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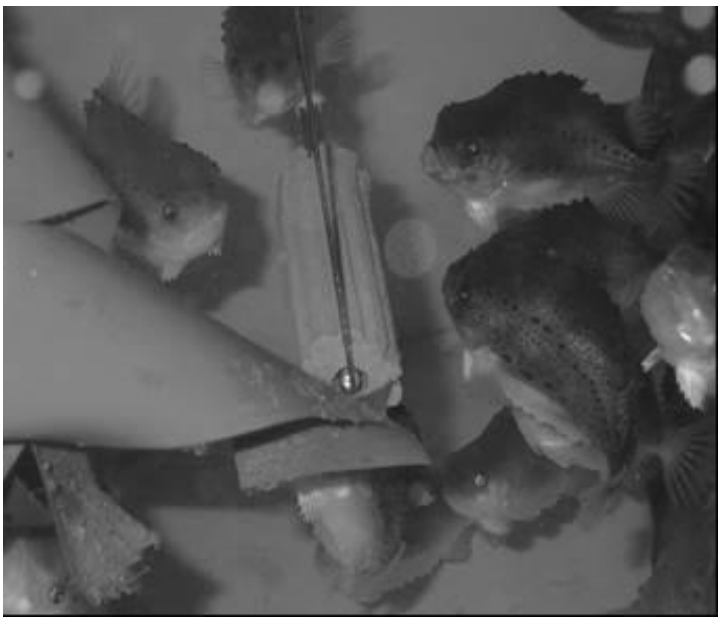
# Comparison of feeding behavior, growth and health of Lumpfish (*Cyclopterus lumpus* L.) fed either feed blocks or pellets commercial feed.



**April 2018**

Version 1.0

**Preface**

This research focus will be an integral part of the research project "**LUCINFER – Optimal bruk av rognkjeks til avulsing av oppdrettslaks" funded by NFR.**

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The objectives of this second in a series of planned studies was to evaluate and compare the effects on growth, cataract development and gut health of fish fed feed blocks or commercially available lumpfish pelleted feed.

Future studies, if lumpfish readily graze from feed blocks will include development of optimal sizes of blocks to use, stability of feed blocks in sea cages and assessing the potential for using feed blocks with varying nutritional profiles.

To this end, the main objectives of this present study are:

1. To evaluate growth and performance of two groups of lumpfish fed either feed blocks or pelleted feed during the study period.
2. To evaluate cataract development.
3. To assess gut health using histopathology.
4. To compare hepatosomatic index (HIS) between the two treatment groups.
5. To evaluate the water stability of feed blocks immersed in seawater.

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**Abstract/Summary:**

Two duplicate groups of individually tagged lumpfish (mean initial weight: 21.5 ± 3.2 g) were fed either a commercially available lumpfish feed or feed blocks for a period of 123 days.

There were significant differences in growth rates between the groups with fish fed pelleted feed having the highest growth rates. Mean weight of fish fed with feed blocks was 52% lower compared to fish fed with pelleted feed whilst maintaining a feeding rate of 2% $BW^{-1}$.

The onset of cataracts was significantly different with fish fed pelleted feed having a cataract prevalence of 87% at the end of the study period whilst fish fed with feed blocks had only 10% prevalence. In addition, fish fed with pellets with cataracts all had severe (score 5-8) cataracts whilst only 5% of fish with cataracts fed with feed blocks had moderate (score 3-4) cataracts. The results suggest a possible dietary effect on cataract alone or in combination with other unknown factors.

The results of the histological examination undertaken in this study showed overall small differences between the two dietary treatments. In some individuals in groups receiving both diets, there was mild to moderate expansion of the lamina propria with tissue most likely to represent fibrous tissue with scattered leucocytes. Changes are most consistent with chronic inflammation. The changes are unspecific, and the cause is uncertain.

Results from the present study show that feeding lumpfish with feed blocks, controlled growth performance without apparently compromising the health status of the fish.

Results from this study show that lumpfish will readily graze from feed blocks and the acclimation period required before the fish will utilize them appears to relatively short, thus potentially allowing for their use in commercial salmon cages.



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## 1.0 Introduction

This project is a continuation of studies designed to assess the use of feed blocks for lumpfish populations. The first study focused mainly on feed block design and deployment to optimize lumpfish utilizing them as a food source. The results from the study indicate that lumpfish require feed blocks with grooves to graze from them and that the acclimation period is relatively short (2 – 4 hours) before the fish will use them as a feed source (Imsland *et al* 2018).

Efficient use of lumpfish in terms of the proportion of fish that graze sea lice, is dependent upon the establishment and maintenance of healthy and robust populations. Recently, there are indications that the incidence of cataracts is prevalent in lumpfish populations. Veterinaries assessing health of lumpfish stocked in salmon cages have observed cataracts in 100% of populations after five months at sea (*pers. com.* Nils Vestvik). In growth studies with lumpfish at different temperatures, cataracts were recorded in varying degrees in fish at high temperatures (13 and 16 °C), but not at low temperatures (Nytrø *et al*. 2014). A recent study undertaken by Jonassen *et al*. 2017 showed cataract development in lumpfish populations was possibly related to disturbed metabolism/ malnutrition, visualized as very high values of selected amino acids in different tissues from sampled fish. This may cause osmotic imbalance in fish tissues and cataract development or is a consequence of osmotic imbalance (Waagbø *et al*, 2016).

If cataracts are associated with sub-optimal nutrition, then further research in nutrition with lumpfish is therefore necessary. One way forward is to test different feed formulations such as marine low energy feed; low protein feed or feed with functional additives. Alternatively, given that juvenile lumpfish display a large ontogenetic variation in optimum temperatures demonstrated by high growth rates (Nytrø *et al*, 2014) and previous studies on Atlantic salmon have shown that cataract development can occur during periods of rapid growth (Bjerkås, *et al*. 2001; Breck & Sveier 2001; Waagbø *et al*. 2010) then controlling the amount and/or type of feed juvenile lumpfish consume may alleviate the potential for cataract development.

It is evident that the supplementary feeding of lumpfish deployed within commercial salmon pens is necessary to maintain their nutritional condition, welfare and sea lice grazing efficacy (Leclercq, Davie, & Migaud, 2014; Leclercq, Graham, & Migaud, 2015) to maintain the nutritional condition, welfare and efficacy of the biological controls over the Atlantic salmon grow-out cycle typically lasting 18–22 months. Presently, lumpfish stocked in commercial salmon pens are being fed extruded pelleted feed usually delivered from feed automats around the edge of the cages. This method is limited as lumpfish have been shown to be opportunistic feeders and readily exploit available food sources (Imsland *et al.*, 2014a, b, c). Such a food source which may be predictable both spatially and temporally may result in most of the lumpfish maintaining position around the periphery of the cages and reduce their potential for grazing sea lice.

In addition, reliance on this food source may result in lumpfish consuming feed pellets to a point where growth rates are enhanced, and cataracts may develop. Therefore, there is a need to develop a feed source adapted to the species grazing feeding habit and to the salmon net-pens rearing environment. Feed blocks have been used in salmon cages stocked with wrasse species (Leclercq *et al*., 2015) and can be positioned in areas of the cage where the wrasse will be in closer proximity to the salmon thus potentially enhancing grazing potential.

Practical feed for lumpfish within salmon net-pens should combine a manufactured base providing a complete and standardised nutrient profile, biosecurity and ease of procurement with high water stability for distribution as a grazing substrate. Further, this methodology has the potential to facilitate lumpfish feeding in sea cages and to allow the monitoring of feed intake to safeguard health, welfare and sea lice grazing activity.

The objectives of this study will be to evaluate and compare the effects on growth and cataract development of fish fed feed blocks or commercially available lumpfish pelleted feed.

Future studies, if lumpfish readily graze from feed blocks will include development of optimal sizes of blocks to use, stability of feed blocks in sea cages and assessing the potential for using feed blocks with varying nutritional profiles.

To this end, the main objectives of this present study are:

1. To evaluate growth and performance of two groups of lumpfish fed either feed blocks or pelleted feed during the study period.
2. To evaluate cataract development.
3. To assess gut health using histopathology.
4. To compare hepatosomatic index (HIS) between the two treatment groups.
5. To evaluate the water stability of feed blocks immersed in seawater.

## 2.0 Methods

### 2.1 Experimental fish and conditions

The lumpfish were produced from fertilized eggs from Senja Akvakultursenter AS, Tromsø. The eggs were transferred to Mørkvedbukta AS, Bodø where they were incubated at 9 – 10°C and the juveniles were initially fed with Gemma Micro (150–500 µm, Skretting, Norway). After 30 days, the juveniles were fed with 500– 800 µm dry feed pellets (Gemma Wean Diamond, Skretting, Norway). The fish were vaccinated with ALPHA JECT Marin micro 5 (Pharmaq AS, Oslo, Norway) on 14$^{th}$ September 2017.

The health status of the fish was assessed immediately prior to transfer to Gifas, Inndyr, Nordland, Norway two weeks after vaccination. Health status was assessed by PCR screening for Vibrio species, atypical furunculosis, pasteurella, moritella, pancreas disease (PD), infectious pancreatic necrosis (IPN), viral hemorrhagic septicemia (VHS), Nodovirus and amoebic gill disease (AGD).

The juveniles were fed a high protein low fat marine feed (Biomar lumpfish grower) using Van Gerven 7/L feeding automats (the Netherlands). A 50% mixture of 1.5 mm and 2 mm pellets was used during this period.

All tanks were supplied with full salinity sea water pumped from 70 m depth at a temperature of between 6.7 and 12.2°C and oxygen saturation was maintained above 80% during the whole experimental period. Water temperature and oxygen concentration was recorded in all experimental tank using a Handy Polaris 2 probe (Oxy- Guard International A/S).

### 2.2 Study design

One-week prior to the start of the trial (week 40, 2017), two groups of lumpfish with an initial mean (±SD) weight of 15.0 ± 2.0g (n = 120; N = 240) were established from the original population. Batches of five fish at a time were sedated (appendix I). Once the suitable level of sedation had been attained, each lumpfish was pit-tagged (appendix II). After tagging, the weight and length of each lumpfish was recorded along with their individual pit-tag ID and the fish transferred into four 3.5 $m^3$ circular flow-through tanks with 60 fish in each tank. The fish were allowed to acclimate for a period of one week prior to the start of the trial during which, all tanks were fed a high protein low fat marine feed (Biomar grower 2.2 mm) using Van Gerven 7 $L^{-1}$ feeding automats (Holland) at a daily feeding rate of 2% $BW^{-1}$. The study period was 123 days.

### 2.3 Feeds and feeding

At trial start (week 41, 2017), the pelleted feed was withdrawn, and feed blocks introduced to two tanks (M3 and M4). The feed blocks were suspended in the water column (image 1A)

Each individual feed block was 26 x 100 mm with a 10 mm hole through the centre and had grooves created on their surface during the extrusion process (image 1B). To increase potential access, the placement of the feed blocks was either at:

- A minimum of 50 cm from then side of the tank and 40 cm from the bottom of the tank
- A minimum of 50 cm from then side of the tank and 70 cm from the bottom of the tank

The blocks were also placed randomly around both tanks allowing for the greatest distance between them.

Feed blocks were weighed prior to placement to ensure sufficient feed was available to maintain a daily feeding rate of 2% $BW^{-1}$. The other two tanks (M5 and M6) received the same daily feeding rate using Biomar grower 2.0mm.

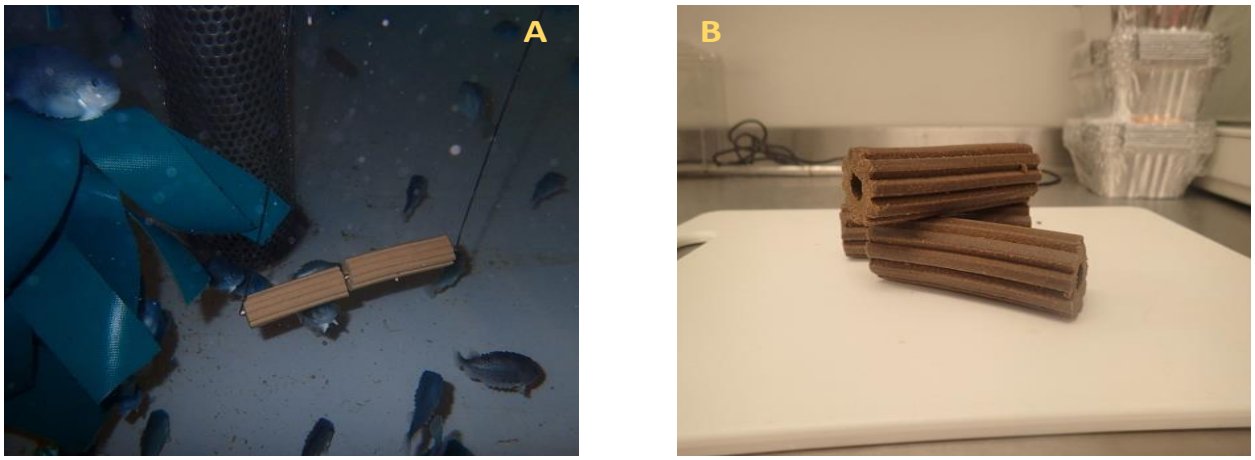



**Image 1 Feed blocks deployed(A) and feed blocks prior to deployment (B).**

### 2.3.1 Feeding behaviour

Feeding behaviour was recorded in both tanks receiving feed blocks using underwater cameras throughout the study period. After deployment, the fish were observed for evidence of eating from the feed blocks at one hour after deployment every day during the study period. Feeding response was evaluated using a frequency distribution table (Table 1). Response was scored from a scale of 0 to 7. Zero equals no evidence of feeding and 7 that more than 50% of the fish in the tank were observed grazing from the blocks. Fresh feed blocks were placed in the tanks every day

**Table 1. Distribution table of recorded feeding response behaviour used during the study period. There were 60 fish in each experimental tank receiving feed blocks.**

| Score | Response |
|---|---|
| 0 | No response to the feed blocks. Fish are distributed and no fish near the blocks. |
| 1 | Fish swimming towards feed blocks or hovering around them. No evidence of grazing. |
| 2 | Periodic grazing by less than 10 fish |
| 3 | Regular grazing by 10 - 19 fish |
| 4 | Regular grazing by 20 - 29 fish |
| 5 | Regular grazing by 30 - 39 fish |
| 6 | Regular grazing by 40 - 49 fish |
| 7 | Regular grazing by over 50 fish |

### 2.3.2 Feed analysis

Two duplicate 300g samples (A and B) of each diet type (feed blocks and pelleted feed) were vacuum packed and stored frozen at – $20^0$C. The two "A" samples were analysed for fatty acid and amino acid profile, crude protein, starch, astaxanthin, moisture and vitamin C content. The "B" samples were retained for potential further analysis.

### 2.4 Growth and performance

All lumpfish from both groups were individually weighed and fork-length recorded at two weekly intervals during the trial period.

The tank was checked for mortalities every day. Any mortalities present were removed, and their weight and length recorded.

