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**Fish meal replacement by plant meals in extruded feeds for
Atlantic salmon, *Salmo salar* L.**

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Abstract

The replacement of fish meal protein with soybean meal (SB) or protein concentrates made from narrow leafed lupin (LP) or field peas (PP) was investigated in extruded feeds for Atlantic salmon. Salmon (47 g) were fed for 63 days on extruded feeds containing each of the plant meals to replace 25 and 33% of the fish meal protein and performance compared against a nutritionally balanced control and a commercial salmon feed formulation (extruded under the same conditions). There were no significant differences in weight gain between the control and feeds containing the plant proteins. The commercial feed produced significantly higher weight gain than the control feed and LP at both replacement levels. Feed consumption was significantly higher for LP at 33% but there were no other significant differences between the other feeds. Feed efficiency ratio and productive protein value were highest for PP and SB and not affected by inclusion level whereas they were significantly lower for LP at 33% inclusion. The weight gain and feed efficiency ratio data showed that soybean meal and pea protein concentrate had the best potential for replacing at least 33% of the fish meal protein in extruded salmon feeds and that lupin protein concentrate was less well utilised at the higher inclusion level. These results support the use of processed plant meals as important replacement protein sources for fish meal in extruded feeds for Atlantic salmon.

Keywords: Aquaculture feeds; Alternative protein sources; Atlantic salmon; Fish meal replacement; Extruded feeds

1. Introduction

As intensive aquaculture continues to expand so does the requirement for high quality protein sources (Barlow, 1989; Hardy, 1996). Fish meal is a major and increasingly expensive component of commercial salmon and trout feeds and numerous studies have investigated the potential of alternative protein sources. The majority of published research on the use of plant proteins in salmonid feeds has focused on the inclusion of soybean meals in feeds for rainbow trout (Hardy, 1982; Tacon et al., 1983; Murai et al., 1989; Pongmaneerat and Watanabe, 1993b; Watanabe and Pongmaneerat, 1993; Watanabe et al., 1993; Oliva-Teles et al., 1994; Kaushik et al., 1995; Olli and Krogdahl, 1995) and, to a lesser extent, for salmon species (Hardy, 1982, 1995, 1996; Arnesen et al., 1989; Carter et al., 1994; Olli et al., 1994a, 1994b, 1995; Refstie et al., 1998). Soybean (dehulled and solvent extracted) meal has a relatively high protein content and also a good balance of essential amino acids and is now widely used to replace some fish meal in salmonid feeds. Less attention has been paid to other plant proteins and although they offer considerable potential they are also associated with negative qualities such as low protein content, less than ideal amino acid balance and the presence of anti-nutritional factors. Their use in high protein / high energy extruded salmon feeds is likely to be limited to processed ingredients with a high protein and lower carbohydrate content.

Field pea and narrow leafed lupin have been shown to have potential for inclusion in Atlantic salmon feeds (Carter, 1998). These experiments used pellets produced by cold pressing in an experimental pellet mill. Since the method of pelletisation has a major influence on the nutritional characteristics of feeds it is important to ensure that ingredients identified as having potential in aquaculture feeds are incorporated into experimental diets that reflect current commercial processing (Bangoula et al., 1993; Gouveia et al., 1993; Pongmaneerat and Watanabe, 1993a; Watanabe and Pongmaneerat, 1993; Oliva-Teles et al., 1994; Olli and Krogdahl, 1995). The aim of the current experiment was to assess the potential of plant protein sources in extruded salmon feeds and with particular emphasis on the use of protein concentrates made from ingredients with low protein contents using air fractionation. Field pea and narrow leafed lupin cultivars were selected as the best candidates for the production of protein concentrates. These were compared with a dehulled and solvent extracted soybean meal, as this is most often used as a plant alternative to fish meal in Atlantic salmon feeds. The plant ingredients were included at two levels to replace 25 and 33% of the protein supplied by fish meal in order to provide an indication of their potential in commercial Atlantic salmon feeds. The pea protein concentrate had the lowest protein content and this determined the maximum replacement of fish meal that maintained the overall feed specifications. All feeds (including a commercial formulation) were made using an experimental extruder configured to match the commercial extruder used to make the majority of Atlantic salmon feeds used in the region.

2. Materials And Methods

2.1. *Experimental diets*

Goodman Fielder Ltd (Summer Hill, NSW, Australia) dehulled, milled and used air separation to produce a narrow leafed lupin (Lupinus angustifolius: Gungurra cultivar) protein concentrate (LP) and a pea (Pisium sativum: Dunndale cultivar) protein concentrate (PP) (Evans, 1998). Feed grade dehulled and solvent extracted soybean (Glycine max) meal (SB) was supplied by Millmaster Feeds (Enfield, NSW, Australia); DL-methionine and α -cellulose by Sigma and bentonite by Commercial Minerals (Granville, NSW, Australia). The remaining ingredients were supplied by Gibson's Ltd (Cambridge, Tas., Australia) and were those used in commercial salmon feeds. Feeds were formulated to contain either a 25 or 33% replacement of fish meal protein from each of the plant meals (Table 1). The diets were formulated, principally, to be isonitrogenous and isoenergetic but consideration was given to the equivalence of other components in the following order of priority: crude fat, NFE and ash. DL-methionine was added to ensure that it was in excess of requirements for salmonids in all diets (NRC, 1993). The control feed contained fish meal as the main protein source with a small amount of protein supplied by wheat flour (12% crude protein) that was added for its binding properties during extrusion. The commercial formulation contained a mix of marine and terrestrial animal and plant meals as the protein sources (chemical composition (g kg^{-1} as is): 925 DM; 430 CP; 262 total fat; 94 ash; 23 MJ GE kg^{-1}). The feeds were manufactured using a twin screw extruder model APV MFP40 (APV-Baker, Peterborough, England) and spray coating of oil following

