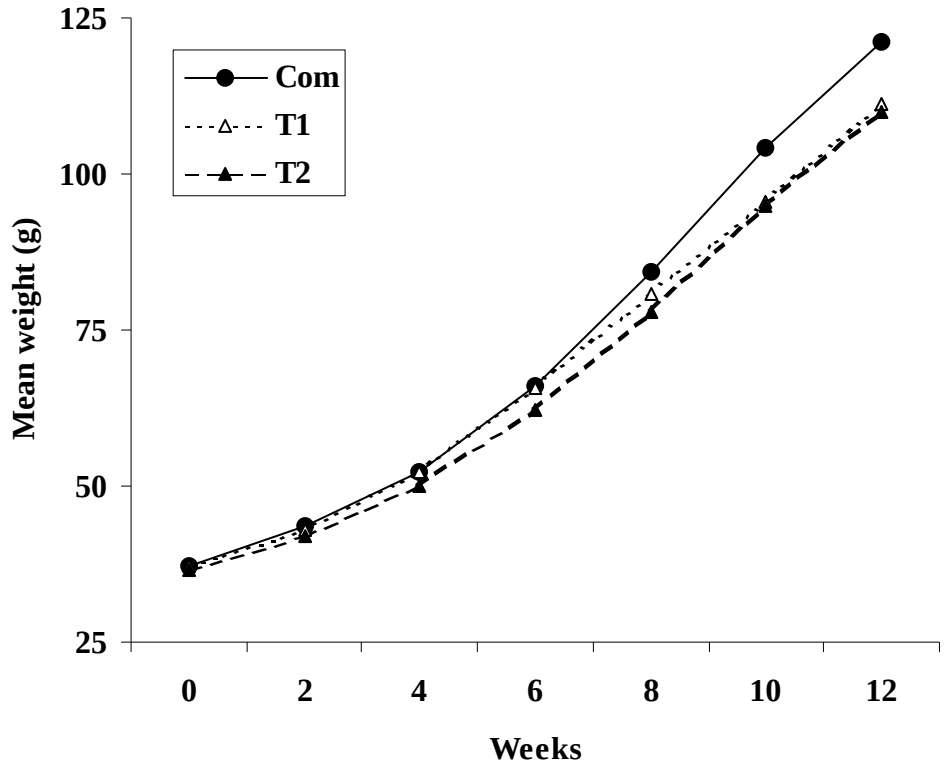

Figure 1
Increase in mean individual wet weight of Atlantic salmon fed three extruded diets for 12 weeks.

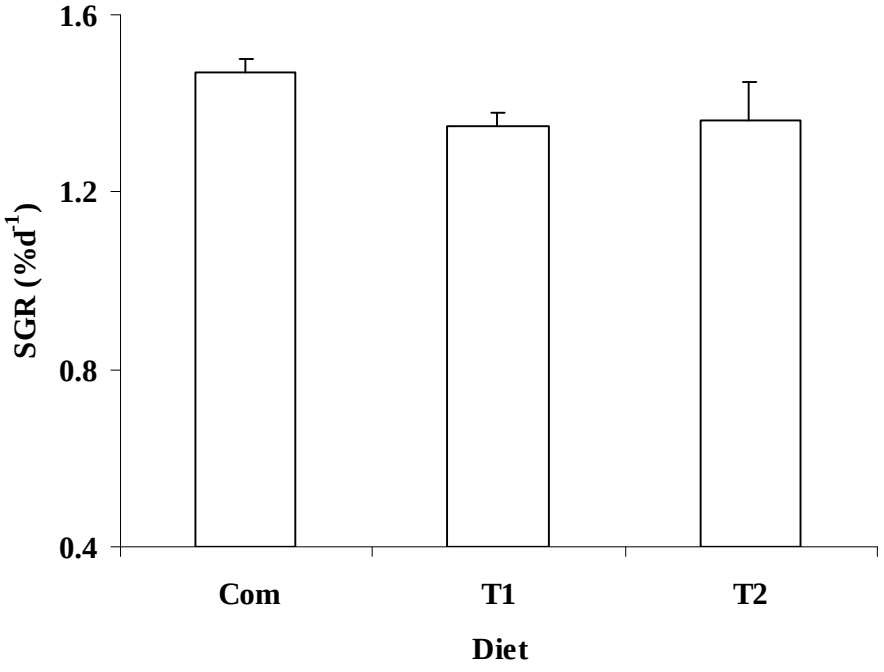

Figure 2
The specific growth rate of Atlantic salmon fed three extruded diets for 10 weeks.

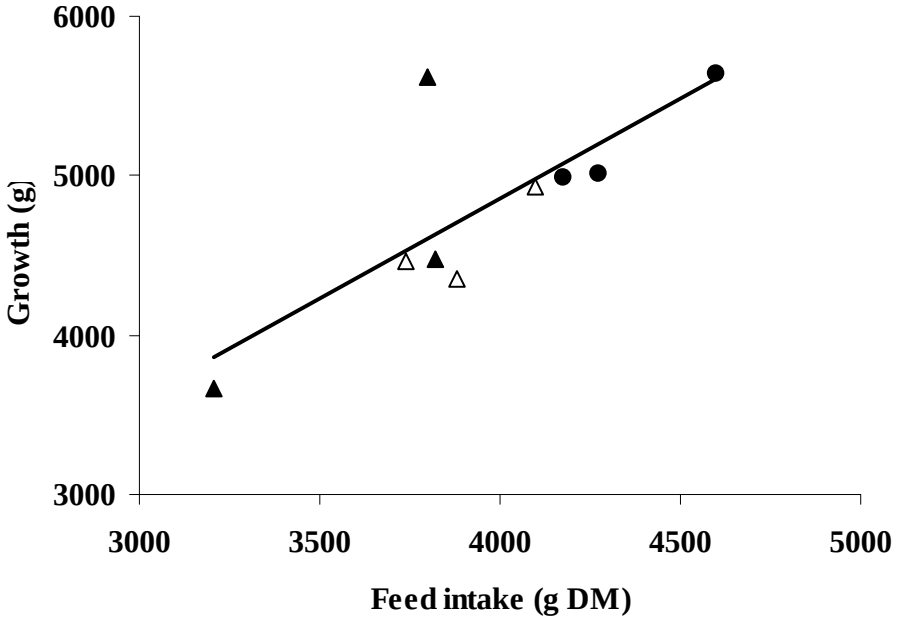

Figure 3
The relationship between feed intake and growth for individual groups of Atlantic salmon fed three extruded diets for 10 weeks (r = +0.77; n = 9; P < 0.05). (●, COM; ^, T1; ▲, T2)

