FRDC FINAL REPORT
(Project No. 93/126)

Development of more cost-effective feeds for the TasmanianAtlantic salmon industry

(May 1996)

Dr. Steve Percival and Mr. Peter Lee

Salmon Enterprises of Tasmania (SALTAS)

CONTENTS

1. PROJECT DETAILS

Project Title:	Development of more cost-effective salmon feeds for the Tasmanian Atlantic salmon industry
Project No:	93/126
Research Organisation:	Salmon Enterprises of Tasmania P/L (SALTAS)
Principal Investigators:	
Name:	Steve Percival
Address:	RSD 755, Allens Rivulet Rd, Allens Rivulet, Tasmania 7150.
Phone: Fax:	(002) 396384 or (002) 976332 (002) 396617 or (002) 976310
Name:	Peter Lee
Address:	Cygnet Rd, Huonville, Tasmania 7109
Phone:	(002) 642596
Funding Corporation:	Fisheries Research and Development Corporation (FRDC)

2. SUMMARY (Non-technical)

This project comprised 5 separate studies.

(i) *Validation of a faecal collection technique for determining apparent digestibility in large (up to 5 kg) Atlantic salmon in sea cages.*

This study was undertaken to assess whether collection of faeces by a stripping method was suitable for measuring digestibility in large Atlantic salmon in sea cages under commercial conditions. Experiments were conducted to determine the significance of a number of factors associated with variable apparent digestibility coefficients (ADCs) when faeces is collected by stripping. Results of these experiments showed that the stripping method was suitable for collecting faeces from these fish for the purpose of calculating apparent digestibility and a robust and practical procedure which takes account of the factors which can cause major variation in data is suggested.

(ii) *Investigation of within day and between day variation in apparent digestibility, with observations on crude fibre as an internal marker.*

This study was undertaken to investigate whether apparent digestibility results determined using the stripping method validated in Study 1 vary within each day and/or between days so that this variation (if present) may be taken into account in digestibility studies. In addition, efficient mechanisms for overcoming such variation were tested during this study so that modifications could be made to the overall technique as appropriate. Results showed that there is variation in apparent digestibility between days and suggest that variation may also occur within each day. Modifications based on results from this study are suggested for the technique developed in Study 1 to overcome this variability.

(iii) *Effect of feeding regime on apparent digestibility results*

The effect of 4 different feeding regimes on apparent digestibility was investigated to determine whether apparent digestibility results derived using one feeding regime can be extrapolated to other feeding regimes and whether the digestibility of the diet may be improved in a commercial situation through manipulation of feeding regime. Short term changes in feeding regime 36 hrs prior to faecal collection were also investigated to assess the potential impact that such changes may have on apparent digestibility results. Within the limits of the feeding regimes tested in this study, it appears that apparent digestibility derived using one feeding regime can be extrapolated to other feeding regimes and there appears to be little potential to improve the digestibility of the diet through manipulation of the feeding regime. However, sudden changes in feeding pattern during digestibility experiments in Atlantic salmon in sea cages may affect apparent digestibility results and these factors must be kept to a minimum during such studies wherever possible.

(iv) *Comparison of apparent digestibility at different stages of the production cycle with further observations on between day variability in apparent digestibility.*

The aim of this study was to compare apparent digestibility at 4 different stages of the production cycle in seawater over a 15 month period with each experiment being conducted over 5 weeks. This was done to investigate whether apparent digestibility determined at one stage can be extrapolated to other stages. Within each stage of the production cycle, apparent digestibility was compared on each of 6 different days over a 12 day period to further evaluate between day variation in results. Significant differences in apparent digestibility were evident between days at all stages of the production cycle tested. Results in large salmon (4.2 kg) in summer were highly variable because many fish became anorexic during the experiment, probably due to a combination of the onset of maturation, high water temperatures and increased handling stress associated with larger fish. Comparison of results between different stages of the production cycle in seawater suggest that apparent digestibility data can be extrapolated across stages, although there may be a slight increase in digestibility during winter.

(v) *Effect of dietary composition on growth rate; apparent digestibility; relative weights of abdominal components; and histology and electron microscopy of the gastrointestinal tract.*

The aim of this study was to compare the growth performance, abdominal composition and apparent digestibility of 3 commercially available Atlantic salmon feeds. Histology and electron microscopy of the gastrointestinal was performed to determine physiological factors that may have contributed to differences in performance. Growth was highest in fish fed the extruded diet with 30% fat, however, the steam pelleted diet with 17% fat performed better than the extruded diet with the same fat content. This is contrary to expectation and may have been associated with reduced intake of the extruded diet. Apparent digestibility was higher for the extruded diets than for the steam pelleted diet and higher in diets with higher fat content. The weight (expressed as a percentage of body weight) of the proximal intestine (includes pyloric caecae and pyloric fat) was higher in fish fed the extruded diet with 30% fat than in fish fed the steam-pelleted diet with 17% fat, however, there were no other differences in abdominal composition between diets. There were no differences found in the histology or electron microscopy of the gastrointestinal tract between different diets.

3. BACKGROUND

Fish feed is the single largest component cost of farming Atlantic salmon in Tasmania, and as such is an area where considerable savings could be made. Small changes in food conversion ratio (FCR) and/or growth rates can significantly affect the cost of production.

Unfortunately, Tasmanian salmon farmers are competing with a much larger overseas Atlantic salmon farming industries, which are supplied by equally large fish feed companies (e.g. BP Nutrition, Skretting and Ewos). These companies, in co-operation with other research bodies, are able to invest considerable funds to research for producing better, more efficient diets. While some insight can be gained by following overseas trends in Atlantic salmon feeds, much of this information is not available to the Tasmanian industry. It is therefore, essential that the Tasmanian industry undertake strategic nutritional research which has the potential to reduce costs of production so that it may remain competitive into the future.

Information on apparent digestibility coefficients (ADCs) is fundamental in developing least-cost feeds using alternative feed ingredients, determining nutrient requirements and in assessing the effects of ingredient quality, manufacturing process and storage conditions on the nutritive value of the diet and therefore in, minimising the impact of feed usage on the environment.

By far the biggest potential for savings is in the seawater phase of the production cycle. However, there is no published information on apparent digestibility in large Atlantic salmon (>1 kg). At present results are extrapolated from experiments conducted overseas in small (< 1 kg) rainbow trout or pacific salmon. Because there are many factors known to affect apparent digestibility in fish, this practice is of doubtful validity. It is therefore essential that a reliable technique be developed for determining apparent digestibility in large Atlantic salmon under local conditions. This technique can then be used to investigate factors that may affect digestibility with the aim of maximising the efficiency of locally produced salmon feeds.

4. OBJECTIVES

(i) To validate a technique for determining apparent digestibility in large Atlantic salmon in seacages

(ii) To compare apparent digestibility in seawater Atlantic salmon at four different stages of the production cycle

(iii) To determine the effect of feeding regime on apparent digestibility results

(iv) To investigate digestibility for currently used commercial feeds

* The objectives of this project were modified during the course of the project in consultation with the FRDC. Modifications were made in light of experimental results, recognition of the limitations of available research facilities and to ensure that FRDC funding was utilised in the most effective manner possible. The initial FRDC funding commitment was reduced during the course of the project in line with these changes in objectives.

5.1 Validation of a faecal collection technique for determining apparent digestibility in large (up to 5 kg) Atlantic salmon (*Salmo salar* L.) in sea cages

5.1.1 Introduction

The accuracy of apparent digestibility measurements depends largely on a capacity to collect faeces which is representative of that voided at the time of normal defaecation. A number of techniques have been used to collect faeces in salmonids, including: intestinal dissection, stripping, a metabolism chamber, anal suction, mechanically rotating screens (Choubert system) and a faecal collection column (Guelph system) (Smith, 1971; Windell et al., 1978; Austreng, 1978; Cho and Slinger, 1979; Cho et al., 1982; Choubert et al., 1982; Cho and Kaushik, 1990; Hajen et al., 1993). The Guelph and Choubert systems have been shown to be suitable techniques for collection of faeces from salmonids in freshwater (Cho and Slinger, 1979; Choubert et al., 1982) and Hajen et al. (1993) have demonstrated the potential of the Guelph system for collection of faeces from chinook salmon (< 60g) in seawater.

All faecal collection methods have been shown to have advantages and disadvantages. However, collection of representative faecal samples in larger fish (up to 5 kg) under commercial conditions is problematic. The use of a metabolism chamber is impractical due to fish size. Dissection is expensive due to the high value of fish, and the use of chromic oxide as an inert marker in experimental diets makes the salvage of these fish for further processing unacceptable. Methods which rely on the extraction of faeces from the water are prone to over-estimation of ADCs as a result of nutrients leaching from the faeces (Smith et al., 1980; Lied et al., 1982; Brown and Robinson, 1989; Spyridakis et al., 1989; Hajen et al., 1993). While Satoh et al. (1992) conclude that faecal retrieval time has little effect on nutrient leaching, Cho et al. (1982) state that faeces is most vulnerable to leaching when the faecal pellets are broken up during the collection process. Vens-Cappell (1985) found that salmon fed commercial feeds produced poorly cohesive faeces which meant that ADCs derived from faeces collected by continuous filtration increased considerably relative to the stripping method. Large seawater Atlantic salmon fed commercial diets often produce faeces which dissipates easily in water. Therefore, leaching is likely to be highly significant when large holding facilities are necessary for maintenance of these fish, due to the distance that faeces would have to travel through the water before it could be collected by settling or physical removal. Collection of faeces by stripping eliminates leaching and this method is more suitable for large seawater Atlantic salmon, especially if commercial diets are compared under commercial conditions. However, before stripping could be used routinely the potential problems with this method (Austreng, 1978; Cho and Kaushik, 1990; Hajen et al., 1993) needed to be addressed.

Thus, the aim of this study was to assess whether the stripping method is suitable for collecting representative faecal samples from large numbers of seawater Atlantic salmon (up to 5 kg) under commercial conditions and with low stress to fish. Experiments were conducted to investigate a number of factors which may affect the reliability of ADC results derived by this method. These factors were: (a) faecal collection technique, (b) the

effect of urine and mucous contamination of faecal samples, (c) difference between a first sample and one taken immediately afterwards from the same fish, (d) the effect of stripping pressure during the collection of faeces, (e) the effect of dorso-flexion of the tail prior to faecal sample collection, (f) variation between individual fish and (g) section of gut sampled.

5.1.2 Materials and Methods

Facilities

All experiments were carried out at Salmon Enterprises of Tasmania's (SALTAS) marine operations at Dover, Tasmania, Australia. Cages were 5 m^2, made from 140 mm polyethylene pipe (with a 1 m handrail). Nets were made of 12 mm nylon mesh which hung 5 m deep from the waterline. Experimental fish were taken from SALTAS' research stock which are husbanded using usual commercial practices for Tasmanian Atlantic salmon farms until they are required for trials. These fish originated from the SALTAS hatchery at Wayatinah, Tasmania. The fish used in each experiment had been transferred directly into sea water as S1 smolts during October of the previous year. Fish used in experiment 3 had been previously used in a feeding regime trial, but had not been sampled. They were reallocated to cages for the purpose of experiment 3.

Diets

All diets were prepared through a commercial steam-pelleting process by Gibsons Ltd, Hobart in Tasmania. Pellet size was 6.5 mm diameter. The composition and proximate analysis of the experimental diets are shown in Table 1. Chromic oxide was used as an inert digestion indicator.

Faecal collection methods

(i) Stripping

Unless otherwise indicated, the stripping method involved the following procedure. Fish were caught (see below) and anaesthetised with benzocaine (30 ppm). Operator A placed the head of each fish into the sleeve of a specially designed plastic apron similar to a commercial spawning apron (faecal collection apron) worn by Operator B. This sleeve supported the head of the fish. The tail was held by Operator B's left hand, while water and mucous were removed gently from the ventral surface of the fish with paper towel held in Operator B's right hand. Operator B then placed the forefinger and thumb of their right hand on either side of the abdomen at the level of the pelvic fins. Moderate pressure was applied to the abdomen as the thumb and forefinger were moved caudally to the anus with a single firm motion. The expressed faeces was collected into a 70 ml polyethylene jar by Operator A. Samples contaminated by visible blood or urine were discarded.

Table 1 Composition of experimental diets

Composition	Experimental diets			
	No. 1	No. 2	No. 3	No. 4
Ingredients (g/kg)[a]				
Triabunna fishmeal	565.2	550.0	550.0	550.0
Triabunna fish oil	119.1	120.0	120.0	120.0
Wheat (ground to 100μm)	154.8	-		
Wheat flour	140.0	155.0	160.0	305.0
Wheat soya starch (50/50)		150.0	150.0	-
Vitamin mix[b]	5.0	7.5	7.5	7.5
Mineral mix[b]	5.0	2.5	2.5	2.5
L-lysine	0.8	2.5	2.5	2.5
DL-methionine		1.0	1.0	1.0
Ascorbic acid	0.1	0.5	0.5	0.5
Carophyll pink		1.0	1.0	1.0
Chromic oxide	10.0	10.0	5.0	10.0
Proximate analysis (dry weight basis)				
Dry matter (%)	92.4	92.38	92.15	93.50
Gross energy (MJ/kg)	22.4	20.83	21.05	20.60
Crude protein (%)	46.7	47.44	47.13	45.00
Ash (%)	9.6	9.40	8.90	8.07
Organic matter (%)	90.4	90.60	91.10	90.93
Chromic oxide (%)	0.78	0.79	0.43	0.88

[a] All feed ingredients supplied by Gibson's Ltd., Cambridge, Tasmania.
[b] Commercial preparations used by the Gibson's feed mill in commercial Atlantic salmon diets in Tasmania.

