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Effects of different feeding regimes on growth, cataract development, welfare, and histopathology of lumpfish (*Cyclopterus lumpus* L.)

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ABSTRACT

To develop specific recommendations on the feeding strategies for farmed lumpfish, four duplicate groups ($n = 70$; $N = 280$) of lumpfish with a mean ± SD start weight of 30.4 ± 5.3 g were distributed among eight sea cages (5 × 5 × 5 m) each stocked with 220 one year old Atlantic salmon with a mean (± SD) weight of 1485.9 ± 213.2 g. The fish were fed either pelleted feed at 3% BW^{-1} or with feed blocks at 1.5%, 2% and 3% BW^{-1} respectively for 66 days. There were clear differences in growth performance between the four treatment groups with lumpfish fed with pelleted feed attaining the highest weight gain. There were only minor histopathological changes observed between the dietary treatments with mainly mild focal and multifocal inflammation observed in sampled tissues. There were no significant differences in liver vacuolisation between the dietary groups and baseline samples with most of the livers evaluated appeared to be vacuolized within what is regarded as normal range for all four treatment groups. Only minor differences in welfare score between the four treatment groups were found. The incidence of cataracts varied between the treatment groups and was correlated to differences observed in growth between the four groups. Lumpfish fed once daily from automatic feeders with pelleted feed had the highest incidence and severity of cataracts. This study suggests that feeding lumpfish with moderate amounts of feed blocks (≤ 2% BW^{-1}) may be advantageous for maintaining slow growth and good welfare in salmon cages, and this should be further tested in large scale studies in commercial salmon farms.

1. Introduction

The biological control of sea lice in Atlantic salmon farming using cleaner fish has recently become an advantageous alternative due to the increased occurrence of resistant lice, the reduced public acceptance of chemotherapeutic use in food production, and the urgent need for an effective and sustainable method of parasite control in Atlantic salmon aquaculture (Denholm et al., 2002; Treasurer, 2002; Boxaspen, 2006). As a cold-water cleaner fish, the common lumpfish (*Cyclopterus lumpus*) has proven to be an effective lice eater at low sea temperatures (Imsland et al., 2014a, 2014b, 2014c; Imsland et al., 2015a, 2015b; Imsland et al., 2016a, 2016b; Imsland et al., 2018a). However, it has become increasingly evident that the supplementary feeding of cleaner fish deployed within commercial salmon pens is necessary to maintain the nutritional condition, welfare, and efficacy of these biological controls, over the duration of the Atlantic salmon grow-out cycle (Leclercq et al., 2014, 2015; Imsland et al., 2018b, c, 2019a, b, 2020). 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. Earlier studies within our research group (Imsland et al., 2018c, 2019a, 2020) suggest that feeding lumpfish with feed blocks may alleviate health issues due to enhanced nutritional intake and better controlled growth compared to fish fed with pelleted feed. The welfare of cleaner fish in cages is a prime concern and lumpfish can lose condition within six weeks of transfer to sea cages (Reynolds et al., 2022). This can be alleviated by the supply of robust fish and also by providing a suitable

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supplementary feed source more suited to the species being fed.

Fast growth is not an aim for lumpfish used as cleaner fish as previous studies have shown that smaller lumpfish tend to consume more sea lice compared to larger lumpfish (Imsland et al., 2016a; Eliasen et al., 2020; Boissonnot et al., 2022a; Engebretsen et al., 2023). Once lumpfish attain a mean weight over 200–300 g, sea lice grazing behaviour generally decreases (Imsland et al., 2016a; Boissonnot et al., 2022a). If growth can be controlled, then the operational use of lumpfish will be extended. Further, Imsland et al. (2019a) showed that lumpfish fed with feed blocks had 40% lower growth rates, 87% less cataracts and better health status compared to fish fed with pelleted feed.

Cataracts may be induced by a variety of factors of a nutritional, environmental, chemical, or infectious nature (Bjerkås et al., 2006). A recent study undertaken by Jonassen et al. (2017) showed cataract prevalence in both farmed and wild lumpfish varied between 20 and 100%. In trial with different families of lumpfish Imsland et al. (2021) found that cataract prevalence differed between the families assessed and varied between 9.8% and 21.2%. In culture rapid cataract occurrence have been demonstrated on deployed lumpfish, which is often associated to sub-optimal feeds (Imsland et al., 2019a) or feeding protocols (Imsland et al., 2019b). This illustrates a welfare issue and if cataracts are associated with sub-optimal nutrition, then further research in nutrition with lumpfish is therefore necessary. Previous studies on Atlantic salmon have also shown that cataract development can occur during periods of rapid growth (Bjerkås et al., 2001; Breck and Sveier, 2001; Waagbø et al., 2010), or nutrient deficiencies (Breck et al., 2003; Bjerkås et al., 2006). Therefore, controlling the amount of feed juvenile lumpfish consume may possibly alleviate the potential for cataract development.

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 (Purushothaman et al., 2016; Imsland et al., 2019b) and is fundamental for learning more about their feeding physiology and habits especially for feed formulation prior to stocking in commercial salmon cages.

This present study is a continuation of studies designed to assess the use of feed blocks for lumpfish populations (Imsland et al., 2018c, 2019a, 2020). The first study focused on feed block design and deployment to optimize lumpfish utilizing them as a food source (Imsland et al., 2018c), and the following studies showed that lumpfish fed with feed blocks have more controlled growth, increased survival, and better overall health status compared to lumpfish fed with pelleted feed in sea pens with Atlantic salmon (Imsland et al., 2019a, 2020). However, to fully maximise their use, more knowledge is required to determine optimal feeding strategies for this feed type. In addition, more detailed internal health monitoring is required to ensure there are no negative effects in gut and liver health and also sub-optimal nutritional stress factors. Accordingly, the aim of this study was to develop specific recommendations on the nutritional and feeding strategies for farmed lumpfish.

2. Materials and methods

2.1. Atlantic salmon

The Atlantic salmon used in the study were under one year old fish from 13G (eleventh generation of the Norwegian breeding program for Atlantic salmon) produced at Sundsfjord Smolt AS (Nordland, Norway) and delivered to Gildeskål Research Station (GIFAS), Nordland, Norway in April 2022. The fish were transferred to small-scale sea pens (5 × 5 × 5 m, 125 m^3) in September 2022 and remained in those sea pens during the trial period. The salmon had an average initial mean (± SD) weight of 1485.9 ± 213.2 g on 11 October 2022. All fish originated from the same group of fish and shared the same genetic and environmental background. These fish had not been used in any previous trials. During the study period the salmon were fed a standard commercial diet (Energy Range, Biomar, Århus, Denmark) from automatic feeders once daily.

2.2. Lumpfish

The lumpfish were produced from fertilized eggs from Nordland Rensefisk AS, Lovund and hatched at 9–10 °C. The juveniles were initially fed with Gemma Micro (150–500 μm, Skretting). After 30 days, the juveniles were fed with 500–800 μm dry feed pellets (Gemma Wean Diamond, Skretting). The fish were vaccinated with Vaxx on marin 3 (Vaxxinova AS). Pathogen status was assessed one week prior to transfer by PCR screening for *Vibrio* species, *Pasteurella* spp., *Paranucleospora*, *Paramoeba, Moritella* spp., *Aeromonas salmonicida*, *pancreas disease* (PD virus), *infectious pancreatic necrosis* (IPN virus), *viral haemorrhagic septicaemia* (VHS virus), *Nodovirus* and *ILA-virus*. Additionally, welfare status ($N = 30$) was assessed using the Lumpfish Operational Welfare Indicator (OWI) model developed by Boissonnot et al. (2022b, 2023). Tissue samples were taken for histopathological analysis. The lumpfish were transferred once they had attained a mean (± SD) weight of 30.4 ± 5.3 g and acclimated in small mesh nets for up to a period of one week at Gifas small-scale facility Langholmen, Inndyr, Nordland. During the period, the fish were fed daily with a mixture of 60.0 g marine feeding blocks (WorldFeeds, UK) and Clean Assist 2 mm pelleted feed (Skretting AS) which were weighed prior to placement to ensure sufficient feed was available to maintain a feeding rate of 3% BW^{-1}.

The following experiment was approved by the local responsible laboratory animal science specialist under the surveillance of the Norwegian Animal Research Authority (NARA) and registered by the Authority.

2.3. Feeding and husbandry

2.3.1. Atlantic salmon

The feeding regime was based on satiation feeding by hand. One distinctive meal was fed each day. When sufficient daylight was available, two meals per day with a four-hour period between meals was offered.

2.3.2. Lumpfish

The lumpfish treatments groups were fed with feed blocks using 60.0 g marine feeding blocks (WorldFeeds, UK) suspended horizontally in the water column. Each individual feed block was an average of 26 × 100 mm with a 10 mm hole through the centre and had grooves created on their surface during the extrusion process. Feed blocks were placed in each of the cages three days per week (Monday, Wednesday, and Friday) and were weighed prior to placement to ensure sufficient feed was available to maintain the experimental feeding rate of 1.5%, 2% and 3% BW^{-1} respectively and were the only supplied source of nutrition. Once feed blocks were placed in the cage, routine monitoring was undertaken to ensure the fish were grazing from them. Usually, all the blocks are eaten over a short period of time (6–12 h in most cases), if any remained this was recorded, and the remaining blocks replaced with fresh ones. The blocks were deployed close to the artificial substrates where the lumpfish rest. Pelleted feed (Skretting Clean Assist 2 mm) was delivered to the fish using either a Van Gerven 7 L^{-1} feeding automats (The Netherlands) for the lumpfish treatment group fed with pelleted feed or by hand when weather prevented their use. The automats were calibrated for the pellets used in the study and programmed to deliver accurate feed amounts at specific times of the day during daylight hours. The feeders were programmed to dispense distinct several small meals on the day of feeding three days per week at 3% BW^{-1} (the same regime as for feed blocks). Feed input was monitored to ensure the lumpfish were actively eating the pellets and if no fish were feeding, the feeders were stopped and reprogrammed to feed the meal later in the day thus ensuring delivery of the prescribed doses over the feeding day. The amount of pelleted feed and feed blocks was calculated and adjusted after each sampling point when all lumpfish are weighed to account for the increased biomass for each cage.

Polyethylene (PE) artificial substrates (NorseAqua, Norway) were placed in each of the cages to allow for attachment by lumpfish when resting. The surface area of each substrate was calculated to ensure sufficient surface area was available for attachment by lumpfish as they grew.