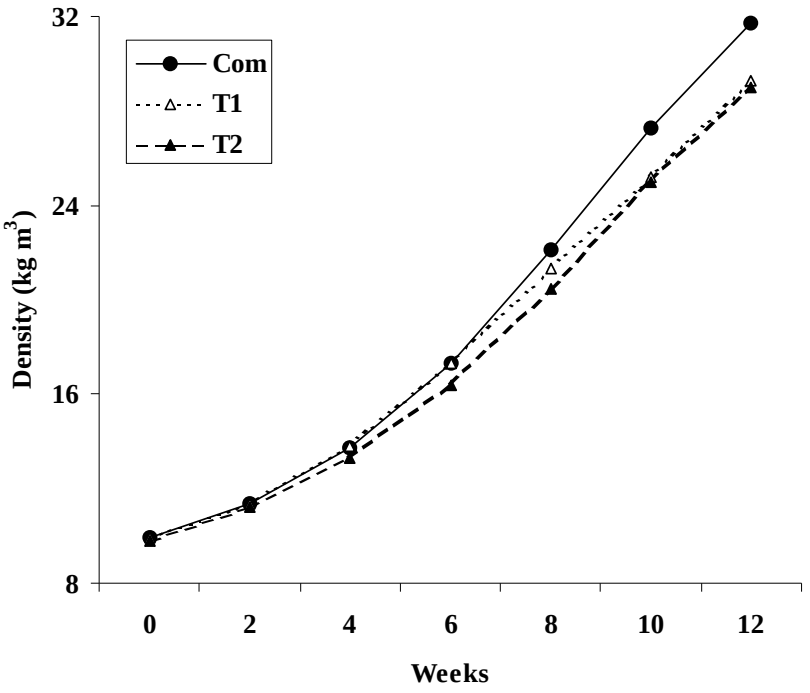

Figure 4
The change in density of groups of Atlantic salmon fed three extruded diets for 12 weeks.

8. Benefits

The major direct benefits of the research are to the Aquafeed industry and Atlantic salmon farming sectors of Australian aquaculture. The original application allocated the flow of benefits to the Fishery (Aquaculture) as 92% to Tasmania salmonid growers and 8% to Victorian trout growers (ie 55% and 5% of the total flow of benefits). The balance of recent salmonid production would suggest a flow of 78.4% (of the benefits to the fishery / aquaculture sector only) to Tasmania, 18.6% to Victoria and also 3% to NSW and South Australia. Of the aquafeed manufactures the principle beneficiary is Skretting Australia who were industry partners in the research. However, availability of the research data in the preliminary 1 year report and in this report to other aquafeed producers means they will also receive considerable benefit. Benefits to feed (ingredient) millers and primary producers were 3 and 1%, respectively, and underestimated the contribution since the research has developed the potential for lupin and field peas. Revised flow of benefits would be 50% to the Fishery (39% to Tasmania; 9% to Victoria; 2% to NSW and South Australia) and 50% to Non-fishery (44% to aquafeeds; 4% to feed millers; 2% to primary producers).

9. Further Development

Atlantic salmon and rainbow trout feeds are extruded and are likely to continue to contain high levels of oil and moderate levels of high quality protein. Given this and the move away from rendered animal products there is a real need for the further development of plant protein meals that have higher protein contents, balanced amino acid composition and lower carbohydrate contents. Further development of fish oil replacement would also be valuable. The replacement of fish meal and fish oil are part of understanding the nutritional physiology of Atlantic salmon at high temperatures. Future research needs to focus on the feed and nutritional requirements, the interactions between nutrition and health and the growth dynamics of sea water salmon held at high (extreme) summer water temperatures.

10. Conclusion

The conclusions are written in relation to the objectives.

1. Expand the database for feed intake, digestibility and utilisation of key nutrients and feed ingredients (principally protein and fat sources) to ensure the optimum balance is used to formulate Atlantic salmon feeds.

   Nineteen ingredients were identified in conjunction with the industry partner as being of most importance for use in aquafeeds. The chemical composition (dry matter, crude protein, crude lipid, ash and gross energy), first limiting amino acid, amino acid score and the apparent digestibility coefficients for crude protein, eighteen amino acids and energy were determined for all of these ingredients. Ingredients that caused a decrease in feed intake at 30% inclusion were identified and tested again at 15% inclusion.

   Particular attention was paid to defining the amino acid requirements in order to ensure a correct basis for an optimum balance of ingredient use. Detailed research into the current literature on amino acid requirements of fish demonstrated major

problems with the information and resulted in a review of the lysine requirements of fish. This review developed into a detailed investigation to define the lysine requirements of Atlantic salmon in relation to the growth performance of the fish. This research enabled more accurate formulation of aquafeeds based on understanding the lysine required for growth and using ingredients selected from the first part of the project.

2. Determine the lowest level of fish meal that can be used by combining alternative protein sources and to investigate the factors limiting inclusion of the most promising of these combinations.

   Several strategies were used to meet this objective. Feed intake was considered the most important factor limiting their inclusion because it reflected the combination of effects of other nutritional factors. Lowing inclusion of single ingredients from 30 to 15% increased the feed intake considerably and was considered one strategy for using alternative protein sources in combination with fish meal. The next step was to select a limited number of important alternative sources that were considered to have the most commercial potential. Corn gluten or dehulled lupin were included with dehulled soybean (the plant protein used most often to replace fish meal in salmon diets) at 15% inclusion levels for each plant protein. This strategy tended to increase the feed intake of the diets. The third strategy was to test selected ingredients in complete diets and compare either directly with a fish meal control diet or compare to expected growth (see Objective 3).