(ii) Dissection

Previously euthanased fish were placed on their right side and the abdominal contents exposed by making a ventral incision with a scalpel from the level of the pectoral fins to the anus, followed by removal of the left abdominal wall with surgical scissors. The gut was clamped immediately proximal to the anus and at the level of the pelvic fins with surgical clamps. This section of the gut was severed with a scalpel and removed from the fish. The contents were gently expressed from the removed segment of gut into a 70 ml polyethylene jar using the thumb and forefinger.

(iii) Rectal suction

Fish were caught (see below) and anaesthetised with benzocaine (30 ppm). The head of each fish was placed into the sleeve of the faecal collection apron and the ventral surface of the fish dried with paper towel as for the stripping method. Faeces was then collected using a suction apparatus. This apparatus consisted of a hand-operated vacuum pump (Nalgene^{TM} Cat. No. 6130-0010) attached by a 2 ft. length of clear plastic tubing (¼ in.

I.D. Nalgene^{TM} 180) to a 70ml polyethylene screw top container (Labserv) through the lid. A 150 mm length of glass tubing (4mm I.D., 7mm E.D.) was also attached to the container through the lid by a 50mm length of clear plastic tubing (¼ in. I.D. Nalgene^{TM} 180). The plastic tubing was secured in place where it passed through the lid of the container using a silicone based sealant (Selleys). Vacuum pressure created by the pump sucked faeces through the glass tube into the container.

The tip of the glass tube was inserted into the anus by Operator A and slowly moved along the gut to the level of the pelvic fins while applying a vacuum pressure consistently between 5 and 10 cm of mercury. The vacuum was then released and the glass tube removed from the fish. The container and attached glass tubing was disconnected from the pump and a new identical section attached. A glass rod (4mm E.D.) was used to remove any faeces remaining in the glass tube. This faeces was added to the sample already in the jar.

Experimental procedures and handling

The mean weight of fish was calculated by dividing the total weight of 60 fish which were weighed *en masse*, by 60. Fish were hand fed 3 times daily to appetite. Fish were fed on a normal commercial diet (Gibsons Ltd) for 5 days following transfer to experimental cages before being weaned onto experimental diets over a period of 4 days by increasing the quantity of the experimental diet in the total ration by 25% per day. Fish were sampled from 0900 hrs after a same day feed at 0800 hrs. Sampling commenced at least 5 days after being weaned onto the experimental diets. Fish were removed from cages by gently crowding them using a crowding net, from where they were dip-netted into a 500 l container of seawater. Fish were transferred as required into a 50 l container of seawater containing the appropriate dose of benzocaine. After each fish was sampled, it was removed from the trial so that no fish was sampled more than once. Faecal samples were pooled as required, weighed and stored frozen (-20°C) until later analysis.

Experiment I

The aim of this experiment was to compare the effects of stripping, rectal suction and dissection on faecal sample wet weight and ADCs. In September 1992, 220 fish (mean weight 2.7 kg) were divided equally between 2 cages. Fish were sampled 5 days after being weaned onto experimental diet no. 1.

Groups of 10 fish were removed from each cage alternately until all fish were sampled. Within each group, faecal samples were collected from each of 9 fish, alternately using the stripping (5 fish) and rectal suction (4 fish) methods. The remaining fish was euthanased with benzocaine (50 ppm) and stored on ice. Faeces were collected from these fish by dissection. For each cage, faecal samples were pooled according to collection method.

Experiment II

The aim of this experiment was to determine the effect of urine contamination of the faecal sample during collection on crude protein ADCs. In June 1993, 360 fish (mean weight 1.8 kg) were divided equally between 6 cages. Fish were sampled 5 days after being weaned onto experimental diet no. 2.

Sixty fish were removed from each of 2 cages. Within each group, faecal samples were collected from individual fish by the stripping method alternately with and without prior urinary catheterisation. Faecal samples collected without catheterisation and which were visibly contaminated by urine were excluded from the sampling sequence and kept separate. Catheterisation was performed by inserting a cat urinary catheter (Promedica, 4 french gauge) approximately 35 mm into the urinary tract. Once inserted by Operator A, the catheter was held in place against the tail of the fish by the left hand of Operator B. Faeces was then collected by the usual stripping method. The catheter was cleared after each fish by attaching a 5 ml syringe of air and expelling any fluid from the catheter, as well as drying the external surface with paper towel. For each cage, faecal samples collected following catheterisation were pooled, while other faecal samples were pooled according to whether there was visible urine contamination of the sample or not.

Thirty fish were removed from each of the other 4 cages. Faecal samples were collected from each fish by the stripping method. For each cage, faecal samples were pooled according to whether there was visible urine contamination or not. No more than 15 samples were pooled for either category of samples.

Experiment III

The aim of this experiment was to (i) compare the faecal wet weight and ADCs for faeces collected in a first stripping action with that collected in an immediately subsequent action and (ii) determine the effect of stripping pressure on faecal sample wet weight and ADCs. In July 1993, 200 fish were equally redistributed into 5 cages subsequent to a separate experiment. Fish had previously been fed on experimental diet no. 3. This diet was continued for 7 days before fish were sampled.

(i) Ten fish were removed from each of the 5 cages. Each fish was stripped twice (i.e. 2 separate stripping actions) with each faecal sample being kept separate. Within each cage, for those fish where significant amounts of faeces (> 0.2g) were expressed by both the first and second stripping action, faeces expressed by the first stripping action was pooled and faeces expressed by the second stripping action was pooled.

(ii) Groups of 10 fish were removed alternately from each of 3 cages until 30 fish had been removed from each cage. Within each group, faeces was collected by the stripping method, alternately using hard (thumb and forefinger compressing the abdominal wall very firmly) or soft (thumb and forefinger compressing the abdominal wall just firmly enough to extract a faecal sample) pressure during the stripping action. For each cage, faecal samples were pooled according to stripping pressure.

Experiment IV

The aim of this experiment was to determine the effect of tail dorso-flexion prior to the collection of faeces by the stripping method on ADCs. In September 1994, 180 fish (mean weight 2.4 kg) were divided equally between 3 cages. Fish were sampled 12 days after being weaned onto experimental diet no. 4.

Forty fish were removed from each cage. Faecal samples were collected alternately using the usual stripping method where there was little dorso-flexion of the tail and a modified stripping method where there was moderate dorso-flexion of the tail prior to stripping. For each cage, faecal samples were pooled according to sampling method.

Experiment V

The aim of this experiment was to compare faecal sample wet weight, and ADCs between (i) individual fish and (ii) the proximal and distal segments of the section of gut located between the level of the pelvic fins and the anus. In September 1994, 120 fish (mean weight 2.4 kg) were transferred to a cage. Fish were sampled 12 days after being weaned onto experimental diet no. 4.

An initial 35 fish were removed from the cage. Fish weight and fork-length was individually measured before faeces was collected by the stripping method. Faecal samples were weighed and stored individually. A subsequent 35 fish were then removed from the cage. Faecal samples were collected from each fish using a modified stripping method whereby the sample was collected in two portions. This was done by placing the thumb and forefinger of the left hand on both sides of the abdomen approximately ½ way between the level of the ventral fins and the anus at the same time as placing the thumb and forefinger of the right hand at the level of the pelvic fins in the usual manner. The first (distal) sample was expressed by the stripping action of the left hand to the anus, while the second (proximal) sample was expressed by the usual stripping action of the right hand to the anus. Faeces from each fish where there was not a significant quantity of faeces in both proximal and distal samples (> 0.5g) was discarded. Remaining proximal and distal faecal samples were weighed and stored separately for each fish.

Chemical analyses

Dry matter was determined by drying to a constant weight at 50°C under vacuum. Ash was determined by heating dried sample at 500° C in a muffle furnace for 16 hrs and organic matter calculated as 100-ash. Total nitrogen was determined using the microkjeldahl method and crude protein calculated as N×6.25. Gross energy was measured by bomb calorimetry. Chromic oxide was determined using the method of Kimura and Miller (1957), except that atomic absorption was substituted for colourimetry (Saha and Gilbreath, 1991; Scott, 1978).

Calculation of ADCs

The ADCs for organic matter, crude protein and gross energy were calculated according to Maynard and Loosli (1969).

$$\text{Apparent digestibility coefficient} = 100 - \left[100 \times \frac{\text{indicator in feed (\%)}}{\text{indicator in faeces (\%)}} \times \frac{\text{nutrient in faeces (\%)}}{\text{nutrient in feed (\%)}} \right]$$

Statistical analysis

All data for the comparison of faecal collection methods and effects of urine contamination was subjected to one-way analysis of variance, with the source of differences between means determined by Newman-Keuls multiple range test (Zar, 1984). Data comparing the effects of hard and soft stripping pressure and the effect of dorso-flexion of the tail prior to faecal stripping were analysed using a Students t-test, while data comparing first and second samples stripped from the same fish and comparing proximal and distal segments of the hindgut were analysed by a paired Students t-test. The regression coefficients for the relationship between fish weight, fork length and condition factor and ADCs for gross energy and crude protein were determined by least-squares regression. Statistical tests other than the Newman-Keuls test, were performed using Microsoft Excel for Windows (Version 4).

5.1.3 Results

Experiment I

There were no significant differences ($P>0.05$) in the mean wet weight of faecal samples collected by each method. The ADC for crude protein was significantly lower ($P<0.05$) for dissection than for the stripping and rectal suction methods. The ADC for gross energy was also lowest for the dissection method, but the difference was not significant ($P>0.05$) (Table 2).

Table 2 Comparison of the average faecal sample wet weight (±s.e.m.) and ADCs (±s.e.m.) for organic matter, gross energy and crude protein using three different faecal sample collection techniques

Parameter	Faecal sample collection technique			Level of significance[a]
	Stripping	Rectal Suction	Dissection	
Average wet weight of faecal sample (g)	2.58 ± 0.07	2.78 ± 0.77	3.55 ± 0.55	(0.50)
ADC (%)				
Gross energy	68.35 ± 0.01	67.81 ± 1.62	66.64 ± 0.40	(0.52)
Crude protein	82.59 ± 0.22^{x}	81.40 ± 0.77^{x}	79.57 ± 0.02^{y}	(0.04)

[a] Within each row, difference between treatment means determined by one-way ANOVA (P value in brackets). Where $P<0.05$, means with different superscripts are significantly different (Newman-Keuls multiple range test).

Experiment II

There were no significant differences ($P>0.05$) between faecal samples collected with or without prior catheterisation or between faecal samples visibly contaminated with urine or not (Table 3).

Table 3 Effect of urine contamination on ADCs (±s.e.m.) for crude protein

Sample	Crude protein ADC (%)
Group 1[a]	
No visible urine[b]	87.17 ± 0.68
Visible urine[c]	86.62 ± 0.70
Level of significance[d]	(0.58)
Group 2[e]	
No visible urine	87.13 ± 0.47
Visible urine	86.91 ± 0.68
Catheterised[f]	86.77 ± 0.01
Level of significance[g]	(0.88)

[a] Six replicate cages.
[b] No visible evidence of urine contaminating the faecal sample.
[c] Visible evidence of urine contaminating the faecal sample.
[d] Students t-test (P value in brackets).
[e] Two replicate cages.
[f] Urinary bladder catheterised prior to stripping of faecal sample.
[g] One-way ANOVA (P value in brackets).

Experiment III

The mean wet weight of the second faecal sample was significantly lower ($P<0.05$) than the first. There were no significant differences ($P>0.05$) however, in ADCs between the two samples, although they were consistently lower in the second sample (Table 4). The wet weight of the faecal sample was significantly higher ($P<0.01$) when hard stripping pressure was applied during sample collection. However, there were no significant differences ($P>0.05$) in ADCs between the 2 levels of stripping pressure (Table 5).

Table 4 Comparison of the average faecal sample wet weight (±s.e.m.) and ADCs (±s.e.m.) for organic matter and crude protein, for a first and immediately subsequent faecal sample stripped from the same fish

Parameter	1st sample	2nd sample	Level of significance[a]
Average wet weight of faeces (gm)	3.88 ± 0.19	1.52 ± 0.22	(0.01)
ADC (%)			
Organic matter	69.61 ± 2.05	62.18 ± 4.20	(0.09)
Crude protein	85.61 ± 1.05	81.18 ± 2.13	(0.09)

[a] Within each row, differences between first and second sample means determined by paired students t-test (P value in brackets).

Table 5 Effect of hard and soft stripping pressure on average faecal sample wet weight (±s.e.m.) and ADCs (±s.e.m.), for organic matter and crude protein

Parameter	Stripping pressure[a]		Level of significance[b]
	Hard	Soft	
Average wet weight of faeces (gm)	5.01 ± 0.27	2.56 ± 0.33	(0.00)
ADC (%)			
Organic matter	68.03 ± 0.26	68.48 ± 0.58	(0.52)
Crude protein	84.45 ± 0.08	82.62 ± 0.76	(0.11)

[a] Stripping pressure was defined as hard (thumb and forefinger compressing the abdominal wall very firmly) and soft (thumb and forefinger compressing abdominal wall just firmly enough to extract a faecal sample).