partial drying (Evans, 1998). Feeds were then dried, bagged and shipped overnight to Tasmania where they were stored at -20°C.

2.2. Growth experiment

The experiment was conducted at the School of Aquaculture, University of Tasmania. Atlantic salmon (Salmo salar L.) parr were obtained from Wayatinah Salmon Hatchery (SALTAS, Tas., Australia) and stocked into 300-l tanks at 21 fish per tank. These fish were acclimated for 2 weeks. The tanks were held in a constant environment room (temperature, $15.7 \pm 0.8^{\circ}\text{C}$; photoperiod, 12:12). The fish were held in a partial freshwater recirculation system. 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 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 was measured. One fish from each tank was killed and 12 used for an assessment of initial chemical composition (see below). The remaining 20 fish were returned to the tanks and distributed to ensure there were no significant differences between group mean weight ($46.6 \pm 0.6 \text{ g}$). The fish were divided into 24 groups so that triplicate groups could be fed one of 8 feeds. The fish were reweighed every 21 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. One day each week total feed consumption (kg DM) was estimated from the amount of feed that was not

eaten and was collected from the settlement collectors. The extruded pellets remained intact prior to collection and uneaten feed was estimated from the number of pellets using the average weight of a pellet for each feed (Helland et al., 1996). For each tank a linear regression of daily feed consumption (g), measured once each week on 8 days over the course of the experiment, against time (day) was determined. All regressions were significant, the lowest R^2 was 0.74 ($P < 0.01$) and 23/24 were above 0.76 ($P < 0.001$). Feed consumption was calculated as the area under the regression. The experiment continued for 63 days when the fish had more than doubled in weight. Specific growth rate (SGR) was calculated as

$$\text{SGR (\% d}^{-1}\text{)} = 100 \times (\ln (W_2 / W_1)) \times d^{-1}$$

where W_1 and W_2 are the weights (g) at two times and d the number of days.

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. Three fish were removed from each tank to determine chemical composition. Fish were killed by transection of the spinal cord after immersion in anaesthetic.

2.3. Apparent digestibility

Apparent digestibility coefficients were measured at the end of the experiment using two tanks from each of the treatments in the growth trial. Faecal samples were collected by settlement (Cho et al., 1982) in settlement collectors attached to the tanks described above. Groups of salmon were fed the experimental feeds containing chromic oxide (10 g. kg^{-1}) for 6 days. On days 5 and 6 faecal samples from two tanks were collected from the settlement collector between 17:00 to 09:00h, freeze-dried and used in the analysis of the marker, chromic oxide, and nutrients (see below). The

apparent digestibility coefficients (ADC) were calculated using the standard formula

$$\text{ADC (\%)} = 100 - [100 \cdot (\% \text{I}_{\text{diet}} / \% \text{I}_{\text{faeces}}) \times (\% \text{N}_{\text{faeces}} / \% \text{N}_{\text{diet}})]$$

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

2.4. Chemical Analysis

Standard methods were used to determine dry matter (freeze dry to constant weight); nitrogen (Kjeldahl using a selenium catalyst); crude fat (Bligh and Dyer, 1959); energy (bomb calorimeter: Gallenkamp Autobomb, calibrated with benzoic acid), ash (AOAC, 1995) and chromium (Furukawa and Tsukahara, 1966).

2.5. Statistical analysis

Mean values are reported \pm standard error of the mean (S.E.M.). Percentage data were arcsin 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.

3. Results

Survival was higher than 96% for all treatments. There were few significant differences in the final weight and weight gain among fish fed the different feeds (Table 2). Mean final weight ranged from 113 g to 127 g for fish fed the fish meal control (CON) and commercial (COM) reference feeds, respectively, and these means were significantly different. Similarly, weight gain ranged from 66 g to 81 g for fish fed the same two feeds. Final weight and weight gain for fish fed the SB and PP diets

were not significantly different from those fed either COM or CON but both LP feeds produced significantly lower weight gain than COM.

Weight gain showed an exponential increase that continued over the period of the experiment and consequently specific growth rates (SGR) were examined for each three week time period over which growth was measured. Repeated-measures ANOVA showed there was no interaction effect between time period and diet on specific growth rate. Differences between feeds became less distinct as the experiment progressed. During the first period SGR was significantly ($P < 0.05$) higher in the groups fed COM ($1.79 \pm 0.07 \% d^{-1}$) than those fed CON ($1.50 \pm 0.05 \% d^{-1}$). There were no significant differences over the second ($P > 0.15$) and third ($P > 0.60$) time periods and the overall SGR (\pm RMSE) were 1.40 ± 0.15 and $1.41 \pm 0.14 \% d^{-1}$, respectively. The differences in final weight and weight gain (Table 2) were therefore explained by differences in the pattern of growth over the first 21 days of the experiment and the relative performance did not change after this.