3. Determine whether at low fish meal inclusion salmon performance is equivalent or better than high fish meal diets.

   Dehulled lupin was selected as the best ingredient for more detailed examination of fish meal replacement. Several experiments were carried out to investigate replacement. Approximately 45% of the fish meal protein was replaced by either dehulled lupin (44% of the diet) or by dehulled lupin and feather meal at a ratio of 1:1. Growth performance was the same as on the fish meal control. Dehulled lupin added at 30% into diets with either 40 or 45% total crude protein resulted in the same growth performance as compared with the fish meal control diets. In the final experiment dehulled lupin was included in a commercially extruded feed at 20% inclusion (see Objective 4)

4. To use the research results to formulate feeds for testing under commercial type conditions.

   Dehulled narrow leafed-lupin meal was selected by the Skretting Australia to test in a commercial type diet. As a result of the complexities of least-cost formulation, ingredient composition and meeting nutrient requirements an extruded feed that contained 20% dehulled lupin meal was produced. This contained 25% fish oil and oil (above the fraction in the fish meal) was added post extrusion by vacuum coating. The feed was used under commercial type conditions at the School of Aquaculture and supported excellent growth rates.

5. To successfully transfer these results to ingredient producers, feed manufacturers, salmon and trout farmers and the scientific community.

Several presentations were given at industry forums including Aquafest (Hobart 2000) and the First and Second Industry Workshops for the FRDC sub-program on Atlantic Salmon (Appendix 3). The summary data on ingredient composition and digestibility was provided to aquafeed producers as a report in 1998. The research was presented at several scientific conferences including Annual International Conference of the World Aquaculture Society (Sydney, 1999), the IXth International Symposium on Fish Nutrition and Feeding (Japan, 2000), Second International Symposium on the Cultivation of Atlantic Salmon (Norway, 2001) (Appendix 3).

11. References

O'Sullivan, D. & Dobson, J. (2001). Status of Australian Aquaculture in 1998/99. *Austasia Aquaculture* Trade Directory 2001, pp. 3-19.

Appendices

Appendix 1: Intellectual Property

Data presented in the report are freely available for use in feed formulations by Australian feed manufacturers.

Appendix 2: Staff

1998-99	Mr Matthew Foale	Higher Education Officer, Level 4 School of Aquaculture Tasmanian Aquaculture and Fisheries Institute University of Tasmania
1999-00	Mr Michael Attard	Higher Education Officer, Level 4 School of Aquaculture Tasmanian Aquaculture and Fisheries Institute University of Tasmania

Appendix 3: Publicity, Presentations and Publications

Publicity

ABC National Radio. Interview on project and salmon feeds for Country Hour (20-Aug-98).

Reports of research in international trade press.

Feed Tech 3(5): 33-35. Page 34, mentions research on use of grains in aquaculture species at University of Tasmania.

Feed Tech 3(7): 46-48. Page 47, mentions ingredient evaluation using *in vitro* digestibility based on salmon and tuna digestive enzymes.

Presentations

Bransden, M.P., Carter, C.G., & Nowak, B.N. 1999. Atlantic salmon (*Salmo salar*) as a surrogate species for the development of an artificial diet for the southern bluefin tuna (*Thunnus maccoyii*). Annual International Conference of the World Aquaculture Society, Sydney, Australia. p99.

Carter, C.G., Hauler, R.C. & Bransden, M.P. 1999. Measurement of protein synthesis in the study of aquaculture nutrition. Annual International Conference of the World Aquaculture Society, Sydney, Australia. p133.

Farhangi, M. & Carter, C.G. 1999. Assessment of Australian grain legumes as fish meal replacements in diet for rainbow trout, *Oncorhynchus mykiss.* Annual International Conference of the World Aquaculture Society, Sydney, Australia. p252

Hauler, R.C. & Carter, C.G. 1999. Phytase treatment of soybean meal increases appetite of Atlantic salmon (*Salmo salar* L.) parr. Annual International Conference of the World Aquaculture Society, Sydney, Australia. p316.

Ward, H., Quinn, K., Lucas, A., Dyer, A., Degger, B., Richardson, N., Carter, C., Carragher, J., Soole, K. & Upton, Z. 1999. Applications of insulin-like growth factors in aquaculture. Annual International Conference of the World Aquaculture Society, Sydney, Australia. p801.

Houlihan, D.F., Carter, C.G., & Conçeicão, L. 1999. Protein synthesis in fish. Annual International Symposium of the Fisheries Society of the British Isles, St.Andrews, Scotland. Environment, Development and Growth of Fishes, p39. [Plenary Paper]

Bransden, M.P., Carter, C.G., & Nowak, B.F. 2000. Effects of dietary protein source on growth, immune function and disease resistance of Atlantic salmon, *Salmo salar* L., parr. IXth International Symposium on Fish Nutrition and Feeding, Miyazaki, Japan. May 21-25.

Bransden, M.P., Carter, C.G., & Nowak, B.F. 2000 Does replacement of marine products with alternative products and oils in feeds for Atlantic salmon, *Salmo salar*, affect immune response. Aquafest Australia, Hobart, Tas. October 6-8.

Carter, C.G., Foale, M., Hauler, R.C., Bransden, M.P., & Ward, D.A. 2000. Nutrition of Tasmanian Atlantic salmon, *Salmo salar* L. Aquafest Australia, Hobart, Tas. October 6-8

Farhangi, .M, & Carter, C.G. 2000. Growth, physiological and immunological responses of rainbow trout to different inclusion levels of dehulled lupin (*Lupinus angustifolius*) in the diet as fish meal replacements', IXth International Symposium on Fish Nutrition and Feeding, Miyazaki, Japan. May 21-25.

Hauler, R.C., & Carter, C.G. 2000. Recent advances in estimating the lysine requirement for Atlantic salmon, *Salmo salar* L. Aquafest Australia, Hobart, Tas. October 6-8.

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Hauler, RC, Carter, CG 2000. Lysine deposition responds linearly to energy intake in Atlantic salmon (*Salmo salar* L) parr. IXth International Symposium on Fish Nutrition and Feeding, Miyazaki, Japan. May 21-25.