2.4. Study protocol

The trial was conducted from 11 October to 17 December 2022. At trial start, the salmon were bulk weighed, counted, and distributed between ten cages of 125 m^3 (5x5x5m) with 220 fish in each cage. To prevent a skewed distribution of treatments with respect to water quality and current, lots were drawn randomly among predetermined duplicate distributions of the cages. At the end of the study period (17 December) all salmon were bulk weighed again. Feed conversion ratio (FCR) was calculated as:

$$FCR = FI\ (B_2 - B_{1+}\ B_{dead})^{-1}$$

where FI is feed consumed, B_1 and B_2 are the biomass at the start and end respectively for the period and B_{dead} is the biomass of dead fish during the period.

Once feeding behaviour was considered normal during the period of acclimation, 35 lumpfish were transferred to one of eight 5x5x5 m cages stocked with the salmon. The remaining two cages received no lumpfish (Control cages). Four duplicate treatment groups of lumpfish were established:

- Group 1: Two cages were fed with a standard commercial pelleted feed (Skretting Clean Assist 2 mm).
- Group 2: Two cages fed with 60 g feed blocks (WorldFeeds UK) at a feeding rate of 1.5% BW^{-1}.
- Group 3: Two cages fed with 60 g feed blocks (WorldFeeds UK) at a feeding rate of 2.0% BW^{-1}.
- Group 4: Two cages fed with 60 g feed blocks (WorldFeeds UK) at a feeding rate of 3.0% BW^{-1}.

Proximal analysis of the two feed types is shown in Table 1. Feeding rates were adjusted according to actual biomass gain for each cage population. All lumpfish stocked into each cage had their weight (W, to nearest g) and fork length (L, to nearest mm) recorded prior to being transferred into the cage. Each cage containing lumpfish had an initial stocking density of 15.9%. All lumpfish had their weight and length recorded at 11–15 days intervals during the trial period. At each period for each cage, the nets were raised, and all visible lumpfish carefully netted from the cage and placed in a container containing well-aerated seawater with continual flow-through. Lumpfish were removed from the container and sedated in batches of five for 10–20 s using Metacaine (200 mg L^{-1}, Scan Aqua AS, Årnes, Norway). Once sedation had been achieved, each individual was placed on a Marel Animal Scale (Marel, Garðabær, Iceland) and the individual weight and length recorded. Subsequently the fish was placed back into the container until fully recovered and then transferred back into their respective cages.

Table 1
Analysed diet composition of the two feeds used in the study.

Composition		Pellets	Feed blocks
Fat		15.4	10.3
Protein (Nx6.25)		52.9	50.1
Moisture content		8.3	23.2
Starch and simple sugars		9.1	8.2
Calc. GE	MJ/kg	20.1	17.3
Calc. DP	%	47.1	44.6
Calc. DE	MJ/kg	17.7	15.2
Calc. DP:DE ratio		26.6	29.4

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.).

Mean weight and specific growth rate (SGR) were calculated at each sampling point. SGR of individual lumpfish and salmon was calculated according to the formula of Houde and Schekter (1981):

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

where $g = (\ln(W2) - \ln(W1)/(t2 - t1)$ andW2 andW1 are weights on days t_2 and t_1, respectively.

Condition was assessed using regression analysis for estimation of length weight parameters. The relationship between weight and length in fishes which has the form:

$$W = aL^b$$

The shape parameter b was calculated using historical weight, length, width, and height data from lumpfish: $N = 3657$). The results were used as part of the welfare scoring system utilised in this study. Each lumpfish from each cage was weighed with a minimum of 1 g precision, and the weight noted to the nearest whole gram. Measurement of length (mm) was performed with a ruler from the snout / mouth to the outermost part of the caudal fin. The condition was automatically calculated in the field form when the measurements of length and weight had been entered. The formula used to calculate the factor is taken from Gutierrez Rabadan et al. (2021):

$$\text{Condition} = \frac{10^{3{,}516} \times V}{(10 \times L)^{2{,}559}}$$

where weight (V) is in g and length (L) is in cm. Lumpfish condition was then automatically scored from 0 to 3, where score 0 indicates good condition and score 3 severe emaciation. The limits are based on the assessments of Gutierrez Rabadan et al. (2021), but an extra limit has been added to distinguish between lumpfish in good condition and those that were slightly emaciated (Boissonnot et al., 2022b, 2023).

2.5. Welfare assessment of lumpfish

Assessment of the welfare status of all the lumpfish was undertaken during routine sampling points using the Lumpfish Operational Welfare Indicator (OWI) model developed by Boissonnot et al. (2022b, 2023). At each sampling point, all lumpfish from each cage were carefully netted and weight, length, and height were recorded for each fish. The status of fins was scored from 0 to 3 along with assessment of body condition, and deformities/malformations.

For cataract/eye ulceration status, the fish were transferred to a darkened room and a hand-held Heine HSL 150, C-002,14,602 (HEINE Optotechnik, Herrschingunder, Germany) slit lamp with a magnifying glass at 10 x magnification 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 cage. Each eye was scored on a scale from 0 to 3 where 0 = no cataract, 1 = cataract covers <10% of the lens, 2 = cataract covers 10–50% of the lens, and 3 = cataract covers over 50 of the lenses. The opacity of each cataract was scored where 0 represents no cataract and 3 represents crystal white pearlescent lens with total loss of translucency.

In addition to the cataract score contributing to the OWI model, the scores per eye was summated for each giving the cataract score per individual (0–6). 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.

The distribution of the individual's welfare score was used to assess the overall welfare status of the population. The overall welfare status was graded from good to severely reduced according Boissonnot et al. (2022b, 2023). For the overall welfare status in the population to be assessed as good, over 60% of the lumpfish must have good welfare (welfare score 0) and none with clearly or severely reduced welfare (welfare score 2 or 3). If >25% of the lumpfish had severely reduced welfare (welfare score 3), the overall welfare status of the population was also considered to be severely reduced. For further information on the welfare scoring see: https://gifas.no/handbok-for-a-sikre-rognkjeksen-god-velferd/

2.6. Liver sampling

Liver biopsies were collected from 10 lumpfish prior to transfer and 3 from each cage at day 41 and day 66 of the project period. Each fish was humanely dispatched with an anaesthetic overdose of Tricaine (800 mg L^{-1} MS-222). The liver of each sampled fish was colour scored in-situ using the scoring index developed by Eliasen et al. (2020). After which, the liver from each fish was carefully removed and weighed. The hepatosomatic index was calculated for each fish using the formula:

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

After weighing, the livers were snap frozen in liquid nitrogen and stored at −20 °C for further analysis of protein and fat content. The protein content was analysed at SINTEF-Norlab using the Kjeldahl method and total fat content analysed using the Schmid-Bondzynski-Ratslaff method (SBR).

2.7. Histopathology

The fish was dissected during liver sampling and the whole intestine carefully removed intact and flushed with sea water using a 1 mL syringe. After flushing, the anterior of the intestines was marked by tying a small section of cord to the end and transferred into a sampling pot containing 4% buffered formalin. The whole pyloric caecae and liver biopsy were also be sampled and transferred to a similar container. Intestine sampling was performed at the same time post feeding and as soon as possible after euthanasia.

Transverse sections of pyloric caecae, liver, midgut and hindgut/distal intestine were sampled from the whole intestinal tracts according to Moldal et al. (2014). Tissue samples were processed at Pharmaq Analytiq 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. Intestinal samples were evaluated semi-quantitatively for inflammatory changes (muscularis, submucosa/lamina propria and epithelial layers), epithelial changes (i.e., degeneration/necrosis, vacuolization, loss) and other pathological changes (Table 2). A description of the pathological changes and their distribution within the tissue (focal/multifocal/diffuse) was noted. Goblet cells stained positive with PAS and Alcian blue in the mid-gut were also counted, and the results given as a count of goblet cells per fold. A total number was recorded, and a comment in the subpopulation (neutral/acidic/mixed) was recorded.

Intestinal fold length was measured in the hindgut/distal intestine 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 layer. The liver was evaluated semi-quantitatively (scoring 0–3) for the presence of pathological changes, i.e., necrosis, haemorrhages, fibrosis, and an assessment of vacuolization was performed (none/minimal – normal – high vacuolization).

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. Statistics

All statistical analyses were conducted using Statistica™ 13.3 software. 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). A two-way nested analysis of variance (ANOVA, Searle et al., 1992) where replicates are nested within feeding frequency groups was applied to calculate the effect of different feeding groups on growth performance, welfare and cataract scores and histological data. The model equation of the nested ANOVA had the form:

$X_{ijk} = \mu + \alpha_i + C_{ij} + \varepsilon_{ijk}$ where μ is the general level; α_i is the feeding group effect; C_{ij} is the contribution caused by replicate (tank) j in feeding frequency i and ε_{ijk} is the error term. We assume that $\varepsilon_{ijk \sim \text{Normal distributed}}$ $(0, \sigma^2)$.

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-β) analysis was performed in Statistica™ using $\alpha = 0.05$.

3. Results

3.1. Growth and mortality of lumpfish

Only one lumpfish died during the study, and this was on day 42. At the start of the study, there were no significant differences in mean weight between the four experimental groups (two-way nested ANOVA, $P > 0.05$, Fig. 1). From day 14 onwards the pelleted feed group had consistently higher mean weights (two-way nested ANOVA, $F_{3,\ 4} \geq 6.2$. $P < 0.05$, Fig. 1A) compared to the other three feed treatment groups. The final mean weight of the pelleted fed group was 21% higher compared to group 2 fed with feed blocks (67.1 g and 53.1 g respectively). There were no significant differences in mean weight between the three feed block groups at any sampling point in the trial period. There were significant differences in specific growth rate (SGR) between