Feed remained in excess and the rates of feed consumption remained below the maximum ration calculated as $25 \text{ mg. g}^{-1} \text{ initial body weight. d}^{-1}$ over each three week period between weighing the fish (Table 2). Feed consumption, expressed as either total feed or a mean daily weight specific rate (FC), differed little among treatments with only salmon fed LP33 having significantly higher feed consumption (Table 2). The similarity in consumption was also reflected in the daily rates of nutrient uptake when expressed in terms of digestible energy or digestible protein. Daily consumption of digestible energy ($\text{kJ DE. g}^{-1} \text{.d}^{-1}$) was significantly higher for LP33 than for the other diets. Similarly, the consumption of digestible crude protein was higher on

LP33 due to the increased DM consumption. There were no differences between CON and the other experimental diets. However, the higher crude protein content of COM resulted in a higher daily consumption of crude protein.

The differences in feed consumption and growth resulted in there being significant differences in feed efficiency ratio (FER) and productive protein value (PPV) (Table 2). The highest FER was for COM, and the other treatments were ranked SB25, PP25 and PP33, SB33, LP25, CON and LP33 (Table 2). FER for LP33 was significantly lower than for all other diets. PPV was highest for PP33 followed by COM, SB25, SB33, PP25, LP25, CON and LP33. The increased inclusion of SB or PP from 25 to 33% had no significant effect on FER or PPV but there were significant reductions when LP was included at 33%. There was little variation in chemical composition of fish among treatments and mean values were 17-18% for crude protein, 11-12% for total lipid and 2% for ash (Table 3). Since the feed had no effect on chemical composition of fish or feed consumption there was a significant correlation between FER and PPV ($n = 8$; $r = 0.86$; $P < 0.001$).

Feed significantly influenced apparent digestibility of dry matter, crude protein (nitrogen) and energy (Table 4). Crude protein digestibility was significantly lower for COM and CON but there were no significant differences among the plant ingredients and no effect of inclusion level. There were more differences in both dry matter and energy digestibility between the treatments due to the variation in the non-protein energy components of the different ingredients (Table 4).

4. Discussion

The present study demonstrated the potential of three plant meals for inclusion in commercial extruded Atlantic salmon feeds. As well as being of immediate importance for feed production in Australia there is little information in the scientific literature concerning the use of plant proteins in Atlantic salmon feeds, particularly feeds produced under commercial conditions. Since global production of Atlantic salmon is in excess of 460 000 tonnes annually (FAO, 1997) there is considerable benefit related to the replacement of part of the fish meal currently used in feeds.

A significant amount of research has been conducted on the replacement of fish meal with soybean meals as protein sources in feeds for rainbow trout (Hardy, 1982; Murai et al., 1989; Pongmaneerat and Watanabe, 1993b; Watanabe et al., 1993; Oliva-Teles et al., 1994; Kaushik et al., 1995; Olli and Krogdahl, 1995). More recently there has been a focus on Atlantic salmon (Carter et al., 1994; Olli et al., 1994b, 1995; Storebakken et al., 1998). The inclusion of four different soybean products at 5 different inclusion levels (0 to 56% of protein) was investigated in Atlantic salmon (300 g) held in sea cages (Olli et al., 1994b). A soybean concentrate gave the best results and could be added to replace 56 % of the dietary protein, mainly supplied from LT fish meal, without any effect on weight gain. Dehulled and solvent extracted, solvent extracted only, and full-fat soybean meals gave similar weight gain at 14% protein replacement but the results suggested that only the full-fat and concentrate could maintain weight gain at 28% protein replacement (Olli et al., 1994b). Atlantic salmon (90 g) growth and growth efficiency decreased when a dehulled and solvent extracted soybean meal replaced 36% of the fish meal protein (Carter et al., 1994). Similar results were obtained for larger Atlantic salmon (900g)

using dehulled solvent extracted soybean meal (Olli et al., 1995). A 20% protein replacement resulted in similar growth to the control but at 40% protein replacement growth was approximately 20% lower (Olli et al., 1995). In the present study protein replacement at 25 and 33% had no effect on growth or growth efficiency compared to a control diet or commercial formulation. The salmon grew at rates of about 1.5 %·d⁻¹ with feed utilisation efficiencies (FER of about 1 and PPV of above 40%) that were comparable with other broadly similar studies (Carter et al., 1993, 1994; Olli et al., 1994a; Refstie et al., 1998).