Carter, C.G., Bransden, M.P., Lewis, T., Attard, M., Nichols, P.D. 2001. The potential of thraustochytrids as a replacement for fish oil in feeds for Atlantic salmon, *Salmo salar* L. International Symposium on the Cultivation of Atlantic Salmon II. Bergen, Norway.

Carter, C.G. 2001. How do fish use protein from feeds? FRDC, Atlantic Salmon First Aquaculture Subprogram Scientific Conference and Workshop, Hobart.

Hauler, R.C. & Carter, C.G. 2001. Lysine utilisation in growing Atlantic salmon. FRDC, First Atlantic Salmon Aquaculture Subprogram Scientific Conference and Workshop, Hobart.

Bransden, M.P., Carter, C.G., & Nichols, P.D. 2001. Dietary n-3/n-6 ratio affects composition and disease resistance in salmon. FRDC, First Atlantic Salmon Aquaculture Subprogram Scientific Conference and Workshop, Hobart.

Ward, D. & Carter, C.G. 2001. Mineral and trace element nutrition in salmonids. FRDC, First Atlantic Salmon Aquaculture Subprogram Scientific Conference and Workshop, Hobart.

Carter, C.G. 2002. Progress in Nutrition Research for Tasmanian Grown Atlantic Salmon. FRDC, Second Atlantic Salmon Aquaculture Subprogram Scientific Conference and Workshop, Hobart.

Related Publications (directly linked with 98/322 in bold)

Bransden, M.P., Carter, C.G. & Nowak, B.F. 2001. Effects of dietary protein source on growth, immune function, blood chemistry and disease resistance of Atlantic salmon (*Salmo salar* L.) parr. Animal Science 73: 105-114

Bransden, M.P., Carter, C.G. & Nowak, B.F. (2001). Alternative methods for nutrition research on the southern bluefin tuna, *Thunnus maccoyii*: use of Atlantic salmon *Salmo salar* L. to screen experimental feeds. Aquaculture Research 32 (Suppl. 1):174-181.

Carter, C.G., Bransden, M.P., Lewis, T.E. & Nichols, P.D. 2003. Potential of thraustochytrids to partially replace fish oil in Atlantic salmon feeds. Marine Biotechnology *in press*

Carter, C.G., Lewis,T.E. & P.D. Nichols. 2003. Comparison of cholestane and yttrium oxide as digestibility markers for lipid components in Atlantic salmon (*Salmo salar* L.) diets. Aquaculture *in press*

Hauler, R.H. & Carter, C.G. 2001. Re-evaluation of the quantitative lysine requirements for fish. Reviews in Fisheries Science 9:133-163

Hauler, R.H. & Carter, C.G. 2001. Lysine deposition responds linearly to lysine intake in Atlantic salmon *Salmo salar* L. parr. Aquaculture Research 32 (Suppl. 1):147-156.

Farhangi, M. & Carter, C.G. 2001. Growth, physiological and immunological responses of rainbow trout (*Oncorhynchus mykiss*) to different dietary inclusion levels of dehulled lupin (*Lupinus angustifolius*). Aquaculture Research 32 (Suppl. 1):329-340.

Appendix 4.

Summary of Ingredient Data

Ingredient	Product Name	Source
Fish meal - 1	Peruvian Fish Meal	Anchovy / Mackerel, Peru
Fish meal - 2	Petuna FAQ	Petuna Seafoods, TAS
Fish meal - 3	Triabunna Fishmeal	Triabunna, TAS
Blood meal	Blood Meal	Peerless Holdings, VIC
Meat meal - 1	HP Meat Meal	Imported
Meat meal - 2	Provine	Aspen By-products, VIC
Meat meal - 3	Lamb Meal	Fletcher International,NSW
Meat & bone - 1	Meat & Bone	Longford Meatworks, TAS
Meat & bone - 2	Meat & Bone	Peerless Holdings, VIC
Poultry meal	Poultry Meal	Edgell, TAS
Feather meal	Feather Meal	Peerless Holdings, VIC
Canola meal - 1	Extracted Canola Meal	Cargill VIC
Canola – whole	Whole Canola	Gibson's, TAS
Corn – Gluten	Corn Gluten	Imported
Corn – Zein	Zein – Corn	Sigma
Lupin – 1	Dehulled Lupin	Milne Feeds, WA
Lupin – whole	Whole Lupin	Milne Feeds, WA
Soybean – 1	Concentrate	Imported
Soybean – 2	Extracted Soybean Meal	Pivot, TAS
Wheat – Gluten	80% Vital Wheat Gluten	Starch Australasia

Ingredient	**Fish Meal**	
Variety	Peruvian 'Hayduk'	
Batch codes	Fish meal - 1 (P/FM/GB/98)	
Local source	Skretting Australia	
Date	12/98	
Fish species	Atlantic Salmon (parr)	
Diets used	ADC98	
	Content	***Digestible***
Proximate (as is) (g/kg)		
DM	969.7	
N	104.9	95.7
CP (N x 6.25)	655.8	598.6
Crude lipid	110.7	
NFE	72.9	
Ash	160.6	
Energy (MJ/kg)	18.84	17.55
IAA(g/kg)		
Arg	52.74	50.02
His	23.91	22.93
Iso	30.90	30.29
Leu	50.74	49.82
Lys	63.33	62.18
Met	20.77	20.07
Cyc	6.49	5.71
Phe	28.39	27.16
Tyr	22.56	21.77
Thr	31.40	30.12
Try	3.81	3.75
Val	35.89	34.99
Ala	42.59	40.34
Asx	63.14	57.58
Glx	87.25	85.57
Gly	45.77	38.69
Pro	28.02	26.04
Ser	29.49	27.50
Nutritional Indices		
First limiting IAA		Try
IAA score (x requirement)		1.88