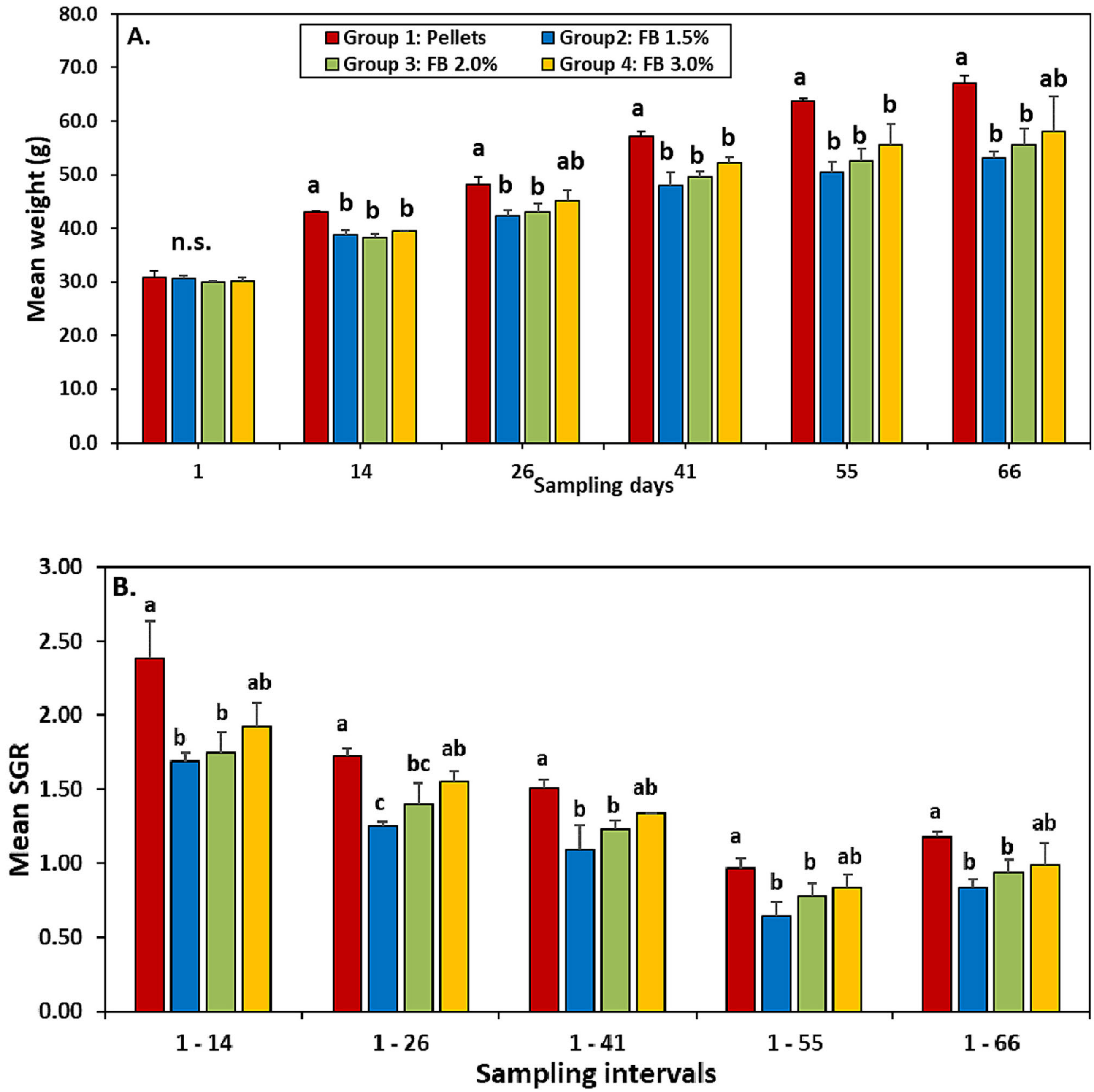

Fig. 1. (A) Mean weight (g) and (B) Specific growth rates (% day $^{-1}$) of lumpfish fed pelleted feed (group 1) or three levels of feed blocks (1.5%, 2.0% and 3.0% BW^{-1}) Values represent means ± SD. Different letters indicate significant differences (SNK test, $P < 0.05$); n.s., not significant.

the experimental groups throughout the study period (two-way nested, ANOVA, $F_{3,\ 4} \geq 5.4$. $P < 0.05$, Fig. 1B). Lumpfish fed with pelleted feed had the highest SGR throughout the study period compared to the three feed block groups and was significantly higher compared to groups 2 and 3, whereas SGR did not differ between the pelleted group and group 4 (FB 3.0%, SNK post hoc test, $P > 0.25$, Fig. 1B).

3.2. Growth, feeding and mortality of salmon

Overall mean weight (± SD) of the Atlantic salmon increased from 1485.9 ± 213.2 g to 2716.1 ± 60.8 g at termination of the trial. No differences in mean weights between the salmon in the experimental cages were seen (two-way nested ANOVA, $F_{9,\ 216} = 1.7$, $P > 0.45$). Specific growth rate of the salmon in the ten sea cages varied between 0.87 and 0.93 and no significant differences were found (two-way nested ANOVA, $P > 0.75$). No difference was seen in the feed conversion rate of the salmon in the ten sea cages which varied between 0.97 and 1.03. Sea lice numbers on the salmon were similar across all cages at the onset of the trial (0.5–0.7 total number of lice per individual).

3.3. Cataracts in lumpfish

At the start of the study period lumpfish in three of the four groups (groups 2, 3 and 4, Fig. 2) had cataracts, with prevalence ranging between 1.7% and 3.3%. There were significant differences at each of the subsequent sampling time points (days 26, 41, 55 and 66) (two-way nested ANOVA, $F_{3,\ 4} \geq 3.07$, $P < 0.05$, Fig. 2). Lumpfish from group 1 (pelleted feed) had the highest prevalence (30–38%) from day 26 onwards, and at the end had a prevalence of 38% compared to the other three treatment groups which had 28%, 31% and 33% for groups 2, 3

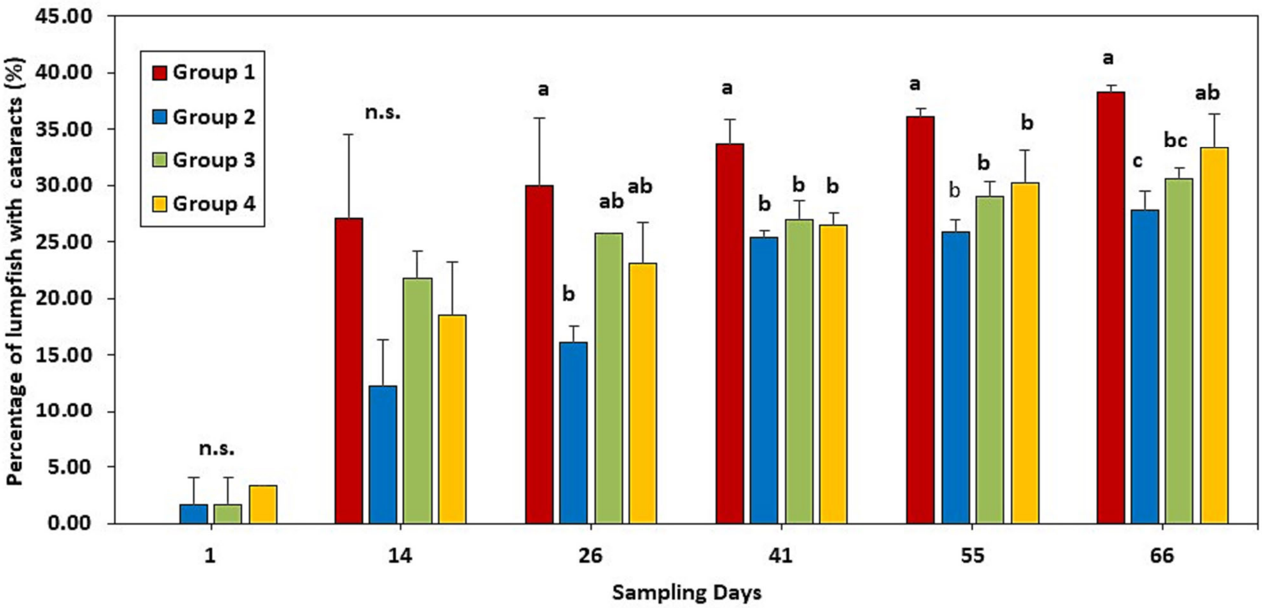

Fig. 2. Occurrence of lumpfish with cataracts (% prevalence) calculated for each of the treatment groups at day 1, 14, 26, 41, 55 and 66. Values represent means ± S. D. Different letters indicate significant differences (SNK test, $P < 0.05$); n.s., not significant.

and 4 respectively. The prevalence of cataract at the end of the trial was not significantly different between group 1 and 4.

As the prevalence of cataract increased over time, so did the severity. There was significantly less lumpfish with no cataracts from group 1 as the study progressed compared to the other three groups (SNK post hoc test, $P < 0.05$, Fig. 3A). There were little differences in in the frequency of mild (score 1–2) and moderate (score 3–4) cataracts between the experimental groups (Fig. 3B-C). There were significant differences between the groups with severe cataracts (score 5–8) from day 41 onwards (SNK post hoc test, $P < 0.05$, Fig. 3D). Lumpfish from group 1 had the highest prevalence increasing from 14% at day 14 to 35% at day 66. Lumpfish from groups 2, 3and 4 had similar scores at day 66 (25.0%, 24.1% and 25.4% respectively).

3.4. Welfare score in lumpfish

The percentage of lumpfish with good welfare scores decreased for each group as the study progressed (Fig. 4). For group 1, the percentage of lumpfish assessed as having good welfare was 83% at pre-transfer and decreased slightly to 75% at day 1 of the study. At day 66, this had decreased to 30% of all fish while 35% of the group were classified as having slight reduction and 35% as having a clear reduction in welfare

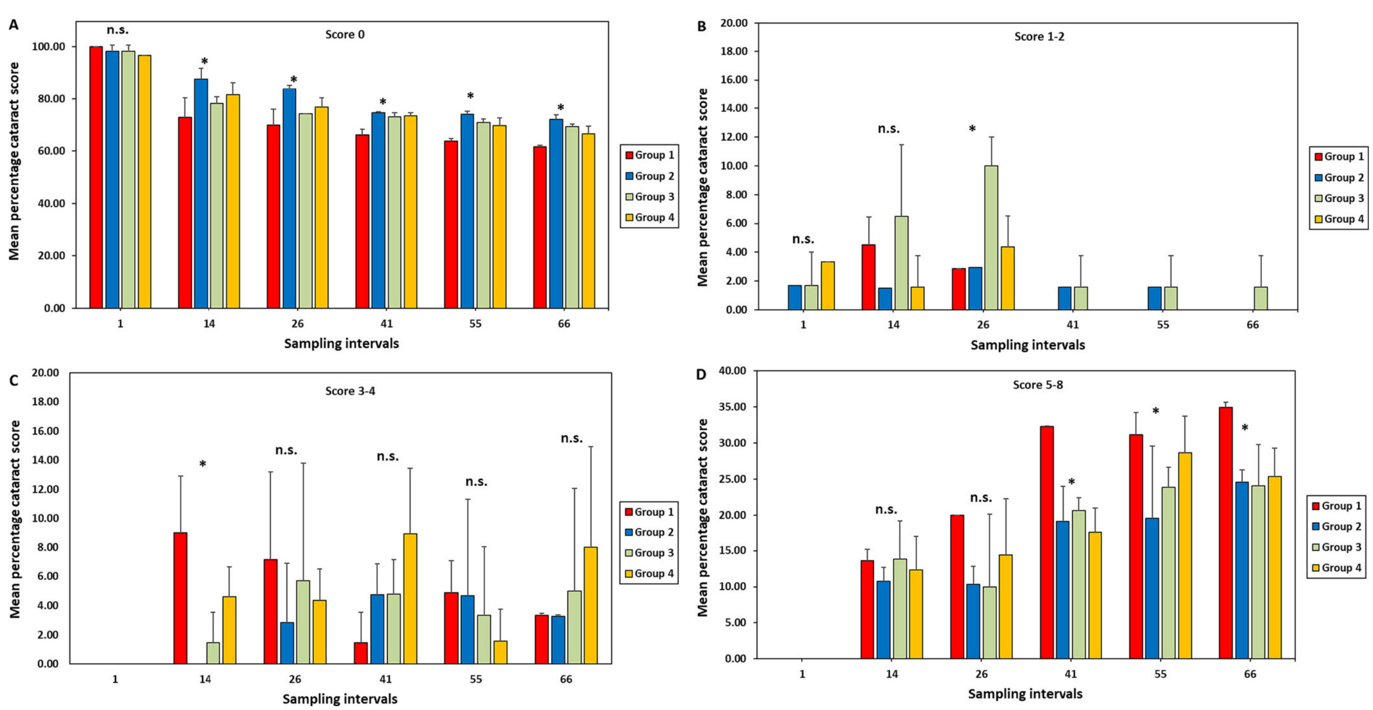

Fig. 3. Percentage of fish form each of the four groups with total cataract score (sum score of both eyes) at day 1, 14, 26, 41, 55 and 66. Scores are classified A: 0, B: 1–2, C: 3–4 and D: 5–8. Values represent means ± SD. Significant difference is indicated by: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$); n.s., not significant.