[b] Within a row, differences between hard and soft means determined using students t-test (P value in brackets).

Experiment IV

There were no significant differences (P>0.05) in the mean ADCs for organic matter, gross energy and crude protein associated with two levels of tail dorso-flexion prior to collection (Table 6).

Table 6 Effect of dorso-ventral flexion of the tail prior to faecal stripping on ADCs (±s.e.m.) for organic matter, crude protein and gross energy.

Parameter	Degree of dorso-flexion[a]		Level of Significance[b]
	Moderate	Minimal	
ADC (%)			
Organic matter	71.08 ± 0.20	70.55 ± 0.14	(0.09)
Gross energy	76.44 ± 0.73	76.78 ± 0.10	(0.41)
Crude protein	88.89 ± 0.27	88.47 ± 0.38	(0.67)

[a] Denotes amount of dorso-flexion of the tail prior to stripping faecal sample.
[b] Within each row, differences between moderate and minimal means determined using students t-test (P value in brackets).

Experiment V

There was no correlation between the mean weight, fork length and condition factor of fish and ADCs for crude protein and gross energy. Mean ADCs for organic matter and crude protein measured for the proximal and distal halves of the gut segment from the level of the pelvic fins to the anus are shown in Table 7. The ADCs for both organic matter (P<0.01) and crude protein (P<0.05) were significantly higher in the distal segment.

Table 7 Comparison of ADCs (%) for organic matter and crude protein between the proximal and distal halves of the section of gut from the level of the pelvic fins to the anus

Parameter	Proximal[a]	Distal[b]	Level of significance[c]
Apparent digestibility coefficient (%)			
Organic matter[d]	65.80 ± 0.54	67.83 ± 0.48	(0.00)
Crude protein[e]	85.32 ± 0.38	86.54 ± 0.35	(0.01)

[a] Denotes results for the faecal sample collected from the proximal half of the section of gut from the level of the pelvic fin to the anus.
[b] Denotes results for the faecal sample collected from the distal half of the section of gut from the level of the pelvic fin to the anus.
[c] Within each row, differences between proximal and distal means determined using paired students t-test (P value in brackets).
[d] n=21
[e] n=18

5.1.4 Discussion

A number of problems have been associated with the stripping method for collection of faeces in digestibility studies. Cho and Slinger (1979), Cho and Kaushik (1990) and Hajen et al. (1993) suggest that ADCs derived from stripped faecal samples may underestimate true digestibility due to contamination of the sample with mucous and urine. However, our results (Table 3) support the conclusions of Austreng (1978) that urine contamination may be ignored as a source of error in digestibility measurements derived by stripping. In addition, urine and mucous contamination during rectal suction or dissection is unlikely, yet the ADC for crude protein for the stripping method was comparable with rectal suction and significantly higher ($P<0.05$) than dissection (Table 2). It could be argued however, that rectal suction and dissection themselves may result in some under-estimation of digestibility values for other reasons. Following euthanasia, faeces may redistribute within the gut, with less digested material passing into the hindgut and/or vice versa before dissection. Rectal suction may draw faeces from further forward in the gut than is anticipated and some dislodgement of intestinal epithelium into the sample may occur. However, despite the loose consistency of faeces in these fish, it was not easily drawn into the rectal suction device and the authors do not believe this source of error is relevant for this method. While urine contamination is not a significant error factor in ADCs determined using the stripping method in large Atlantic salmon, slight dorso-flexion of the tail can express urine without any concurrent expression of faeces. Because this procedure does not affect ADCs (Table 6), it may be used to minimise urine contamination of faeces in large Atlantic salmon.

ADCs will also underestimate true digestibility if faeces is collected prior to complete digestion. Austreng (1978) and Lied et al. (1982) found that protein digestion was occurring throughout the rectum. Our findings (Table 7) support their results, with ADCs for organic matter ($P<0.01$) and crude protein ($P<0.05$) being significantly higher in the distal portion of the gut segment examined. Although the absolute difference is small, more accurate ADCs will be derived from samples collected as close to the anus as possible and the collection site should remain consistent if direct comparisons are to be made between experiments.

Cho and Kaushik (1990) suggest that forced evacuation of faeces by stripping could result in the addition of intestinal epithelium to rectal contents. However, stripping pressure which may be expected to affect the level of intestinal epithelial dislodgement was not found to affect ADCs in this study (Table 4).

Our results indicate no correlation between fish size and ADCs. This suggests that digestibility studies in large Atlantic salmon will be valid regardless of whether evenly sized experimental fish are used or not. In addition, ADCs may be extrapolated across a range of fish sizes (same age class) for any particular set of environmental conditions. This is important as large numbers of evenly sized large fish are not always readily available for experimental purposes and in commercial salmon seacages where there is likely to be a range of fish sizes, ADCs can be applied to all fish in the cage.

Additional faeces could be collected by stripping each fish twice. However, contents from further forward in the gut may move down into the rectum once emptied and therefore ADCs derived from a second sample could underestimate true digestibility. While not statistically significant ($P>0.05$), ADCs for faecal samples collected second in this study (Table 5) were lower. The ultimate effect on ADCs derived from a combination of these 2 faecal samples would be small due to the smaller mean weight of the second sample and the effect of mixing. However, only one stripping action is recommended.

While the stripping method is not without potential errors, our findings indicate that it is suitable for collecting faeces from large seawater Atlantic salmon for the purpose of calculating ADCs. Faecal samples should be collected as close to the anus as possible, moderate stripping pressure used, and only one sample stripped from each fish. Slight dorso-flexion of the tail to express urine would appear to be a useful means of minimising urine contamination of stripped faeces and while the use of evenly sized fish in research trials is ideal, there appears to be no correlation between fish size (same age class) and apparent digestibility results for a particular set of environmental conditions.

5.2 Investigation of within day and between day variation in apparent digestibility, with observations on crude fibre as an internal digestion indicator.

5.2.1 Introduction

Digestibility measurements are known to vary both within a day and between days in a number of species (Inaba et al., 1962; Kotb and Luckey, 1972; De Silva and Perera, 1983, 1984; De Silva et al., 1990; Saha and Gilbreath, 1991). This variation may result from differential passage of chromic oxide along the gut in relation to the digesta (Tacon and Rodrigues, 1984; Saha and Gilbreath, 1991), reflect the existence of a natural rhythmicity in digestion (De Silva and Perera, 1984) or be associated with day to day fluctuations in other factors, including food intake (Henken et al., 1985; Jobling, 1986). Storebakken and Austreng (1987) found however, that apparent protein digestibility in rainbow trout (*Oncorhynchus mykiss*) was not influenced by food intake.

In salmonids, automatic faecal collection methods (Choubert et al., 1982; Cho et al., 1982) usually involve the collection of faeces over a number of days. Thus, mean ADCs derived by these methods, will to some extent overcome any between day variation in apparent digestibility. However, these methods rely on the collection of faeces from the water column when fish are not being fed so that samples are not contaminated by feed. Therefore, they may not be an accurate measure of true digestibility if within day variation exists. In addition, comparisons between these methods, dissection and stripping may be misleading if faeces is collected at different times in the day.

The aim of this study was to further refine a method for determining digestibility in large seawater Atlantic salmon by assessing whether ADC results vary within each day and/or between days in these fish. Chromic oxide and crude fibre were compared as inert markers to determine whether differential passage of chromic oxide through the gut in relation to other nutrients is responsible for variation in apparent digestibility results. In addition, experiments were conducted to determine whether fish could be removed from the same cage on a number of occasions within a day or on each of a number of successive days without affecting ADCs derived from these fish so that appropriate procedures could be developed for overcoming within day and between day variation in ADCs, if relevant.

5.2.2 Materials and Methods

Facilities, diets and experimental procedures

All facilities and experimental procedures were as described for Project 1, as was the stripping faecal collection method except that slight dorso-flexion of the tail was performed prior to stripping the faecal sample in an attempt to express urine. Ingredient composition of experimental diets was as for diet no. 1 and no. 2 in Project 1, however the proximate analysis of diet no. 1 was slightly different (Table 8).

Table 8 Proximate analysis of experimental diets.

Composition	Experimental diets	
	No. 1	No. 2
Proximate analysis (dry wt. basis)		
Dry matter (%)	93.98	92.38
Gross energy (MJ/kg)	21.13	20.83
Crude protein (%)	46.31	47.44
Ash (%)	10.27	9.40
Organic matter (%)	89.73	90.60
Crude fibre (%)	1.47	
Cr_2O_3 (%)	0.74	0.79

Experiment I

The aim of this experiment was to determine the variation in ADCs for organic matter, gross energy and crude protein on three different days so that this factor can be accounted for, if necessary in digestibility studies. ADCs calculated using crude fibre as the inert marker were compared with those using chromic oxide to assess whether the dynamics of chromic oxide in the gut was contributing to between day variation in ADCs and to determine the suitability of crude fibre as an internal inert digestion indicator. In July 1993, 720 fish (mean wt. 2.4 kg) were divided equally between 6 cages. Fish were sampled 7 days after being weaned onto experimental diet 1. Groups of 5 fish were stripped alternately from each of 2 cages until 60 fish had been sampled from each cage. Faecal samples were pooled for each cage. This procedure was repeated 3 and 5 days later. On each of these days fish were stripped from 2 new cages.

Experiment II

The aim of this experiment was to determine the variation in daily ADCs for organic matter and crude protein over a 10 day period. On each sampling day, comparisons were made between results from duplicate cages used at every sampling time and those from 2 new cages or cages previously sampled only once. This was done to determine whether fish could be removed from the same cage on a number of consecutive days without affecting ADCs derived from these fish. In September 1993, 1800 fish (mean wt. 2.8 kg) were divided equally between 12 cages. Fish were sampled 7 days after being weaned onto experimental diet 2.

Fish were sampled on each of 9 days of a 10 consecutive day period, with no samples being collected on day 5. On each sampling day, groups of 5 fish were stripped alternately from each of 4 cages until 15 fish had been sampled from each cage. The 4 cages sampled on each sampling day comprised (i) duplicate cages which were sampled on all 9 sampling days and (ii) 2 previously unsampled cages on days 1,2,3,4 and 6, while on days 7,8,9 and 10 these 2 cages were the same as those sampled on days 1,2,3 and 4 respectively.

Experiment III

The aim of this experiment was to determine the variation in ADCs for organic matter, gross energy and crude protein at intervals of 6 hrs throughout the day so that this factor can be accounted for, if necessary in digestibility studies. At each sampling time comparisons were made between results from duplicate cages used at every sampling time and those from 2 previously unsampled cages. This was done to determine whether fish could be removed from the same cage on a number of occasions within a day without affecting ADCs derived from these fish. In August 1993, 960 fish (mean wt. 2.6 kg) were divided equally between 8 cages. Fish were sampled after 10 days on experimental diet 2.

At midnight, 5 fish were stripped alternately from each of 2 cages until 15 fish had been sampled from each cage. At each of 0600, 1200 and 1800 hrs of the same day a further 15 fish were stripped from these same duplicate cages as well as from 2 previously unsampled cages. At each sampling time, faecal samples were pooled for each cage.

Chemical analyses and calculation of ADCs

As for Project 1, except that in addition, crude fibre was determined by the AOAC method used by Wolters et al. (1992).

Statistical analysis

One-way ANOVA was used to analyse differences in ADC results on different days and at different times of the day in Experiments I, II and III. A paired students t-test was used to analyse differences between chromic oxide and crude fibre as inert digestion indicators in experiment II and differences between results when different duplicate cages were sampled on each day during each period and when the same duplicate cages were sampled repeatedly in that period in experiment III. All the above tests were performed using the Microsoft Excel for Windows Version 4 computer program. In experiment II, the source of differences between means was determined using the Newman-Keuls multiple range test (Zar, 1984) when significant differences were evident with one-way ANOVA.

5.2.3 Results

Experiment I

ADCs for organic matter ($P<0.01$), gross energy ($P<0.01$) and crude protein ($P<0.05$) were significantly different on the 3 days of sampling when chromic oxide was used as the inert marker (Table 9). However, only the ADCs for organic matter were significantly different ($P<0.05$) when crude fibre was used. All results calculated with crude fibre as the inert marker were significantly lower ($P<0.05$) than results using chromic oxide.

Table 9 Variation in mean ADCs (±s.e.m.) for organic matter, gross energy and crude protein (calculated using chromic oxide and crude fibre as inert digestion indicators) on three different days (n= 2 cages, 60 fish/ cage)

ADC (%)	Inert marker	Day of collection						Level of Significance	
		Day 1		Day 4		Day 6			
Organic matter	CO[a]	67.38	± 0.30	73.23	± 0.60	66.81	± 0.11	(0.00)[c]	(0.03)[d]
	CF[b]	58.70	± 1.05	56.32	± 0.47	51.08	± 1.19	(0.02)	
Gross energy	CO	73.39	± 0.08	78.54	± 0.63	74.66	± 0.17	(0.00)	(0.03)
	CF	66.31	± 0.63	64.98	± 0.61	62.65	± 1.04	(0.10)	
Crude protein	CO	87.67	± 0.24	90.13	± 0.26	88.08	± 0.09	(0.01)	(0.03)
	CF	84.38	± 0.54	83.89	± 0.22	82.43	± 0.51	(0.11)	

[a] Denotes chromic oxide as inert marker.
[b] Denotes crude fibre as inert marker.
[c] Within each row, differences between daily means determined by one-way ANOVA (P value in brackets).
[d] For each ADC parameter, differences between means measured using chromic oxide and crude fibre as inert markers determined by paired student's t-test (P value in brackets).