Ingredient	**Fish Meal**	
Variety	Fish meal – Petuna FAQ	
Batch codes	Fish meal – 2	
Local source		
Date	12/98	
Fish species	Atlantic Salmon (parr)	
Diets used	ADC98, 99.1, 99.2	
	Content	***Digestible***
Proximate (as is) (g/kg)		
DM	965.8	
N	104.9	92.3
CP (N x 6.25)	655.7	577.1
Crude lipid	87.8	
NFE	75.2	
Ash	181.3	
Energy (MJ/kg)		
IAA(g/kg)		
Arg	59.11	53.85
His	16.34	15.27
Iso	36.2	34.39
Leu	60.64	57.56
Lys	61.97	58.86
Met	26.49	24.68
Cyc	8.11	7.35
Phe	32.9	30.69
Tyr	27.86	26.32
Thr	38.53	35.78
Try	7.41	6.82
Val	40.01	37.64
Ala	51.1	46.42
Asx	76.34	69.31
Glx	121.4	114.1
Gly	56.94	48.16
Pro	38.6	34.55
Ser	42.7	38.27
Nutritional Indices		
First limiting IAA		His
IAA score (x requirement)		2.18

Ingredient	**Fish Meal**	
Variety	Triabunna – Jack Mackerel	
Batch codes	Fish meal – 3	
Local source	Skretting Australia	
Date	12/98	
Fish species	Atlantic Salmon (parr)	
Diets used	ADC98	
	Content	***Digestible***
Proximate (as is) (g/kg)		
DM	975.3	
N	112	111
CP (N x 6.25)	702	694
Crude lipid	123.3	
NFE	52.1	
Ash	122.6	
Energy (MJ/kg)	19.92	19.67
IAA(g/kg)		
Arg	57.19	56.74
His	24.5	24.18
Iso	33.68	33.00
Leu	54.17	53.31
Lys	61.34	61.14
Met	20.15	19.63
Cyc	8.02	8.02
Phe	29.77	29.06
Tyr	24.9	24.21
Thr	34.62	34.13
Try	8.69	8.69
Val	37.83	37.28
Ala	45.84	44.67
Asx	68.23	68.00
Glx	93.1	92.08
Gly	46.53	45.08
Pro	29.49	27.90
Ser	32.04	31.41
Nutritional Indices		
First limiting IAA		Met + Cyc
IAA score (x requirement)		2.77

Ingredient	**Blood Meal**	
Variety	Blood meal	
Batch codes	Blood – meal (P/BM/P/98)	
Source	Peerless Holdings, Vic.	
Date	12/98	
Fish species	Atlantic Salmon (parr)	
Diets used	ADC98	
	Content	***Digestible***
Proximate (as is) (g/kg)		
DM	954.1	
N	140.2	129.5
CP (N x 6.25)	876.6	810.3
Crude lipid	35.1	
NFE	69.9	
Ash	18.4	
Energy (MJ/kg)	21.73	20.80
IAA(g/kg)		
Arg	43.86	40.61
His	54.99	50.87
Iso	15.00	14.78
Leu	113.3	108.64
Lys	80.91	78.96
Met	14.52	13.91
Cyc	13.88	12.38
Phe	64.93	61.92
Tyr	30.45	29.21
Thr	49.22	47.07
Try	6.00	3.81
Val	78.98	75.84
Ala	72.53	69.15
Asx	97.21	92.72
Glx	86.11	83.64
Gly	39.26	36.63
Pro	35.55	32.75
Ser	50.62	47.68
Nutritional Indices		
First limiting IAA		Iso
IAA score (x requirement)		1.64

Ingredient	**Meat Meal**	
Variety	'Daka' meat solubles	
Batch codes	Meat meal – 1 (P/MS/D/98)	
Local source	Skretting Australia	
Date	12/98	
Fish species	Atlantic Salmon (parr)	
Diets used	ADC98	
	Content	***Digestible***
Proximate (as is) (g/kg)		
DM	982.8	
N	106.9	101.3
CP (n x 6.25)	668.2	640.9
Crude lipid	51.8	
	180.9	
Ash	99.1	
Energy (MJ/kg)	19.94	17.20
IAA(g/kg)		
Arg	54.15	52.75
His	11.14	10.53
Iso	18.52	17.95
Leu	39.33	38.49
Lys	30.89	30.28
Met	8.38	8.11
Cyc	3.08	2.31
Phe	19.04	18.20
Tyr	14.29	13.60
Thr	21.53	20.53
Try	4.49	4.12
Val	26.74	25.88
Ala	48.08	46.53
Asx	47.95	42.56
Glx	92.25	90.55
Gly	92.03	88.08
Pro	54.16	52.37
Ser	37.14	34.89
Nutritional Indices		
First limiting IAA		Met + Cyc
IAA score (x requirement)		1.04

Ingredient	**Meat Meal**	
Variety	Provine	
Batch codes	Meat meal – 2 (P/PV/A/99.1)	
Source	Aspen Byproducts, Vic.	
Date	2/99	
Fish species	Atlantic Salmon (parr)	
Diets used	ADC99.1	
	Content	***Digestible***
Proximate (as is) (g/kg)		
DM	990.0	
N	120.8	106.3
CP (N x 6.25)	755.3	664.8
Crude lipid	143.2	
NFE	71.2	
Ash	30.3	
Energy (MJ/kg)	23.71	19.29
IAA(g/kg)		
Arg	58.93	5.34
His	15.06	13.25
Iso	34.02	28.71
Leu	59.37	51.72
Lys	50.37	45.79
Met	12.69	10.45
Cyc	9.75	7.73
Phe	37.12	31.97
Tyr	28.01	23.79
Thr	34.12	29.25
Try	7.32	6.06
Val	39.06	33.64
Ala	44.98	40.88
Asx	65.94	54.62
Glx	89.27	80.60
Gly	70.82	66.75
Pro	38.53	34.24
Ser	33.94	29.09
Nutritional Indices		
First limiting IAA		Arg
IAA score (x requirement)		0.36

Ingredient	**Meat Meal**	
Variety	Lamb meal	
Batch codes	Meat meal – 3 (P/LB/F/99.1)	
Source	Fletcher International, NSW	
Date	2/99	
Fish species	Atlantic Salmon (parr)	
Diets used	ADC99.1	
	Content	***Digestible***
Proximate (as is) (g/kg)		
DM	995.0	
N	83.6	59.1
CP (N x 6.25)	522.6	382.2
Crude lipid	81.3	
NFE	82.2	
Ash	313.9	
Energy (MJ/kg)	15.11	10.52
IAA(g/kg)		
Arg	44.38	32.50
His	10.00	7.98
Iso	17.45	13.56
Leu	35.20	27.71
Lys	30.03	23.84
Met	6.97	5.05
Cyc	7.72	4.77
Phe	19.06	14.70
Tyr	14.84	11.34
Thr	21.88	16.24
Try	4.80	3.72
Val	23.94	18.44
Ala	39.29	28.14
Asx	43.66	31.18
Glx	70.79	53.63
Gly	69.51	46.29
Pro	39.60	26.92
Ser	25.47	17.70
Nutritional Indices		
First limiting IAA		Met + Cyc
IAA score (x requirement)		0.98