Fig. 4. Percentage of lumpfish assessed as having either good, slight reduction, clear reduction or severe reduction for overall group welfare score, caudal fins, other fins and condition for each of the four experimental groups.

score. No lumpfish were assessed as having severely reduced welfare during the project period.

Caudal fin damage/erosion decreased through time for each of the groups (Fig. 4). However, were no significant differences between the groups. Between 52% of fish from group 1 and 67% of fish from group 2 had no damage of the caudal fin when examined at the end of the study period (day 66). Only 1.7% of fish from groups 1, 2 and 4 had severe erosion observed at day 66 (Fig. 4). The majority of lumpfish had intact fins at the end of the study period (Fig. 4) and no significant differences in damage was seen.

For all four groups, condition of the lumpfish was classified as good for the majority during the project period (Fig. 4). For group 1, 98% of the fish were in good condition and 2% had slight emaciation at the end of the study while for group 2, 90% had good condition and 10% slightly emaciated (Fig. 4). A similar trend was noted for group 3 while lumpfish from group 4 had 5% slightly emaciated, 2% clearly and 2% severely emaciated (Fig. 4).

3.5. Lumpfish liver weight, HSI, liver colour, fat and protein liver content

There were significant differences in mean liver weight between the treatment group at intermediate sampling (day 41) and end sampling (day 66) (two-way ANOVA, $F_{3,\,20} > 3.9$, $P < 0.005$, Fig. 5A). At day 41, mean liver weights were significantly lower (SNK post hoc test, $P < 0.01$) for groups 1 and 2 (0.7 and 0.8 g respectively) compared to groups 3 (1.4 g) and 4 (1.6 g). At day 66, lumpfish from group 1 had significantly lower mean liver weight compared to group.

Mean HSI varied significantly at each of the 2 sampling time points with groups 1 and 2 having lower HSI values compared to the other two groups at day 41 (SNK post hoc test, $P < 0.05$, Fig. 5B), and group 1 having the lowest HSI at day 66 (SNK post hoc test, $P < 0.05$, Fig. 5B).

There were no significant variations in liver colour score between treatment groups for the two sampling time points (SNK post hoc test, $P > 0.15$, Fig. 5C). Liver colour for all groups was higher than that calculated for the baseline at day 1.

No significant (two-way nested ANOVA, $P > 0.35$, Fig. 6) in mean liver fat content and mean liver protein content were found between the four treatment groups at day 41 and day 66.

3.6. Histopathology

All lumpfish assessed at the start of the study (baseline) had healthy tissues apart from one fish which had mild, multifocal necrosis in the epithelium and an increased number of Intraepithelial lymphocytes (IELs). At intermediate sampling (day 26), one fish from group 1 and three from group 2 were assessed as having mild focal or multifocal inflammation in the lamina propria and one fish from group 2 with an increased number of IELs (Table 3, Fig. 7). For tissue samples drawn from the mid-gut section of the intestine showed no evidence of inflammation on any of the samples throughout the project period. There was evidence of dilation at day 26, when two fish showed evidence of dilation and seven fish (43.8%>) had evidence of dilation at day 66 (Table 3, Fig. 8). For hind gut tissue samples, only one fish from day 26 and one from day 66 had evidence of mild multifocal inflammation in the lamina propria. Three fish from day 26 and three from day 66 had evidence of dilation int the hind gut.

Mean mid-gut fold length varied between and withing the treatment groups (two-way nested ANOVA, $P < 0.05$). There were no significant differences at day 26 between the treatment groups with mean values ranging between 469.8 µm for group 2 and 676.7 µm for group 4. There were significant differences in fold length at day 66 with sample fish from group four having the highest fold length of 540.0 µm compared to the other three treatment groups (Fig. 9A). The number of goblet cells did not vary within and between treatment groups (Fig. 9B).

The degree of liver vacuolisation was assessed as normal in most of the samples from all sampling time points (Table 3, Fig. 10A showing normal vacuolisation). Three sample fish showed evidence of having less vacuolisation compared to the normal vacuolisation seen in Fig. 10A.

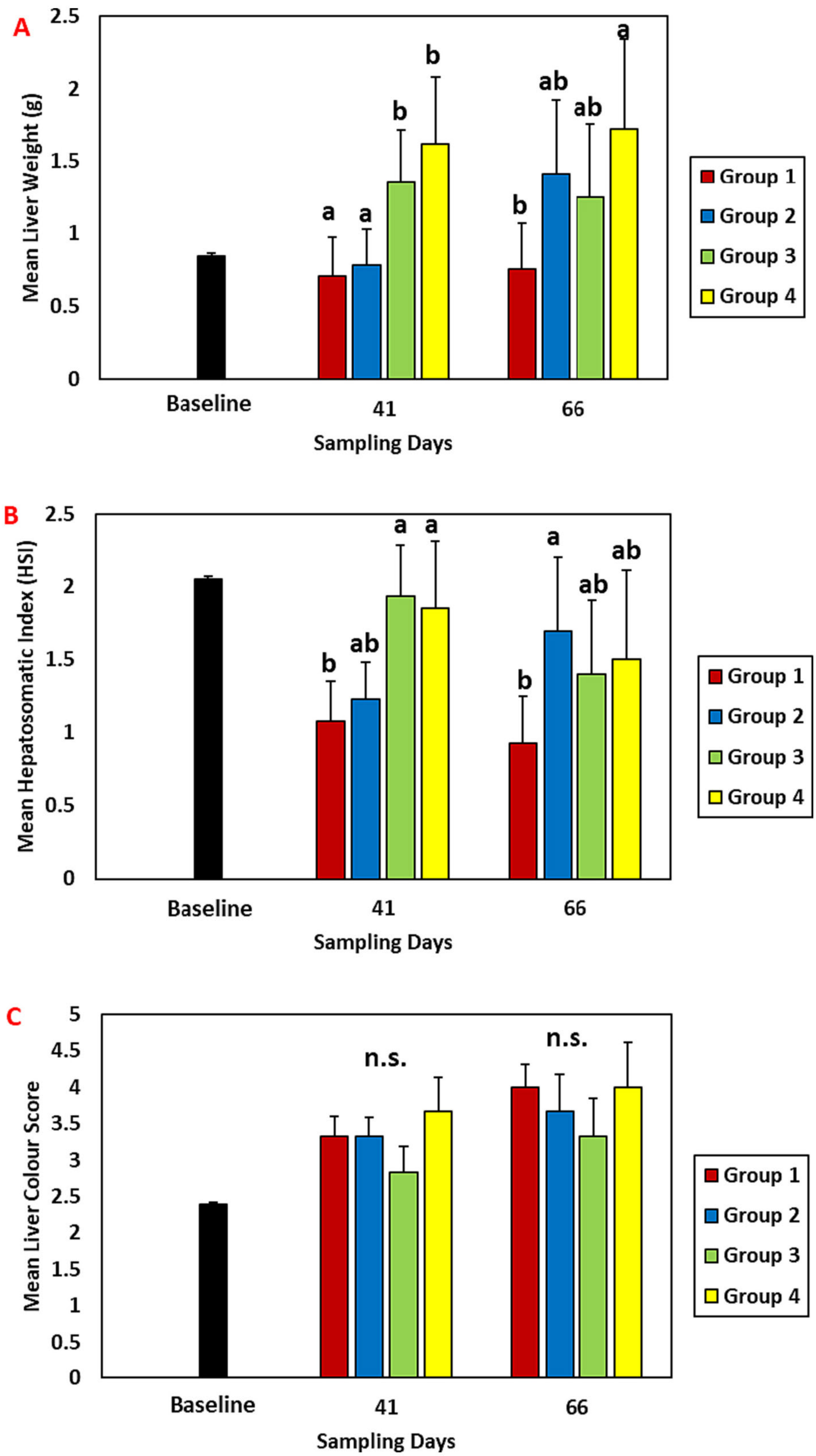

Fig. 5. A) Mean liver weights (g); B) mean Hepatosomatic index (HIS) and C) mean liver colour score for each of the four treatment groups. Values represent means ± S.D. Mean values which do not share a letter were found to be significantly different (SNK post hoc test, $P < 0.05$). Baseline = day 1.