Experiment II

ADCs varied between days , but were not influenced by taking repeat samples from the same cage compared to using different cages. There were significant differences ($P<0.05$) in the daily ADCs for both organic matter and crude protein during period 1 (days 1,2,3,4 and 6) (Table 10). There was also a significant difference ($P<0.05$) between the daily ADCs for organic matter in period 2 (days 7,8,9 and 10), but not for crude protein. There were no significant differences ($P<0.05$) in either period 1 or 2 between the ADCs measured when new cages were sampled on each day during the period and those measured from the same cages which were sampled on every sampling day.

Table 10 Mean daily ADCs (±s.e.m.) for organic matter and crude protein over a 10 day period derived from fish sampled from new cages and the same (repeat) cages (n= 2 cages, 15 fish/ cage)

Day of Collection[a]	ADC (%)			
	Organic matter		Crude protein	
	New[b]	Repeat[c]	New[b]	Repeat[c]
Period 1				
1	65.26 ± 4.95	66.77 ± 1.88	88.65 ± 1.46	89.03 ± 0.32
2	67.64 ± 1.26	64.73 ± 2.89	88.99 ± 0.68	88.26 ± 0.78
3	65.29 ± 1.53	67.66 ± 2.31	88.88 ± 0.10	89.32 ± 0.87
4	64.29 ± 1.21	65.53 ± 1.28	87.43 ± 0.71	88.07 ± 0.22
6	60.00 ± 0.31	62.11 ± 0.09	85.80 ± 0.14	87.35 ± 0.21
Level of significance[d]				
[e]	(0.02)		(0.01)	
[f]	(0.57)		(0.58)	
Period 2				
7	61.38 ± 1.89	60.67 ± 2.25	87.52 ± 0.71	87.37 ± 0.41
8	56.09 ± 0.98	55.48 ± 2.50	84.70 ± 0.55	84.27 ± 1.00
9	60.15 ± 1.51	59.74 ± 1.54	86.03 ± 0.58	86.25 ± 0.79
10	61.05 ± 3.52	60.27 ± 1.20	86.56 ± 0.94	86.12 ± 0.84
Level of significance[g]				
[e]	(0.04)		(0.17)	
[f]	(0.34)		(0.29)	

[a] Denotes position of day of collection within a 10 consecutive day period.
[b] Denotes sampling from different duplicate cages on each of days 1,2,3,4 and 6, while the cages sampled on days 7,8,9 and 10 were the same as on days 1,2,3 and 4 respectively.
[c] Denotes sampling from the same duplicate cages on all days.
[d] For days 1,2,3,4 and 6.
[e] Within each row, differences between the daily means (single) determined by one-way ANOVA (P value in brackets).
[f] For each ADC parameter, differences between repeat and new means determined by a paired students t-test (P value in brackets).
[g] For days 7,8,9 and 10.

Experiment III

When new cages were sampled at each interval, there was a significant difference in the ADC results for gross energy (P=0.047), however the source of this difference could not be determined (Table 11). There were no other significant differences (P<0.05). When the same cages were sampled at each interval, there were significant differences (P<0.05) in the ADCs also for gross energy, with both the midday and afternoon results being significantly lower than the midnight and morning results and significantly different from each other. There were no significant differences (P<0.05) in ADCs for crude protein. In all cases however, the ADCs for organic matter, gross energy and crude protein were higher at midnight than at midday.

Table 11 Variation in mean ADCs (±s.e.m.) for organic matter, crude protein and gross energy at intervals of 6 hrs throughout a day derived from fish sampled from new cages and the same (repeat) cages (n=2 cages, 15 fish/ cage)

ADC (%)	Time of day				Level of significance[a]
	Midnight	Morning (6AM)	Midday	Afternoon (6PM)	
New[b]					
Organic matter	78.65 ± 0.86	78.23 ± 0.98	73.70 ± 1.81	75.84 ± 1.36	(0.15)
Crude protein	92.38 ± 0.27	91.34 ± 0.08	90.01 ± 0.43	90.64 ± 1.27	(0.23)
Gross energy	83.37 ± 0.34	82.91 ± 0.53	80.24 ± 0.84	79.93 ± 0.85	(0.05)
Repeat[c]					
Crude protein	92.38 ± 0.27	91.91 ± 0.97	88.47 ± 0.09	89.45 ± 1.89	(0.15)
Gross energy	83.37 ± 0.34[x]	84.09 ± 0.48[x]	80.32 ± 0.42[y]	81.89 ± 0.71[z]	(0.02)

[a] With each row, differences between means at different times of the day were determined using a one-way ANOVA (P value in brackets). Where $P<0.05$, means with different superscripts are significantly different (Newman-Keuls multiple range test).
[b] Fish stripped from different duplicate cages at each sampling time.
[c] Fish stripped from the same duplicate cages at each sampling time.
[bc] There were no significant differences between new and repeat means (paired students t-test, $P<0.05$)

5.2.4 Discussion

Reliable ADCs cannot be measured until both the inert marker and nutrients reach an equilibrium in the gut. Representative faecal samples can then be collected. Saha and Gilbreath (1991) suggest that due to temporary sequestration, chromic oxide excretion takes 2-4 days to equilibrate in pigs. Windell and Norris (1969) suggest that 2 days is necessary to achieve representative faecal samples in rainbow trout (*Oncorhynchus mykiss*) if the water temperature is higher than 8°C. Cho et al. (1982) suggest that collection commence once daily feed intake becomes uniform, while Austreng (1978), Vens-Cappell (1985) and Hajen et al. (1993b) fed experimental diets for 7, 7 and 3 days respectively before collection was commenced. There were significant differences ($P<0.05$) in ADCs between days in this study when chromic oxide was used as the inert marker (Table 9 and 10). In experiment I, fish were stripped 7, 10 and 12 days after being weaned onto the experimental diet while in experiment 2 it was 14-26 days. In both experiments the period between weaning fish onto experimental diets and commencement of sampling should have been adequate to allow chromic oxide and digesta to equilibrate within the gut. The validity of these between day differences is further supported by the significant differences ($P<0.05$) in ADCs for organic matter calculated using crude fibre as an internal marker. However, it may be that both crude fibre and chromic oxide pass differentially along the gut in relation to other digesta.

In experiment I, ADCs calculated using crude fibre as the inert marker were all significantly lower ($P<0.05$) than those using chromic oxide (Table 9). Tacon and Rodrigues (1984) found that ADCs calculated using crude fibre were significantly higher

than those using chromic oxide for one diet and significantly lower for another. While both chromic oxide and crude fibre appear to be suitable as inert markers in this study, it is not possible to determine which marker more closely reflects true digestibility from these results.

Regardless of whether between day variability in ADCs is due to the dynamics of chromic oxide in the gut or real variation in digestibility, faecal samples should be collected on a number of days in digestibility studies. When collecting faeces at different times, some workers have disregarded the impact of stripping on further digestibility measurements in the same fish (Austreng, 1978; Hajen, 1993a). However, intestinal damage caused by stripping may interfere with digestion and since digestion continues to occur close to the anus (Austreng, 1978; Lied et al., 1982) future digestibility measurements in these fish may be unreliable. Therefore, repeated stripping of the same fish is not advisable. Our findings (Table 10), suggest however, that the crowding associated with sampling different fish from the same cage over a number of days does not significantly affect apparent digestibility values ($P<0.05$). It is therefore unnecessary to have different cages for each sampling day.

Our results (Table 11) also show variation in ADCs for gross energy ($P<0.05$) within a 24 hr period and while not significant ($P<0.05$), a pattern was also evident for the ADCs of organic matter and crude protein with the highest values being at midnight and lowest being at midday. These results are consistent with those of Vens-Cappell (1985) in rainbow trout where ADCs for crude protein were lowest between 09.45 and 14.00 and highest between 17.30 and 07.30 and De Silva et al. (1990) in *Oreochromis aureus* (Steindachner) where 18 of 20 ADCs for crude protein whether derived using chromic oxide or crude fibre as the inert marker were lower between 0930 and 1630 than between 1630 and 0900. While further research is needed to clarify the significance of these results, important ramifications for the timing of faecal collection in digestibility research are evident. Four possible scenarios exist with regard to variation in ADCs within each day: (i) no variation exists, (ii) variation is random, (iii) variation follows a pattern which is not synchronised to each 24 hr period or (iv) variation is diurnal. Collection of faecal samples at the same time each day would overcome (i) to (iii) regardless of the actual time of sampling. However, if a diurnal pattern exists where ADCs are highest at midnight and lowest at midday as this and other studies suggest, then collection of samples early in the morning or late in the day would be the most appropriate. Without further information, comparisons of ADCs derived from faecal samples collected at different times of the day should be treated with caution.

Apparent digestibility in large seawater Atlantic salmon (2-3 kg) varies both within each day and between days. Therefore, faeces should be collected at consistent times of the day, with 0700-0900 probably being the most appropriate period in case a diurnal rhythm in apparent digestibility exists. In addition, when the stripping method is used, faeces should be collected from different fish on a number of days to overcome day to day variation in apparent digestibility. However, these fish may come from the same cage without any significant effect on results.

5.3 Effect of feeding regime on apparent digestibility results

5.3.1 Introduction

A number of feeding strategies are used in studies of apparent digestibility in salmonids and in commercial aquaculture. These include feeding fish twice daily to satiation (Vens-Cappell, 1985; Hajen et al, 1993), 3 times daily to satiation (Cho and Slinger, 1978), continuously or semi-continuously (Austreng, 1978; Storebakken and Austreng, 1987) or at restricted levels (Tacon and Rodrigues, 1984; Storebakken and Austreng, 1987).

Feeding regime has been shown to affect ADCs in pigs (Saha and Gilbreath, 1991) and African catfish (Henken et al., 1985) however other workers found that ration level had no significant effect on the ADC for crude protein in 0.5-1.0 kg rainbow trout (Storebakken and Austreng, 1987). Apparent digestibility results are often compared between workers using different feeding regimes and applied to commercial aquaculture across a range of commercial feeding strategies. Further investigation of the effects of feeding regimes on ADCs is necessary before the above assumptions can be used broadly.

The aim of this study was to assess the effects of a number of feeding regimes and sudden changes in feeding regime on the ADCs for 1.6-1.8 kg seawater Atlantic salmon. Results would determine whether ADCs calculated using one feeding regime can be extrapolated to other feeding regimes and whether digestibility may be improved through manipulation of feeding regime. In addition, the contribution of sudden changes in feed intake to variability in ADCs was assessed.

5.3.2 Materials and Methods

Facilities and experimental procedures

All facilities and experimental procedures were as described for Project 1. The stripping faecal collection method was as described in Project 2.

Diets

Diets were as for Project 1, except for composition and proximate analysis (Table 12).

Experiment I

The aim of this experiment was to determine the effect of 4 different feeding regimes on ADCs for organic matter, gross energy and crude protein. In June 1994, 768 fish (mean wt. 1.6 kg) were divided equally between 8 cages. Fish were sampled 14 days after being weaned onto the experimental diet. Each of 4 different feed regimes was allocated randomly to 2 of the 8 cages, including (i) once daily (0800 hrs) to appetite, (ii) twice

daily (0800 and 1600 hrs) to appetite, (iii) three times daily (0800, 1200 and 1600 hrs) to appetite, and (iv) twice daily (0800 and 1600 hrs) restricted to half of expected daily intake. Groups of 16 fish were stripped from each cage on each of 6 days over a 12 day period (Day1, Day3, Day5, Day8, Day 10, Day12). The sequence of sampling was varied between days. All faecal samples were pooled for each cage on each day.

Table 12 Composition and proximate analysis of the experimental diet.

Composition	
Ingredients (g/kg)[a]	
Triabunna fishmeal	550.0
Triabunna fish oil	120.0
Wheat flour	305.0
Vitamin supplement[b]	7.5
Mineral supplement[b]	2.5
L-lysine	2.5
Dl-methionine	1.0
Ascorbic acid	0.5
Carophyll pink	1.0
Chromic oxide	10.0
Proximate analysis (dry wt. basis)	
Dry matter (%)	93.50
Crude protein (%)	45.00
Ash (%)	9.00
Organic matter (%)	91.00
Chromic oxide (%)	0.88
Gross energy (MJ/kg)	20.60

[a] All feed ingredients supplied by Gibson's Ltd., Cambridge, Tasmania.
[b] Commercial preparations used by the Gibson's feed mill in commercial Atlantic salmon diets in Tasmania.

Experiment II

The aim of this experiment was to determine the effect of a sudden change in feeding regime over a 36 hr period on ADCs for organic matter, gross energy and crude protein. In July 1994, 360 fish (mean wt. 1.8 kg) were divided equally between 12 cages.