In contrast to soybean meal there is less information available on the use of other legumes in feeds for salmonids. Legumes such as peas (Gomes et al., 1993; Gouveia et al., 1993; Pfeffer et al., 1995), lupin (De la Higuera et al., 1988; Hughes, 1988, 1991; Moyano et al., 1992; Bangoula et al., 1993; Gouveia et al., 1993), faba beans (Gouveia et al., 1993; Pfeffer et al., 1995), rapeseed and canola (Teskeredzic et al., 1995; Stickney et al., 1996) and others (Alexis et al., 1985; Alexis, 1990; Moyano et al., 1992; Watanabe et al., 1993; Morales et al., 1994; Sanz et al., 1994) have been used in trout feeds. The inclusion of a co-extruded plant protein made from rapeseed and field peas (Colzapro) had no effect at up to 15% replacement of the protein but at 45% inclusion growth performance of rainbow trout was significantly lower than the control diet (Gomes et al., 1993). The low digestible energy from both raw and autoclaved field peas was predicted to limit their use in rainbow trout feeds (Pfeffer et al., 1995). However, digestibility measurements were made for an unspecified number of days following 4 days adaptation and this period may not have been long enough for complete adaptation of the digestive enzymes (Gouveia and Davies, 1998). Comparison of the crude protein digestibility of the extruded pea protein concentrate,

used in the present study in Atlantic salmon, after 2 and 4 weeks of feeding, showed an increase from 90 to 97 % (Carter et al., 1999).

In the present study, dietary crude protein digestibility values were above 90% and comparison with complete diets supported the observation that pea protein is well digested by salmonids especially following extrusion (Gomes et al., 1993; Gouveia et al., 1993). Crude protein digestibility values of the individual ingredients used in PP33 (Carter et al., 1999) were used to calculate a value for the complete feed. This gave a similar digestibility value to the measured value and confirmed the validity of the measurements. Energy digestibility of legumes is considerably lower than for protein due to their high carbohydrate content. However, extrusion and the use of pea and lupin protein concentrates in the present study explains the higher energy digestibility found in the present study compared to previous studies (Hughes, 1988; Gouveia et al., 1993; Carter, 1998; Gouveia and Davies, 1998). There was no correlation between crude protein digestibility and either energy digestibility or dietary α -cellulose content (added as a non-nutrient bulking agent). This suggested that the addition of a non-digestible carbohydrate did not influence protein digestibility.

Few studies on Atlantic salmon have investigated the use of plant protein sources other than soybean meal. Previous experiments using cold pressed pellets for Atlantic salmon suggested that 40% fish meal protein replacement with pea or lupin protein concentrates was feasible (Carter, 1998). The present research confirmed that at least 33% fish meal protein replacement with pea protein concentrate was possible. The situation was less clear using lupin, partly due to the higher feed intake and lower feed conversion efficiency at the higher lupin inclusion. Except for LP33 (and the

commercial feed that had a higher protein content) the daily intakes of digestible crude protein and energy were not different among the diets. Since feed intake was below the set ration it is of interest that salmon fed LP33 were satiated at higher energy and protein intakes than the other feeds. This suggests feed intake was influenced by factors other than macro-nutrient intake. Analysis of the essential amino acid intake showed that only phenylalanine was present in the protein component at less than the requirement for trout (NRC, 1993) but its content was similar for LP (80-82% requirement) and PP (82-83% requirement).

A major problem in the use of plant meals is their relatively low protein content which prevents them being used commercially as ingredients in current salmon feeds that are typically formulated to contain relatively high protein and oil contents. The protein concentrates used in the present study were produced by air separation and resulted in lupin and pea concentrates with 46 and 49 % crude protein, respectively. This represented an increase of between 44 and 133 % over the protein in the raw lupin and peas, respectively (Pettersen et al., 1997). Protein at this level is comparable to soybean meals but still far lower than fish meals. Plant meals also contain significant amount of carbohydrates that may have detrimental effects on Atlantic salmon performance (Waagbø et al., 1994; Hemre et al., 1995a, 1995b). The use of extrusion is important in increasing the nutrient availability of plant meals especially in relation to increasing the amount of digestible energy available through greater gelatinisation of starch (Watanabe and Pongmaneerat, 1993). However, an increase in digestible energy from carbohydrates does not necessarily result in an increase in growth performance (Pongmaneerat and Watanabe, 1993a) and will depend on the ability of Atlantic salmon to use dietary carbohydrates (Hemre et al., 1995a; Grisdale-

Helland and Helland, 1997). The digestible energy content of the experimental diets used in the present study was similar as was growth and growth performance on most of the experimental feeds. It is possible that the higher levels of non-starch polysaccharides from lupin may partly explain differences. Plant meals also contain various anti-nutritional factors of which trypsin inhibitors are of particular concern (Hendriks et al., 1990; Van den Ingh et al., 1991; Olli et al., 1994a). However, extrusion reduces the efficacy of the majority of these (Pongmaneerat and Watanabe, 1993a; Rumsey et al., 1993).

Extruded salmon feeds that contained up to 27% pea protein concentrate or 22% lupin protein concentrate had no significant effect on the growth performance of Atlantic salmon parr when compared to fish meal and solvent extracted soybean meal, ingredients more often used in salmon feeds. Further improvements in the use of these plant meals are likely to involve their combination with other protein sources rather than as single meals (Watanabe and Pongmaneerat, 1993).