Ingredient	**Meat & Bone**	
Variety	Meat & bone meal	
Batch codes	Meat & bone – 1 (P/MB/G/98)	
Local source	Skretting Australia	
Date	12/98	
Fish species	Atlantic Salmon (parr)	
Diets used	ADC98	
	Content	***Digestible***
Proximate (as is) (g/kg)		
DM	977.9	
N	84.3	65.3
CP (N x 6.25)	527.0	425.6
Crude lipid	140.3	
NFE	111.8	
Ash	220.9	
Energy (MJ/kg)	15.80	11.93
IAA(g/kg)		
Arg	44.03	3.45
His	11.61	8.99
Iso	16.02	14.03
Leu	38.54	33.40
Lys	35.44	31.62
Met	8.76	7.85
Cyc	5.57	3.00
Phe	21.36	18.28
Tyr	13.95	11.87
Thr	21.37	17.89
Try	2.95	0.80
Val	27.64	24.17
Ala	44.53	35.90
Asx	43.98	36.04
Glx	67.94	56.38
Gly	80.20	60.21
Pro	43.23	31.97
Ser	26.19	19.92
Nutritional Indices		
First limiting IAA		His
IAA score (x requirement)		0.23

Ingredient	**Meat & Bone**	
Variety	Meat & bone meal	
Batch codes	Meat & bone –2 (P/MB/P/98)	
Source	Peerless Holdings, Vic.	
Date	12/98	
Fish species	Atlantic Salmon (parr)	
Diets used	ADC98	
	Content	***Digestible***
Proximate (as is) (g/kg)		
DM	981.1	
N	85.1	69.4
CP (N x 6.25)	532.0	441.3
Crude lipid	139.8	
NFE	47.7	
Ash	280.5	
Energy (MJ/kg)	18.17	13.56
IAA(g/kg)		
Arg	41.85	34.03
His	8.27	7.30
Iso	18.53	16.73
Leu	37.32	33.31
Lys	26.33	23.14
Met	8.34	7.77
Cyc	8.42	6.27
Phe	21.33	18.63
Tyr	14.92	13.37
Thr	21.4	18.62
Try	4.94	4.27
Val	27.09	23.99
Ala	38.03	30.29
Asx	41.31	33.52
Glx	65.3	56.20
Gly	65.07	46.53
Pro	42.86	33.07
Ser	30.96	26.26
Nutritional Indices		
First limiting IAA		His
IAA score (x requirement)		1.04

Ingredient	**Poultry Meal**	
Variety	Poultry meal	
Batch codes	Poultry – meal (P/P/E/98)	
Local source	Skretting Australia	
Date	12/98	
Fish species	Atlantic Salmon (parr)	
Diets used	ADC98	
	Content	***Digestible***
Proximate (as is) (g/kg)		
DM	965.2	
N	76.8	69.8
CP (N x 6.25)	480.1	450.5
Crude lipid	416.7	
NFE	40.0	
Ash	63.2	
Energy (MJ/kg)	26.18	23.89
IAA(g/kg)		
Arg	41.25	39.65
His	9.26	8.58
Iso	22.58	21.43
Leu	40.31	38.44
Lys	28.46	27.98
Met	9.00	8.97
Cyc	13.72	10.77
Phe	23.51	22.18
Tyr	16.41	15.65
Thr	23.69	22.12
Try	4.30	4.54
Val	29.89	27.99
Ala	32.34	30.79
Asx	42.24	36.43
Glx	65.07	63.46
Gly	46.80	43.74
Pro	38.91	35.93
Ser	37.60	34.52
Nutritional Indices		
First limiting IAA		His
IAA score (x requirement)		1.23

Ingredient	**Feather Meal**	
Variety	Feather meal	
Batch codes	Feather meal (P/FM/P/98)	
Source	Peerless Holdings, Vic.	
Date	12/98	
Fish species	Atlantic Salmon (parr)	
Diets used	ADC98	
	Content	***Digestible***
Proximate (as is) (g/kg)		
DM	976.7	
N	120.9	104.3
CP (N x 6.25)	755.6	651.9
Crude lipid	115.4	
NFE	103.5	
Ash	25.5	
Energy (MJ/kg)	23.22	18.58
IAA(g/kg)		
Arg	64.19	58.93
His	3.84	2.75
Iso	43.99	40.66
Leu	72.25	65.60
Lys	19.3	15.64
Met	6.17	5.16
Cyc	36.95	28.33
Phe	45.49	41.29
Tyr	27.22	23.70
Thr	42.36	33.85
Try	4.16	3.12
Val	67.75	61.84
Ala	38.57	34.63
Asx	55.37	41.92
Glx	88.82	78.63
Gly	65.93	58.78
Pro	85	75.01
Ser	105.41	94.43
Nutritional Indices		
First limiting IAA		His
IAA score (x requirement)		0.39

Ingredient	**Thraustochytrid**
Variety	ACEM 6063
Batch codes	ACEM 6063
Source	Tom Lewis
Date	2000
Fish species	Atlantic salmon (parr)
Diets used	CTH (7.6)

	Content	***Digestible***		***Content***
Proximate (as is) (g/kg)			**Fat fractions (g/kg)**	
DM	979			
N	33		18:3 n-3	0.23
CP (N x 6.25)	207		18:2 n-6	0.14
Crude lipid	589		20:4 n-6	0.53
NFE	119		20:5 n-3	2.63
Ash	85		22:6 n-3	92.21
Energy (MJ/kg)	20.7			
IAA(g/kg)				
Arg	14.6			
His	5.3			
Iso	9.1			
Leu	16.9			
Lys	13.0			
Met	5.5			
Cyc				
Phe	10.4			
Tyr				
Thr	10.9			
Try	2.6			
Val	20.9			
Ala				
Asx				
Glx				
Gly				
Pro				
Ser				
Nutritional Indices				
First limiting IAA				
IAA score (x requirement)				
n-3/n-6				