Specific growth rate (SGR) from mean weights at each weighing interval per tank was calculated according to the formula of Houde and Schekter (1981):

$$SGR = (e^{g}-1) \times 100$$

where g = (ln ($W_2$)-ln ($W_1$) / ($t_2$-$t_1$) and $W_2$ and $W_1$ are weights on days $t_2$ and $t_1$, respectively.
Condition factor (K) of individual lumpfish (calculated at each weighing interval) was defined as:

$$K = 100 * W = L3$$

where W is the weight (g) of the fish and L the corresponding total length (cm).
Actual feed intake data could not be determined per treatment as each tank could not be fitted with feed collection apparatus. However, biological feed conversion ratio (*b*FCR) per tank was calculated based on feed presented/ (biomass gain + mortality biomass) for each duplicate group.

**2.5 Cataract scoring**

During weighing and counting of lumpfish throughout the study period, the cataract score of all fish was recorded. Each fish was transferred to a darkened room and a handheld slit lamp with a magnifying glass at 10x magnification (Heine HSL 150, C-002,14,602) was used to examine both eyes. After scoring, the fish were transferred to a holding tank containing well-aerated seawater until fully recovered before being placed back in its respective tank.
Each eye was scored on a scale from 0 to 4 in accordance with Wall and Bjerkås (1999) where 0 = no cataract, 1 = cataract covers less than 10% of the lens, 2 = cataract covers 10-50 % of the lens, 3 = cataract covers 50-75% of the lens and 4 = cataract covers 75-100% of the lens. The scores per eye were summated for each giving the cataract score per individual (0 – 8). In addition, mean scores (cataract index) of all examined individuals within the experimental groups was calculated. Both affected and non-affected individuals were included in calculated average group scores.

**2.6 Histopathology**

For histological evaluation, ten fish were sampled immediately prior to the start of the study and ten fish from each dietary treatment (5 from each replicate) were sampled at the end of the study period. All fish were humanely dispatched with an overdose of Benzoak (Bensokain 200mg/ml (20%)) and PIT-tag ID, weight and length were recorded along with cataract score. The fish were then dissected and the whole intestine was carefully removed intact and flushed with 4% buffered formalin. After flushing, the intestines were transferred into a sampling pot containing 4% buffered formalin. The whole pyloric caeca and a liver biopsy were also sampled and transferred to a similar container. Additionally, both eyes from 4 fish per dietary treatment were removed at the end of the study period. Fish were selected based on the cataract status indicative of both groups.
Transverse sections of pyloric caeca, liver and mid-gut and hind-gut were sampled from the whole intestinal tracts according to Moldal *et al* (2014) and all whole eye samples. Tissue samples were processed for histology and embedded in paraffin. Tissue sections (1–2 μm) were stained with haematoxylin and eosin (HE), periodic acid-Schiff (PAS) (stains neutral mucin) and Alcian blue (stains acid mucin), scanned with an Aperio Scan Scope AT Turbo slide scanner and examined by digital light microscopy using Aperio eSlide Manager.
Samples were evaluated semi-quantitatively for inflammatory changes in the muscularis, submucosa/lamina propria and epithelial layers according to the criteria in Table 2. Epithelium was evaluated for degeneration/necrosis and vacuolisation. Goblet cells stained positive with PAS and Alcian blue in the mid-gut were also assessed semi-quantitatively according to criteria in Table 1.
Intestinal fold length was measured in the mid-gut using Aperio eSlide Manager by measuring the height of all intact folds in the cross section of the loop with the most optimal orientation. Measurements were taken from the tip of the fold immediately under the epithelium until the start of the muscularis. Similarly, eyes were evaluated semi-quantitatively.

**2.7 Hepatosomatic Index and liver weight**

At trial start, 10 lumpfish were randomly selected from the initial population and humanely dispatched with an overdose of Benzoak. For each fish, individual weight and total length was recorded after which each fish was carefully dissected to reveal the internal organs and a digital

picture taken. The liver from each fish was carefully removed and weighed. The hepatosomatic index was calculated for each fish using the formula:

$$HSI\ (\%) = 100 \times (\text{liver weight[g]} / \text{whole fish weight [g]}).$$

This process was repeated at three regular intervals (intermediate 1 & 2 and end) after trial start with 10 fish from each of the two established groups (five fish per tank).

**Table 2 Evaluation criteria used for histological analysis.**

| Score | Criteria |
|---|---|
| | **Inflammation muscularis, submucosa/lamina propria** |
| **0** | normal |
| **1** | focal or mild diffuse inflammation |
| **2** | multifocal or moderate diffuse inflammation |
| **3** | severe diffuse inflammation |
| | **Epithelial degeneration/necrosis, epithelial vacuolization** |
| **0** | normal |
| **1** | mild changes |
| **2** | moderate changes |
| **3** | severe changes |
| | **Epithelial inflammation** |
| **0** | <2 leukocyte per 20 epithelial cells |
| **1** | 2-4 leukocytes per 20 epithelial cells |
| **2** | 5-6 leucocytes per 20 epithelial cells |
| **3** | >6 leukocytes per 20 epithelial cells |
| | **Goblet cells stained positive with PAS** |
| **0** | <1 positive cell per 20 epithelial cells |
| **1** | 1-2 positive cells per 20 epithelial cells |
| **2** | 2-5 positive cells per 20 epithelial cells |
| **3** | > 5 positive cells per 20 epithelial cells |
| | **Goblet cells stained positive with Alcian blue** |
| **0** | <1 positive cell per 20 epithelial cells |
| **1** | 1-2 positive cells per 20 epithelial cells |
| **2** | 2-7 positive cells per 20 epithelial cells |
| **3** | > 7 positive cells per 10 epithelial cells |
| | **Liver vacuolization** |
| **0** | none or minimal |
| **1** | mild |
| **2** | moderate |
| **3** | severe |

## 2.8 Determination of water stability of feed blocks

The water stability of the feed blocks (WS%) was determined over a period of 0.5, 2, 4, 6, 12, 18 and 24 hours by wet durability tests using a modified version of the methods described by Jayaram and Shetty (1981), Aquacop (1978) and Adedeji *et al* (2015).

For each exposure time, triplicate 20g samples of feed blocks were placed into 3 pre-weighed 600 ml borosilicate glass beakers (VWR, Oslo, Norway) which contained fresh seawater. After immersion, the undissolved solids and water were filtered using a vacuum pump fitted with pre-weighed glass microfibre filters, grade GF/C (Whatman (GE Healthcare), Oslo, Norway). After filtering, the remaining solids and filter paper were dried in an oven at 105 $^0$C for 30 minutes followed by further drying at 65 $^0$C until constant weight. The mean differences in weights of beakers containing the feed blocks before immersion and after drying were used to calculate the percentage dry matter loss, which is a measure of the water stability of the feed blocks for the corresponding time intervals. Water stability was calculated according to the formula:

$$WS\% = \frac{m_i - m_w}{m_i} \times 100$$

were:
$m_i$ = weight of blocks before immersion.
$m_w$ = dry weight of remaining solids

### 2.9 Statistics
All statistical analyses were conducted using Statistica™ 12.0 software. A Kolmogorov-Smirnov test (Zar, 1984) was used to assess for normality of distributions. The homogeneity of variances was tested using the Levene's F test (Zar, 1984). Possible differences in feeding behaviour, mean weights, condition factor and growth rates between the experimental groups were tested with two-way nested analysis of variance (ANOVA), where replicates are nested within feeding types. Significant differences revealed in ANOVA were followed by Student–Newman–Keuls (SNK) post-hoc test to determine differences among experimental groups. A significance level (α) of 0.05 was used if not stated otherwise. In cases with non-significant statistical tests, power (1–b) analysis was performed in Statistica using α = .05.

## 3.0 Results
### 3.1 Diet composition
Two different diet types were used during the study period. Feed blocks (World Feeds Limited. UK) and pelleted feed (Biomar Grower NO.). Analysed diet composition for each of the diets can be seen in Table 3. Crude protein varied between the diets with the pelleted feed having higher inclusion levels compared to feed blocks (56.5 and 50.1% respectively). Similarly, the pelleted feed had higher inclusion levels of crude fat compared to feed blocks (15.8 and 10.3% respectively). Starch content was lowest in pelleted feed (6.3%) and higher in feed blocks (8.2%). Moisture content was higher in feed blocks (23.2%) compared to pelleted feed (6.6%). Vitamin C inclusion levels in the pelleted feed (1020.0 mg $kg^{-1}$) was higher compared to feed blocks (613.0 mg $kg^{-1}$). The pelleted feed had an astaxanthin ester inclusion level of 11.9 mg $kg^{-1}$ whilst feed blocks had an astaxanthin level of 133.0 mg $kg^{-1}$. Both gross energy (GE) and dietary energy (DE) was highest in the pelleted feed compared to feed blocks (Table 3).

**Table 3. Analysed diet composition of feed blocks and pellets used in the study.**

| *Composition* | | Pellets | Blocks |
|---|---|---|---|
| **Fat** | **%** | **15.8** | **10.3** |
| **Protein (Nx6.25)** | **%** | **56.5** | **50.1** |
| **Moisture content** | **%** | **6.6** | **23.2** |
| **Starch and simple sugars** | **%** | **6.3** | **8.2** |
| **Astaxanthin** | **mg/kg** | **-** | **133.0** |
| **Astaxanthin esters** | **mg/kg** | **11.9** | **-** |
| **Vitamin C (L-ascorbyl-2-phosphate) (mg $kg^{-1}$)** | **mg/kg** | **1020.00** | **613.00** |
| **Calc. GE** | **MJ/kg** | **20.7** | **17.3** |
| **Calc. DP** | **%** | **50.3** | **44.6** |
| **Calc. DE** | **MJ/kg** | **18.3** | **15.2** |
| **Calc. DP:DE ratio** | | **27.4** | **29.4** |

**GE = Protein*23.7 + Lipid*39.5 + Starch*17.2. DE was calculated from analysed protein, lipid and starch content, caloric values for each nutrient, and digestibility's of 89%, 93% and 60% for protein, lipid and starch, respectively (Bendiksen, E.Å., AquaNutrition, Levanger, Norway, pers. comm.).**

The pelleted feed had higher levels of both essential (EAA) and non-essential (NEAA) amino acids compared to feed blocks (Table 4). The pelleted feed had an EAA inclusion level of 23.6 g 100 $g^{-1}$

whilst the feed bocks had an inclusion level of 16.8 g 100 $g^{-1}$. The pelleted feed and feed blocks had NEAA inclusion levels of 28.1 and 19.9 g 100 $g^{-1}$ respectively.
There was variation in most of the individual fatty acids (FA) between the two diets (Table 5). Inclusion levels of saturated fatty acids (SAFAs) was highest (27.3%) in the pelleted feed compared to the feed blocks (24.9%). Whilst, the sum of monounsaturated fatty acids (MUFAs) was highest in feed blocks compared to the pelleted feed (38.2 and 31.8% respectively). There were higher inclusion levels of n-3 PUFAs in the feed blocks (35.8%) compared to 24.3% for the pelleted feed. The sum of n−6 PUFA was lowest in feed blocks and highest in pelleted feed whilst the n−3/n−6 ratio was highest in feed blocks (9.2) compared to pelleted feed (2.7).

**Table 4. Analysed amino acid profile of both diets used in the study.**

| Amino acids (g 100 $g^{-1}$) | Pellets | Blocks |
|---|---|---|
| **Valine** | 2.56 | 1.87 |
| **Isoleusine** | 2.27 | 1.56 |
| **Leucine** | 4.04 | 2.85 |
| **Phenylalanine** | 2.46 | 1.51 |
| **Histidine** | 1.32 | 0.74 |
| **Lysine** | 3.44 | 2.93 |
| **Arginine (total)** | 3.41 | 2.53 |
| **Cystine + Cystein** | 0.62 | 0.27 |
| **Methionine** | 1.33 | 0.87 |
| **Threonine** | 2.13 | 1.64 |
| **Tryptophan** | *n.a.* | *n.a.* |
| **SUM EAA** | **23.6** | **16.8** |
| **Asparagine** | 4.77 | 3.64 |
| **Serine** | 2.45 | 1.63 |
| **Glutamic acid** | 9.99 | 5.12 |
| **Proline** | 3.12 | 1.87 |
| **Glycine** | 2.97 | 3.18 |
| **Alanine** | 2.83 | 2.72 |
| **Tyrosine** | 1.86 | 1.06 |
| **hydroxyproline** | 0.15 | 0.61 |
| **Ornithine** | <0.05 | 0.06 |
| **SUM NEAA** | **28.1** | **19.9** |

### 3.2 Growth and feed conversion ratio.

Mortality rates for fish fed feed blocks or pelleted feed was 6.7% and 0.8% respectively. Mortality rates included fish removed with obvious wound and/or poor condition. Mean weight and specific growth rates (SGR) varied between the diet groups from day 14 onwards (SNK post hoc test, $P < 0.05$, Figure 1 & Figure 2). Fish fed pellets was largest and displayed the highest SGR from day 14 and throughout the study period. There was a trend for fish fed pellets for SGR to decrease from day 14 more so compared to fish fed feed blocks where SGR changed little from day 28 onwards.
At the end of the study period, fish fed pellets achieved a mean (± SD) weight of 169.67 ± 41.69g whilst fish fed with feed blocks had a mean (± SD) weight of 290.45 ± 57.86g. This represents a difference of 52.5% difference between the two experimental groups.

**Table 5. Analysed fatty acid profile of the diets used in the study.**

| Fatty acid profile | Pellets | Blocks |
|---|---|---|
| C 14:0 | 6.0 | 4.7 |
| C 15:0 | 0.4 | 0.5 |
| C 16:0 | 17.1 | 16.6 |
| C 18:0 | 2.8 | 2.3 |
| Sum C 20:0, C 22:0 and C 24:0 isomers | 0.6 | 0.4 |
| **Sum SAFAs (Saturated fatty acids)** | **27.3** | **24.9** |
| C 16:1 n-7 | 5.7 | 4.9 |
| C 18:1 n-9 | 16.8 | 17.1 |
| C 20: 1 n-9 | 3.3 | 5.7 |
| C 22:1 and C24:1 isomers | 5.8 | 10.3 |
| **Sum MUFAs (Monosaturated)** | **31.8** | **38.2** |
| C 18:3 n-3 | 1.5 | 13.0 |
| C 18:4 n-3 | 2.0 | 2.1 |
| C 20:3 n-3 | 0.1 | 0.2 |
| C 20:4 n-3 | 0.5 | 0.5 |
| C 20:5 n-3 (EPA) | 10.0 | 7.3 |
| C 22:6 n-3 (DHA) | 9.0 | 11.9 |
| C 22:5 n-3 | 1.2 | 0.8 |
| **Sum n-3 PUFAs (Polyunsaturated) (Omega 3)** | **24.3** | **35.8** |
| C 18:3 n-6 | 0.2 | 0.2 |
| C 18:2 n-6 | 7.9 | 2.6 |
| C 20: 4 n-6 (ARA) | 0.6 | 0.5 |
| C 20: 2n-6 | 0.2 | 0.4 |
| C 22:5 n-6 | 0.2 | 0.2 |
| **Sum n-6 PUFAs (Polyunsaturated) (Omega 6)** | **9.1** | **3.9** |
| **Sum PUFAs** | **33.7** | **28.1** |
| Total fatty acids | 92.7 | 91.2 |
| Unidentified components | 7.3 | 8.8 |
| **n-3:n-6 ratio** | **2.7** | **9.2** |

The condition factor (K) in both groups varied between 3.6 and 4.3 (Figure 3). The mean K tended to be higher in the fish fed pelleted feed throughout the trial period being significantly higher from day 46 onwards (SNK test, $p < .05$).

