Comparison of diet composition, feeding, growth and health of lumpfish (*Cyclopterus lumpus* L.) fed either feed blocks or pelleted commercial feed

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Running title: Feeding lumpfish with feed blocks

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Abstract

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. The aim was to evaluate and compare the effects of these feed types on growth, cataract development and histopathology in lumpfish. There were significant differences in growth rates between the groups with fish fed pelleted feed having the highest growth rates. The development 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 10% prevalence. 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. Overall, the findings of the present study show that lumpfish will readily graze from feed blocks and although growth is lower the prevalence of cataract is greatly reduced using feed blocks.

Keywords*:* Lumpfish; growth; feeding; cataract; feed blocks

1 | INTRODUCTION

Efficient use of lumpfish in terms of the proportion of fish that graze sea lice (Imsland et al., 2014a-c), 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. 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 (10°C or lower, Nytrø et al., 2014). A recent study undertaken by Jonassen, Hamidi, Remø & Waagbø (2017) showed cataract development in lumpfish populations that was possibly related to disturbed metabolism/malnutrition, visualized as high levels of several amino acids. It was hypothesized that the cataracts in these fish groups were caused by osmotic imbalance in the fish lenses (Jonassen et al., 2017). If cataracts are associated with sub-optimal nutrition, further research of lumpfish nutrition is 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, Bjørnestad, Breck & Waagbø, 2001; Breck & Sveier, 2001; Waagbø, Trösse. Koppe, Fontanillas & Breck, 2010), then controlling the amount and/or type of feed juvenile lumpfish consume may alleviate the potential for cataract development.

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, Davie & Migaud, 2014; Leclercq, Graham & Migaud, 2015). 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. In an earlier study (Imsland et al., 2018a) investigated if lumpfish could be fed using feed blocks and what design of the feed block would facilitate optimal feeding. Results from the study of Imsland et al. (2018a) show that lumpfish will readily graze from feed blocks if they are presented in a way that allows them to (feed blocks with grooves). In addition, the acclimation period required before the fish will utilize them appears to relatively short, thus potentially allowing for their use in commercial salmon cages. 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 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., 2017) 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., 2018a). The first study focused on feed block

design and deployment to optimize lumpfish utilizing them as a food source (Imsland et al., 2018a), while effect on growth and welfare remains to be investigated.

The aim of the current study was to compare growth rates, feeding conversion rate, cataract development and gut health of juvenile lumpfish fed feed blocks or commercially available lumpfish pelleted feed. Consequently, the objective of the study is to help develop an optimal feeding strategy to maintain healthy lumpfish populations in the hatchery as well as in commercial salmon cages. Based on the positive effect of feed blocks in earlier studies in lumpfish (Imsland et al., 2018a) and wrasse (Leclercq et al., 2015) we predict that use of feed blocks will have lead to improved growth, lower prevalence of cataract and improved gut health in juvenile lumpfish.

2 | MATERIALS AND METHODS

2.1 | Experimental fish and conditions

The lumpfish were produced from fertilised eggs from Senja Akvakultursenter AS, Tromsø. The eggs 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 September 2017. The health status of the fish was assessed immediately prior to transfer to Gifas, Inndyr, Nordland, Norway in early January 2017. For further details about the experimental fish see Imsland et al. (2018a).

All tanks were supplied with full salinity sea water pumped from 70 m depth at a temperature of between 5.4 and 8.5 °C and oxygen saturation was maintained above 80%

during the whole experimental period. Water temperature and oxygen concentration was recorded in each tank for both studies using a Handy Polaris 2 probe (OxyGuard International A/S).

2.2 | Experimental design

One-week prior to the start of the trial in October 2017, two groups of lumpfish with an initial mean (±SD) weight of 15.0 ± 2.0 g were established. All fish were tagged intraperitoneally with a Trovan® Passive Integrated Transponder (Melton, United Kingdom) in order to monitor their growth. After tagging, the weight and length of each lumpfish was recorded along with their individual pit-tag ID and the fish randomly distributed into four 3.5 m^3 circular flow-through tanks with 60 fish in each tank (N_{total}= 240). 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 (The Netherlands) at a daily feeding rate of 2% BW^{-1} over a 12 hr period using 6 distinct meals.

At trial start (8 October 2017), the pelleted feed was withdrawn from two tanks, and feed blocks (Imsland et al., 2018a) introduced those tanks whereas the lumpfish in the two other tanks remained on pelleted feed. The chemical composition of the feed blocks was: 50.1 % protein; 10.3 % lipid; 12.6 % carbohydrate; 1.7 fibre % and 20.8 % moisture. The energy content of the feed block feed was 17 MJ/kg. The composition of the pelleted feed was: 55 % protein; 15 % lipid; 11 % carbohydrate; 2.5 % and 7 % moisture. The energy content of the feed pellets was 20.7 MJ/kg.

Feeding response in the feed block tanks was scored using a frequency distribution table (Table 1, Imsland et al., 2018a) on a scale from 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 for each design.