Ingredient	**Canola**	
Variety	Dehulled canola meal	
Batch codes	Canola meal – 1 (P/C/G/98)	
Local source	Skretting Australia	
Date	12/98	
Fish species	Atlantic Salmon (parr)	
Diets used	ADC98	
	Content	***Digestible***
Proximate (as is) (g/kg)		
DM	961.6	
N	56.6	38.4
CP (N x 6.25)	353.7	240.0
Crude lipid	56.5	
NFE	528.3	
Ash	61.5	
GE (MJ/kg)	17.60	7.44
IAA(g/kg)		
Arg	24.67	20.54
His	8.92	6.94
Iso	16.09	13.20
Leu	27.73	23.37
Lys	18.79	15.33
Met	7.51	6.75
Cyc	10.10	7.52
Phe	15.93	12.92
Tyr	12.82	10.36
Thr	18.05	13.63
Try	2.70	1.89
Val	20.60	16.42
Ala	17.23	12.45
Asx	27.84	20.28
Glx	67.67	60.60
Gly	19.00	10.73
Pro	23.82	17.70
Ser	18.82	13.52
Nutritional Indices		
First limiting IAA		Lys
IAA score (x requirement)		0.85

Ingredient	**Canola**	
Variety	Whole canola meal	
Batch codes	P/WC/G/99.1	
Source	Skretting Australia	
Date	2/99	
Fish species	Atlantic Salmon	
Diets used	ADC99.1	
	Content	***Digestible***
Proximate (as is) (g/kg)		
DM	980.2	
N	35.4	34.1
CP (N x 6.25)	221.8	213.1
Crude lipid	414.4	
NFE	326.0	
Ash	37.8	
Energy (MJ/kg)	26.13	21.04
IAA(g/kg)		
Arg	15.72	15.70
His	6.43	6.34
Iso	9.96	9.07
Leu	17.13	15.46
Lys	14.41	14.27
Met	3.69	3.73
Cyc	5.91	4.08
Phe	9.69	8.98
Tyr	7.64	6.98
Thr	11.37	10.36
Try	4.34	4.28
Val	12.52	11.39
Ala	10.79	10.65
Asx	18.39	19.13
Glx	40.46	40.05
Gly	12.23	13.30
Pro	12.93	11.06
Ser	11.89	11.47
Nutritional Indices		
First limiting IAA		Lys
IAA score (x requirement)		0.79

Ingredient	**Corn**	
Variety	Corn gluten	
Batch codes	P/CG/G/98	
Source	Skretting Australia	
Date	12/98, 2/99	
Fish species	Atlantic Salmon (parr)	
Diets used	ADC98, ADC99.1	
	Content	***Digestible***
Proximate (as is) (g/kg)		
DM	952.6	
N	96.4	84.9
CP (N x 6.25)	603.0	530.6
Crude lipid	70.8	
NFE	310.1	
Ash	16.1	
Energy (MJ/kg)	21.25	17.74
IAA(g/kg)		
Arg	22.78	20.73
His	13.38	11.91
Iso	28.43	25.97
Leu	112.37	104.41
Lys	9.16	8.05
Met	16.25	14.83
Cyc	12.21	10.71
Phe	43.27	39.76
Tyr	37.68	34.70
Thr	24.09	21.63
Try	1.52	1.19
Val	30.88	28.10
Ala	59.81	55.04
Asx	41.92	37.48
Glx	142.08	132.65
Gly	18.23	14.49
Pro	59.32	54.45
Ser	40.02	36.24
Nutritional Indices		
First limiting IAA		Lys
IAA score (x requirement)		0.45

Ingredient	**Corn**	
Variety	Corn – Zein	
Batch codes	P/CZ/S/99.1	
Source	Sigma N.S.W	
Date	2/99	
Fish species	Atlantic Salmon	
Diets used	ADC99.1	
	Content	***Digestible***
Proximate (as is) (g/kg)		
DM	983.5	
N	140.0	135.4
CP (N x 6.25)	875.3	846.3
Crude lipid	64.6	
NFE	48.0	
Ash	12.1	
Energy (MJ/kg)	23.28	22.17
IAA(g/kg)		
Arg	15.63	15.98
His	3.20	3.08
Iso	43.51	41.16
Leu	196.54	191.45
Lys	**0.70**	**0.00**
Met	1.52	1.45
Cyc	6.01	0.54
Phe	69.58	66.43
Tyr	54.41	52.51
Thr	30.23	29.04
Try	0.46	0.65
Val	37.30	35.15
Ala	97.27	95.69
Asx	54.26	51.45
Glx	224.74	217.95
Gly	12.00	14.64
Pro	89.51	87.91
Ser	59.34	57.14
Nutritional Indices		
First limiting IAA		Lys
IAA score (x requirement)		0

Ingredient	**Lupin**	
Variety	Dehulled lupin meal (*Lupinus angustofolius*)	
Batch codes	Lupin Meal – 1 (P/DL/G/99.1)	
Source	Skretting Australia	
Date	2/99	
Fish species	Atlantic Salmon	
Diets used	ADC99.1	
	Content	***Digestible***
Proximate (as is) (g/kg)		
DM		
N	61.2	59.1
CP (N x 6.25)	382.6	369.4
Crude lipid	98.1	
NFE	495.5	
Ash	23.8	
Energy (MJ/kg)	18.52	11.68
IAA(g/kg)		
Arg	48.69	49.18
His	11.44	11.09
Iso	17.61	17.12
Leu	27.67	26.64
Lys	16.24	15.69
Met	2.84	3.09
Cyc	5.55	5.54
Phe	16.62	16.17
Tyr	16.77	16.66
Thr	15.31	15.11
Try	2.56	2.74
Val	16.57	16.20
Ala	13.32	13.69
Asx	40.82	40.56
Glx	81.73	81.11
Gly	16.25	17.34
Pro	15.57	15.17
Ser	22.53	22.35
Nutritional Indices		
First limiting IAA		Lys
IAA score (x requirement)		0.87