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References

- Alexis, M.N., 1990. Comparative evaluation of soybean meal and carob seed germ meal as dietary ingredients for rainbow trout fingerlings. *Aquat. Living Resour.* 3, 235-241.
- Alexis, M.N., Papaparaskeva-Papoutsoglou, E., Theochari, V., 1985. Formulation of practical diets for rainbow trout (*Salmo gairdneri*) made by partial or complete substitution of fish meal by poultry by-products and certain plant by-products. *Aquaculture* 50, 61-73.
- AOAC, 1995. Official Methods of Analysis of AOAC International. AOAC International, Arlington, VA.
- Arnesen, P., Brattas, L.E., Olli, J., Kroghdahl, Å., 1989. Soybean carbohydrates appear to restrict the utilization of nutrients by Atlantic salmon (*Salmo salar* L.). In: Takeda, M. and Watanabe, T. (Eds.), *The Current Status of Fish Nutrition in Aquaculture (Proceedings of the Third International Symposium on Nutrition and Feeding in Fish)*. Tokyo University of Fisheries, Toba, Japan, pp. 273-280.
- Bangoula, D., Parent, J.P., Vellas, F., 1993. Nutritive value of white lupin (*Lupinus albus* var luto) fed to rainbow trout (*Oncorhynchus mykiss*) - effects of extrusion cooking. *Reprod. Nutr. Dev.* 33, 325-334.
- Barlow, S., 1989. Fish meal - World outlook to the year 2000. *Fish Farmer*, September / October, (1989), pp. 40-43.
- Bligh, E.G., Dyer, W.G., 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* 37, 911-917.
- Carter, C.G., 1998. Fish Meal Replacement in Aquaculture Feeds for Atlantic Salmon. 93/120. Fisheries Research and Development Corporation, Deakin, Australia.
- Carter, C.G., Houlihan, D.F., Buchanan, B., Mitchell, A.I., 1993. Protein-nitrogen flux and protein growth efficiency of individual Atlantic salmon (*Salmo salar* L.). *Fish Physiol. Biochem.* 12, 305-315.
- Carter, C.G., Houlihan, D.F., Buchanan, B., Mitchell, A.I., 1994. Growth and feed utilization efficiencies of Atlantic salmon fed a diet containing supplementary enzymes. *Aquacult. Fish. Manage.* 25, 37-46.
- Carter, C.G., Bransden, M.P., van Barneveld, R.J., Clarke, S.M., 1999. Alternative methods for nutrition research on the southern bluefin tuna, *Thunnus maccoyii*: In vitro digestibility. *Aquaculture* 179, 57-70.

- Cho, C.Y., Slinger, S.J., Bayley, H.S., 1982. Bioenergetics of salmonid fishes: energy intake, expenditure and productivity. *Comp. Biochem. Physiol.* 73B, 25-41.
- De la Higuera, M., Garcia-Gallego, M., Sanz, A., Cardenete, G., Suarez, M.D., Moyano, F.J., 1988. Evaluation of lupin seed meal as an alternative protein source in feeding of rainbow trout (*Salmo gairdneri*). *Aquaculture* 71, 37-50.
- Evans, T.J., 1998. Replacement of Fish Meal in Aquaculture Diets - Feed Processing. Fisheries Research and Development Corporation, Deakin, Australia.
- FAO, 1997. Review of the State of World Aquaculture. FAO Fisheries Circular No. 886 FIRC/C886 (Rev.1). FAO, Rome.
- Furukawa, A., Tsukahara, H., 1966. On the acid digestion method for the determination of chromic oxide as an index substance in the study of digestibility of fish feed. *Bull. Jpn. Soc. Sci. Fish.* 32, 502-506.
- Gnaiger, E., Bitterlich, G., 1984. Proximate biochemical composition and caloric content calculated from elemental CHN analysis: a stoichiometric concept. *Oecologia (Berlin)* 62, 289-298.
- Gomes, E.F., Corraze, G., Kaushik, S., 1993. Effects of dietary incorporation of a co-extruded plant protein (rapeseed and peas) on growth, nutrient utilization and muscle fatty acid composition of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 113, 339-353.
- Gouveia, A., Davies, S.J., 1998. Preliminary nutritional evaluation of pea seed meal (*Pisum sativum*) for juvenile European sea bass (*Dicentrarchus labrax*). *Aquaculture* 166, 311-320.
- Gouveia, A., Oliva Teles, A., Gomes, E., Rema, P., 1993. Effect of cooking / expansion of three legume seeds on growth and food utilisation by rainbow trout. In: Kaushik, S.J. and Luquet, P. (Eds.), *Fish Nutrition in Practice*. INRA, Paris, pp. 933-938.
- Grisdale-Helland, B., Helland, S.J., 1997. Replacement of protein by fat and carbohydrate in diets for Atlantic salmon (*Salmo salar*) at the end of the freshwater stage. *Aquaculture* 152, 167-180.
- Hardy, R.W., 1982. The use of soybean meal in trout and salmon diets. NOAA Technical Report. NMFS Circular 477, 15-19.
- Hardy, R.W., 1995. Current issues in salmonid nutrition. In: Lim, C. and Sessa, D.J. (Ed.), *Nutrition and Utilization Technology in Aquaculture*. ACCS Press, Champaign, Illinois, pp. 26-40.
- Hardy, R.W., 1996. Alternate protein sources for salmon and trout diets. *Anim. Feed Sci. Technol.* 59, 71-80.