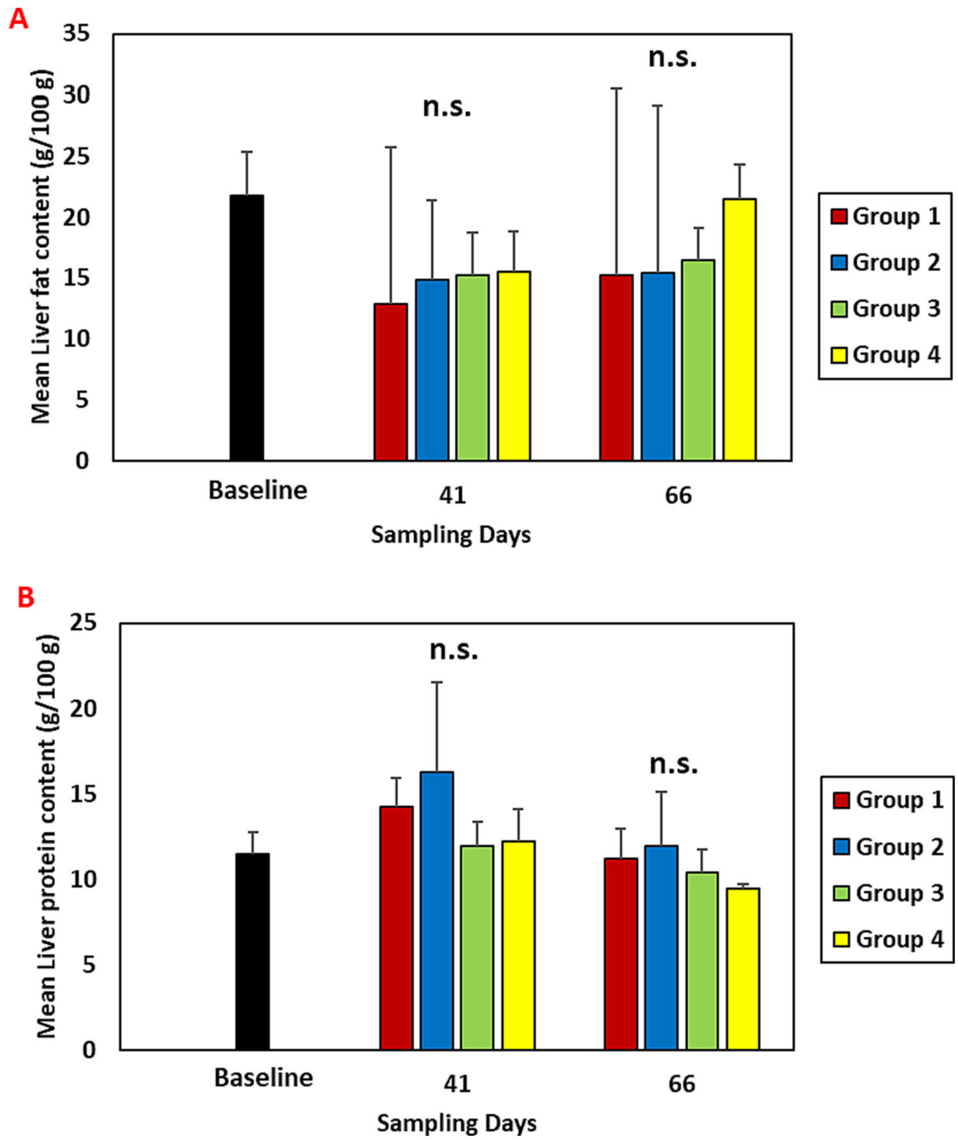

Fig. 6. A) Mean liver fat content and B) mean liver protein content for each of the four experimental groups. Values represent means ± S.D. Mean values which do not share a letter were found to be significantly different (SNK post hoc test, $P < 0.05$). Baseline = day 1.

1. An example of liver sample showing less vacuolisation (fish from group 2) is shown in Fig. 10B.
2. Two sampled fish showed evidence of focal aggregation of eosinophilic granulocytes (group 3, Fig. 10C: highlighted area) and five fish exhibiting mild inflammation (group 2, Fig. 10D).

4. Discussion

Overall, the present results indicate that the type and amount of feed have an effect on growth and cataract development in lumpfish, while only minor effect on welfare score and histopathology were found.

4.1. Growth of lumpfish

Lumpfish fed with commercial pelleted feed (group 1) exhibited significantly higher growth at each sampling time point from day 14 onwards compared to the three groups fed with feed blocks. At the end of the study period, the mean weight of group 1 was between 14% and 21% higher when compared to the three feed block groups. High growth is not an aim for lumpfish used as cleaner fish. Imsland et al. (2016a) found that small lumpfish (initial size approx. 39 g) have a higher overall preference for natural food items, including sea lice, compared to larger conspecifics (initial size 67 g). Similar size-related sea lice grazing of lumpfish were presented in Boissonnot et al. (2022a). 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. Further, earlier studies have indicated that sexual maturation in lumpfish can occur from around 200 g onwards (Imsland et al., 2015a) and adaptations in the feeding behaviour of the fish may be suppressed (Davenport, 1985) along with an observed reduction in sea lice grazing efficacy. Consequently, they may stop foraging for food sources which require an output of energy and shift towards consuming readily available salmon pellets which require much less energy expenditure. Controlling growth rates of lumpfish in commercial sea cages may allow for the prolongation of sea lice grazing behaviour and allow salmon farmers to alter their stocking strategies and potentially reduce the number of times restocking of lumpfish occurs as well as perhaps enhancing sea lice grazing potential.

The lower growth observed in treatments groups 2, 3 and 4 was attributed to the use of feed blocks to maintain the lumpfish during the study period. Previous studies have shown that using feed blocks controls growth in lumpfish without apparently compromising the health

Table 3
Mean histological scoring results from samples drawn from pyloric caeca, mid-gut, hindgut and liver at start, day 26 and day 66. Values represent means ± S.D.

Tissues sampled	Baseline	Intermediate sampling (day 26) Group 1	Group 2	Group 3	Group 4	End sampling (day 66) Group 1	Group 2	Group 3	Group 4
Pyloric caeca score	0.33 0.58	0.25 0.50	0.75 0.50	0.00 0.00	0.00 0.00	0.00 0.00	0.25 0.50	0.00 0.00	0.00 0.00
Mid-gut Fold length (μm)	507.19 42.70	540.42 90.47	469.84 36.74	572.55 57.94	676.67 161.13	447.06 26.34	398.04 97.50	402.34 67.93	539.96 33.99
Mid-gut No. goblet cell/fold	46.50 6.50	37.55 6.07	44.17 4.19	38.80 6.32	48.26 10.41	43.26 12.35	37.45 3.43	36.98 2.88	45.08 10.57
Mid-gut score	0.00 0.00	0.00 0.00	0.00 0.00	0.00 0.00	0.00 0.00	0.00 0.00	0.00 0.00	0.00 0.00	0.00 0.00
Hind-gut score	0.00 0.00	0.00 0.00	0.25 0.50	0.00 0.00	0.00 0.00	0.00 0.00	0.00 0.00	0.25 0.50	0.00 0.00
Liver vacuolisation	0.00 0.00	0.25 0.50	0.25 0.50	0.25 0.50	0.00 0.00	0.50 0.58	0.25 0.50	0.25 0.50	0.00 0.00

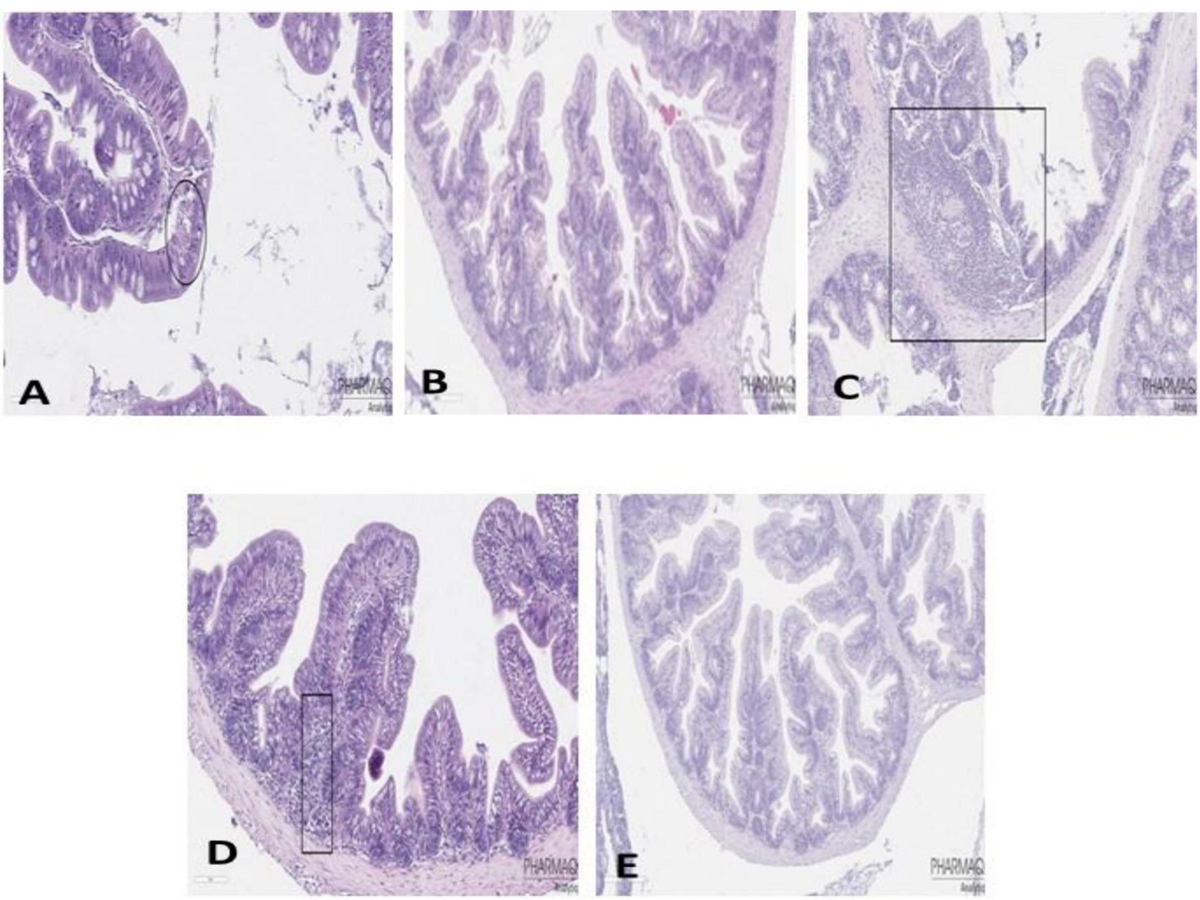

Fig. 7. Micrographs of tissue samples of pyloric caeca drawn at A) Baseline showing mild, multifocal necrosis in the epithelium, increased number of Intraepithelial lymphocytes (IELs); B) day 26 from group 3 showing normal pathology; C & D) day 26 from group 2 Intermediate sampling Group 2 showing mild, multifocal inflammation in lamina propria, increased number of Intraepithelial lymphocytes (IELs) and E) day 66 from group 3 showing normal pathology.

status of the fish and may facilitate enhanced grazing efficacy (Imsland et al., 2018c, 2019a, 2020). Lumpfish feeding from feed blocks may have to expend more energy to maintain position when grazing from them or due to more competition between conspecifics compared with fish fed with pellets possibly relating to feeding hierarchies (Imsland et al., 1998, 2009). Interestingly, no significant difference in growth was found between the three feed block groups. Since the different feeding quantities were achieved by setting various number of blocks per feeding station, keeping the same amount of feeding stations in each cage, the same competition factor may have applied for the three groups.