Thermal growth coefficient (TGC) was significantly higher in fish fed pelleted feed compared to fish fed feed blocks throughout the study period (SNK test, $p < .05$, Figure 4). TGC values ranged from 1.1 to 2.2 for fish fed with feed blocks whilst values ranged from 3.1 to 3.2 for fish fed with pelleted feed.

The dietary group fed with pelleted feed had a significantly lower biological FCR (1.24) compared to fish fed with feed blocks (1.79) (SNK post hoc test, $P < 0.01$) at the end of the study period.

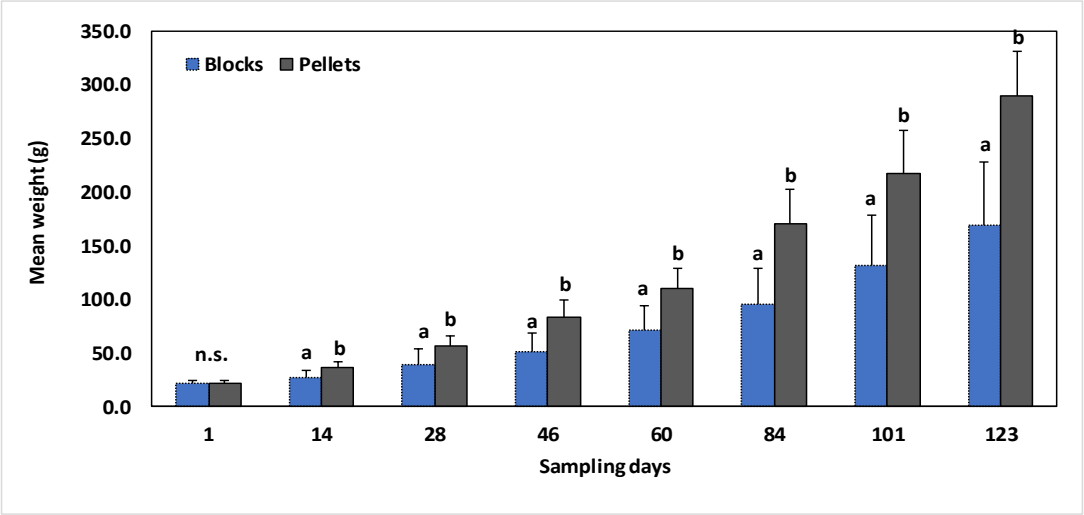



**Figure 1 Mean weight (g) of lumpfish fed either feed blocks or pelleted feed. Values represent means ± SD. Different letters indicate significant differences (SNK test, $P < 0.05$); n.s., not significant.**

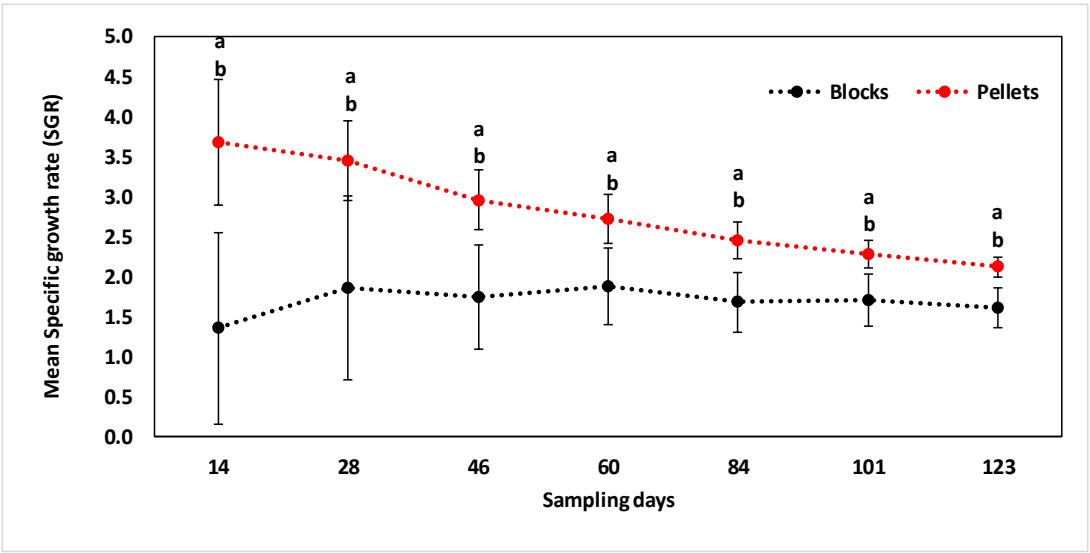



**Figure 2 Specific growth rates (% day $^{-1}$) of lumpfish fed either feed blocks or pelleted feed. Values represent means ± SD. Different letters indicate significant differences (SNK test, $P < 0.05$); n.s., not significant.**

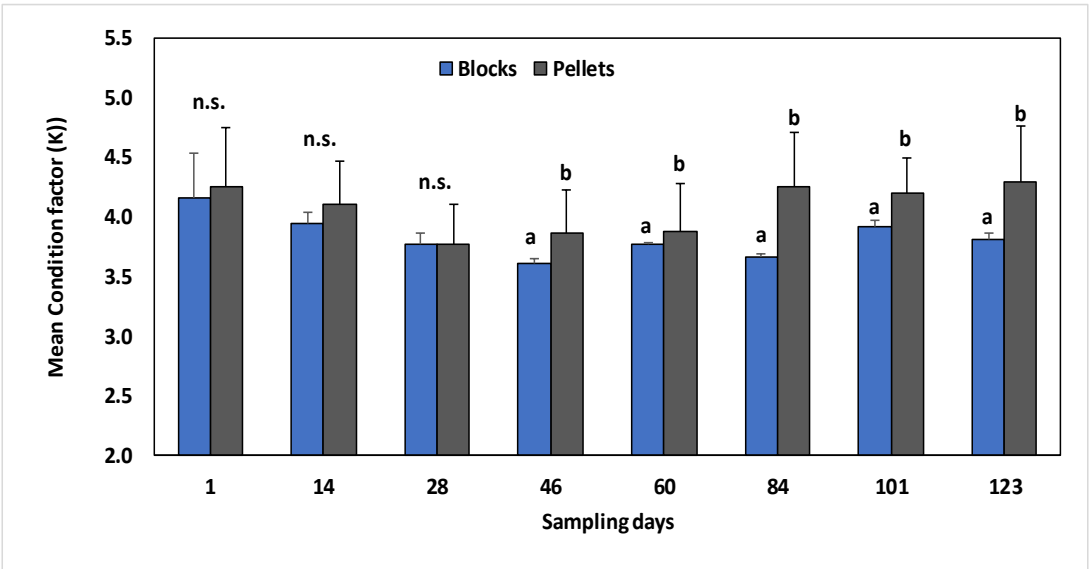



**Figure 3 Mean condition factor (K) of lumpfish fed either feed blocks or pelleted feed. Values represent means ± SD. Different letters indicate significant differences (SNK test, $P < 0.05$); n.s., not significant.**

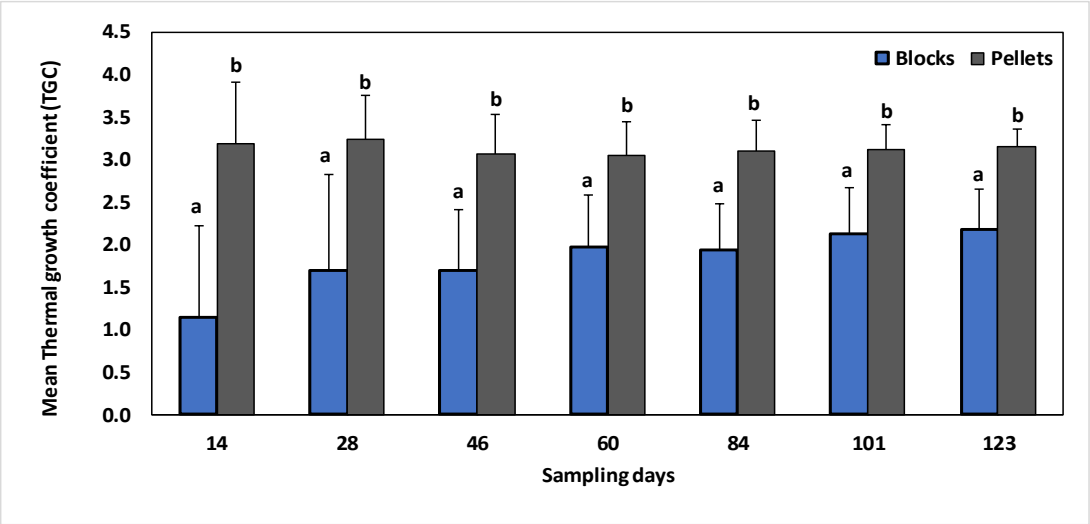



**Figure 4 Mean Thermal growth (TGC) of lumpfish fed either feed blocks or pelleted feed. Values represent means ± SD. Different letters indicate significant differences (SNK test, *P* < 0.05); n.s., not significant.**

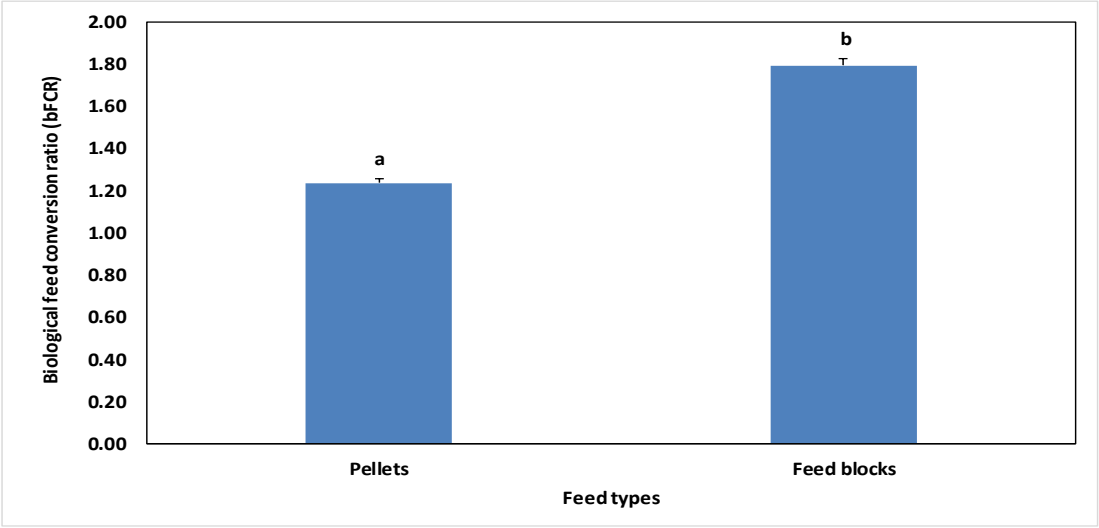



**Figure 5 Mean biological Feed conversion ratio (*b*FCR) of lumpfish fed either feed blocks or pelleted feed. Values represent means ± SD. Different letters indicate significant differences (SNK test, *P* < 0.05); n.s., not significant.**

### 3.3 Feeding behaviour

Feeding response was similar for both replicate tanks receiving feed blocks throughout the study period (figure 6). During week 1 (3-day period) the mean feed response was similar for both tanks (Regular grazing by 20 - 29 fish (33% to 48% of the population). By week 2, feed block grazing intensity had increased to regular grazing by 30 - 39 fish (50% to 65%) in each tank. Form week 3 onwards, both replicate groups of fish exhibited regular similar grazing intensity with regular grazing by 40 - 49 fish (67% to 82% of the population) recorded during 5 weeks of the study period and regular grazing by over 50 fish (83% > of the population) observed for 10 weeks of the study period.

Grazing behaviour from feed blocks decreased for a short period after each sampling in both tanks. The day after each sampling, fish feeding from feed blocks decreased to an average of regular grazing by 30 - 39 fish (50% to 65%) for both tanks. However, feeding intensity increased to previous higher levels generally within 2 days after sampling.

At the start of the study period when feed blocks were first introduced to each of the 2 replicate tanks, fish were observed feeding from the feed blocks within 1 to 2 hours after deployment.

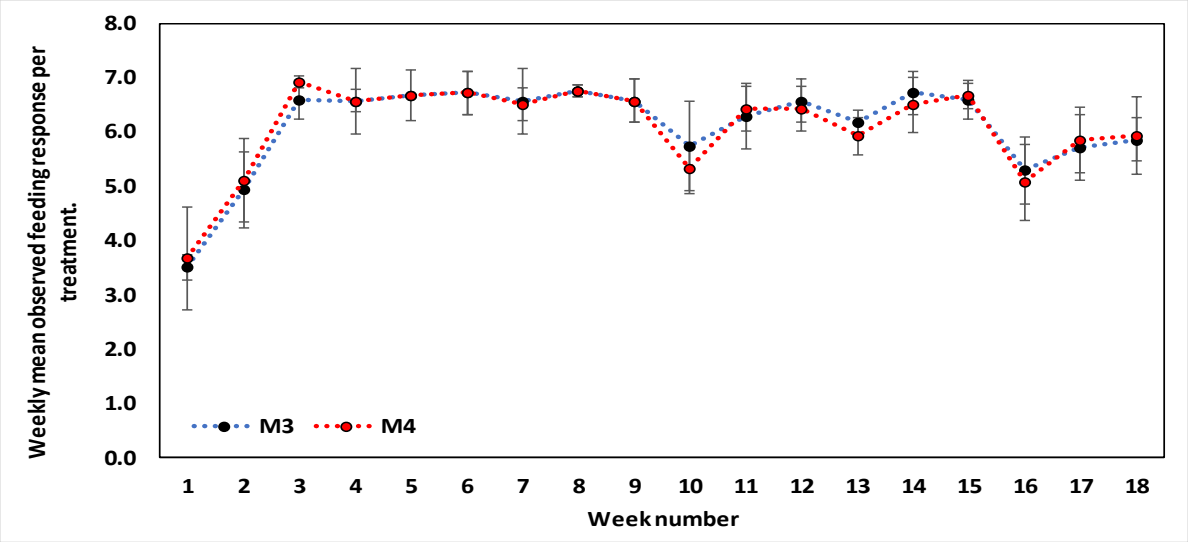



**Figure 6 Mean behavioural observations recorded for each of the two tanks (M3 and M4) which received feed blocks. Scoring was based on a constructed frequency distribution (Table 1) for each feed block deployed in each tank. Values represent means ± SD.**

### 3.4 Feed input and biomass

Mean weekly calculated feed input (kg) for each treatment group can be seen in figure 7. There was significantly more feed required for fish fed with pellets compared to fish fed feed blocks after week 1. Feed inputs required was based on the biomass of each tank and at a daily feeding rate of 2% $BW^{-1}$.