Two duplicate 300 g samples of each diet (e.g. pellets and feed blocks) were vacuum packed and stored frozen at – 20°C. The feed samples were analysed for fatty acid and amino acid profile, crude protein, lipid, starch, astaxanthin, moisture and vitamin C content.

2.3 | Feed block design and placement in tanks

The feed blocks were suspended in the water column (Figure 1A). Each individual feed block was 26 x 100 mm with a 10 mm hole through the centre (Figure 1B). The surface structure had grooves (3 – 5 mm wide) cut in them. The blocks were extruded under cool temperature (no external heat source used) and were relatively dense although they are of sufficient consistency to enable grazing. In order to increase the potential access to the blocks, their placement of the feed blocks was either at a minimum of 50 cm from the side of the tank and at either 40 or 70 cm from the bottom of the tank. The blocks were also placed randomly around the tank allowing for the greatest distance between them. Each block was weighed to calculate the number required based on the biomass. Thus for each tank 4 blocks were placed at 2 different depths at the start increasing to 5/6 blocks towards the end of the study.

2.4 | 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 Adedeji et al. (2017). For each exposure time, triplicate 20 g 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 °C for 30 min followed by further drying at 65°C until constant weight. Water stability was calculated according to the formula: WS (%) = $((m_i - m_w) / m_i)\ 100$

were:

m_i = weight of blocks before immersion.

m_w = dry weight of remaining solids

2.5 | Growth and feed conversion

All fish were individually weighted (g) and their total length (cm) measured every second week during the study period. Specific growth rate (SGR) of individual lumpfish was calculated according to the formula of Houde & 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.

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.

2.6 | Cataract scoring

During weighing of lumpfish throughout the study period all fish were examined for cataract by slit lamp biomicroscopy at 10 x magnification using a portable hand-held Heine HSL 150, C-002,14,602 (HEINE Optotechnik, Herrschingunder, Germany) under darkened conditions. All cataract examinations were performed by the same person, and each lens was scored on a scale from 0 to 4 according to the procedure of Wall and Bjerkås (1999) where 0 represented no opacity and 4 represented an opacity of more than 75% of the cross section of the lens. The score for each lens was summarized, giving a total cataract score in the range of 0–8 for individual fish. 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

2.7 | Histopathology

For histological evaluation, six fish were sampled immediately prior to the start of the study and four fish from each dietary treatment (2 from each replicate) were sampled at the end of the study period. All fish were humanely dispatched with an overdose of Benzoak (Bensocain 200 mg ml^{-1} (20%)) and PIT-tag ID, weight and length were recorded along with cataract score. The fish were 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 four 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. As 87% of the fish fed pellets had cataract sores of 4:4 (bilateral) so these were sampled from the pellet group, whereas 90% of fish fed feed blocks had no cataracts so 4 of these were sampled. This was done to compare between potential histological differences to the obvious the visual ones. It was also done to assess whether it was possible to develop a histological sampling scoring system for eye health in lumpfish (work in progress).

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). 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 2.

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.

2.8 | 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 response, growth performance, cataract scores and histological data between the experimental groups were tested with two-way nested analysis of variance (ANOVA) where replicates were nested within feeding types (i.e. pellets or feed blocks). The model equation of the nested ANOVA had the form:

(1) $$X_{ijk} = \mu + \alpha_i + C_{ij} + \varepsilon_{ijk}$$

where μ is the general level; α_i is the feeding type (pelleted feed or feed blocks) effect; C_{ij} is the contribution caused by replicate (tank) j in feeding type i and ε_{ijk} is the error term. We assume that $\varepsilon_{ijk} \sim$ 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 ($1-\beta$) analysis was performed in Statistica using $\alpha = 0.05$.

3 | 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).

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).

3.2 | Growth and feed conversion ratio

Overall mortality for fish fed feed blocks or pelleted feed was 1.7% and 0.8%, respectively. Mean weight and specific growth rates (SGR) was significantly higher in the pellet group from day 14 onwards (SNK post hoc test, $p < 0.05$, Figure 2A-B). Average final weights were 52.5% higher in the pellets group (Figure 2A). The group fed pelleted feed had a significantly lower biological FCR (1.24) compared to fish fed feed blocks (1.79, SNK post hoc test, $p < 0.01$) at the end of the study period.

3.3 | Feeding response in feed block group

Feeding response was similar for both replicate tanks receiving feed blocks throughout the study period. The average response (±SE) rose from 3.68 ± 0.32 (regular grazing by 33-48% of the lumpfish) to 6.75 ± 0.17 (67-82% of the lumpfish population grazing on feed blocks) in week 3 and remained between 5.52 and 6.75 throughout the study period.

3.4 | Water stability of feed blocks

Water stability of feed blocks decreased through time (Figure 3). 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.