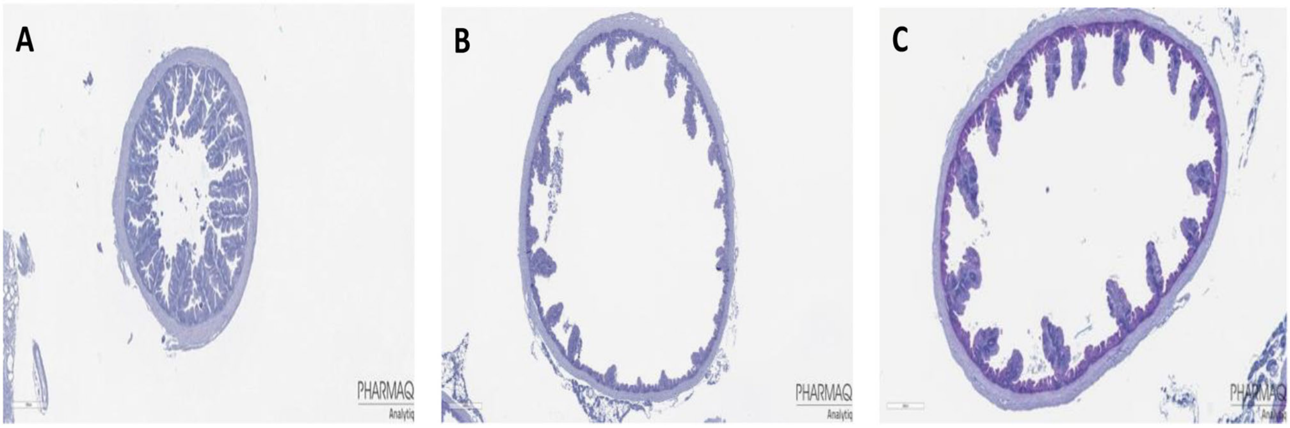

Fig. 8. Micrographs of transverse sections of mid-gut tissue at A) baseline showing normal pathology; B) day 26 from group 2 showing dilated segment and C) day 66 from group 2 showing dilated section.

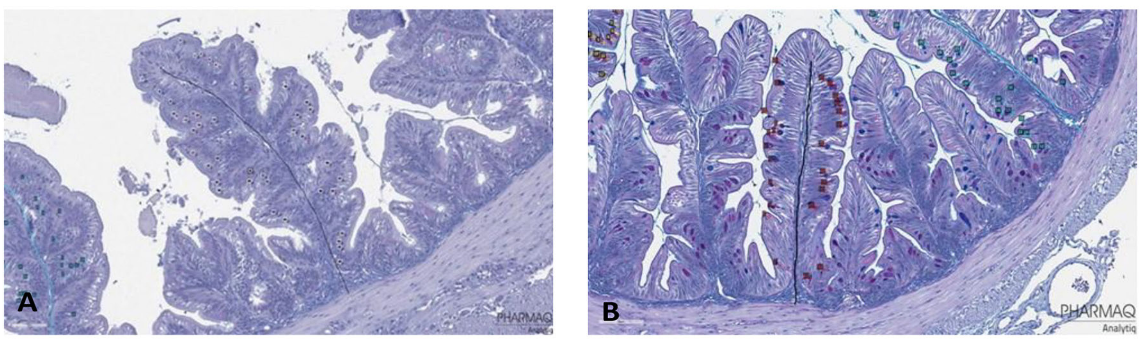

Fig. 9. Micrographs of sections of mid-gut from A) day 26, group 4 highlighting measurement of fold and counting of goblet cells. AB-Pas stain and B) day 66 from group 3 showing mixed goblet cell population. AB-Pas stain.

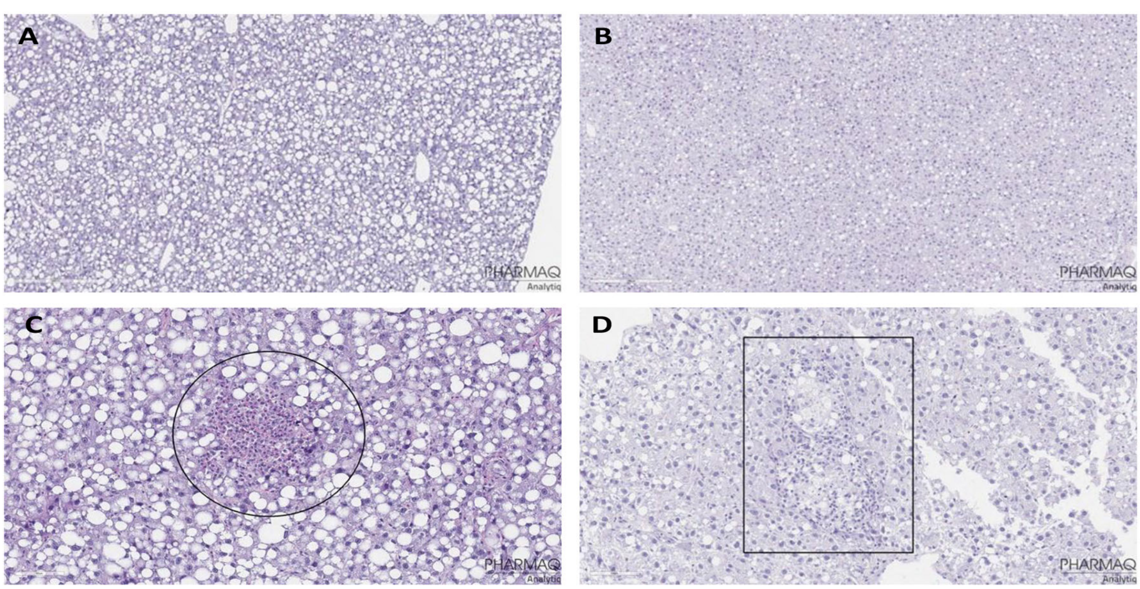

Fig. 10. Micrographs of sections of liver from A) baseline showing normal degree of vacuolisation: B) day 26, group 2 showing less vacuolisation; C) day 26 group 3 showing focal aggregation of eosinophilic granulocytes (highlighted area) and D) day 26 group 2 showing inflammation in highlighted area. All tissues prepared with HE stain.

The establishment of multiple feeding stations for lumpfish in tanks or sea cages could influence growth as it would assist in the prevent formation of such feeding hierarchies. In addition, fragments of feed block fall off when the lumpfish graze from them thus not all the block is consumed (Imsland et al., 2018c, 2019a). The feed blocks currently being used in commercial salmon farms generally generate waste as uneaten material and this can be as high as 50% of the total pre-immersed weight (P. Reynolds, pers. obs.). The feed blocks have surface grooves to allow lumpfish to graze on them, but these grooves are too shallow and once they are absent, lumpfish show reduced block grazing intensity. The feed blocks used in this study are the smaller type (typically circa 60–70 g) and have much deeper grooves. These blocks are generally consumed entirely within the same day of deployment. These findings indicate that a process of development is required for the larger commercial cage blocks in order for them to be more available to lumpfish and ensure waste is reduced and nutrient uptake safeguarded.

It is important that lumpfish populations have access to a regular food source particularly in wintertime when naturally occurring food items become scarce. This food source is vital to maintain healthy and robust populations. Presently, most commercial farms using lumpfish feed them with pelleted feed (Imsland et al., 2015a, 2018a; Powell et al., 2018) which usually is delivered from the edge of the cage, manually or by 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.

4.2. Cataracts

The incidence of cataracts varied between the treatment groups and seem to be correlated to differences observed in growth between the four groups. Lumpfish fed with pelleted feed had the highest incidence of cataracts (38%) and the highest incidence of severe cataract (score 5–8). The only difference between the four treatment groups was dietary as the fish for each treatment group were established from the same base population and none of the groups were treated differently before and during the study. There were differences between the two feed types used in the study, but dietary analysis was not undertaken and thus no direct diet profile comparison can be made. A previous study comparing lumpfish fed with feed blocks or pelleted feed (Imsland et al., 2019a), showed 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, the present study revealed that lumpfish fed with the highest amount of feed blocks (group 4, 3% BW) exhibited higher incidence of cataract than lumpfish fed with the lowest amount of feed blocks (group 1, 1.5% BW). It is known that high or rapid growth can increase the risk of cataracts in salmon (Ersdal et al., 2001) and previous studies on lumpfish (Imsland et al., 2018b, 2019b) found that fish with high growth rates also had the highest incidence of cataracts. In the present study, growth was higher among lumpfish fed with 3% BW than among those fed with 1.5% BW, but the difference was not statistically significant. It is possible that the nutrients offered in feed blocks are not optimal for lumpfish, and that high feeding on those contribute to cataract development.

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 cataract is only comparable with the highest incidences previously found in farmed Atlantic salmon caused by a histidine-deficient diet. It is possible that a similar unbalance in nutrients affects cataracts in lumpfish fed with either pellets or feed blocks. In farmed salmon, it has been shown that even moderate degrees of cataract can result in reduced growth (Breck and Sveier, 2001). Previous studies have shown cataract development in lumpfish does not seem to be related to insufficient histidine in the feed as previously reported in Atlantic salmon. However, it has been highlighted that cataracts in lumpfish may be related to disturbed metabolism / malnutrition, visualized as exceedingly high values of selected amino acids in different tissues (Jonassen et al., 2017). This can cause osmotic imbalance in fish tissues and cataract development or is a consequence of osmotic imbalance. It is known that high or rapid growth can increase the risk of cataracts in salmon (Ersdal et al., 2001). Further, previous studies on lumpfish (Jonassen et al., 2017; Imsland et al., 2018b) found that high SGR increased risk of developing cataracts (as observed in Atlantic salmon). Although, some of these effects may be partially attributed to differences in food sources consumed, there may well be an additional genetic factor which manifests as certain lumpfish populations being less predisposed developing cataracts (Imsland et al., 2021).

Further, lumpfish may be predisposed in developing cataracts due to rapid growth during the hatchery phase. Recent anecdotal evidence from our research group (P. Reynolds, GIFAS, pers. comm.) has suggested that most hatcheries producing lumpfish have cataracts present during the pre-vaccination period of production with prevalence varying greatly between them. It is also known that there are many different production strategies employed by hatcheries, some of which may contribute to cataract development. Currently, there is no detailed research being undertaken during this phase to fully elucidate the causal factors of cataract development. Importantly, if lumpfish are stocked in commercial salmon and have some degree of cataracts (Jonassen et al., 2017) this may lead to reduced vision reducing the fish ability to graze sea lice from salmon.

4.3. Histopathology and liver characteristics

The physiological condition of the fish is one of the key factors that determine the health status of fish (Saravia et al., 2015; Ahmed et al., 2020). Thus, monitoring the physiological status of fish by using histopathological examination (Saravia et al., 2015) can lead to a good understanding of the functional morphology of the lumpfish alimentary canal (Imsland et al., 2019b) and can be an important tool for learning more about their feeding habits especially for feed formulation prior to stocking in commercial salmon cages as the size of fish used at the start of this study (30 g) is representative and within the size range at which these fish are stocked in commercial salmon cages. At intermediate sampling and end sampling, there were very little histopathological changes observed and no obvious differences between the dietary treatments. In one sample, from the hindgut there was mild, multifocal inflammation in the lamina propria. 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 66 days (the duration of the study) inflammation to be more pronounced as seen in Atlantic salmon fed diets containing >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 and Krogdahl, 1996).