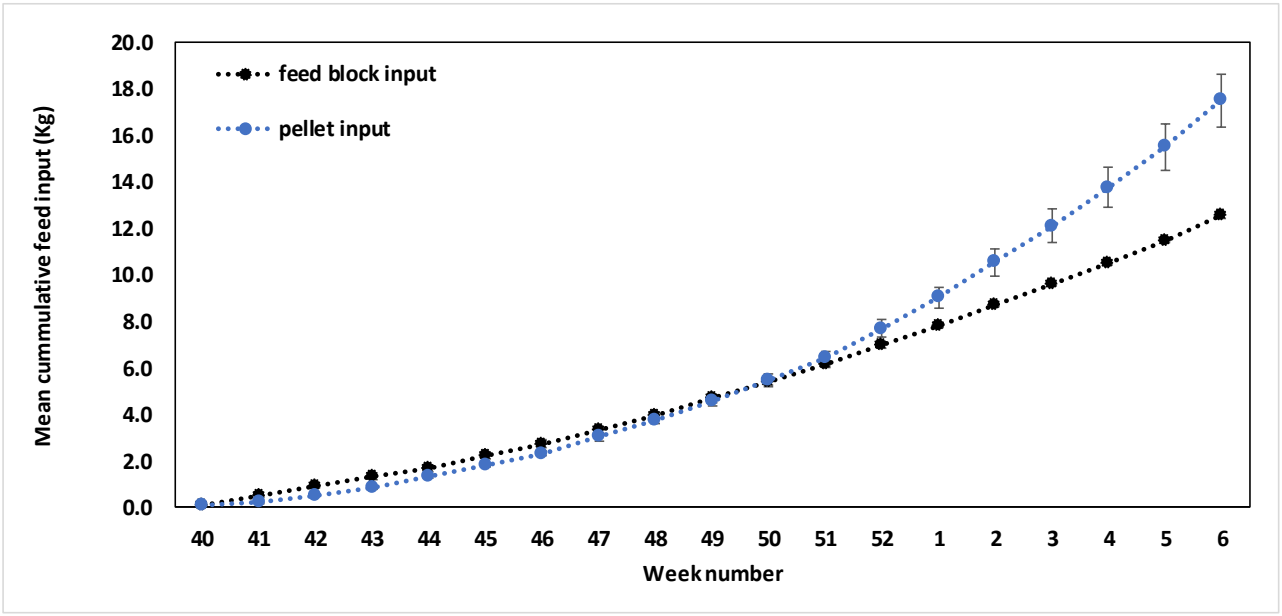



**Figure 7 Mean cumulative feed input (kg) for fish fed either pelleted feed or feed blocks during the experimental period. Values represent means ± SD.**

Fish fed with pelleted feed at 2% $BW^{-1}$ had a higher biomass gain throughout the study period. The differences in biomass between fish fed pellets and fish fed feed blocks was significant from week 44 onwards (SNK post hoc test, $P < 0.05$; figure 8).

There were differences between the amount of feed blocks required and the amount delivered to both treatment groups (figure 9) from week 41 onwards. There was a 28% difference between the actual and estimated amount required at the end of the study period (week 6).

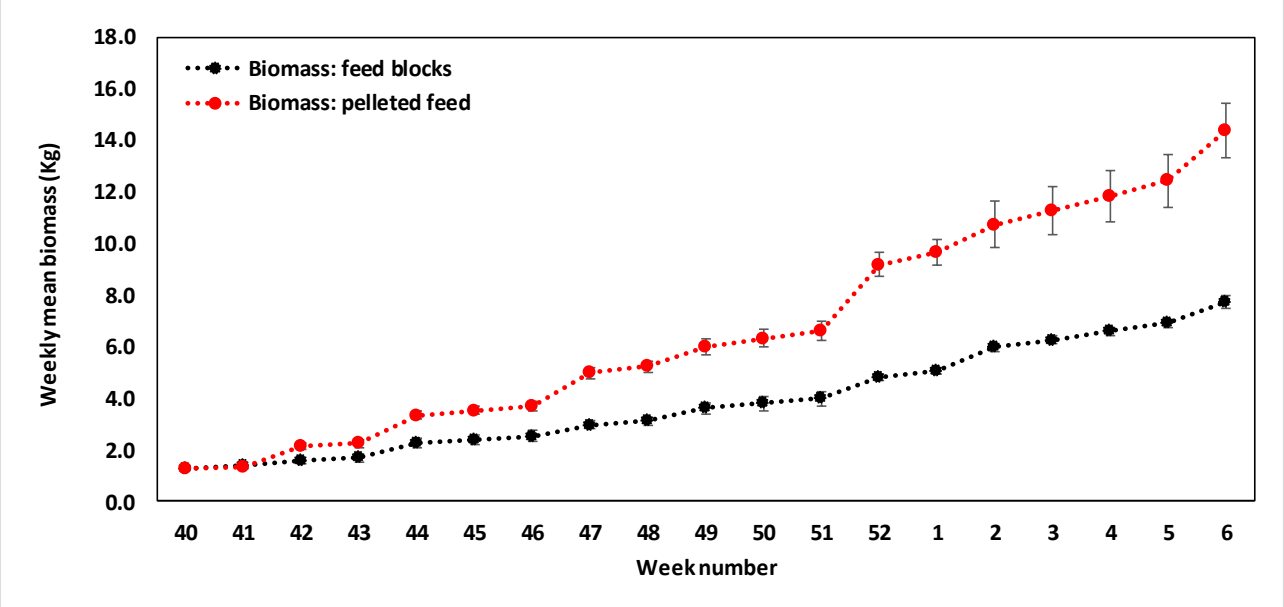



**Figure 8 Mean weekly biomass (kg) for fish fed either feed blocks or pelleted feed. Values represent means ± SD.**

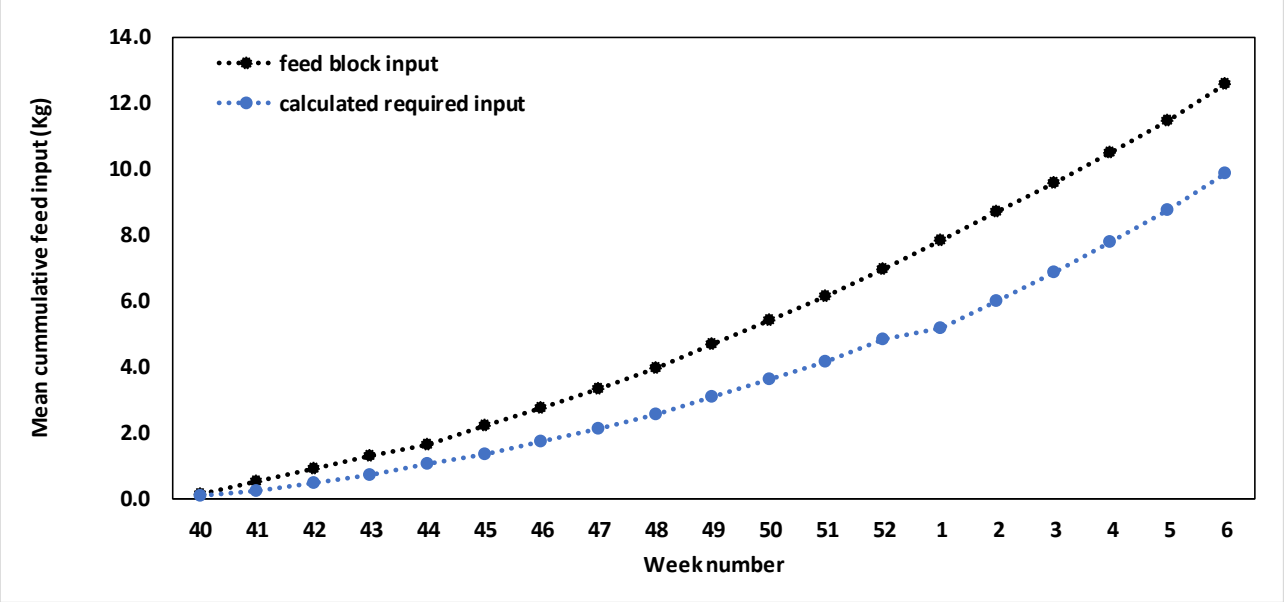



**Figure 9 Comparison of actual cumulative feed block input and estimated feed blocks required based on fish biomass at a daily feeding rate of 2% $BW^{-1}$. Values represent means ± SD.**

### 3.5 Water stability of feed blocks

Water stability of feed blocks decreased through time (figure 10). For the first 24 hours, feed blocks lost from 8.5% of their initial mass after 30 minutes immersion to 30.6% after 24 hours immersion. At 48 hours immersion, 90% of feed block mass was found to be lost.

### 3.6 Cataracts

Occurrence of lumpfish from each dietary treatment with cataracts (% prevalence) throughout the study period can be seen in figure 11. At the start of the study period all three groups had a cataract prevalence of 4%. However, from day 14 onwards there was a trend towards significantly higher increasing prevalence in fish fed with pellets compared to fish fed feed blocks. This difference was significantly different from day 28 onwards (two-way nested ANOVA, $P < 0.001$. At the end of the study period the percentage prevalence of fish with cataracts fed pellets was 87% compared to 10.0% for fish fed with feed blocks (image 2).

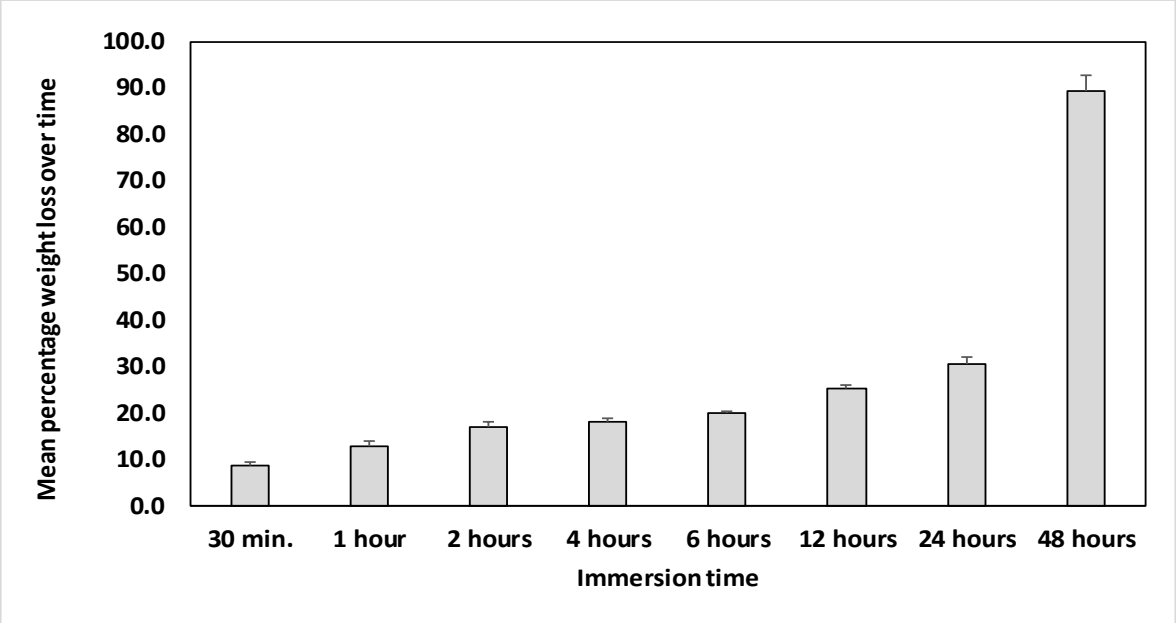



**Figure 10 Percentage weight loss of feed blocks after immersion at different time intervals.**

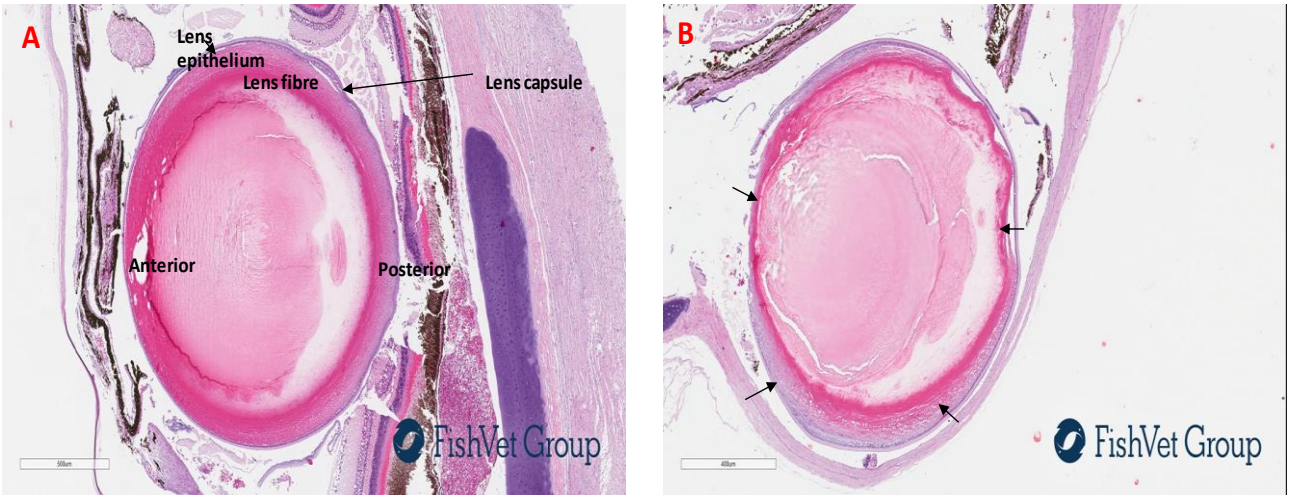



**Image 2 Micrographs of eyes from (A) fish fed with feed blocks showing normal lens, a thin (unicellular) layer and epithelium deep to the fibrous lens capsule with regular, even lens fibres and (B) fish fed with pelleted feed showing swollen hyperrosinophilic lens fibres (arrows). Lens capsule missing at the posterior pole (possible artefact).**

There was an increasing severity of cataracts for fish fed with pelleted feed compared to fish fed with feed blocks (figure 12). There were significant differences in the frequency of no cataracts (score 0-0) from day 28 onwards, fig 12A) whilst there were significant differences in the frequency of mild (score 1-2) from day 60 onwards (fig 12B) with fish fed with feed blocks having a higher frequency. (SNK post hoc test, $P < 0.05$). No fish fed with feed blocks had a medium score (3-4) until day 101whilst fish fed with pelleted feed had a medium score between day 14 and day 84 (fig 12C). There were increasingly significantly more fish with severe cataract (score 5-8) from day 28 onwards whilst there were no fish fed with feed blocks having severe cataracts (fig12D; SNK post hoc test, $P < 0.05$).