- Helland, S.J., Grisdale-Helland, B., Nerland, S., 1996. A simple method for the measurement of daily feed intake of groups of fish in tanks. *Aquaculture* 139, 157-163.
- Hemre, G.I., Sandnes, K., Lie, Ø., Torrissen, O., Waagbø, R., 1995a. Carbohydrate nutrition in Atlantic salmon, *Salmo salar* L.: growth and feed utilization. *Aquacult. Res.* 26, 149-154.
- Hemre, G.I., Torrissen, O., Krogdahl, Å., Lie, Ø., 1995b. Glucose tolerance in Atlantic salmon, *Salmo salar* L., dependence on adaption to dietary starch and water temperature. *Aquacult. Nutr.* 1, 69-75.
- Hendriks, H.G.C.J.M., van den Ingh, T.S.G.A.M., Krogdahl, Å., Olli, J., Koninkx, J.F.J.G., 1990. Binding of soybean agglutinin to small intestinal brush border membranes and brush border membrane enzyme activities in Atlantic salmon (*Salmo salar*). *Aquaculture* 91, 163-170.
- Hughes, S.G., 1988. Assessment of lupin flour as a diet ingredient for rainbow trout (*Salmo gairdneri*). *Aquaculture* 71, 379-385.
- Hughes, S.G., 1991. Use of lupin flour as a replacement for full-fat soy diets for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 93, 57-62.
- Kaushik, S.J., Craedi, J.P., Lalles, J.P., Sumpter, J., Fauconneau, B., Laroche, M., 1995. Partial or total replacement of fish meal by soybean protein on growth, protein utilization, potential estrogenic or antigenic effects, cholesterolemia and flesh quality in rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* 133, 257-274.
- Maynard, L.A., Loosli, J.K., 1969. *Animal Nutrition*. McGraw-Hill, New York.
- Morales, A.E., Cardenete, G., De la Higuera, M., Sanz, A., 1994. Effects of dietary protein source on growth, feed conversion and energy utilisation in rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* 124, 117-126.
- Moyano, F.-J., Cardenete, G., de la Higuera, M., 1992. Nutritive value of diets containing a high percentage of vegetable proteins for trout, *Oncorhynchus mykiss*. *Aquat. Living Resour.* 5, 23-29.
- Murai, T., Ogata, H., Villaneda, A., Watanabe, T., 1989. Utilization of soy flour by fingerling rainbow trout having different body size. *Nippon Suisan Gakkaishi* 55, 1067-1073.
- NRC, 1993. *Nutrient Requirements of Fish*. National Academy Press, Washington, D.C.
- Oliva-Teles, A., Gouveia, A.J., Gomes, E., Rema, P., 1994. The effect of different processing treatments on soybean meal utilization by rainbow trout, *Oncorhynchus mykiss*. *Aquaculture* 124, 343-349.

- Olli, J.J., Krogdahl, Å., 1995. Nutritive value of four soybean products as protein sources in diets for rainbow trout (*Oncorhynchus mykiss*, Walbaum) reared in freshwater. Acta Agric. Scand. Sect. A, Animal Sci. 44, 185-192.
- Olli, J.J., Hjelmeland, K., Krogdahl, Å., 1994a. Soybean trypsin inhibitors in diets for Atlantic salmon (*Salmo salar* L) - effects on nutrient digestibilities and trypsin in pyloric caeca homogenate and intestinal content. Comp. Biochem. Physiol. 109A, 923-928.
- Olli, J.J., Krogdahl, Å., Van den Ingh, T.S.G.A.M., Brattås, L.E., 1994b. Nutritive value of four soybean products in diets for Atlantic salmon (*Salmo salar*, L.). Acta Agric. Scand. Sect. A, Animal Sci. 44, 50-60.
- Olli, J.J., Krogdahl, Å., Våbenø, A., 1995. Dehulled solvent-extracted soybean meal as a protein source in diets for Atlantic salmon, *Salmo salar* L. Aquacult. Res. 26, 167-174.
- Petterson, D.S., Sipas, S., Mackintosh, J.B., 1997. The Chemical Composition and Nutritive Value of Australian Pulses. Grains Research and Development Corporation, Canberra.
- Pfeffer, E., Kinzinger, S., Rodehutsord, M., 1995. Influence of the proportion of poultry slaughter by-products and of untreated or hydrothermically treated legume seeds in diets for rainbow trout, *Oncorhynchus mykiss* (Walbaum), on apparent digestibilities of their energy and organic compounds. Aquacult. Nutr. 1, 111-117.
- Pongmaneerat, J., Watanabe, T., 1993a. Effect of extrusion processing on the utilisation of soybean meal diets for rainbow trout. Nippon Suisan Gakkaishi 59, 1407-1414.
- Pongmaneerat, J., Watanabe, T., 1993b. Nutritional evaluation of soybean meal for rainbow trout and carp. Nippon Suisan Gakkaishi 59, 157-163.
- Refstie, S., Storebakken, T., Roem, A.J., 1998. Feed consumption and conversion in Atlantic salmon (*Salmo salar*) fed diets with fish meal, extracted soybean meal or soybean meal with reduced content of oligosaccharides, trypsin inhibitors, lectins and soya antigens. Aquaculture 162, 301-312.
- Rumsey, G.L., Hughes, S.G., Winfree, R.A., 1993. Chemical and nutritional evaluation of soya protein preparations as primary nitrogen sources for rainbow trout (*Oncorhynchus mykiss*). Anim. Feed Sci. Tech. 40, 135-151.
- Sanz, A., Morales, A.E., Delahiguera, M., Cardenate, G., 1994. Sunflower meal compared with soybean meal as partial substitutes for fish meal in rainbow trout (*Oncorhynchus mykiss*) diets - protein and energy utilization. Aquaculture 128, 287-300.