There were no significant differences in liver vacuolisation between the dietary groups and baseline samples with most of the livers evaluated appeared to be vacuolized withing what is regarded as normal range for all four treatment groups with a low number appearing less vacuolized and denser. 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 mid-gut between the two diet groups compared to the baseline 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 feed blocks at 3% BW^{-1} had a slightly longer intestinal fold height compared to the other three groups, which was significant at day 66. Fold height can be increased by addition of supplements to the diet (Dimitroglou et al., 2009). The fold height for fish fed at 3% was longer perhaps because of the higher amount of vitamin C being absorbed compared to the other three groups although the actual amount in the pelleted feed is unknown. 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.

There were differences in HSI values between the groups with lumpfish fed with pelleted feed having the lowest scores at each sampling timepoint. 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). Atlantic cod are known to deposit large quantities of the dietary fat in the liver when fed to satiety (Lie et al., 1988). >80% of the fat content of the Atlantic cod can be found in the liver, whereas skeletal muscle contains <2% fat in farmed Atlantic cod (Aksnes et al., 2006).

There were no significant differences in liver colour scores between the treatment groups with most scoring in the 3 to 4 category which indicates good nutritional status according to the scoring system developed by Eliasen et al. (2020). The authors found that a lumpfish liver that is dark reddish-brown (score 5–6), had a very low content of fat (triacylglycerides). This indicates that the lumpfish has/is using its fat reserves, which in turn can mean reduced welfare and suboptimal feeding conditions. Boissonnot et al. (2022b) also showed that lumpfish livers with low fat levels had dark reddish-brown colourisation (score 5–6). Both liver scores 1–2 (pale liver) and 3–4 (bright orange) appear to indicate a lumpfish with good nutritional status and good feeding conditions (Eliasen et al., 2020; Boissonnot et al., 2022a-b). Based on this, one should therefore pay extra attention to liver colour 5–6 with regard to feeding and feeding regimes. Eliasen et al. (2020) also suggest that light / pale liver (score 1–2) may indicate impaired welfare. This has not been confirmed and must be seen in connection with other autopsy findings, as well as any test results from PCR, bacteriology, and histology.

4.4. Welfare status

As cleaner fish are produced for their delousing behaviour as a pest management strategy rather than any physical characteristics, good welfare is essential to promote their natural behaviours (Brooker et al., 2018). For any new species in aquaculture such as lumpfish, it is important to develop indicators to define and monitor welfare. Such OWIs should be based on physiological and physical status and behaviour. Welfare was assessed during this study using the Lumpfish Operational Welfare Indicator (OWI) model developed by Boissonnot et al. (2022b, 2023). There was a trend for the percentage of lumpfish with good welfare scores to decrease for each group as the study progressed. These findings are in line with a study by Boissonnot et al. (2023) who monitored welfare and survival of lumpfish from four commercial farms over a 6 to 12-month period. Further, there were little differences between the four treatment groups. This is in contrast to a previous study by Imsland et al. (2020) which showed that there was a slight deterioration in condition manifested by increasing welfare scores with fish fed with feed blocks having consistently lower average scores indicating better health condition compared to pelleted fed fish. The trend for welfare to be affected in salmon cages is perhaps universal and expected given the type of environment that lumpfish are expected to operate in. Thus, mitigation methods are of critical importance to maintain and/or improve welfare status.

The Norwegian Fish Health Report for 2021 (Sommerset et al., 2022) reported feedback from fish health personnel on welfare challenges related to handling and delousing, but there is to date very little published material on whether production conditions have an effect on the welfare of lumpfish. Recently Reynolds et al. (2022) investigated causes of mortality and loss of lumpfish from both small- and large-scale studies in Northern Norway. Results showed that causes of mortality varied within and between sites. For lumpfish deployed at small-scale sea pens, the primary cause of mortality was identified as pathogenic, while for lumpfish deployed at large-scale sea pens, transporting, grading and mechanical delousing were the primary causes of mortality. The results indicated that more research is required to clarify best rearing practices of lumpfish both in commercial hatcheries and salmon cages.

5. Conclusions

There were significant differences in growth performance between the four treatment groups with lumpfish fed with pelleted feed attaining the highest weight gain. The incidence of cataracts varied between the treatment groups and was correlated to differences observed in growth between the four groups. Lumpfish fed with pelleted feed had the highest incidence and severity of cataracts, followed by lumpfish fed with highest amount of feed blocks. Lumpfish welfare (measured by OWI) decreased for each group as the study progressed. Little histopathological changes between groups were found with mainly mild focal and multifocal inflammation observed in sampled tissues. This study suggests that feeding lumpfish with moderate amounts of feed blocks ($\leq$ 2% BW^{-1}) may be advantageous for maintaining slow growth and good welfare in salmon cages, and this should be further tested in large scale studies in commercial salmon farms.

CRediT authorship contribution statement

Albert K.D. Imsland: Conceptualization, Funding acquisition, Writing – original draft, Writing – review & editing. **Patrick Reynolds:** Investigation, Conceptualization, Writing – original draft, Writing – review & editing. **Lauris Boissonnot:** Project administration, Funding acquisition, Investigation, Conceptualization, Writing – review & editing.

Declaration of Competing Interest

There is no conflict of interest in relation to this study.

Data availability

Data will be made available on request.

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References

Ahmed, I., Reshi, Q.M., Fazio, F., 2020. The influence of the endogenous and exogenous factors on hematological parameters in different fish species: a review. Aquac. Int. 28, 869–899.
Aksnes, A., Hope, B., Høstmark, Ø., Albrektsen, S., 2006. Inclusion of size fractionated fish hydrolysate in high plant protein diets for Atlantic cod, *Gadus morhua*. Aquaculture 261, 1102–1110.
Baeverfjord, G., Krogdahl, Å., 1996. Development and regression of soybean meal induced enteritis in Atlantic salmon, *Salmo salar* L, distal intestine: a comparison with the intestines of fasted fish. J. Fish Dis. 19, 75–87.
Bjerkås, E., Bjørnestad, E., Breck, O., Waagbø, R., 2001. Water temperature regimes affect cataract development in smelting Atlantic salmon, *Salmo salar* L. J. Fish Dis. 24, 281–291.
Bjerkås, E., Breck, O., Waagbø, R., 2006. The role of nutrition in cataract formation in farmed fish. CAB Reviews: Persp. Agric., Vet. Sci. Nutr. Nat. Res. 1, 1–16.
Boissonnot, L., Kharlova, I., Iversen, N.S., Staven, F.R., Austad, M., 2022a. Characteristics of lumpfish (*Cyclopterus lumpus*) with high cleaning efficacy in commercial Atlantic salmon (*Salmo salar*) production. Aquaculture 560, 738544.