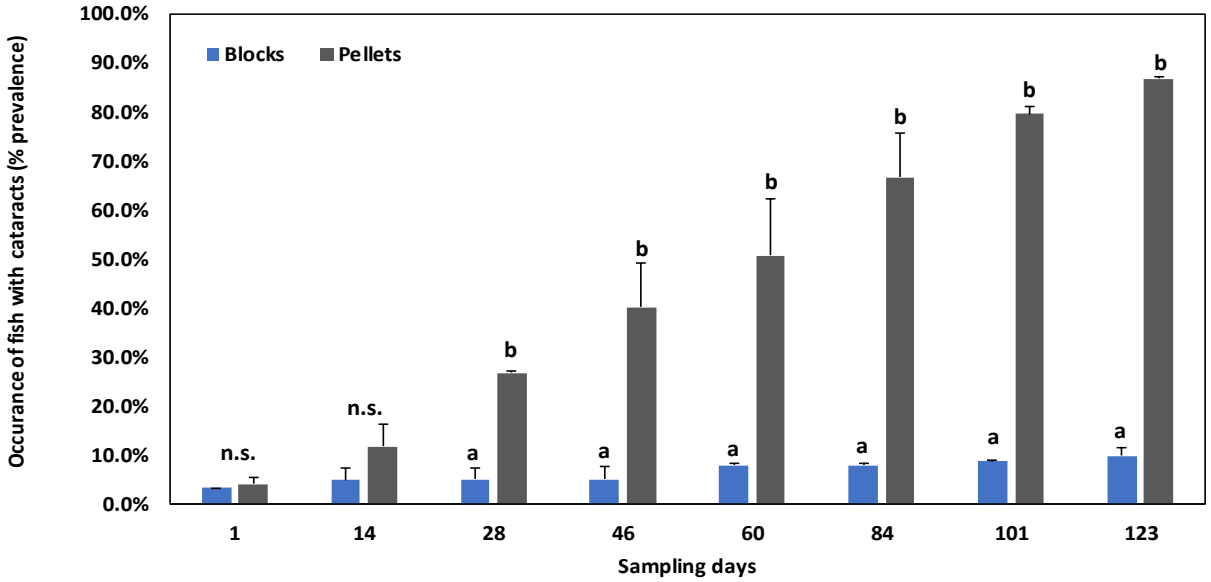



**Figure 11 Occurrence of lumpfish with cataracts (% prevalence) calculated at each of the sampling days. Values represent means ± SD. Different letters indicate significant differences (SNK test, *P* < 0.05); n.s., not significant.**

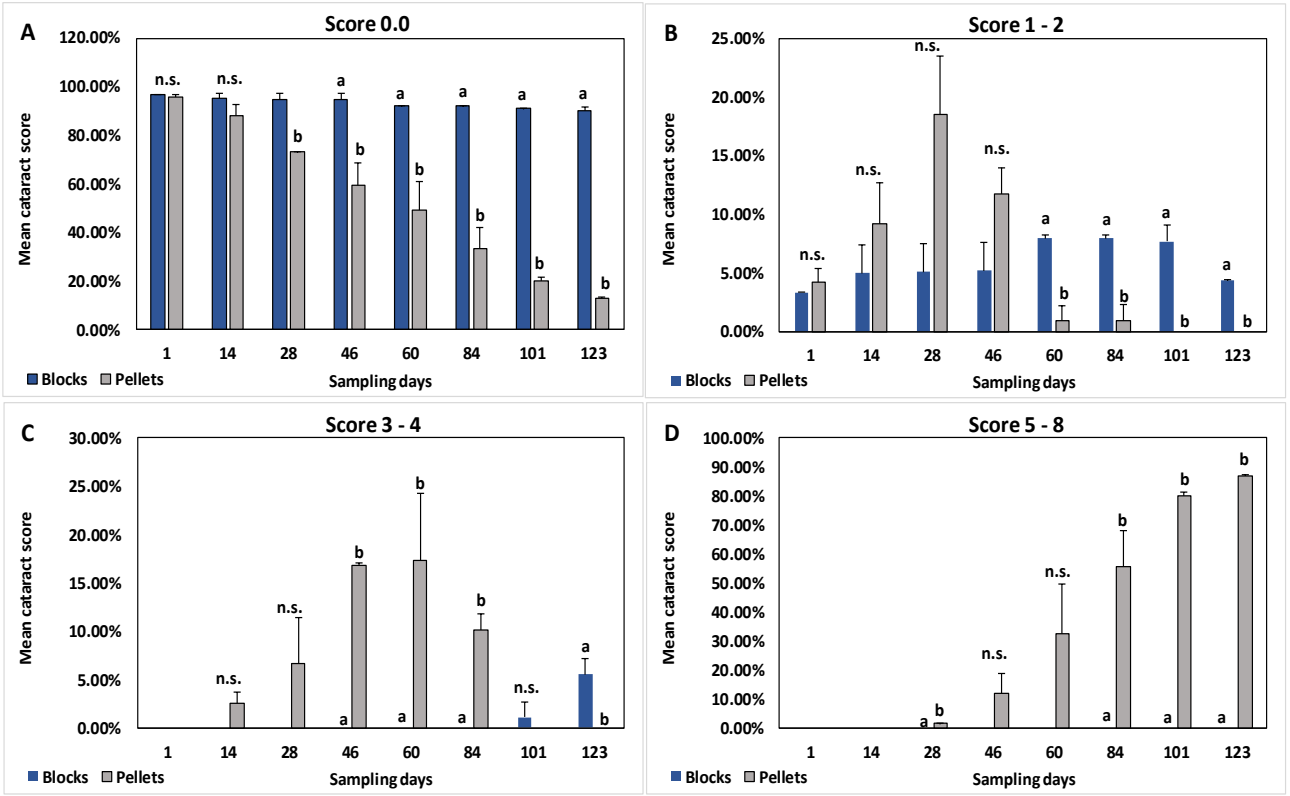



**Figure 12 Percentage of fish with total cataract score (sum score of both eyes) at each sampling point. Scores are classified A: 0, B: 1-2, C: 3-4 and D: 5-8. Values represent means ± SD. Different letters indicate significant differences (SNK test, P < 0.05); n.s., not significant.**

The majority of cataracts was bilateral, while incidences of unilateral cataract was low and found only until day 84 (decreasing from 5% at day 14 to 1% at day 84) (figure 13). There were a significantly increasing frequency for fish fed with pelleted feed to have bilateral cataracts (increasing from 4% at day 14 to 87% at the end of the study period) whilst fish fed with feed blocks increased from 3% at day 28 to 10% at the end of the study period.

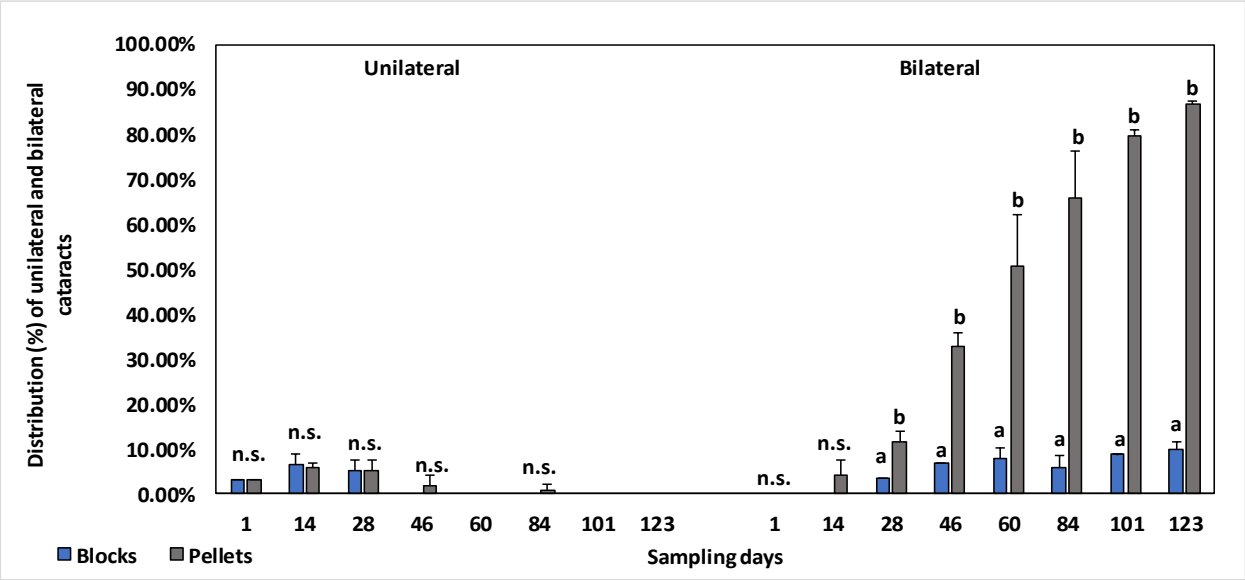



**Figure 13 Percentage distribution of lumpfish fed either feed blocks or pellets with unilateral and Bilateral cataracts throughout the study period. Values represent means ± SD. Different letters indicate significant differences (SNK test, P < 0.05); n.s., not significant.**

## 3.7 Histopathology

Evaluation results for histological analysis of pyloric caeca, intestine (mid and hind gut) and liver samples drawn prior to trial start (baseline) and from each of the two dietary treatments at the end of the study period can be seen in table 6.

**Table 6 Evaluation results (mean ± S.D) for histological analysis of pyloric caeca, intestine (mid and hind gut) and liver samples drawn prior to trial start (baseline) and from each of the two dietary treatments at the end of the study period. Mean values not sharing a letter were found to be significantly different by ANOVA and by Student-Newman-Keuls multiple range post hoc test.**

| Tissue | Analysis | Mean values | | | ANOVA | |
|---|---|---|---|---|---|---|
| | | Baseline | Feed blocks | Pellets | *F* | *p* |
| | ***Inflammation*** | | | | | |
| **Pyloric caeca** | **muscularis** | 0.0 | 0.0 | 0.0 | - | - |
| | **submucosa/lamina propria** | 0.10 ± 0.32 | 0.00 | 0.20 ± 0.63 | 0.6 | n.s. |
| | **Epithelium** | 0.5 ± 0.53***a*** | 0.00***b*** | 0.00***b*** | 9.0 | 0.001 |
| **Midgut** | **muscularis** | 0.0 | 0.0 | 0.0 | - | - |
| | **submucosa/lamina propria** | 0.20 ± 0.42 | 0.20 ± 0.42 | 0.40 ± 0.70 | 0.47 | n.s. |
| | **epithelium** | 0.20 ± 0.42 | 0.0 | 0.0 | 2.25 | n.s. |
| **Hindgut** | **muscularis** | 0.0 | 0.0 | 0.0 | - | - |
| | **submucosa/lamina propria** | 0.30 ± 0.48 | 0.30 ± 0.48 | 0.40 ± 0.70 | 0.10 | n.s. |
| | **epithelium** | 0.30 ± 0.48***a*** | 0.0***b*** | 0.0***b*** | 3.86 | 0.034 |
| | ***Epithelium degeneration /necrosis*** | | | | | |
| **Pyloric caeca** | | 0.0 | 0.0 | 0.0 | - | - |
| **Midgut** | | 0.0 | 0.0 | 0.0 | - | - |
| **Hindgut** | | 0.0 | 0.0 | 0.0 | - | - |
| | ***Vacuolisation*** | | | | | |
| **Pyloric caeca** | | 0.6 ± 0.52*a* | 0.0*b* | 0.0*b* | 8.45 | 0.001 |
| **Midgut** | | 0.0 | 0.0 | 0.0 | - | - |
| **Liver** | | 2.20 ± | 2.20 ± | 2.40 ± | 0.64 | n.s. |
| **Midgut** | **Goblet cells: PAS** | 2.0 ± 0.0 | 2.0 ± 0.0 | 2.0 ± 0.0 | | n.s. |
| | **Goblet cells: Alcian blue** | 2.0 ± 0.0 | 2.0 ± 0.0 | 2.1 ± 0.32 | 1.00 | n.s. |

For baseline samples, there was mild inflammation in submucosa/lamina propria and epithelial tissue sampled from the pyloric caeca, midgut and hindgut (Table 6). In addition, there was no epithelium degeneration/necrosis or vacuolisation in pyloric caeca, midgut and hindgut tissue samples. There was evidence of mild vacuolisation in epithelial tissue from the pyloric caeca and moderate vacuolisation in liver tissue (score 2.2). Midgut tissue stained with either PAS or Alcian blue was scored as 2.0 (2-5 to 2.7 positive cells per 20 epithelial cells).

At the end of the study period there was no inflammation evident in submucosa/lamina propria and epithelial tissue sampled from the pyloric caeca, midgut and hindgut for fish fed either with feed blocks or pelleted feed (table 6). Nor was there was no evidence of epithelium degeneration/necrosis or vacuolisation in pyloric caeca, midgut and hindgut tissue samples for both treatment groups. There was no evidence of vacuolisation in epithelial tissue from the pyloric caeca for both dietary treatments whilst the degree of vacuolisation was scored as moderate in liver tissue which was similar for both groups at the end of the study period and comparable to the degree of vacuolisation in baseline samples (Image 3).

There was little or no change in the number of goblet cells from the start to the end of the study for both test diets (Table 6; Image 4).

Figure 14 shows mean intestinal fold height (µm) measured for the start and end of the study period. Mean fold height at the start of the study period was 440.6 µm (± 59.5 µm) whilst at the end of the trial there was a significant increase in mean fold height for both dietary treatments compared to baseline samples. Fish fed with pelleted feed had a higher fold height (778.5 ± 197.7 µm) compared to fish fed with feed blocks (698.8 ± 260.0 µm) but not significantly so.