On the morning of the 13th day after fish were weaned onto the experimental diet, 9 cages were randomly changed to 1 of 3 different feed regimes for 36 hrs, including (i) feeding to appetite once only in the morning, (ii) feeding to appetite once only in the morning and then stressing the fish during the day by raising the nets to a 1 metre depth for the whole day and disrupting the water in the cage with a dipnet 3 times during the day and (iii) starvation. The other 3 cages continued to be fed 3 times daily to appetite. On the

morning of the 14th day, groups of 30 fish were stripped from each cage. All faecal samples were pooled for each cage.

Chemical analyses and calculation of ADCs

As for Project 1.

Statistical analysis

One-way ANOVA was used to analyse differences in ADCs and faecal components for different feed regimes. The Newman-Keuls multiple range test was used to determine the source of differences identified by ANOVA.

5.3.3 Results

Experiment I

Average daily feed intake, faecal sample wet weight and ADCs for organic matter, gross energy and crude protein are shown in Table 13. The average wet weight of faeces collected from fish on different feed regimes was not significantly different ($P<0.05$), however fish being fed once daily to appetite produced noticeably less faeces than fish on the other regimes. The ADCs for organic matter were significantly higher in fish fed 2 or 3 times daily ($P<0.05$) than in fish fed once daily. There were no other significant differences ($P<0.05$).

Table 13 Effect of 4 different feeding regimes on ADCs (±s.e.m.) for organic matter, gross energy and crude protein

Parameter	Feeding Regime				Level of
	1 × Daily(A)[a]	2 × Daily(A)	3 × Daily(A)	2 × Daily(R)	Significance[b]
Average daily feed intake (g)	1143 ± 123	1527 ± 47	1995 ± 40	1000	
Average faecal wet weight (g)	2.52 ± 0.10	2.90 ± 0.25	3.05 ± 0.37	2.98 ± 0.14	(0.48)
ADCs (%)					
Organic matter[c]	70.30 ± 0.21^{x}	71.51 ± 0.28^{y}	71.20 ± 0.18^{y}	70.47 ± 0.16^{xy}	(0.04)
Gross energy	78.38 ± 0.09	78.78 ± 0.29	78.36 ± 0.27	78.22 ± 0.07	(0.37)
Crude protein	87.44 ± 0.00	88.07 ± 0.39	87.34 ± 0.20	87.31 ± 0.01	(0.18)

[a] Letter in brackets denotes level of feeding at each meal. (A) denotes to appetite. (R) denotes half of expected feed intake.

[b] Within rows, differences between means determined by one-way ANOVA (P value in brackets).

[c] Where $P<0.05$, means with the same superscript are not significantly different (Newman-Keuls multiple range test).

Experiment II

The average faecal sample wet weight, faecal composition and ADCs for organic matter, gross energy and crude protein are shown in Table 14. The average wet weight of faeces collected from fish fed once or 3 times daily to appetite for the 36 hrs prior to collection was significantly higher ($P<0.05$) than for fish on starve. Fish fed 3 times daily to appetite also produced more faeces than fish fed once and then stressed throughout the day prior to collection. Faecal content of organic matter, gross energy, crude protein and chromic oxide was significantly lower ($P<0.01$) in fish on starve for the 36 hrs prior to stripping. Faecal content of ash was significantly higher ($P<0.01$) in these fish. The ADC for organic matter was also significantly lower ($P<0.01$) in fish on starve, however there were no other significant differences ($P<0.05$) in ADCs for organic matter, gross energy or crude protein.

Table 14 Effect of a sudden change in feed regime for 36 hrs on the ADCs (±s.e.m.) for organic matter, gross energy and crude protein

Parameter	Feeding regime changed to				Level of
	3 × Daily (A)[a]	1 × Daily (A)	1 × Daily (AS)	No Feed	Significance[b]
Average faecal wet weight	4.19 ± 0.25^{x}	3.65 ± 0.44^{xy}	2.82 ± 0.32^{yz}	2.03 ± 0.22^{z}	(0.01)
Faecal composition (%)					
Organic matter	73.07 ± 0.09^{x}	72.33 ± 0.30^{x}	72.87 ± 0.46^{x}	67.77 ± 0.63^{y}	(0.00)
Gross energy	12.83 ± 0.16^{x}	12.18 ± 0.20^{x}	$12\ 95 \pm 0.39^{x}$	10.57 ± 0.25^{y}	(0.00)
Crude protein	16.35 ± 0.15^{x}	14.15 ± 0.38^{y}	15.54 ± 0.50^{x}	11.92 ± 0.44^{z}	(0.00)
Ash	26.93 ± 0.09^{x}	27.67 ± 0.30^{x}	27.13 ± 0.46^{x}	32.23 ± 0.63^{y}	(0.00)
Chromic oxide	2.58 ± 0.04^{x}	2.42 ± 0.01^{x}	2.37 ± 0.09^{x}	1.91 ± 0.01^{y}	(0.00)
ADCs (%)					
Organic matter	72.46 ± 0.38^{x}	70.98 ± 0.21^{x}	70.14 ± 1.22^{x}	65.53 ± 0.14^{y}	(0.00)
Gross energy	78.63 ± 0.57	78.42 ± 0.25	76.52 ± 1.50	76.25 ± 0.53	(0.17)
Crude protein	87.54 ± 0.16	88.52 ± 0.35	87.10 ± 0.83	87.74 ± 0.46	(0.33)

[a] Letter in brackets denotes level of feeding at each meal. (A) denotes to appetite. (R) denotes half of expected feed intake.

[b] Within rows, differences between means determined by one-way ANOVA (P value in brackets). Where $P<0.05$, means with the same superscript are not significantly different (Newman-Keuls multiple range test).

5.3.4 Discussion

There is considerable debate over the appropriateness or otherwise of different feed regimes for optimising growth and food conversion in farmed seawater Atlantic salmon (Talbot, 1993). Blyth et al. (1993) suggest that Atlantic salmon display preferential feeding patterns at different times of the year which relate to both endogenous and exogenous factors. Feeding of commercial diets to appetite, apart from increasing the potential for feed wastage, can also cause gastrointestinal overload (Jobling, 1986) which may result in a reduction in absorption efficiency. This may be due to gut enzymes having a shorter period in which to act on gut contents or reduced ability of gut enzymes to come in contact with all parts of the gut contents.

In this study, fish fed once daily to appetite had significantly lower ADCs ($P<0.05$) for organic matter than fish fed 2 or 3 times daily to appetite. Storebakken and Austreng (1987), suggest that variation in ADCs for crude protein may be more likely at different ration levels when fish are fed fewer times through the day. Usher et al. (1990) found that feeding discrete meals rather than feeding continuously improved digestion efficiency and suggested this was due to a slower gut evacuation rate with discrete meals. It is difficult to explain our result for organic matter however, when it was not reflected in the ADCs for gross energy and crude protein. Lower ADCs for organic matter in the absence of changes in ADCs for gross energy and crude protein may also be explained by the ingestion of extraneous poorly digestible material as discussed later in this section.

When comparing sudden changes in feeding regime 36 hrs before faecal collection, the faecal content of chromic oxide and crude protein was less for starved fish than for other feeding regime changes. Faecal chromic oxide (%) ranged from 1.88 - 1.93 for starved fish vs 2.26 - 2.65 for other fish, while faecal crude protein (%) ranged from 11.06 - 12.50 in starved fish vs. a range of 14.13 - 16.56 for other fish. The fact that the ADC for crude protein for starved fish is not different from other fish (in fact it is second highest), would suggest that lower faecal chromic oxide and crude protein percentages are a dilution effect. This dilution effect always occurs in sea water studies as stated by Hajen et al. (1993a) because ash ingested with sea water (Usher et al., 1988) contributes to the ash content of the faeces. Because the additional ash dilutes both the chromic oxide and organic components in the faeces equally, there is no net effect on ADCs for organic components of the diet.

In starved fish in this study however, there appears to be differential dilution of faecal components, with organic matter being diluted less than chromic oxide, crude protein or gross energy. This may be due to the ingestion, or internal excretion into the gut of non-protein organic matter which has a low energy value. In starved fish it is possible that they ingested extraneous material such as macro algae from the nets, or the water column, or accidentally ingested such material in an attempt to eat other animals living on the nets. This material would be relatively indigestible, and so would result in a higher percentage of organic matter in faeces. This in turn may lower the ADC for organic matter, while not influencing ADCs for crude protein or gross energy. There is anecdotal evidence that farmed salmonids do ingest naturally occurring prey organisms while in sea cages.

Seal or dolphin attack and bad weather conditions may occur during digestibility studies in sea cages. The stress regime used in experiment 2, of shallowing nets and disturbing the fish throughout the day was designed to approximate the stress which may be associated with these events. While there were no significant differences ($P<0.05$) created by these procedures, the standard error of the mean was consistently greatest in the cages of fish which were stressed. This is expected as it is difficult to stress different cages to exactly the same extent, and consequently any effect of the stress is likely to be more variable. Although all factors that have potential to cause stress in fish during a digestibility study should be minimised and regular feeding strategies should be maintained, it is not possible in a sea cage situation to always maintain environment influences within narrow ranges as is possible in tank facilities. However, it is apparent from the results of experiment 2 that some of these factors may significantly affect apparent digestibility results, probably due more to changes in feed intake rather than the stress itself.

5.4 Comparison of apparent digestibility at different stages of the production cycle with further observations on between day variability in ADCs

5.4.1 Introduction

There are no published ADCs for larger Atlantic salmon (> 1.0 kg) in seawater and therefore, many commercial Atlantic salmon feed manufacturers without in-house research facilities do not have access to ADCs for feed ingredients used in diets for these fish. Consequently ADCs measured in smaller fish or other species (e.g. rainbow trout) are often extrapolated to large Atlantic salmon in seawater. Windell et al. (1978) found that apparent digestibility results were influenced by both size and age in rainbow trout, however there is no published information on the effect of stage of the production cycle in seawater on ADCs in Atlantic salmon.

The aim of this study was to compare apparent digestibility in Atlantic salmon (300-4200 g) at 4 different stages of the production cycle in seawater, including (a) smolt during summer, (b) salmon during winter, (c) salmon during spring and (d) salmon during their second summer at sea. These stages were selected because they represent distinctly different phases in relation to feeding pattern and growth while the fish are in seawater. Smolt during their first summer at sea are growing rapidly (up to 2.5% per day) and have a high feed rate (up to 3.0% of body weight per day). The water temperature is high (up to 19°C) and daylength long (up to 16 hrs). Salmon during winter grow more slowly (0.6% per day), feed rate is lower (0.8% of body weight per day) and water temperature (down to 9°C) and daylength (down to 10 hrs) are less. Salmon in spring undergo a surge in growth associated with increasing daylength, onset of early maturation and in some cases increasing water temperature. Salmon in their second summer at sea are growing more slowly due to the stresses of high summer water temperatures and advancing maturation. Further investigation of daily variation in ADCs at each stage of the production cycle tested was also undertaken.

5.4.2 Materials and Methods

Facilities and experimental procedures

All facilities and experimental procedures were as described for Project 1. The stripping faecal collection method was as described in Project 2.

Diets

Diets were as for Project 1, except for composition and proximate analysis (Table 15) and pellet size was 4 mm for smolt (300-320 g) and 8 mm for salmon (>1.5 kg). Both diets were prepared in January 1994, and stored frozen until being used.

Table 15 Composition of the experimental diet

Ingredients[a]	Content (g/kg)
Triabunna fishmeal	550.0
Triabunna fish oil	120.0
Wheat/Soya starch 50/50	150.0
Wheat flour	155.0
Vitamin supplement[b]	7.5
Mineral supplement[b]	2.5
L-lysine	2.5
Dl-methionine	1.0
Ascorbic acid	0.5
Carophyll pink	1.0
Chromic oxide	10.0

a All feed ingredients supplied by Gibson's Ltd., Cambridge, Tasmania.
b Commercial preparations used by the Gibson's feed mill in commercial Atlantic salmon diets in Tasmania.

Table 16 Proximate analysis of the experimental diet during the study.

Parameter	Experiment No.				
	I	I	II	III	IV
Pellet size	4mm	8mm	8mm	8mm	4mm
Proximate analysis (dry weight basis)					
Dry matter (%)	93.80	92.03	91.86	93.48	93.48
Organic matter (%)	90.90	90.93	89.99	90.93	90.23
Gross energy (MJ/kg)	21.68	21.43	20.63	20.60	21.50
Crude protein (%)	45.29	43.42	43.56	43.40	45.35
Ash (%)	9.10	9.07	10.01	9.07	9.77
Chromic oxide (%)	0.77	0.84	0.86	0.86	0.80

Experiment I

In January 1994, 1200 fish (mean weight 320 g) were divided equally between 4 cages and 480 fish (mean weight 4.2 kg) were divided equally between another 4 cages. At each collection time, faeces was stripped from 50 fish from each of the 4 cages containing smolt and from 20 fish from each of the 4 cages containing salmon.

Experiment II

In July 1994, 480 fish (mean weight 1.5 kg) were divided equally between 4 cages. At each collection time, faeces was stripped from 20 fish from each of the 4 cages.

Experiment III

In September 1994, 480 fish (mean weight 2.2 kg) were divided equally between 4 cages. At each collection time, faeces was stripped from 20 fish from each of the 4 cages.

Experiment IV

In January 1995, 1200 fish (mean weight 300 g) were divided equally between 4 cages. At each collection time, faeces was stripped from 50 fish from each of the 4 cages.

Chemical analyses and calculation of ADCs

As for Project 1.