- Stickney, R.R., Hardy, R.W., Koch, K., Harrold, R., Seawright, D., Massee, K.C., 1996. The effects of substituting selected oilseed protein concentrates for fish meal in rainbow trout *Oncorhynchus mykiss* diets. J. World Aquacult. Soc. 27, 57-63.
- Storebakken, T., Kvein, I.S., Shearer, K.D., Grisdale-Helland, B., Helland, S.J., Berge, G.M., 1998. The apparent digestibility of diets containing fish meal, soybean meal or bacterial meal to Atlantic salmon (*Salmo salar*): evaluation of different faecal collection methods. Aquaculture 169, 195-210.
- Tacon, A.G.J., Haaster, J.V., Featherstone, P.B., Kerr, K., Jackson, A.J., 1983. Studies on the utilization of full-fat soybean and solvent extracted soybean meal in a complete diet for rainbow trout. Nippon Suisan Gakkaishi 49, 1437-1443.
- Teskeredzic, Z., Higgs, D.A., Dosanjh, B.S., McBride, J.R., Hardy, R.W., Beames, R.M., Jones, J.D., Simell, M., Vaara, T., Bridges, R.B., 1995. Assessment of undephytinized and dephytinized rapeseed protein concentrate as sources of dietary protein for juvenile rainbow trout (*Oncorhynchus mykiss*). Aquaculture 131, 261-277.
- Van den Ingh, T.S.G.A.M., Krogdahl, Å., Olli, J., Hendriks, H.G.C.J.M., Koninkx, J.F.J.G., 1991. Effects of soybean-containing diets on the proximal and distal intestine in Atlantic salmon (*Salmo salar*): a morphological study. Aquaculture 94, 297-305.
- Waagbø, R., Glette, J., Sandnes, K., Hemre, G.I., 1994. Influence of dietary carbohydrate on blood chemistry immunity and disease resistance in Atlantic salmon, *Salmo salar* L. J. Fish Dis. 17, 245-258.
- Watanabe, T., Pongmaneerat, J., 1993. Potential of soybean meal as a protein source in extruded pellets for rainbow trout. Nippon Suisan Gakkaishi 59, 1415-1423.
- Watanabe, T., Pongmaneerat, J., Sato, S., Takeuchi, T., 1993. Replacement of fish meal by alternative protein sources in rainbow trout diets. Nippon Suisan Gakkaishi 59, 1573-1579.
- Wedemeyer, G.A., 1996. Physiology of Fish in Intensive Culture Systems. Chapman and Hall, New York.

Table 1
Ingredient and chemical composition of experimental feeds

	Diet						
	CON	SB25	SB33	LP25	LP33	PP25	PP33
Ingredient composition (g.kg ⁻¹)							
Fish meal	601.5	451.1	400.0	451.1	400.0	451.1	400.0
Soybean meal	0	203.7	272.9	0	0	0	0
Lupin protein concentrate	0	0	0	217.9	291.9	0	0
Pea protein concentrate	0	0	0	0	0	205.8	275.7
DL-methionine	0	3.0	5.0	4.0	6.0	5.0	6.0
Fish oil	154.6	160.0	159.2	157.6	156.4	166.9	168.9
Wheat flour	115.0	115.0	115.0	115.0	115.0	115.0	115.0
Wheat starch	23.2	23.2	23.2	23.2	23.2	23.2	23.2
α-Cellulose	50.0	36.5	17.2	23.7	0	42.7	26.7
Bentonite	48.2	0	0	0	0	0	0
Vitamin & mineral premix ¹	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Chemical composition (g.kg ⁻¹ DM)							
Dry matter (g.kg ⁻¹)	941	948	943	925	910	927	933
Nitrogen	67	66	66	68	68	66	65
Crude fat	263	258	268	272	260	260	258
NFE ²	215	276	266	250	272	284	302
Ash	130	80	80	80	70	70	60
Gross energy (MJ.kg ⁻¹ DM)	21.86	22.66	22.94	22.81	22.65	22.76	22.90
Digestible crude protein	363	368	370	380	381	368	363
Digestible energy (MJ.kg ⁻¹ DM)	19.21	20.16	20.58	20.82	20.79	20.22	20.42

¹ Added to supply in excess of vitamin and mineral requirements for salmonids (NRC, 1993).