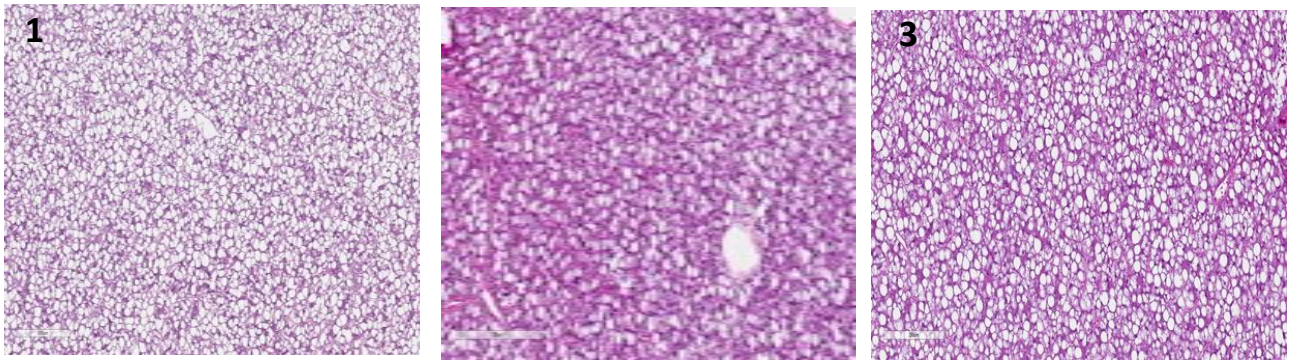



**Image 3 Micrographs of liver tissue from 1) baseline; 2) fish fed feed blocks and 3) fish fed pelleted feed. All images show moderate vacuolization (score 2).**

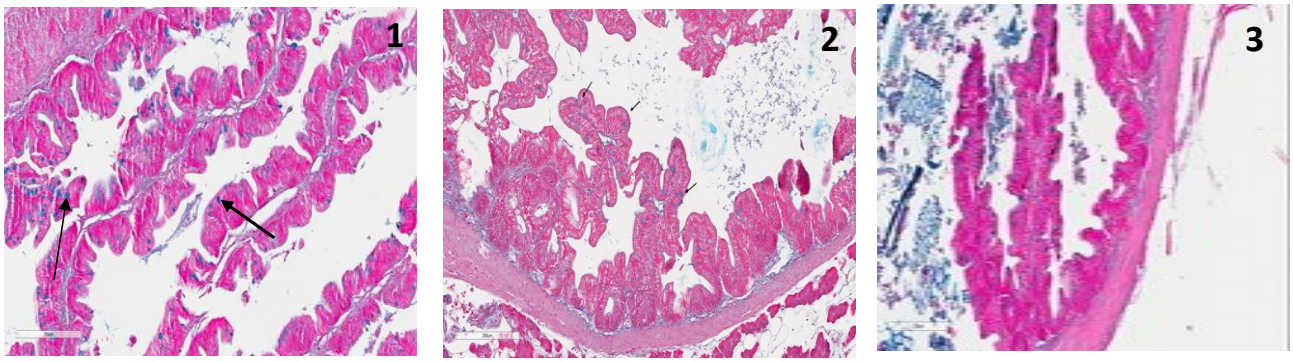



**Image 4 Micrographs of goblet cells from midgut tissue stained with Alcian blue: 1) baseline; 2) fish fed feed blocks and 3) fish fed pelleted feed. All three tissue samples show 2-7 positive cells per 20 epithelial cells (Score 2).**

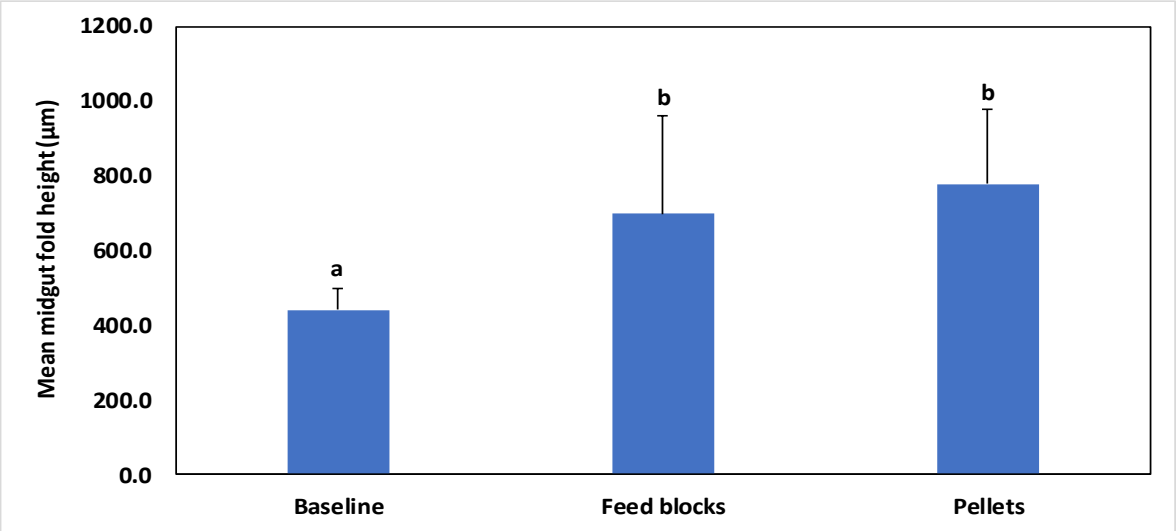



**Figure 14 Mean midgut intestinal fold height of lumpfish prior to trial start (baseline) (n = 10) and for fish fed either feed blocks or pellets sampled at the end of the study period (n = 10; N = 20). Values represent means ± SD. Different letters indicate significant differences (SNK test, P < 0.05); n.s., not significant.**

### 3.8 HSI and liver weight

Fish fed with pelleted feed had a higher mean HSI compared to fish fed with feed blocks at each sampling time point (figure 15). This difference was significant at days 79 and 117(SNK test, p < .05). There was a tendency for fish fed with feed blocks for the mean HIS to decrease at each sampling point (from 1.8 ± 0.7 at day 32 to 1.52 ± 0.7 at day 117) whilst fish fed with pelleted feed showed an increase in HSI from 1.9 ± 0.4 at day 32 to 2.3 ± 0.5 at day 117. Mean HSI values for both dietary treatments were lower compared to the baseline sample (2.5 ± 0.3).

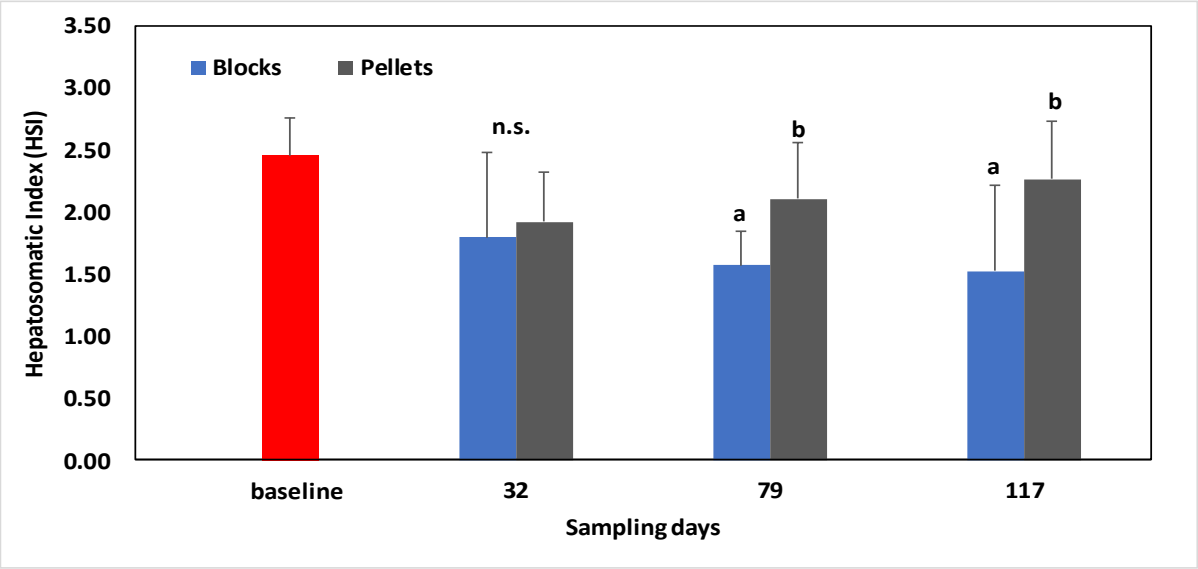



**Figure 15 Mean Hepatosomatic Index (HSI) of lumpfish fed either feed blocks or pelleted feed. Values represent means ± SD. Different letters indicate significant differences (SNK test, P < 0.05); n.s., not significant.**

Fish fed with pelleted feed had a significantly higher mean liver weight (g) compared to fish fed with feed blocks at each sampling time point (SNK test, p < .05; figure 16). Mean liver weight for fish fed with pelleted feed increased from 1.4 ± 0.4g at day 32 to 5.6 ± 2.1g at day 117 whilst mean liver weight for fish fed with feed blocks increased from 1.8 ± 0.5 to 2.5 ±1.4 over the same period.

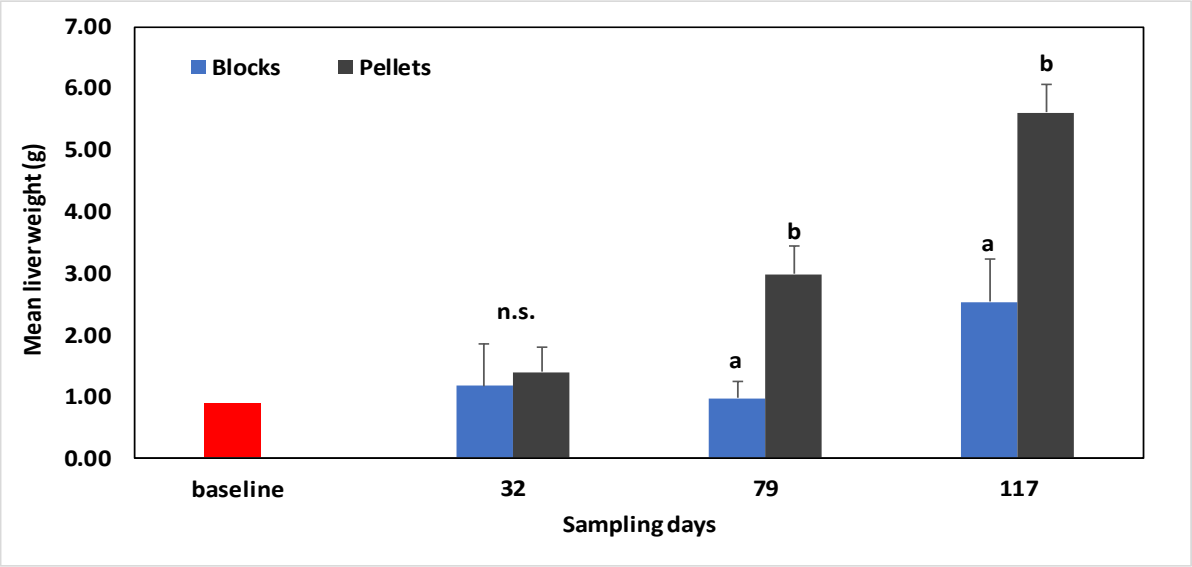
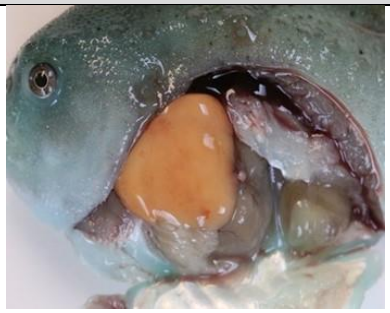
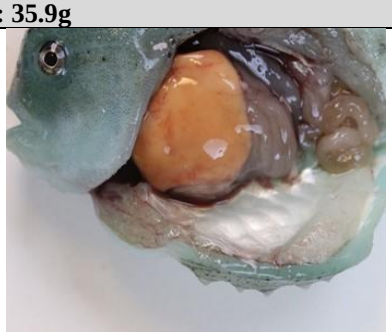
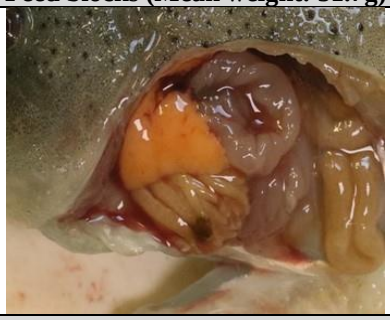
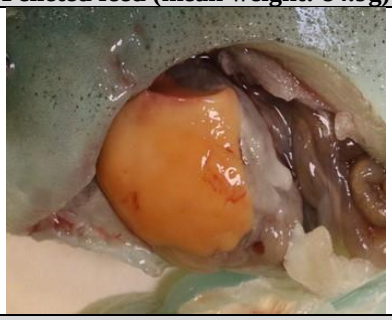
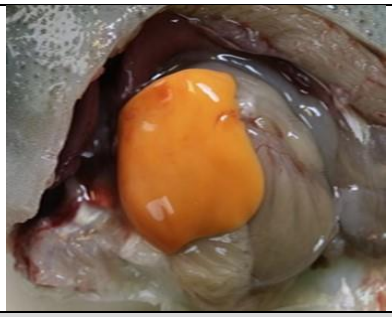
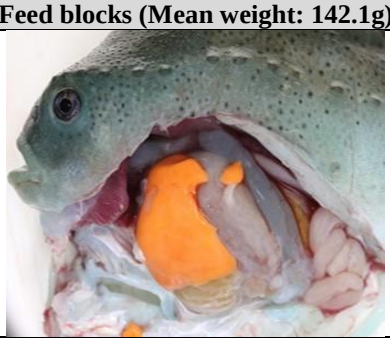
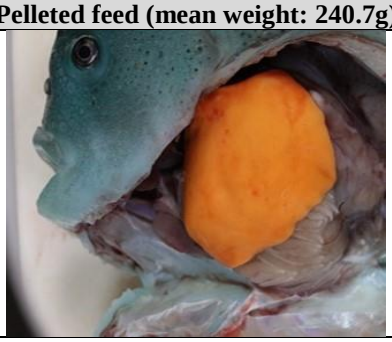



**Figure 16 Mean liver weight (g) of lumpfish fed either feed blocks or pelleted feed. Values represent means ± SD. Different letters indicate significant differences (SNK test, P < 0.05); n.s., not significant.**

A comparative study of livers was made throughout the study period (table 7). Baseline samples showed less pigmentation compared to fish sampled at day 32. There was similar colourisation of livers for fish fed either feed blocks or pelleted feeds at this time. At day 79, colourisation appeared more pronounced in fish fed with pelleted feed compared to fish fed with feed blocks. In addition, fish fed with pelleted feed had significantly a higher HIS compared to fish fed with feed blocks (figure 14). At day 117, liver colourisation appeared similar between the two dietary treatments with fish fed with pelleted feed having a significantly higher HIS.

**Table 7 Images of livers of lumpfish fed either feed blocks or pelleted feed recorded at day 1, 32, 79 and 117 during the study period. Images are representative of each treatment group at each sampling time point.**

| | | |
|---|---|---|
| **Baseline Day 1** | **Mean weight: 35.9g** | |
| |  |  |
| **Day 32** | **Feed blocks (Mean weight: 51.7g)** | **Pelleted feed (mean weight: 84.9g)** |
| |  |  |
| **Day 79** | **Feed blocks (Mean weight: 97.1g)** | **Pelleted feed (mean weight: 171.2g)** |

| | | |
|---|---|---|
| |  |  |
| **Day 117** | **Feed blocks (Mean weight: 142.1g)** | **Pelleted feed (mean weight: 240.7g)** |
| |  |  |

## 4.0 Discussion

The main objectives in this second of a series of planned studies was to evaluate and compare the effects on growth, cataract development and gut health of fish fed feed blocks or commercially available lumpfish pelleted feed. The following sections will discuss the results of the present study.

### 4.1 Growth, diets and feeding behaviour

The growth rates for fish fed with pelleted feed observed during this study were similar to growth rates from previous studies (Imsland *et al.,* 2015ab). However, growth rates were significantly lower for fish fed with feed blocks even though both feed types were offered a daily feeding rate of 2% /BW based on biomass gain. This difference in growth performance may be attributed to the lumpfish not eating all the offered feed blocks. It was also observed that as the fish grazed on them, small pieces would break off and sink to the bottom of the tank. Some of these fragments would be eaten by fish near the bottom whilst some were lost through the flow-through system thus reducing the feed available to the fish. Alternatively, the higher growth in the group fed pelleted feed could be linked to higher energy and lipid content of pellets (17 vs. 21 MJ/kg). Lumpfish fed with pelleted feed attained a mean weight which was 52% higher compared to the group fed with feed blocks. Other differences between the diets may also have contributed to the observed differences in growth. From analysed composition, the pelleted feed is a much more nutritious feed with a higher nutrient and energy content that supports substantially higher and more efficient growth than feed blocks. In addition, the pelleted feed contained substantially (ca. 50%) more EPA and DHA than the feed blocks.