Statistical analysis

Differences in apparent digestibility of organic matter, crude protein and gross energy were analysed using one-way ANOVA. The Neuman-Keuls multiple range test was used to determine the source of any differences identified by ANOVA.

5.4.3 Results

Daily variation in mean ADCs for organic matter, gross energy and crude protein measured at each of 4 different stages of the production cycle in seawater are shown in Table 17. There were significant differences ($P<0.01$) between the mean ADCs for organic matter, gross energy and crude protein over the 12 day collection period in experiments II-IV, except that the significance of this difference was lower for gross energy in experiment II ($P<0.05$). There were also significant differences ($P<0.05$) between the mean ADCs for organic matter and crude protein measured for smolt in experiment I, however there was no significant difference ($P<0.05$) in the mean ADCs for crude protein in these fish. There were no significant differences ($P<0.05$) in ADCs for gross energy and crude protein in salmon (4.2 kg) in experiment I.

The results for salmon (4.2 kg) in experiment I were not included in statistical analyses as they are known to be inaccurate. Results tended to be lower and more variable for these fish. Many became anorexic probably due to a combination of factors, including: onset of maturation, relatively high water temperatures (< 19° C on day 3 of collection) and handling stress associated with large fish. Faecal samples with no obvious chromic oxide present were discarded. However, onset of inappetence may not have been obvious in fish where the period of anorexia was insufficient to visibly reduce chromic levels in faecal samples.

Mean ADCs for organic matter, gross energy and crude protein measured in fish at 4 different stages of the production cycle in seawater are compared in Table 18. There were significant differences between stages for organic matter, gross energy ($P<0.01$) and crude

protein ($P<0.05$). Smolt (320 g) during experiment I had a significantly lower ADC for organic matter than all other stages. The low mean value for these smolt was mostly a result of very low organic matter ADC values on 1 day (i.e. 62.31, 56.38, 59.54 and 60.81). Smolt (300 g) during experiment IV had ADCs for organic matter that were significantly lower ($P<0.05$) than salmon in winter but not significantly different from salmon in spring. ADCs for gross energy were significantly higher (0.01) in salmon in winter compared to all other stages, however there were no other significant differences. ADCs for crude protein were significantly higher ($P<0.05$) in salmon in winter compared to smolt (300 g) in experiment IV. There were no other significant differences in crude protein ADCs.

Although ADCs for organic matter were significantly different between smolt in summer 1994 and summer 1995, ADCs for gross energy and crude protein were not significantly different. This suggests that storage of the experiment diets at -5°C did not cause any effect on apparent digestibility over the 15 month period of the experiments.

Table 17 Daily variation in ADCs (±s.e.m.) for organic matter, gross energy and crude protein at 4 different stages of the Atlantic salmon production cycle in seawater

Parameter	Day of Collection[a]						Level of Significance[b]
	Day 1	Day 3	Day 5	Day 8	Day 10	Day 12	
Smolt - February 1994 (320 g)							
Organic matter	65.68 ± 1.78	63.73 ± 4.18	69.57 ± 1.19	67.38 ± 1.47	59.76 ± 1.26	65.82 ± 1.19	(0.02)
Gross energy	76.05 ± 1.60	74.53 ± 2.98	76.80 ± 1.13	76.55 ± 0.33	73.28 ± 0.73	76.47 ± 0.64	(0.33)
Crude protein	88.44 ± 1.56	85.74 ± 1.13	89.58 ± 0.64	89.66 ± 0.12	87.71 ± 0.68	85.99 ± 0.80	(0.03)
Salmon - February 1994 (4.2 kg)[c]							
Gross energy	76.89 ± 1.94	71.23 ± 3.54	66.64 ± 3.74	74.87 ± 1.42	74.82 ± 1.35	74.21 ± 2.53	(0.12)
Crude protein	89.17 ± 1.03	77.94 ± 8.17	80.10 ± 3.87	87.62 ± 0.41	86.12 ± 1.14	82.64 ± 1.73	(0.29)
Salmon - July 1994 (1.5 kg)							
Organic matter	71.29 ± 0.57	69.66 ± 0.47	69.52 ± 0.44	71.93 ± 0.35	72.77 ± 0.18	71.75 ± 0.17	(0.00)
Gross energy	77.42 ± 0.57	76.66 ± 0.35	76.60 ± 0.37	78.37 ± 0.46	78.45 ± 0.18	77.83 ± 0.06	(0.01)
Crude protein	89.16 ± 0.61	87.29 ± 0.20	86.26 ± 0.16	89.18 ± 0.27	89.05 ± 0.17	88.40 ± 0.10	(0.00)
Salmon - September 1994 (2.2 kg)							
Organic matter	69.33 ± 0.50	67.54 ± 0.20	71.15 ± 0.18		66.50 ± 0.81	70.35 ± 0.18	(0.00)
Gross energy	75.57 ± 0.39	74.44 ± 0.11	77.13 ± 0.22		73.32 ± 0.67	76.67 ± 0.10	(0.00)
Crude protein	87.33 ± 0.36	87.31 ± 0.13	89.08 ± 0.41		86.95 ± 0.47	88.28 ± 0.31	(0.00)
Smolt - February 1995 (300 g)							
Organic matter	68.57 ± 0.42	67.39 ± 0.39	66.60 ± 0.32	71.33 ± 0.67	66.98 ± 0.65	71.50 ± 0.16	(0.00)
Gross energy	75.31 ± 0.15	74.51 ± 0.38	73.64 ± 0.10	77.09 ± 0.60	75.37 ± 0.31	77.81 ± 0.17	(0.00)
Crude protein	87.11 ± 0.26	85.80 ± 0.35	85.69 ± 0.24	89.51 ± 0.46	84.63 ± 0.26	88.34 ± 0.26	(0.00)

[a] Denotes collection day sequence starting 14 days after fish were weaned onto the experimental diet.
[b] Within rows, level of significance of differences between means is presented as P value (in brackets) (one-way ANOVA).

Table 18 Comparison of ADCs (±s.e.m) for organic matter, gross energy and crude protein at 4 stages of the Atlantic salmon production cycle in seawater

Stage of the production cycle	ADCs (%)		
	Organic matter	Gross energy	Crude protein
Salmon - summer 1994 (4.2 kg)[a]		73.22 ± 1.54	83.93 ± 0.67
Smolt - summer 1994 (320 g)	65.42 ± 0.81^{x}	75.66 ± 0.50^{x}	87.85 ± 0.55^{xy}
Salmon - winter 1994 (1.5 kg)	71.15 ± 0.19^{z}	77.55 ± 0.18^{y}	88.22 ± 0.14^{x}
Salmon - spring 1994 (2.2 kg)	69.97 ± 0.91^{yz}	75.42 ± 0.15^{x}	87.81 ± 0.17^{xy}
Smolt - summer 1995 (300 g)	68.73 ± 0.15^{y}	75.62 ± 0.06^{x}	86.85 ± 0.15^{y}
Level of significance[b]	(0.00)	(0.00)	(0.03)

[a] These results were not included in statistical analyses

[b] Within each column, level of significance between different means is presented as P value (in brackets) (one-way ANOVA). Means with different superscripts are significantly different (Newman-Keuls multiple range test, $P<0.05$).

5.4.4 Discussion

There has been a trend by overseas salmonid producers in recent years to the use of high energy feeds with lower digestible protein to digestible energy ratios. These feeds are produced by increasing levels of fish oil in the feed at the expense of protein and carbohydrate ingredients. Whilst the growth and food conversion benefits of these feeds is apparent at all stages of the production cycle, evidence suggests that the cost efficiency of high energy diets is greatest in colder water conditions (pers. com. Slinning, 1994). Suggested reasons for this include (i) that higher energy feeds enable fish to consume more energy in a given quantity of feed during a period when feed intake is low and (ii) that salmon consume more feed due to the greater palatability of higher energy feeds.

In contrast to other workers (Cho and Slinger, 1979) where apparent digestibility decreased with reducing water temperature in rainbow trout (<150 g) in freshwater, results from this experiment suggest that improved performance of high energy feeds during colder water conditions may also be assisted by an improvement in digestibility as ADCs for organic matter, gross energy and crude protein were always highest for salmon in winter. The significantly higher ($P<0.01$) ADC for gross energy in salmon in winter compared with fish at other stages may reflect a differential improvement in digestibility favouring lipid and/or perhaps carbohydrate over protein in colder water temperatures.

ADCs for harvest size salmon in summer in this experiment are not meaningful due to the level of anorexia encountered in these fish. Despite this however, the ADCs for crude protein and gross energy are only a few % points lower than for salmon tested at other stages of the production cycle. Therefore, ADCs for these fish may be comparable with other stages if all the samples collected from anorexic fish could be eliminated from the final pooled faecal sample.

While significant differences ($P<0.05$) were found between differents stages of the production cycle in seawater for Atlantic salmon in this study, the small absolute differences suggest that for commercial purposes ADCs can be extrapolated across all stages. It would be valuable however, to further investigate the apparent increase in digestibility found for fish during winter in this study.

5.5 Effect of dietary composition on growth rate; apparent digestibility; relative weight of abdominal components and histology and electron microscopy of the gastrointestinal tract

5.5.1 Introduction

Significant reductions in feed conversion ratio and increases in growth have been achieved in farmed Atlantic salmon overseas by feeding high energy extruded diets and through genetic selection programs. Despite both these factors however, Atlantic salmon farmed in Tasmania and fed lower energy steam-pelleted feeds grow as fast as these fish. This is mainly due to ideal water temperatures and the absence of significant diseases. It is likely that even faster growth could be achieved in Tasmanian farmed Atlantic salmon if the industry changed to higher energy feeds as has occurred overseas. However, while there would be advantages for the Tasmanian industry in achieving faster growth (e.g. earlier harvesting) in a percentage of stock, many fish already grow too large by the end of the harvest season in March. In addition, there have been problems with flesh quality associated with the feeding of high energy feeds overseas and these diets come at a cost premium to farmers.

In view of the above, the reliance on the Tasmanian industry to produce a high quality product and the fact that no research has been conducted in localy farmed Atlantic salmon to determine their optimum nutritional requirements, it is essential that the Tasmanian industry adopt high energy feeds cautiously. The aim of this study was to undertake a preliminary comparison of 3 commercially available feeds, including: steam-pelleted (17% fat), extruded (17% fat) and extruded (30%) fat. A number of factors were assessed, including: growth rate, apparent digestibility, relative weights of abdominal components, and histology and electron microscopy of the gastrointestinal tract. An extruded diet (17% fat) containing 1% chromic oxide was also compared with these feeds to assess whether chromic oxide in the diet affected any of the above.

5.5.2 Materials and Methods

Facilities and experimental procedures

All facilities and experimental procedures were as described for Project 1. The stripping faecal collection method was as described in Project 2.

Diets

Diets were prepared through a commercial steam or extrusion-pelleting process by Gibsons Ltd, Hobart, Tasmania. Pellet size was 6.5 mm diameter. The composition and proximate analysis of the experimental diets are shown in Table 19. Chromic oxide was used as an inert digestion indicator.

Table 19 Proximate analysis of experimental diets[a]

Proximate analysis (dry weight basis)	Experimental diets					
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
Dry matter (%)	97.57	97.71	98.09	97.83	97.45	97.61
Gross energy (MJ/kg)	22.53	21.84	22.70	22.82	24.29	24.25
Crude fat (%)	17.50	17.25	17.55	17.65	29.60	29.50
Crude protein (%)	47.5	45.56	45.25	45.15	46.80	45.63
Ash (%)	9.45	10.07	9.33	9.99	9.69	9.64
Organic matter (%)	90.55	89.93	90.67	90.01	90.31	90.36
Chromic oxide (%)		0.67		0.60		0.60

[a] Commercial feeds (ingredient composition not available)

Experimental procedures and fish handling

The aim of this experiment was to compare growth rate, ADCs for organic matter, gross energy and crude protein, relative weights of abdominal components, and histology and electron microscopy of the gastrointestinal tract for 3 commercially available diets. In June 1995, 2400 fish (mean weight 1.5 kg) were divided equally between 12 cages. Fish were fed twice daily to appetite at 0800 hrs and 1600 hrs. Each of the 4 experimental diets were allocated randomly to 3 cages. Fish were weaned onto experimental diets over a 4 day period after being fed a normal commercial diet for 1 week.

After 8 weeks 50 fish were removed from each cage and weighed *en masse*. Average weight was determined by dividing the total weight by 50. Once weighed, fish were removed from the trial.

Five fish were removed from each cage. Fish weight and length was measured after being individually euthanased in benzocaine (50 ppm). The fish was then placed on its right side and the abdominal contents exposed by making a ventral incision with a scalpel from the level of the pectoral fins to the anus, followed by removal of the left abdominal wall with surgical scissors. The gut was clamped and severed where the oesophagus entered the abdominal cavity and immediately proximal to the anus. The entire abdominal contents excluding the kidney and urinary system were removed from the fish and the following components dissected free and weighed individually: stomach, gut from the distal end of the stomach to level of the last pyloric caecae (included fat between pyloric caecae), gut from the level of the last pyloric caecae to the anus, liver, spleen and abdominal fat (excluding fat between pyloric caecae).