Ingredient	**Lupin**	
Variety	Whole lupin meal (*Lupinus angustofolius*)	
Batch codes	Lupin – whole (P/L/G/99.1)	
Source	Skretting Australia	
Date	2/99	
Fish species	Atlantic Salmon (parr)	
Diets used	ADC99.1	
	Content	***Digestible***
Proximate (as is) (g/kg)		
DM	951.8	
N	54.0	52.7
CP (N x 6.25)	338.0	329.4
Crude lipid	89.7	
NFE	546.9	
Ash	25.4	
Energy (MJ/kg)	18.16	11.93
IAA(g/kg)		
Arg	43.38	44.49
His	8.94	8.86
Iso	14.90	14.60
Leu	23.42	22.98
Lys	15.43	15.57
Met	2.24	2.26
Cyc	3.95	4.04
Phe	14.17	13.71
Tyr	13.41	13.33
Thr	12.41	12.48
Try	3.15	3.16
Val	13.99	13.74
Ala	11.31	11.61
Asx	35.22	35.77
Glx	73.16	73.34
Gly	13.95	15.54
Pro	14.28	12.94
Ser	18.78	18.67
Nutritional Indices		
First limiting IAA		Met + Cyc
IAA score (x requirement)		0.63

Ingredient	**Pea**	
Variety	Field pea meal (*Pisium sativum*: Dunndale)	
Batch codes	(ADC97 Pea PC)	
Source	Goodman Fielder, NSW	
Date	1997	
Fish species	Atlantic Salmon	
Diets used	ADC97	
	Content	***Digestible***
Proximate (as is) (g/kg)		
DM		
N		
CP (N x 6.25)		
Crude lipid		
NFE		
Ash		
Energy (MJ/kg)		
IAA(g/kg)		
Arg	50.38	49.41
His	7.43	7.23
Iso	18.82	18.22
Leu	32.42	31.52
Lys	28.75	28.02
Met	4.07	3.90
Cyc	5.92	5.53
Phe	21.96	21.27
Tyr	15.83	15.37
Thr	16.96	16.49
Try	N/A	
Val	20.76	19.99
Ala	18.77	17.99
Asx	49.65	49.17
Glx	78.10	76.67
Gly	18.24	17.50
Pro	17.70	16.55
Ser	23.78	23.00
Nutritional Indices		
First limiting IAA		Met + Cyc
IAA score (x requirement)		0.94

Ingredient	**Soybean**	
Variety	Concentrate - Soycomil	
Batch codes	Soybean –1 (P/SbM/ADM/98)	
Source	Gibson's (ADM) N.S.W	
Date	12/98	
Fish species	Atlantic Salmon (parr)	
Diets used	ADC98	
	Content	***Digestible***
Proximate (as is) (g/kg)		
DM	962.4	
N	96.2	93.4
CP (N x 6.25)	601.5	584.3
Crude lipid	56.1	
NFE	277.1	
Ash	65.3	
Energy (MJ/kg)	17.82	14.15
IAA(g/kg)		
Arg	51.89	51.52
His	16.88	16.41
Iso	32.95	31.6
Leu	52.66	52.10
Lys	45.10	45.82
Met	9.25	9.41
Cyc	10.24	9.48
Phe	34.66	33.98
Tyr	25.99	25.97
Thr	29.55	28.68
Try	5.57	5.58
Val	34.16	33.74
Ala	29.39	28.26
Asx	78.20	75.49
Glx	122.02	122.07
Gly	29.40	26.37
Pro	32.25	30.60
Ser	38.48	37.22
Nutritional Indices		
First limiting IAA		Met + Cyc
IAA score (x requirement)		1.89

Ingredient	**Soybean**	
Variety	Extracted soybean	
Batch codes	Soybean – 2 (P/SbM/P/99.1)	
Source	Skretting Australia	
Date	2/99	
Fish species	Atlantic Salmon (parr)	
Diets used	ADC99.1	
	Content	***Digestible***
Proximate (as is) (g/kg)		
DM	975.8	
N	74.0	75.6
CP (N x 6.25)	462.6	472.5
Crude lipid	58.3	
NFE	417.7	
Ash	61.4	
Energy (MJ/kg)	17.81	15.91
IAA(g/kg)		
Arg	37.56	39.34
His	12.45	12.56
Iso	23.45	23.25
Leu	38.33	37.76
Lys	30.50	31.26
Met	5.11	5.44
Cyc	8.04	8.22
Phe	25.12	24.92
Tyr	18.58	18.58
Thr	20.95	20.89
Try	4.76	5.01
Val	24.37	24.33
Ala	21.05	21.72
Asx	57.40	59.14
Glx	88.76	89.44
Gly	20.60	23.01
Pro	23.08	22.68
Ser	27.40	27.70
Nutritional Indices		
First limiting IAA		Met + Cyc
IAA score (x requirement)		1.37

Ingredient	**Wheat**	
Variety	Wheat gluten – 80% CP	
Batch codes	Wheat – gluten (P/WG8/ST/98)	
Source	Starch Australasia, NSW.	
Date	12/98	
Fish species	Atlantic Salmon (parr)	
Diets used	ADC98	
	Content	***Digestible***
Proximate (as is) (g/kg)		
DM	969.6	
N	124.6	123.4
CP (N x 6.25)	779.0	778.5
Crude lipid	67.8	
NFE	146.6	
Ash	6.6	
Energy (MJ/kg)	20.97	18.80
IAA(g/kg)		
Arg	30.5	30.76
His	12.62	11.79
Iso	31.67	32.48
Leu	57.34	58.60
Lys	10.32	12.06
Met	13.54	13.88
Cyc	19.84	19.88
Phe	41.89	42.29
Tyr	30.88	31.40
Thr	23.86	24.43
Try	3.57	3.67
Val	34.21	35.06
Ala	21.84	21.74
Asx	27.77	28.70
Glx	306.00	307.93
Gly	28.51	27.01
Pro	99.53	98.77
Ser	46.15	46.07
Nutritional Indices		
First limiting IAA		Lys
IAA score (x requirement)		0.67