The pelleted feed had a very different moisture content compared to the feed blocks (7 vs. 23%). This may have influenced nutrient composition and availability of the two feeds, including essential or semi-essential components. For example, it has been shown in Atlantic cod *Gadus morhua*, the specific growth rates increased with an increasing dietary water content of 0% to 50% (Otterå et al., 1994), while Grove et al. (2001) reported that the growth of turbot *Scophthalmus maximus* increased when fed a moist rather than a dry diet. Marine fish may require dietary water for osmoregulation between the body and medium (Ruyet et al., 1982; Higgs et al., 1985), and the growth-promoting effect of moisturized diets could be attributable to a faster nutrient release from the stomach, leading to higher digestibility (Grove et al., 2001). Other reasons for the improved growth when receiving moist diets include an improved acceptance of soft versus hard feed particles (Stradmeyer et al., 1988). These results are in direct contrast to the observed growth performance of lumpfish fed with feed blocks in this study. It may well be that the observed lower

growth may be more attributed to the loss of feed particles or the potentially higher energy expenditure required to orientate and maintain position at the feed blocks. In addition, it was observed that the water stability of the feed blocks decreased with increase immersion time in seawater. Results showed that feed blocks steadily lost 30% dry matter after to 24 hours post-immersion but after 48 hours had lost 90%. The higher moisture content of feed blocks results in a softer texture feed profile which in turn results in higher leaching rates compared to extruded commercial pelleted feed which are denser. However, from observations made during the study, it was evident that when feed blocks were deployed in the tanks, they would be completely gone after 3 to 4 hours after deployment as the fish were observed eating intensely from them.

The differences observed may also be attributed to the feeding behaviour observed dependant on what type of feed was offered. For fish fed with pelleted feed, the fish were observed swimming slowly towards when pellets were introduced to the tanks or else waiting for the pellets to be transported in the water current to reach them. There were very few fish showing a rapid feeding response which may indicate feeding competition within the population and there were few instances of aggression observed during feeding. In contrast, fish fed with feed blocks were often observed competing to maintain position at the feed blocks when they were deployed. Lumpfish to feed from feed blocks may have to expend more energy to maintain position when grazing from them or due to more competition between conspecifics compared to fish fed with pellets. Feeding hierarchies (Imsland, Folkvord, & Nilsen, 1998; Imsland, Jenssen, Jonassen, & Stefansson, 2009) may have been established resulting in some fish being less able to compete for food when available as this behaviour was observed on several occasions throughout the study when the feed blocks were deployed. The presence of dominant fish is not such a surprise with fish fed feed blocks as this food source is available in single persistent locations within the tanks whilst pelleted feed is delivered by automatic feeders and is more spread throughout the tank thus resulting in less chance of dominant hierarchies forming. To prevent dominant fish controlling areas where feed blocks are deployed in commercial cages it will be necessary to establish multiple feeding stations within the cage. However, it should be noted that high growth is not an aim for lumpfish used as cleaner fish. Imsland et al. (2016) found that small lumpfish (initial size approx. 20 g) have a higher overall preference for natural food items, including sea lice, compared to larger conspecifics. This makes slow to moderate and uniform growth of lumpfish more desirable than fast growth for its optimal use as cleaner fish in salmon aquaculture.

There were variations in K throughout the study period with fish fed feed pelleted feed having significantly higher K values from day 46 onwards. Lumpfish exhibit a high degree of opportunistic feeding behaviour as seen in previous studies (Imsland *et al*., 2014a; Imsland *et al*., 2014ab, 2015ab) and individual fish exhibit different food selection choices within populations. These selection differences can greatly affect body condition due to differences in the nutritional quality of different food sources. The variation in K values observed in this study may in part be attributed to the ease of access of the food source and /or competition between conspecifics as mentioned earlier. The use of K is the assumption of isometric growth, which has been observed in previous studies with lumpfish (Imsland *et al* 2018. In prep). The result is that K increases with fish length when $b > 3$ (i.e., fish become more rotund with increased length).

An important factor in using feed blocks in commercial salmon cages is that the lumpfish will readily graze from them. In addition, it is important that lumpfish used as cleaner fish in salmon cages have access to a regular food source particularly in winter time when naturally occurring food items become scarce. This food source is vital to maintain healthy and robust populations. The study recorded feeding behaviour of the fish offered feed blocks and the results suggest that lumpfish require very little time to acclimate to eating feed blocks and after three weeks, over 60% of the population readily grazed from them. In addition, when feed blocks were first introduced, lumpfish were observed eating from them after only 1-2 hours post-deployment and the time taken for initiation of a feeding response to be observed when the blocks were deployed reduced to a few seconds after one week.

Pelleted feed is normally used to feed these fish in commercial cages, however, its availability is generally limited to the periphery of the cage and lumpfish which have regular access to pelleted feed would also compete for salmon feed once they have grown larger (Imsland et al., 2015a). Feed blocks offer the advantage that they can be deployed anywhere in the cage and used as a maintenance rather than a self-sustaining food source.
The overall aim is to deploy feed blocks in commercial salmon cages. To achieve this goal, the lumpfish must be able to access them and readily graze from them. One advantage of using such a feed type is that they can be deployed in areas in the cage where they would be in close proximity to the salmon and thus enhance their lice grazing potential. Presently, most commercial farms using lumpfish fed them with pelleted feed (Imsland *et al*., 2015a; Powell *et al*., 2017) which usually is delivered from the edge of the cage using automatic feeders. This limits their ability to deliver feed away from the edges of the cage and thus encourage lumpfish to colonize these areas due to feed availability. By using feed blocks, lumpfish can be encouraged to occupy areas of the cage where the salmon are predominantly found, thus increasing the interaction between salmon and lumpfish. A prerequisite for successful use of lumpfish is that they need attachment areas to rest when not actively grazing or looking for food. This is particularly important when the fish are first introduced into cages. In the wild, juvenile lumpfish are typically found among algae, both attached and free floating during their first year of life (Ingólfsson 2000; Ingólfsson & Kristjánsson 2002), but are also found attached to substrates (Moring, 1989). In general, members of the family Cyclopteridae use their ventral adhesive disc to adhere to rocks, vegetation and other available substrates (Brown, 1986; Moring, 1989). Small juvenile lumpfish (c. 15–20 g) are routinely deployed in salmon cages and previous studies have shown that they require areas of attachment to rest when not foraging for food (Imsland et al., 2014; Imsland et al., 2015b). The lack of suitable attachment sites is likely to result in increased stress thus increasing the probability of disease particularly bacterial agents. Ongoing research in our research group is focussed on using feed blocks in combination with artificial substrates thus providing stand-alone units which can be deployed in any area inside commercial cages. These "lumpfish stations" provide suitable habitats for the fish which in turn may enhance their lice grazing efficacy.

### 4.2 Cataract development and eye health

The incidence of cataracts increased as the study progressed for both groups. However, there was extreme significant differences observed between the treatments. Cataract prevalence for fish fed with feed blocks only increased from 3% to 9% over the whole study period whilst prevalence for fish fed with pelleted feed increased from 4% to 87% over the same period. These differences may be attributed to dietary effects as both groups shared the same husbandry and environmental conditions throughout the project period. In addition, fish fed with pelleted feed all developed severe cataracts (score 5-8; fig 12) towards the end of the study whilst the low number of fish fed with feed blocks which developed cataracts scored no higher than 3-4. These differences were also manifested when the overall condition of the eyes where compared between the two groups. Fish fed with feed blocks generally had very clean and healthy-looking eyes compared to fish fed with pelleted feed (image 5).
In farmed salmon, it has been shown that even moderate degrees of cataract can result in reduced growth (Breck & Sveier 2001). Development of cataract means that less light passes to the retina and vision becomes impaired or disappears (Shariff *et al*. 1980; Bjerkås & Sveier 2001). Especially the more severe degrees of cataract may then reduce feed intake (Savino *et al*. 1993) and competition for food (Barber *et al*. 2000), thus reducing growth. In post-smolts an average cataract index of 5.4 is considered enough to impair vision (Bjerkås *et al*. 2003).
A previous study has shown that the prevalence of cataracts can vary between 20% and 100% in lumpfish populations (Jonassen *et al.* 2017) Such high prevalence of severe cataract is only comparable with the highest incidences previously found in farmed Atlantic salmon caused by a histidine-deficient diet. However, dietary histidine was circa 80% higher in pelleted feed compared to feed blocks (circa 50% higher on a dry-matter basis). In salmonids, cataract development has

especially been associated with suboptimal dietary levels of histidine during sensitive production periods such as after seawater transfer and high temperatures (Breck, *et al* 2003; Waagbø *et al*., 2010) and increasing dietary histidine inclusion at levels from 0.9% to 1.4% of diet had a positive effect on eye lens protein turn-over and n-acetyl histidine(NAH) content of lens, which protect the lens against osmotic pressure and oxidative stress in salmon smolts.
Any nutritional requirements for histidine in lumpfish, and how histidine requirement changes at higher growth rates is not determined for lumpfish. However, a study by Jonassen *et al* (2017), showed lumpfish lens contained N-acetylhistidine (NAH), of which low concentrations were strongly related to cataract severity. However, no correlation between lens NAH and cataract severity was found. The authors speculate that cataract in farmed lumpfish may be related to primary or secondary disturbed nutrient metabolism or malnutrition, shown by the high levels of specific amino acids in different tissues, which may cause osmotic imbalance and cataract development. Given that, it may be assumed that histidine and its derivatives have the same function in lumpfish as in salmon, and that similar mechanisms exist, however, further research is required to fully elucidate the role of histidine in lumpfish. Results from the present study showed that even with histidine at 1.3% of feed seems to be inadequate to eliminate risk of cataracts developing.

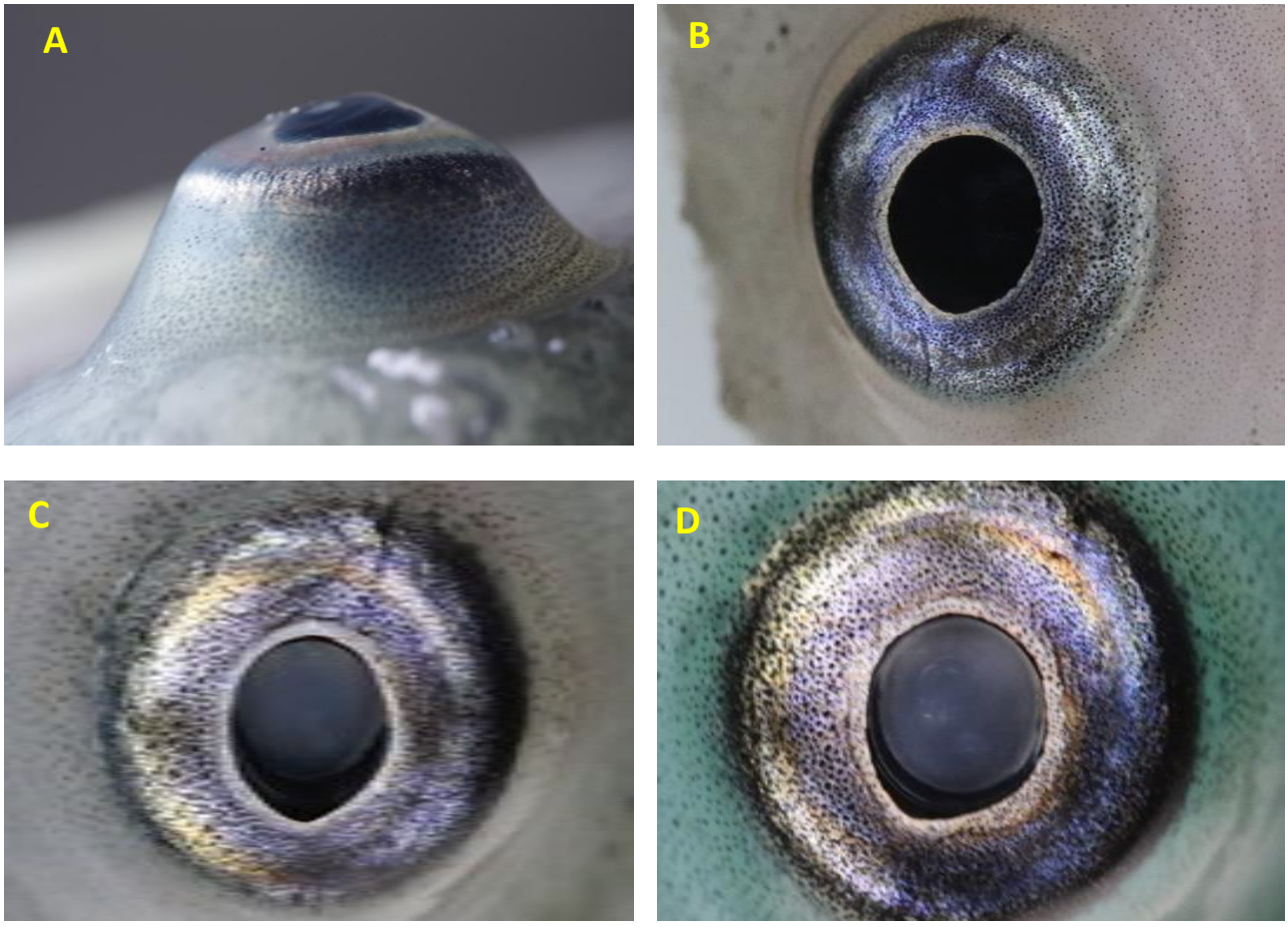



**Image 5 (A) Eye of lumpfish fed feed blocks showing small cataract and (B) showing no cataracts. C and D are from fish fed with pelleted feed. Both images show severe cataracts (score 4).**

It is known that high or rapid growth can increase the risk of cataracts in salmon (Ersdal *et al.* 2001). However, previous studies on lumpfish (Jonassen *et al*. 2017; Imsland *et* al 2018: in press) found that high SGR increased risk of developing cataracts (as has been observed in salmon). In contrast, the results from this study show that fish fed with pelleted feed had significantly higher SRG compared to fish fed with feed blocks and that these fish had a very high incidence of cataracts. It is known that growth rates of small lumpfish are generally high and thus one cannot

rule out the possibility that high growth rates observed in lumpfish populations may contribute to the development of cataracts. It may be that cataracts may be caused by not just high growth rates but also how efficiently the fish utilize the nutrients in the feed. If cataracts are caused not by excess amino acids but rather than how they metabolize nutrients in the feed, then perhaps optimizing feeding regimes with the commercial diets available now may reduce the onset of cataracts. Further studies are planned to examine this theory in more detail. However, the results from this study strongly suggest that feed blocks would be the preferred choice of diet for lumpfish in commercial cages due to the lower growth rates observed in this study in combination with the maintained eye health. These factors combined may enhance sea lice grazing behavior for a more prolonged period of time compared to what is achieved presently. Further, fish with severe cataracts have reduced ability to locate food items and as a result this affects feed intake, growth and weakened immunity and robustness of the fish (reduced stress tolerance) (Breck & Sveier 2001). This has been observed in a recent study with over 80% of lumpfish which developed severe cataracts and of these, 50% were found to have weight loss (Reynolds, unpublished data).