One sample (approximately 1 cm^3) and a number of samples (approximately 1 mm^3) of liver, stomach, pyloric fat (including pancreas), pyloric caecae and gut (at level of middle of pyloric caecae and 2 cm from the anus) were cut from the abdominal contents. The larger sample was placed in 10% neutral buffered formalin and the smaller samples in

a mixture of 2% glutaraldehyde and 0.1 M cacodylate buffer. The remainder of each tissue sample was individually frozen at -20 °C.

Histology

Tissue samples were embedded in paraffin wax, sectioned at 5 μm and stained with haematoxyllin and eosin.

Electron Microscopy

Tissue samples were washed 3 times in 0.2M cacodylate buffer for 15 minutes each time. Samples were then placed in 1% OsO_4 in distilled water for 1 hr and washed with distilled water. Tissue samples were then dehydrated by placing them in increasing concentrations of alcohol (30%, 50%, 70%), each for 1 hr.

Chemical analyses and calculation of ADCs

As for Project 1.

Statistical Analysis

All data was analysed using one-way analysis of variance, with the source of differences between means determined by the Newman-Keuls multiple range test (Zar, 1984). One-way analysis of variance was performed using Microsoft Excel for Windows (version 4).

5.5.3 Results

Mean weight gain was significantly higher ($P<0.05$) in fish fed an extruded diet containing 30% fat than in fish fed extruded diets containing 17% fat, either with or without chromic oxide. The mean weight gain of fish fed the steam pelleted diet with 17% fat was not significantly different from other diets, although it was higher than for fish fed on extruded diets containg the same fat level. There were no significant differences in the weights of individual abdominal components expressed as a percentage of total body weight, other than for the proximal intestine (with pyloric caecae and caecal fat). This component was significantly higher ($P<0.05$) in fish fed on an extruded diet with 30% fat than in fish fed the steam pelleted diet with 17% fat (Table 20).

Table 20 Effect of feed manufacturing process, dietary fat level and chromic oxide on weight gain and weight of abdominal components as a percentage of total body weight

Experimental diet	Weight gain (g)	Tissue weight (% of body weight)					
		Stomach	Proximal intestine[a]	Distal intestine	Liver	Spleen	Other fat
Steam (17% fat)	673 ± 32^{xy}	0.60 ± 0.03	3.19 ± 0.01^{x}	0.45 ± 0.01	0.90 ± 0.01	0.11 ± 0.00	0.94 ± 0.08
Extruded (17% fat)	573 ± 47^{x}	0.58 ± 0.02	3.21 ± 0.05^{xy}	0.41 ± 0.01	0.89 ± 0.06	0.12 ± 0.00	1.01 ± 0.03
Extruded (17% fat) + chromic oxide	559 ± 31^{x}	0.59 ± 0.02	3.41 ± 0.09^{xy}	0.42 ± 0.02	0.95 ± 0.01	0.14 ± 0.02	0.91 ± 0.13
Extruded (30% fat)	758 ± 36^{y}	0.54 ± 0.01	3.55 ± 0.13^{y}	0.44 ± 0.01	0.95 ± 0.02	0.12 ± 0.01	1.01 ± 0.07
Level of significance[b]	(0.02)	(0.22)	(0.04)	(0.18)	(0.50)	(0.18)	(0.78)

[a] denotes proximal intestine plus pyloric caecae and pyloric fat.

[b] Within each column, difference between experimental diet means determined by one-way ANOVA (P value in brackets). Where $P<0.05$, means with different superscripts are significantly different (Newman-Keuls multiple range test).

Mean ADCs for organic matter and gross energy were significantly lower ($P<0.01$) in fish fed the steam pelleted diet with 17% fat compared to fish on extruded diets. The mean ADC for organic matter was significantly higher ($P<0.01$) in fish fed the extruded diet with 30% fat than those fed the extruded diet with 17% fat. There was no significant difference in the ADC for gross energy between these extruded diets. There were no significant differences in mean ADCs for crude protein (Table 21).

Table 21 Effect of manufacturing process and dietary fat level on the ADCs for organic matter, gross energy and crude protein

Parameter	Experimental diet			Level of significance[a]
	Steam (17% fat)	Extruded (17% fat)	Extruded (30% fat)	
ADCs (%)				
Organic matter	72.37 ± 0.66^{x}	75.10 ± 0.47^{y}	79.93 ± 1.03^{z}	(0.00)
Gross energy	79.20 ± 0.30^{x}	82.72 ± 1.29^{y}	85.16 ± 0.73^{y}	(0.01)
Crude protein	89.42 ± 0.48	89.60 ± 0.38	89.68 ± 0.45	(0.91)

[a] Within each row, differences between experimental diet means determined by one-way ANOVA (P value in brackets). Where $P<0.05$, means with different superscripts are significantly different (Newman-Keuls multiple range test).

There were no apparent differences in the histology or electron microscopy of gastrointestinal tract components. Good staining in electron microscopy sections was not achieved in this experiment. This was thought to be due to the high oil levels in the tissues as oil is known to interfere with the staining process.

5.5.4 Discussion

The higher weight gain in fish fed the extruded diet with 30% fat is as expected. The digestible protein to digestible energy ratio in this diet is lower compared to other diets, with the greater energy component producing a protein sparingly effect resulting in added growth. The higher mean weight gain for fish fed the steam pelleted feed compared to fish fed on extruded diets with the same fat level is unexpected. The extrusion process is normally associated with an improvement in growth. This is due to effects such as the gelatinisation of starches which increases the digestibility of carbohydrate components of the diet. In this experiment the ADCs for organic matter and gross energy in extruded diets containing 17% fat were higher than the steam pelleted diet with the same fat level as expected, yet the weight gains in these fish did not reflect this increase in digestibility.

The diets used in this experiment were produced through commercial feed manufacturing equipment as the main purpose of the experiment was to trial commercially available feeds. However, there are 2 outcomes of this that may explain the anomalous results above. Firstly, the ingredients used in the extruded diets with 17% fat were slightly different to those used in the steam pelleted diet. This was necessary to achieve an experiment pellet of acceptable physical quality. However, this resulted in the extruded diet containing a small percentage of soyabean meal (<10%) not in the steam pelleted diet. It is therefore possible that feed consumption in fish fed this diet was reduced due to pallatibility problems associated with this feed ingredient. This is unlikely though, as higher levels of soyabean meal have been used in commercially available diets for salmonids in Europe. The second complicating factor was that the extruded diets containg 17% fat had a percentage (up to 10%) of pellets that would not sink. This problem arises in the manufacturing process and was unable to be resolved. All fish were fed in excess of appetite, however it is possible that the presence of soyabean meal as an ingredient or floating pellets in some way affected the feeding pattern of fish fed on these diets. This result very clearly demonstrates however, the complexity of the relationship between the feed and fish performance.

The extrusion process and fat levels in the diet had no effect on the ADC for crude protein. Higher ADCs for organic matter and gross energy are a reflection mostly of the higher level of fat in the diet, as fat is usually more digestible than protein in salmonid diets. The higher weight of proximal intestine (including pyloric caecae and pyloric fat) in fish fed the extruded diet with 30% fat compared to the steam pelleted diet is most likely due to an increase in pyloric fat.

In this experiment, fish increased in weight by approximately 30% of their body weight over the duration of the experiment. Ideally this type of experiment should run over a period long enough for experimental fish to double in weight, so that trends in results can become more clear, however this was not possible.

6. REFERENCES

Austreng, E., 1978. Digestibility determination in fish using chromic oxide marking and analysis of contents from different segments of the gastrointestinal tract. Aquaculture, 13: 265-272.

Blythe, P.J., Purser, G.J. and Russell, J.F., 1993. Detection of feeding rhythms in seacaged Atlantic salmon using new feeder technology. In: Fish Farming Technology, Reinertsen, Dahle, Jørgensen, Tvinnereim (Eds), Balkema, Rotterdam: 209-216.

Brown, P.B. and Robinson, E.H., 1989. Nutrient concentrations of catfish feces and practical diets after immersion in water. J. World Aqua. Soc., 20: 245-249.

Cho, C.Y. and Slinger, S.J., 1979. Apparent digestibility measurement in feedstuffs for rainbow trout. In: J.E Halver and K. Tiews (Editors), Finfish Nutrition and Fishfeed Technology, Vol II. Heenemann Verlagsgesellschaft MbH, Berlin, pp. 239-247.

Cho, C.Y. and Kaushik S.J., 1990. Nutritional energetics in fish: Energy and protein utilisation Rainbow trout (*Salmo gairdneri*). In: Bourne, G.H. (Editor), Aspects of Food Production, Consumption and Energy Values. World Rev. Nutr. Diet., Basel, Karger, 61: 132-172.

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

Choubert, G., De La Noüe, J. and Luquet, P., 1982. Digestibility in fish: Improved device for the automatic collection of faeces. Aquaculture, 29: 185-189.

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

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

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

Hajen, W.E., Beames, R.M., Higgs, D.A. and Dosanjh, B.S., 1993a. Digestibility of various feedstuffs by post-juvenile chinook salmon (*Oncorhynchus tshawytscha*) in sea water. 1. Validation of technique. Aquaculture, 112: 321-332.

Hajen, W.E., Beames, R.M., Higgs, D.A. and Dosanjh, B.S., 1993b. Digestibility of various feedstuffs by post-juvenile chinook salmon (*Oncorhynchus tshawytscha*) in sea water. 2. Measurement of digestibility. Aquaculture, 112: 333-348.

Henken, A.M., Kleingeld, D.W. and Tjissen, P.A.T., 1985. The effects of feeding level on apparent digestibility of dietary dry matter, crude protein and gross energy in the African catfish *Clarias gariepinus* (Burchell 1822). Aquaculture, 51: 1-11.

Inaba, D., Ogino, C., Takamatsu, C., Sugano, S. and Hata, H., 1962. Digestibility of dietary components in fishes. I. Digestibility of dietary protein in rainbow trout. Bull. Jpn. Soc. Sci. Fish., 29: 242-244.

Jobling, M., 1986. Gastrointestinal overload - a problem with formulated feeds. Aquaculture, 51: 257-263.

Kimura, F.T. and Miller, V.I., 1957. Improved determination of chromic oxide in cow feed and faeces. J. Agric. Food. Chem., 5: 216

Kotb, A.R. and Luckey, T.D., 1972. Markers in nutrition. Nutr. Abstr. Rev., 42: 813-845.

Lied, E., Julshamn, K. and Braekkan, O.R., 1982. Determination of protein digestibility in Atlantic cod (*Gadus morhua*) with internal and external indicators. Can. J. Fish. Aquat. Sci., 39: 854-861.

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

Saha, D.C. and Gilbreath, R.L., 1991. Analytical recovery of chromium from diet and faeces determined by calorimetry and atomic absorption spectrophotometry. J. Sci. Food Agric., 55: 433-446.

Saha, D.C. and Gilbreath, R.L., 1991. Faecal recovery and diurnal variation in excretion of dietary chromium by mature swine. J. Sci. Food Agric., 56: 407-418.

Satoh, S., Cho, C.Y. and Watanabe, T., 1992. Effect of fecal retrieval timing on digestibility of nutrients in rainbow trout diet with the Guelph and TUF feces collection systems. Nippon Suisan Gakkaishi, 58: 1123-1127

Scott, K., 1978. Cause and control of losses of chromium during nitric, perchloric acid oxidation of aquatic sediments. Analyst, 103: 754.

Smith, R.R., 1971. A method for measuring digestibility and metabolizable energy of feeds. Prog. Fish-Cult., 33: 132-134

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

Spyridakis, P., Metallier, R., Gabaudin, J. and Riaza, A., 1989. Studies on nutrient digestibility in European sea bass (*Dicentrarchus labrax*). 1. Methodological aspects concerning faeces collection. Aquaculture, 77: 61-70

Storebakken, T. and Austreng, E., 1987. Ration level for salmonids II. Growth, feed intake, protein digestibility, body composition, and feed conversion in Rainbow trout weighing 0.5-1.0 kg. Aquaculture, 60: 207-221.

Tacon, A.G.J. and Rodrigues, A.M.P., 1984. Comparison of chromic oxide, crude fibre, polyethylene and acid-insoluble ash as dietary markers for the estimation of apparent digestibility coefficients in Rainbow trout. Aquaculture, 43: 391-399.

Talbot, C., 1993. Some biological and physical constraints to the design of feeding regimes for salmonids in intensive cultivation. In: Fish Farming Technology, Reinertsen, Dahle, Jørgensen, Tvinnereim (Eds), Balkema, Rotterdam: 19-26.

Usher, M.L., Talbot, C. and Eddy, F.B., 1988. Drinking in Atlantic salmon smolts transferred to seawater and the relationship between drinking and feeding. Aquaculture, 73: 237-246.

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

Vens-Cappell, B., 1985. Methodical studies on digestion in trout. 1. Reliability of digestion coefficients in relation to methods for faeces collection. Aquacultural Engineering, 4: 33-49.

Windell, J.T., Foltz, J.W. and Sarokon, J.A., 1978. Methods of fecal collection and nutrient leaching in digestibility studies. Prog. Fish-Cult., 40: 51-55.

Windell, J.T. and Norris, D.O., 1969. Gastric digestion and evacuation in rainbow trout. Prog. Fish Cult., 31: 20-26.