² Calculated as the remainder of Crude Protein + Crude Fat + Ash and assuming Crude Protein = 5.85 x N (Gnaiger and Bitterlich, 1984)

Table 2
The performance of Atlantic salmon fed diets containing different protein sources

Parameter	Unit	Diet						P	
		COM	CON	SB25	SB33	LP25	LP33	PP25	PP33
Initial weight	(g)	46.5	46.7	46.4	46.8	46.3	46.4	46.8	46.6
		0.7	0.2	0.4	0.7	0.8	0.5	0.7	0.4
Final weight	(g)	127.3 ^a	113.1 ^b	120.7 ^{ab}	116.9 ^{ab}	114.0 ^b	113.9 ^b	123.4 ^{ab}	118.4 ^{ab}
		2.0	1.7	0.7	4.6	5.2	0.4	2.2	1.9
Weight gain	(g)	80.8 ^a	66.4 ^b	74.3 ^{ab}	70.0 ^{ab}	67.7 ^b	67.6 ^b	76.6 ^{ab}	71.8 ^{ab}
		2.4	1.5	0.3	4.2	4.5	0.9	1.5	2.2
Total feed consumption	(kg DM)	1.458 ^b	1.411 ^b	1.403 ^b	1.425 ^b	1.370 ^b	1.697 ^a	1.467 ^b	1.396 ^b
		0.036	0.064	0.020	0.040	0.066	0.039	0.043	0.028
FC	(mg DM.g ⁻¹ .d ⁻¹)	13.31 ^b	14.59 ^b	13.56 ^b	13.82 ^b	13.79 ^b	17.10 ^a	14.15 ^b	13.65 ^b
		0.20	0.32	0.24	0.29	0.11	0.60	0.04	0.10
FER	(g . g ⁻¹)	1.11 ^{abc}	0.91 ^{de}	1.04 ^{abc}	0.98 ^{bcde}	0.97 ^{bcde}	0.78 ^f	1.01 ^{abcde}	1.01 ^{abcde}
		0.02	0.01	0.02	0.04	0.02	0.03	0.01	0.03
PPV	(%)	41.71 ^{ab}	38.01 ^{bc}	41.54 ^{ab}	41.22 ^{ab}	38.88 ^{ab}	30.23 ^c	40.88 ^{ab}	45.10 ^a
		0.46	1.31	2.88	1.04	1.51	1.03	0.28	0.88
Overall survival	(%)	100	96.7	98.3	100	98.3	98.3	96.7	98.3
			1.67	1.67		1.67	1.67	1.67	1.67

Each value is the mean (\pm S.E.M.) of three replicates

Means with same letter are not significantly different (Tukey-Kramer HSD multiple comparison)

FC. feed consumption = total feed consumption (g DM) / Σ individual mid-weight (g) / 63 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 3
Proximate composition (% wet weight) of Atlantic salmon

Parameter	Diet						P	
	COM	CON	SB25	SB33	LP25	LP33	PP25	PP33
Dry matter	30.6	30.8	29.9	30.6	30.4	30.4	30.7	31.6
	0.6	0.8	1.1	0.2	0.7	0.2	0.1	0.6
Crude protein	18.0	17.9	17.2	17.7	17.8	17.6	17.5	18.4
	0.3	0.2	0.7	0.2	0.2	0.2	0.1	0.4
Total lipid	11.38	11.57	11.41	11.33	11.45	11.41	11.79	12.15
	0.56	0.56	0.54	0.25	0.54	0.23	0.06	0.32
Ash	1.96	2.13	1.99	2.03	1.93	2.03	2.07	2.01
	0.05	0.04	0.14	0.03	0.06	0.09	0.06	0.10

Each value is the mean (\pm S.E.M.) of three replicates (3 fish per replicate)
Initial group (mean \pm sd; n = 11): 28.7 \pm 0.9 % DM; 16.8 \pm 0.5 % crude protein; 10.17 \pm 1.07 % total lipid; 2.69 \pm 0.28 % ash.

Table 4
Apparent digestibility coefficients (%) for dry matter (DM), crude protein (N) and energy (kJ) for diets used and collected using settlement system

Parameter	Diet						P	
	COM	CON	SB25	SB33	LP25	LP33	PP25	PP33
ADCDM	82.75 ^{bc}	76.52 ^e	82.15 ^{cd}	83.05 ^{bc}	84.36 ^{ab}	85.5 ^a	81.52 ^{cd}	82.51 ^{cd}
	0.30	0.71	0.43	0.09	0.17	0.55	0.25	0.33
ADCN	93.62 ^b	92.71 ^b	95.31 ^a	95.86 ^a	95.65 ^a	95.90 ^a	95.22 ^a	95.48 ^a
	0.16	0.38	0.23	0.17	0.03	0.14	0.09	0.29
ADC _{kj}	90.52 ^{abc}	87.86 ^d	88.98 ^{bcd}	89.73 ^{abcd}	91.26 ^{ab}	91.78 ^a	88.84 ^{cd}	89.19 ^{bcd}
	0.34	0.19	0.65	0.01	0.20	0.59	0.20	0.69

Each value is the mean (\pm S.E.M.) of four replicates

Means with the same letter are not significantly different (Tukey-Kramer HSD multiple comparison)