The results also indicate that the development of cataracts from mild to severe can occur over a relatively short period of time, particularly for lumpfish fed with pellets. This is similar to previous studies which have shown both an increase in prevalence and severity of cataracts (Reynolds, 2017; Imsland *et al* 2016). Given that lumpfish populations have some degree of cataracts (Jonassen *et al* 2017), there is the issue that if the fish have reduced vision then they lose their ability to graze sea lice from salmon. It is known that cataracts can affect how efficiently fish catch natural feed, such as in arctic char where fish with no cataracts caught zooplankton more effective than fish with cataracts (Voutilainen *et al*. 2008). It has been shown from a previous study (Imsland *et al*. (2016) that lumpfish with a low degree of cataracts does not affect their ability to detect and consume sea lice nor affect their overall feed intake and growth negatively. However, fish with severe cataracts lose their ability to locate food and as a result this affects feed intake, growth and weakened immunity and robustness of the fish (reduced stress tolerance) (Breck & Sveier 2001). This has been observed in a recent study with over 80% of lumpfish which developed severe cataracts and of these, 50% were found to have weight loss (Reynolds, unpublished data).

There was a higher incidence of lumpfish with bilateral cataracts compared to fish with unilateral cataracts in both groups throughout the study. This indicates that the development of cataracts may be systemic. Bilateral cataracts have been shown to have generally systemic causes such as nutrition, in Atlantic salmon (Breck *et al*. 2003).) whilst unilateral cataracts are generally associated with external mechanical stresses on fish, such as different types of handling that can create friction or damage to the eyes (Jonassen *et al*. 2017).

In addition, at the end of the study period, it was noted that other issues regarding eye health were evident. Fish that were assessed as having cataracts which covered more than 75% in both eyes would be considered to be visually impaired to a point where locating food items (pelleted feed in this instance) would be challenging and as a result growth performance would be limited to a point where the fish would starve and suffer weight loss. However, the results from the study show that all fish with severe cataracts in both eyes showed positive growth throughout the study period and some fish with severe cataracts even showed SGR values which were comparable to fish with no cataracts. The results indicate that even with the eye completely covered with cataracts, the fish were still able to locate food. Upon closer examination, cataracts scored as 4 (covering over 75% of the eye surface) varied in their level of opacity and it may be the degree of opacity which is the limiting factor in fish being able to find food items. A scoring system has now been developed and will be implemented in all future studies with lumpfish.

Several fish were also noted to have corneal ulcers (descemetocele) or possible corneal perforation with marked oedema present generally in one eye (image 6). Lesions such as these may be painful and may lead to complete loss of vision both of which must raise concerns from an animal welfare perspective. Corneal ulceration in mammals is most commonly caused by trauma and then secondary infections occur, but primary infection may also lead to ulceration (Liv Østevik pers comm.).

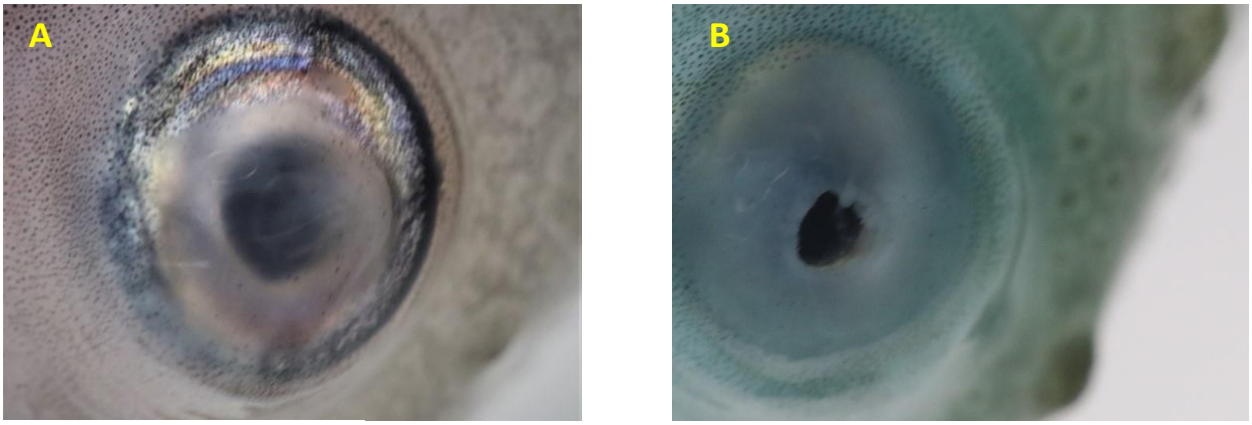



**Image 6 Images of eyes showing mild (A: fish fed with feed blocks) to severe (B: fish fed with pelleted feed) corneal ulceration (descemetocele) or possibly corneal perforation, with marked oedema and possible inflammation of the surrounding cornea.**

Previous studies have shown ulcers to be periodically present in lumpfish populations and in some instances, fish with corneal ulceration have shown complete recovery even after two weeks (Reynolds, *per comm*). Image 7 shows an individual fish from a previous study developing corneal ulceration in the right eye through time. It appears that after 22 days, there was partial recovery at day 42 followed by a further relapse at day 62. Future studies will include histology to assess severity of corneal damage, inflammation, presence/absence of infectious agents, inflammation/damage to other ocular structures i.e. iris/anterior chamber, retina etc. and to see if cataracts are present in these fish as well, as previous loss of vision may predispose the fish to ocular trauma.

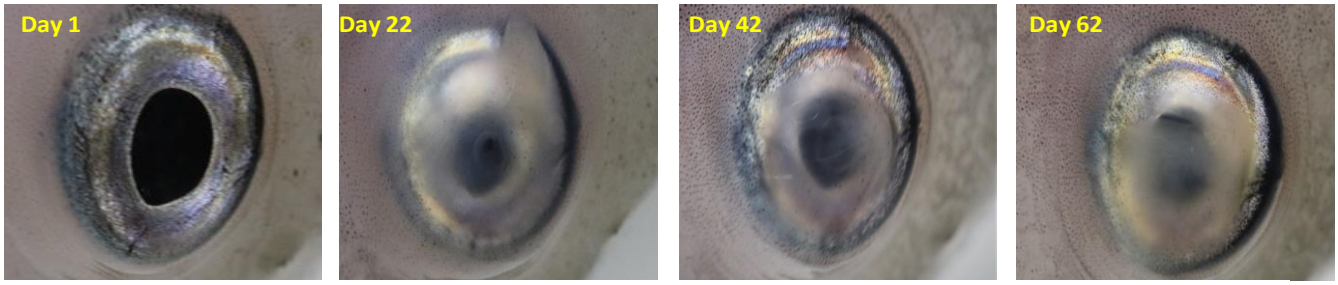



**Image 7 Right eye of lumpfish fed feed blocks showing development of corneal ulceration at each sampling time point.**

### 4.3 Histopathology

The physiological condition of the fish is one of the key factors that determine the health status of fish. Thus, monitoring the physiological status of fish by using histopathological examination leads to a good understanding of the functional morphology of the lumpfish alimentary canal and is fundamental for learning more about their feeding physiology and habits especially for feed formulation prior to stocking in commercial salmon cages as the size of fish used at the start of this study (20g) is representative and within the size range at which these fish are stocked in commercial salmon cages. The results of the histological examination undertaken in this study showed overall small differences between the two dietary treatments. In some individuals in groups receiving both diets, there was mild to moderate expansion of the lamina propria with tissue most likely to represent fibrous tissue with scattered leucocytes. Changes are most consistent with chronic inflammation. The changes are unspecific, and the cause is uncertain.

The level of inflammation observed may indicate dietary effect, although the mild inflammation observed does not indicate any negative effects which may affect growth and health of the fish. However, if the diets fed were causing an inflammatory response then it would be expected that after 123 days (the duration of the study) inflammation to be more pronounced as seen in Atlantic salmon fed diets containing more than 5-10% full fat or defatted (extracted) soybean meal (SBM)

develop inflammation in the distal part of the intestine (van den Ingh *et al* 1991). The first histological signs of inflammation are apparent after 2-5 days of SBM feeding and the severity escalates with extended exposure time (van den Ingh *et al* 1991; Baeverfjord & Krogdahl 1996).
Differences in moisture content between the two dietary treatments resulted in no significant differences in inflammation and/or growth. Several studies have been undertaken on the effects of dietary water content on growth performance (Oehme *et al* 2014; Hughes, 1989). Storebakken and Austreng (1988) reported increased dry matter intake in rainbow trout fed moist pellets compared to dry pellets. Increased FI was reported in fish fed moist pellets with approximately 60-70% dry matter at falling seawater temperature (Gabrielsen and Austreng, 1998). Results indicate that high moisture in the pellet stimulated the appetite resulting in greater feed intake. Thus, if the higher moisture content of the feed blocks stimulates appetite then this may be offset by fragmentation and leaching from the feed blocks resulting in not all of the offered feed being consumed by the fish. It is commonly accepted that marine fish drink much more water than do fresh-water fish for osmoregulation. Hughes (1989) showed the differences in the ability of several fish species to moisturize the digestive tract, and they suggested that this ability might indicate the suitable dietary moisture level. It may well be that lumpfish require higher moisture dietary content to maintain gut health and further research is required to fully elucidate this.
There were no significant differences in liver vacuolisation between the dietary groups and baseline samples, but fish fed pellets had slightly higher levels of vacuolisation. These results indicate that the fat content of both diets was not in excess. It is known that excess fat is stored in the liver (Caballero, *et al* 2004) and this can be manifested as increased vacuolisation.
There was no increase in the number of goblet cells present in the hindgut between the two diet groups compared to the baseline samples for both Alcian Blue and PAS stained samples. The relatively high number of goblet cells in the posterior intestine appears to be a universal feature in fishes and is probably useful for increased mucous production to safeguard the intestinal lining and aid faecal expulsion (Machado *et al*., 2013).
Fish fed with pelleted feed had a slightly longer intestinal fold height compared to fish fed feed blocks but not significantly so. Fold height can be increased by addition of supplements to the diet (Dimitroglou *et al* 2009). The fold height for fish fed pellets was longer perhaps because of the higher amount of vitamin C in the diet compared to the amount in feed blocks. It is known that vitamin C plays an important role in certain aspects of protein metabolism (Shiau and Jans 1992) and is an essential molecule in the overall health of animals. Given this, the fish fed with pellets did have significantly better growth to fish fed feed blocks which may have in part been attributed to increased total surface area of the intestine and hence better nutrient absorption.
Histological examination of the eyes was undertaken at the end of the study period to assess the potential for using this type of analysis in future studies. The results indicate that histology can be used to score semi-quantitatively the degree of swelling of lens fibers, proliferation of lens epithelium, lens capsule rupture and ocular inflammation and a scoring system has been developed and will be used in future studies.

### 4.4 HSI

Lumpfish fed pelleted feed had higher HSI throughout the study period. Differences in HIS occurred over a relatively short period of time with lumpfish fed pelleted feed having larger livers as early as 32 days after the study commenced. The differences in fat content between the two diets was only 6% and it appears that even this small difference in fat content was sufficient for lumpfish not to be able to utilise the fat as an energy source for somatic growth and the excess fat was stored in the liver. It has been shown that for other marine species such as farmed Atlantic cod, the hepatosomatic index (HSI) is closely related to the dietary lipid level (Lie *et al.* 1988; Jobling *et al.* 1991) and that they deposit large quantities of the dietary fat in the liver when fed to satiation (Lie *et al.* 1988). However, results from histology show that fish fed pellets had only a small higher difference in the level of vacuolization compared to fish fed feed blocks and whether this difference resulted in the observed differences in HSI remains unclear.

There was also little difference in the visual colour of the livers for all sampling time points apart for at day 79 when the livers of fish fed with feed blocks appeared paler compared to the pelleted fed fish even though feed blocks had 133.0 mg kg whilst pelleted feed had an inclusion of 12.0 mg kg. Astaxanthin is the major carotenoid used in commercial salmon feeds which gives the flesh its red colour. As well as used to colour the flesh of farmed Atlantic salmon, astaxanthin has been found to improve growth of Atlantic salmon fry (Torrissen and Ingebrigsten. 1992) and that astaxanthin was essential for growth and survival during start-feeding periods. In addition, astaxanthin also is a strong antioxidant and plays a role in protecting lipid. Further, carotenoids such as astaxanthin have been shown to enhance both the specific and non-specific immune systems of many fish species (Bendich 1989). There has been no research undertaken in the role of astaxanthin in lumpfish, however, given its positive role in both anadromous and marine species, addition of astaxanthin in the diet may prove to be beneficial.

**5.0 Conclusions**

Results from the present study show that feeding lumpfish with feed blocks, controlled growth performance without apparently compromising the health status of the fish. Mean weight was 52% lower compared to fish fed with a commercially available lumpfish feed whilst maintaining a feeding rate of 2% $BW^{-1}$.

The onset of cataracts was significantly different with fish fed pelleted feed having a cataract prevalence of 87% at the end of the study period whilst fish fed with feed blocks had only 10% prevalence. In addition, fish fed with pellets with cataracts all had severe (score 5-8) cataracts whilst only 5% of fish with cataracts fed with feed blocks had moderate (score 3-4) cataracts.

Results from this study show that lumpfish will readily graze from feed blocks and the acclimation period required before the fish will utilize them appears to relatively short, thus potentially allowing for their use in commercial salmon cages.

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## Appendix I and II

### 1. The use of Benzoak (Bensokain 200mg/ml (20%)) for sedating Lumpfish

- 25 litres of well aerated seawater is placed into a suitable container.
- 10 ml of Benzoak added giving a concentration of 80 mg/l.
- 1 fish is then added to the sedation bath and once starting to become sedated another fish is then added.
- Exposure to a concentration of 80 mg/l results in stage II plane 1 of anaesthesia after 3 minutes' exposure using the classification as proposed by McFarland (1959). The fish after this time show reduced motion and partial loss of equilibrium.
- **At no time must more than two fish be in sedation at the same time as it takes time for stomach pumping and identifying contents.**

### 2. Insertion and placement of passive integrated transponder (PIT) tags for lumpfish

- A suitable work surface is prepared in advance. The surface must be disinfected regularly throughout the procedure. A small tub of alcohol-based disinfectant is placed on the work surface along with a flame source (candle). All pit tags used have been thoroughly disinfected prior to use and had been allowed for the disinfectant to have evaporated off prior to use. Once the fish were ready for tagging, a pit tagging gun fitted with an individual tag will be used to insert the tag.
- The fish are tagged intraperitoneally with a Trovan® Passive Integrated Transponder
- After each tagging, the needle end of the gun is dipped into the tub of disinfectant and then passes the blade through a flame to burn off the excess alcohol before another tag is fitted to the gun. This procedure is repeated after each tagging. The work surface is regularly disinfected throughout the operation to ensure that potential contamination was kept to a minimum.
- After tagging, weight and length of each fish is recorded along with cage number allocation.
- **Fish are handled as little as possible during the tagging operation.**

**After sampling and/or tagging**

- After gastric lavage or tagging each fish is placed into the recovery tank containing fresh well aerated seawater to allow for the sedation effects to dissipate and for the fish to fully recover before being placed into its respective cage.