Wolters, M.G.E., Verbeek, C., Van Westerop, J.J.M., Hermus, R.J.J. and Voragen A.G.J., 1992. Comparison of different methods for determination of dietary fiber. J. Assoc. Off. Anal. Chem., 68: 626-634.

Zar, J.H., 1984. Biostatistical Analysis. (2nd Ed.) Prentice-Hall, New Jersey: 718 pp.

7. IMPLICATIONS AND RECOMMENDATIONS

This project was undertaken as a basis for a long-term strategy by SALTAS to develop more cost-effective salmon feeds for the Tasmanian Atlantic salmon industry.

It has successfully validated a reliable technique for investigating apparent digestibility in large Atlantic salmon (*Salmo salar* L.) in sea cages under commercial conditions. This methodology can be utilised with confidence by aquaculture feed manufacturers and industry to determine apparent digestibility coefficients for both feed ingredients and complete feeds used in the seawater salmonid industry in Australia. These measurements are essential for:

* Formulation of least-cost rations

* Nutrient requirement research

* Screening of potential nutritive value in relation to raw material quality and processing and storage conditions (e.g. testing potential alternative protein sources)

* Formulation of feeds to minimise water pollution

Using the above validated technique, this project has also investigated a number of factors which may affect apparent digestibility results, including: within day and between day variation, effect of feeding regimes and effect of stage of the production cycle. The results of these studies have further improved the digestibility technique itself and provided potential mechanisms for maximising the information obtained in digestibility studies through extrapolation across feed regimes and stages of production in seawater. In addition, the results have highlighted a number of areas where direct comparison of apparent digestibility results either within a single study or between different studies must be treated with caution, thereby identifying limitations in this exercise. A number of results in this study may also prove relevant to digestibility studies in other aquaculture species.

The technique was also used to compare the performance of 3 commercially available salmonid feeds. Results from this trial have highlighted the importance of controlled nutrition experiments in evaluating the cost-effectiveness of commercial feeds.

Nutrition research in large salmon is expensive and the facilities necessary to conduct these trials scarce. It is therefore recommended that feed manufacturers and salmonid growers co-operate in building on the information gained in this study. If co-operation is achieved in conducting future nutrition research, information will be obtained in the most cost-effective manner possible.

In summary, this study has provided an excellent foundation for further nutrition research to improve the cost-effectiveness of salmon feeds for the Tasmanian Atlantic salmon industry.

8. INTELLECTUAL PROPERTY

N/A

9. TECHNICAL SUMMARY

This project was comprised of 5 separate studies.

(i) *Validation of a faecal collection technique for determining apparent digestibility in large (up to 5 kg) Atlantic salmon (Salmo salar L.) in sea cages*

This study was undertaken to assess whether collection of faeces by a stripping method was suitable for measuring digestibility in large seawater Atlantic salmon (up to 5 kg). Experiments were conducted to determine the significance of a number of factors associated with variable ADCs when faeces is collected by stripping. These factors were: (a) faecal collection technique, (b) the effect of urine and mucous contamination of faecal samples, (c) difference between a first sample and one taken immediately afterwards from the same fish, (d) the effect of stripping pressure during the collection of faeces, (e) the effect of dorso-flexion of the tail prior to faecal sample collection, (f) variation between individual fish and (g) section of gut sampled.

Two faecal collection techniques were compared with the stripping method. Rectal suction produced comparable results to stripping, however ADCs for crude protein were significantly lower ($P<0.05$) for dissection. This may be due to the loose consistency of the faeces produced by large salmon on commercial diets which allows it to redistribute along the gut after euthanasia. Urine and mucous contamination, stripping pressure, number of samples from the same fish and dorso-flexion of the tail did not appear to affect ADCs. However, there was a significant difference in the ADCs for organic matter ($P<0.01$) and crude protein ($P<0.05$) derived from faeces collected from different halves of the gut between the level of the pelvic fins and the anus. There was no relationship between fish size and ADCs for gross energy and crude protein.

The stripping method used appears to be suitable for collecting faeces from large seawater Atlantic salmon (300-5000g) for the purpose of calculating apparent digestibility. A robust and practical procedure which takes account of the factors which can cause major variation in data is suggested.

(ii) Investigation of within day and between day variation in apparent digestibility, with observations on crude fibre as an internal digestion indicator.

This study was undertaken to investigate whether ADCs vary within each day and/or between days so that this variation (if present) may be taken into account in digestibility studies. Experiments were also conducted to determine whether fish could be removed at intervals of 6 hours within a day from the same cage or on each of a number of successive days from the same cage without affecting the ADCs derived from these fish. This was done to evaluate potential mechanisms for overcoming daily variability in ADCs during digestibility studies. Crude fibre was compared with chromic oxide as an inert digestion indicator to investigate whether the gut dynamics of chromic oxide contributes to variability in ADCs and to evaluate the appropriateness of crude fibre as an internal digestion marker.

Significant variation was found in the ADCs for organic matter ($P<0.01$), gross energy ($P<0.01$) and crude protein ($P<0.05$) on 3 different days. The existence of between day variation was further evident in experiment 2, with significant differences ($P<0.05$) occurring in the ADCs for organic matter and crude protein between days over a 10 day period. In addition, ADCs for organic matter, gross energy and crude protein were always lower at midday than at midnight. However, this difference was only significant ($P<0.05$) for gross energy in fish removed from the same cage at each sampling time. Repeated removal of fish from the same cage either within a day or over a number of successive days did not significantly ($P<0.05$) affect apparent digestibility. ADCs calculated using crude fibre as the inert marker were also significantly different ($P<0.05$) between days and were always significantly lower ($P<0.05$) than those using chromic oxide. Therefore between day variation in ADCs is considered to be a real phenomenon, however which marker reflects digestibility more accurately is difficult to determine from this study.

Sampling regimes for determining apparent digestibility in large Atlantic salmon must account for variability in ADCs within each day and between days, otherwise results will be unreliable and the ability to compare results from different experiments diminished. Modifications to the technique developed in Study 1 are suggested to overcome this variability.

(iii) Effect of feeding regime on apparent digestibility results

The effects of 4 different feeding regimes on ADCs was investigated to determine whether ADCs derived using a particular feeding regime could be extrapolated to other feeding regimes. Feeding regimes trialled, included (a) once daily (0800 hrs) to appetite, (b) twice daily (0800 hrs and 1600 hrs) to appetite, (c) three times daily (0800, 1200 and 1600 hrs) to appetite and (d) twice daily (0800 and 1600 hrs) restricted to half of expected daily intake. Three sudden changes in feeding regime 36 hrs prior to faecal collection were also investigated to assess the potential impact on ADCs of a number of external factors (e.g. predator attack) which may affect feeding during digestibility studies in sea cages. Sudden changes trialled, included (a) feeding once daily (0800 hrs) to appetite, (b) As for

(a) plus stressing the fish during the day, (c) starvation and (d) continuation of feeding 3 times daily (0800, 1200 and 1600 hrs) to appetite (i.e. no change).

When comparing 4 different feeding regimes, there were no significant differences ($P<0.05$) in the average wet weight of faeces collected from fish. However, fish being fed once daily to appetite produced noticeably less faeces than fish on other regimes. ADC's for organic matter were significantly higher ($P<0.05$) in fish fed 2 or 3 times daily than in fish fed once daily. There were no other significant differences ($P<0.05$).

When comparing 3 different sudden changes in feeding strategy with continuation of the prescribed feeding regime (i.e. 3 times daily to appetite), the average wet weight of faeces collected from fish fed once or 3 times daily to appetite for the 36 hrs prior to collection was significantly higher ($P<0.05$) than for starved fish. Fish fed 3 times daily to appetite also produced more faeces than fish fed once and then stressed throughout the day prior to collection. The ADCs for organic matter were significantly lower ($P<0.01$) in for starved fish, however there were no other significant differences ($P<0.05$) in ADCs for organic matter, gross energy or crude protein.

Within the limits of the feeding regimes tested in this study, it appears that ADCs derived using one feeding regime can be extrapolated to other feeding regimes. However, sudden changes in feeding pattern during digestibility experiments may affect ADCs and these factors must be kept to a minimum during digestibility studies in sea cages wherever possible.

(iv) Comparison of apparent digestibility at different stages of the production cycle with further observations on between day variability in ADCs

The aim of this study was to compare ADCs at 4 different stages of the production cycle in seawater. This was done to investigate whether ADCs determined at one stage can be extrapolated to other stages of the production cycle in seawater. Within each stage of the production cycle ADCs were compared on each of 6 different days over a 12 day period to further evaluate between day variation in results.

There were significant differences ($P<0.01$) in ADCs for organic matter, gross energy and crude protein over the 12 day collection period in experiments II-IV, except that the significance of this difference was lower for gross energy in experiment II ($P<0.05$). There were also significant differences ($P<0.05$) in ADCs for organic matter and crude protein measured for smolt in experiment I, however there was no significant difference ($P<0.05$) in the mean ADCs for crude protein in these fish. There were no significant differences ($P<0.05$) in ADCs for gross energy and crude protein in salmon (4.2 kg) in experiment I. These results further support the evidence in Study 2 that there is significant between day variation in ADCs.

When comparing ADCs between 4 different stages of the production cycle in seawater, there were significant differences for organic matter ($P<0.01$), gross energy and crude

protein ($P<0.05$). Smolt (320 g) in experiment I had a significantly lower ADC for organic matter than all other stages. The low mean value for these smolt was mostly due to low organic matter ADC values on 1 particular day. However, ADC results on this day were consistently low for each of the 4 cages tested, being 62.31, 56.38, 59.54 and 60.81% compared with mean ADCs for organic matter of 65.68, 63.73, 69.57, 67.38 and 65.82% on the other 5 days of faecal collection. ADCs for gross energy were significantly different ($P<0.05$) between salmon in experiment I and experiment 2. There were no other significant differences in gross energy ADCs. ADCs for crude protein were significantly different ($P<0.05$) between salmon in experiment I and all other experiments, except smolt in experiment IV. There were no other significant differences in crude protein ADCs.

Results tended to be lower and more variable for salmon (4.2 kg) in experiment I compared with other size fish and other stages of the production cycle in seawater. Many fish became anorexic during this experiment probably due to a combination of factors, including: onset of maturation, relatively high water temperatures (up to 19° C on day 3 of collection) and increased handling stress associated with larger fish.

These results suggest that ADCs can be extrapolated across stages of the production cycle in seawater, although there may be an a slight increase in ADCs during winter. While ADCs in adult fish during summer were variable in this study and lower than other stages, it seems likely that this was due to reasons other than real changes in apparent digestibility (e.g. feeding behaviour).

(v) Effect of dietary composition on growth rate; apparent digestibility; relative weight of abdominal components; and histology and electron microscopy of the gastrointestinal tract

The aim of this study was to compare the growth performance and apparent digestibility of 4 commercially available Atlantic salmon feeds. Histology and electron microscopy of the gastrointestinal tract was performed to try and determine factors which may have contributed to differences in performance.

Growth was significantly higher ($P<0.05$) in fish fed on an extruded diet with 30% fat compared to that in fish fed on extruded diet with 17% fat. There was no significant difference however, between the steam-pelleted diet and extruded diets. Chromic oxide in the extruded diet with 17% fat had no affect on growth. The weight (expressed as a percentage of body weight) of the proximal intestine (includes pyloric caecae and caecal fat) was significantly higher ($P<0.05$) in fish fed on an extruded diet with 30% fat compared to fish fed on the steam-pelleted diet with 17% fat. There were no other significant differences ($P<0.05$) in proximal intestine weights or for stomach, distal intestine, liver, spleen and other abdominal fat.

There were no significant differences ($P<0.05$) in the ADCs for crude protein between the diets. All ADCs for organic matter were significantly different ($P<0.05$) with results for steam-pelleted feed with 17% fat, extruded feed with 17% fat and extruded feed with

30% fat being 72.37%, 75.10% and 79.93% respectively. The same trend in results was evident for ADCs for gross energy being 79.20, 82.72 and 85.16 respectively, however the 2 extruded diets were not significantly different from each other ($P<0.05$). No differences in the histology or electron microscopy of the gastrointestinal tract were found between diets.

Higher ADCs for organic matter and gross energy are expected in diets with higher fat level due to the higher digestibility of the fat component of the diet. This was reflected in the significant differences in growth between extruded diets with 17% fat and those with 30% fat. However, it is not clear why the growth in fish fed the steam-pelleted diet with 17% fat was higher than that for extruded diets with the same level of fat when as expected the ADCs for organic matter and gross energy were higher in these extruded diets. This may have resulted from a reduced feed intake for the extruded diets. There was no evidence of differences in the histology or electron microscopy of the gut, liver or pancreas between diets.

10. ACKNOWLEDGEMENTS

We wish to thank Svein Oddsson, Eric Brain, Harry King and staff at SALTAS' marine operations for their assistance in maintaining research trials and the staff at Gibsons Ltd for preparation of experimental diets. We would also like to thank Brian Hoare and Regina Maloney at the Department of Primary Industry and Fisheries and Ken O'Brien and staff at the Wollongbar Agricultural Institute for their assistance with chemical analyses as well as Dr. Chris Carter for his assistance in conducting the final trial and preparation of this report. In addition to the Australian Fisheries Research and Development Corporation (FRDC), this research was funded by the Tasmanian Atlantic salmon industry, and S.B. Percival was the recipient of an Australian Post Graduate Research Award (Industry).