Boissonnot, L., Austad, M., Karlsen, C., Reynolds, P., Stensby-Skjærvik, S.A.I., 2022b. Welfare Assessment of Lumpfish in Sea Cages - Manual. Version 2. URL. https://aqua-kompetanse.no/lumpfish/.
Boissonnot, L., Austad, M., Reynolds, P., Karlsen, C., Remen, M., Imsland, A.K.D., 2023. Welfare and survival of lumpfish (*Cyclopterus lumpus*) in Norwegian commercial Atlantic salmon (*Salmo salar*) production. Aquaculture 572, 739496.
Boxaspen, K., 2006. A review of the biology and genetics of sea lice. ICES J. Sea Res. 63, 1304–1316.
Breck, O., Sveier, H., 2001. Growth and cataract development in two groups of Atlantic salmon (*Salmo salar* L) post smolts transferred to sea with a four-week interval. Bull. Europ. Ass. Fish Pathol. 21, 91–103.
Breck, O., Bjerkås, E., Campbell, P., Arnesen, P., Haldorsen, P., Waagbø, R., 2003. Cataract preventative role of mammalian blood meal, histidine, iron and zinc in diets for Atlantic salmon (*Salmo salar* L.) of different strains. Aquacult. Nutr. 9, 341–350.
Brooker, A.J., Papadopoulou, A., Gutierrez, C., Rey, S., Davie, A., Migaud, H., 2018. Sustainable production and use of cleaner fish for the biological control of sea lice: recent advances and current challenges. Vet. Rec. 183, 12.
Caballero, M.J., Izquierdo, M.S., Kjørsvik, E., Fernández, A.J., Rosenlund, G., 2004. Histological alterations in the liver of seabream, *Sparus aurata* L., caused by short- or long-term feeding with vegetable oils. Recovery of normal morphology after feeding fish oil as the sole lipid source. J. Fish Dis. 27, 531–541.
Davenport, J., 1985. Synopsis of Biological Data of the Lumpsucker *Cyclopterus lumpus* (L 1758). FAO Fisheries synopsis No. 147.31 pp.
Denholm, I., Devine, G.J., Horsberg, T.E., Sevatdal, S., Fallang, A., Nolan, D.V., Powell, R., 2002. Analysis and management of resistance to chemotherapeutants in salmon lice *Lepeophtheirus salmonis* (Krøyer) (Copepoda: Caligidae). Pest Manag. Sci. 58, 528–536.
Dimitroglou, A., Merrifield, D.L., Moate, R., Davies, S.J., Spring, P., Sweetman, J., Bradley, G., 2009. Dietary mannan oligosaccharide supplementation modulates intestinal microbial ecology and improves gut morphology of rainbow trout, *Oncorhynchus mykiss* (Walbaum). J. Anim. Sci. 10, 3226–3234.
Eliasen, K., Patursson, E.J., McAdam, B.J., Pino, E., Morro, B., Betancor, M., Baily, J., Rey, S., 2020. Liver colour scoring index, carotenoids and lipid content assessment as a proxy for lumpfish (*Cyclopterus lumpus* L.) health and welfare condition. Sci. Rep. 10, 8927.
Engebretsen, S., Aldrin, M., Qviller, L., Stige, L.C., Rafoss, T., Danielsen, O.R., Lindhom, A., Jansen, P.A., 2023. Salmon lice (*Lepeophtheirus salmonis*) in the stomach contents of lumpfish (*Cyclopterus lumpus*) sampled from Norwegian fish farms: relationship between lice grazing and operational conditions. Aquaculture 563, 738967. https://doi.org/10.1016/j.aquaculture.2022.738967.
Ersdal, C., Midtlyng, P.J., Jarp, J., 2001. An epidemiological study of cataracts in seawater farmed Atlantic salmon *Salmo salar*. Dis. Aquat. Organ. 45, 229–236.
Gutierrez Rabadan, C., Spreadbury, C., Consuegra, S., Garcia de Leaniz, C., 2021. Development, validation and testing of an operational welfare score index for farmed lumpfish *Cyclopterus lumpus* L. Aquaculture 531, 735777.
Houde, E.D., Schekter, R.C., 1981. Growth rates, rations and cohort consumption of marine fish larvae in relation to prey concentrations. Rapp. P.-v. Réun. Cons. Int. Explor. Mer. 178, 441–453.
Imsland, A.K., Folkvord, A., Nilsen, T., 1998. Stochastic simulation of size-variation in turbot: possible causes analysed with an individual based model. J. Fish Biol. 53, 237–258.
Imsland, A.K., Jenssen, M.D., Jonassen, T.M., Stefansson, S.O., 2009. Best among unequals? Effect of different size grading and social environments on the growth performance of juvenile Atlantic halibut. Aquac. Int. 17, 217–227.
Imsland, A.K., Reynolds, P., Eliassen, G., Hangstad, T.A., Foss, A., Vikingstad, E., Elvegård, T.A., 2014a. The use of lumpfish (*Cyclopterus lumpus* L.) to control sea lice (*Lepeophtheirus salmonis* Krøyer) infestations in intensively farmed Atlantic salmon (*Salmo salar* L.). Aquaculture 425-426, 18–23.
Imsland, A.K., Reynolds, P., Eliassen, G., Hangstad, T.A., Nytrø, A.V., Foss, A., Vikingstad, E., Elvegård, T.A., 2014b. Notes on behaviour of lumpfish in sea pens with and without Atlantic salmon. J. Ethol. 32, 117–122.
Imsland, A.K., Reynolds, P., Eliassen, G., Hangstad, T.A., Nytrø, A.V., Foss, A., Vikingstad, E., Elvegård, T.A., 2014c. Assessment of growth and sea lice infection levels in Atlantic salmon stocked in small-scale cages with lumpfish. Aquaculture 433, 137–142.
Imsland, A.K., Reynolds, P., Eliassen, G., Hangstad, T.A., Nytrø, A.V., Foss, A., Vikingstad, E., Elvegård, T.A., 2015a. Feeding preferences of lumpfish (*Cyclopterus lumpus* L.) maintained in open net-pens with Atlantic salmon (*Salmo salar* L.). Aquaculture 436, 47–51.
Imsland, A.K., Reynolds, P., Eliassen, G., Hangstad, T.A., Nytrø, A.V., Foss, A., Vikingstad, E., Elvegård, T.A., 2015b. Assessment of suitable substrates for lumpfish in sea pens. Aquac. Int. 23, 639–645.
Imsland, A.K., Reynolds, P., Eliassen, G., Mortensen, A., Hangstad, T.A., Jónsdóttir, Ó.D. B., Emaus, P.A., Elvegård, T.A., Lemmens, S.C.A., Rydland, R., Nytrø, A.V., Jonassen, T.M., 2016a. Effects of lumpfish size on foraging behaviour and co-existence with sea lice infected Atlantic salmon in sea cages. Aquaculture 465, 19–27.
Imsland, A.K., Reynolds, P., Eliassen, G., Mortensen, A., Hansen, Ø.J., Puvanendran, V., Hangstad, T.A., Jónsdóttir, Ó.D.B., Emaus, P.A., Elvegård, T.A., Lemmens, S.C.A., Rydland, R., Nytrø, A.V., Jonassen, T.M., 2016b. Is cleaning behavior in lumpfish (*Cycloptherus lumpus*) parentally controlled? Aquaculture 459, 156–165.
Imsland, A.K., Hanssen, A., Reynolds, P., Nytrø, A.V., Jonassen, T.M., Hangstad, T.A., Elvegård, T.A., Urskog, T.C., Mikalsen, B., 2018a. It works! Lumpfish can significantly lower sea lice infections in large scale salmon farming. Biol. Open 7. https://doi.org/10.1242/bio.036301 bio036301.
Imsland, A.K., Reynolds, P., Jonassen, T.M., Hangstad, T.A., Elvegård, T.A., Urskog, T.C., Mikalsen, B., 2018b. Effects of three commercially available diets on growth, cataract development and health of lumpfish (*Cyclopterus lumpus* L.). Aquacult. Res. 49, 3131–3141.
Imsland, A.K., Reynolds, P., Jonassen, T.M., Hangstad, T.A., Jónsdóttir, Ó.D.B., Stefansson, S.O., Noble, T., Wilson, W., Mackie, J.A., Elvegård, T.A., Urskog, T.C., Mikalsen, B., 2018c. Feeding behaviour and growth of lumpfish (*Cyclopterus lumpus* L.) fed with feed blocks. Aquacult. Res. 49, 2006–2012.
Imsland, A.K., Reynolds, P., Jonassen, T.M., Hangstad, T.A., Noble, T., Wilson, W., Mackie, J.A., Elvegård, T.A., Urskog, T.C., Mikalsen, B., 2019a. Comparison of diet composition, feeding, growth and health of lumpfish (*Cyclopterus lumpus* L.) fed either feed blocks or pelleted commercial feed. Aquacult. Res. 50, 1952–1963.
Imsland, A.K., Reynolds, P., Jonassen, T.M., Hangstad, T.A., Elvegård, T.A., Urskog, T.C., Hanssen, A., Mikalsen, B., 2019b. Effects of different feeding frequencies on growth, cataract development and histopathology of lumpfish (*Cyclopterus lumpus* L.). Aquaculture 501, 161–168.
Imsland, A.K.D., Reynolds, P., Lorentzen, M., Eilertsen, R.A., Micallef, G., Tvenning, R., 2020. Improving survival and health of lumpfish (*Cyclopterus lumpus* L.) by the use of feed blocks and operational welfare indicators (OWIs) in commercial Atlantic salmon cages. Aquaculture 527, 735476.
Imsland, A.K.D., Reynolds, P., Hangstad, T.A., Madura, S., Hagen, S., Jónsdóttir, Ó.D.B., Spetland, F., Lindberg, K.S., 2021. Quantification of grazing efficacy, growth and health score of different lumpfish (*Cyclopterus lumpus* L.) families: possible size and gender effects. Aquaculture 530, 735925.
van den Ingh, T.S.G.A., Krogdahl, Å., Olli, J.J., Hendriks, H.G.C.J., Koninkx, J.G.J.F., 1991. Effects of soybean-containing diets on the proximal and distal intestine in Atlantic salmon (*Salmo salar*): a morphological study. Aquaculture 94, 297–305.
Jobling, M., Knudsen, R., Pedersen, P.S., Dossantos, J., 1991. Effects of dietary-composition and energy content on the nutritional energetics of cod. *Gadus morhua*. Aquaculture 92, 243–257.
Jonassen, T.M., Hamadi, M., Remø, S.C., Waagbø, R., 2017. An epidemiological study of cataracts in wild and farmed lumpfish (*Cyclopterus lumpus* L.) and the relation to nutrition. J. Fish Dis. 40, 1903–1914.
Leclercq, E., Davie, A., Migaud, H., 2014. Delousing efficiency of farmed ballan wrasse (*Labrus bergylta*) against Lepeophtheirus salmonis infecting Atlantic salmon (*Salmo salar*) post-smolts. Pest Manag. Sci. 70, 1274–1282.
Leclercq, E., Graham, P., Migaud, H., 2015. Development of a water-stable agar-based diet for the supplementary feeding of cleaner fish ballan wrasse (*Labrus bergylta*) deployed within commercial Atlantic salmon (Salmo salar) net-pens. Ani. Feed Sci. Tech. 208, 98–106.
Lie, O., Lied, E., Lambertsen, G., 1988. Feed optimization in Atlantic cod (*Gadus morhua*) – fat versus protein-content in the feed. Aquaculture 69, 333–341.
Machado, M.R.F., De Oliveira Souza, H., De Souza, V.L., De Azevedo, A., Goitein, R., Nobre, A.D., 2013. Morphological and anatomical characterization of the digestive tract of *Centropomus parallelus* and *Centropomus undecimalis*. Acta Sci. Biol. Sci. 35, 467–474.
Moldal, T., Løkka, G., Wiik-Nielsen, J., Austbø, L., Torstensen, B.E., Rosenlund, G., Dale, O.B., Kaldhusdal, M., Koppang, E.O., 2014. Substitution of dietary fish oil with plant oils is associated with shortened mid intestinal folds in Atlantic salmon (*Salmo salar*). BMC Vet. Res. 10, 60.
Powell, A., Treasurer, J.W., Pooley, C.L., Keay, A.J., Lloyd, R., Imsland, A.K., Garcia de Leaniz, C., 2018. Cleaner fish for sea-lice control in salmon farming: challenges and opportunities using lumpfish. Rev. Aquac. 10, 683–702.
Purushothaman, K., Lau, D., Saju, J.M., Musthaq, S.K.S., Lunny, D.P., Vij, S., Orban, L., 2016. Morpho-histological characterisation of the alimentary canal of an important food fish, Asian seabass (*Lates calcarifer*). PeerJ 4, e2377.
Reynolds, P., Imsland, A.K.D., Boissonnot, L., 2022. Causes of mortality and loss of lumpfish *Cyclopterus lumpus*. Fishes 7, 328.
Saravia, A., Costa, J., Serrao, J., Eiras, J.C., Cruz, C., 2015. Study of the gill health status of farmed sea bass (*Dicentrarchus labrax* L., 1758) using different tools. Aquaculture 441, 16–20.
Searle, S.R., Casella, G., McCulloch, C.E., 1992. Variance components. John Wiley & Sons, New York, p. 501.
Shiau, S.Y., Jans, F.I., 1992. Dietary ascorbic acid requirement of juvenile tilapia *Oreochromis niloticus* × *O. aureus*. Nippon. Suisan Gakkaishi 58, 671–675.
Sommerset, I., Walde, C., Bang Jensen, B., Wiik-Nielsen, J., Bornø, B., Oliveira, V., Haukaas, A., Brun, E., 2022. Fish Health Report 2021. Technical Report 2a/2022. Norwegian Veterinary Institute. URL. www.vetinst.no.
Treasurer, J.W., 2002. A review of potential pathogens of sea lice and the application of cleaner fish in biological control. Pest Managem. Sci. 58, 546–558.
Waagbø, R., Trösse, C., Koppe, W., Fontanillas, R., Breck, O., 2010. Dietary histidine supplementation prevents cataract development in adult Atlantic salmon, *Salmo salar* L., in seawater. Brit. J. Nutr. 104, 1460–1470.
Zar, J.H., 1984. Biostatistical Analysis, 2nd edition. Prentice-Hall, Inc., Englewood Cliffs, N.J, p. 718.