3.5 |Cataracts

At the start of the study period both experimental groups had a cataract prevalence of 4% (Figure 4). However, from day 14 onwards the cataract prevalence was significantly higher in fish fed pellets compared to fish fed feed blocks (two-way nested ANOVA, $p < 0.001$). At the end of the study the cataract prevalence of fish fed pellets was 87% compared to 10.0% for fish on feed blocks (Figure 4). Cataract severity increased throughout the experiment for fish fed pelleted feed, while there was no clear increase for the fish onfeed blocks (Figure 5). There were significant differences in the frequency of unaffected fish (score 0) from day 28 onwards (Figure 5A). In addition, there were significant differences in the prevalence of mild cataract (score 1-2) from day 60 onwards (Figure 5B), where fish on feed blocks had a higher prevalence. (SNK post hoc test, $p < 0.05$). No fish on feed blocks had a medium score (3-4) until day 101 (Figure 5C). In the pellet group there was a significant increase in fish with severe cataract (score 5-8) from day 28 onwards, while no fish on feed blocks showed severe cataracts (Figure 5D, SNK post hoc test, $p < 0.05$).

3.3 | Histopathology

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 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 any 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; however, the degree of vacuolisation was scored as moderate in liver tissue which was similar for both groups at the end of the study and comparable to the degree of vacuolisation in baseline samples. 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, Figure 6). Mean intestinal fold height at the start of the study was 440.6 µm (± 59.5 µm) with a significant increase in mean fold height for both dietary treatments compared to baseline samples at the end of the trial. 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.

4 | DISCUSSION

4.1 | Growth, diets and feeding response

The growth rates for fish fed with pelleted feed observed during this study were similar to growth rates from previous studies (Imsland et al., 2014a, c; 2015a-b, 2016). However, growth rates were significantly lower for fish fed feed blocks although both feed types were offered at a daily feeding rate of 2% BW^{-1} based on biomass gain. This is in contract to the prediction put forward in the introduction. This difference in growth performance may be attributed to the lumpfish not eating all the offered feed blocks as reported in Imsland et al. (2018a). 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^{-1}). From analysed composition, the pelleted feed was found to be 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 eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) than 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.

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 to fish fed with pellets possible relating to feeding hierarchies (Imsland, Folkvord, & Nilsen, 1998; Imsland, Jenssen, Jonassen, & Stefansson, 2009) that could lead to higher variation in growth in the feed block group. This is apparent when looking at the coefficient of variation of the mean weights ($C_V = \sigma/\mu$) in Fig. 2 where the CV is about 200% higher in the feed pellet group from November onwards. To prevent formation of such feeding hierarchies it is recommended to establish multiple feeding stations for lumpfish in tanks or sea cages.

Normal practice is to feed lumpfish a pelleted feed (Imsland et al., 2015a; Powell et al., 2017), usually fed by automatic feeders placed close to the side of the cage. This reduces the feed dispersal to the centre of the cage and thus encourage lumpfish to colonize the sides of the cages away from the salmon. However, by using feed blocks that can be deployed in the centre of the cage, 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 | Cataract development

The incidences of cataract increased as the study progressed for both groups. However, there were significant differences observed between the treatments. Cataract prevalence for fish fed with feed blocks only increased from 3% to 10% over the whole study period whilst prevalence for fish fed with pelleted feed increased from 4% to 87% over the same period. This difference is in line with the initial predictions set out for the study and may be

attributed to dietary effects as both groups shared the same husbandry and environmental conditions throughout the experimental period. In addition, fish fed pelleted feed all developed severe cataract (score 5-8) towards the end of the study, whilst fish on feed blocks only reached a cataract severity of 3-4. 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 as seen in Jonassen et al. (2017) is only comparable with the highest incidences previously found in farmed Atlantic salmon caused by a histidine-deficient diet (Breck & Sveier, 2001; Waagbø et al., 2010). However, dietary histidine was approx. 80% higher for fish on pelleted feed compared to fish on feed blocks (approx. 50% higher on a dry-matter basis). For lumpfish, the requirements for histidine and how histidine requirement changes at higher growth rates has not been determined. However, a study by Jonassen et al. (2017), revealed that 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 fed a high energy and high protein pelleted feed 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.

It is known that high or rapid growth can increase the risk of cataracts in salmon (Ersdal et al. 2001). Previous studies on lumpfish (Jonassen et al., 2017; Imsland et al., 2018b) have also found that high SGR increased risk of developing cataracts. The results from this study show that fish fed with pelleted feed had significantly higher growth 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.

4.3 | Histopathology

The results of the histological examination undertaken in this study showed overall small differences between the two dietary treatments. In some individuals, irrespective of diet, there was mild to moderate expansion of the lamina propria with tissue most likely to represent fibrous tissue with scattered leucocytes. Changes were most consistent with chronic inflammation. The changes were unspecific, and the cause uncertain. The level of inflammation observed may indicate dietary effects, although the mild inflammation observed did not indicate any negative effects compromising growth and health of the fish. However, if the diets were causing an inflammatory response, it would be expected that after 123 days (the duration of the study) inflammation would 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, Krogdahl, Olli, Hendriks & Koninkx, 1991). The first histological signs of inflammation were apparent after 2-5 days of SBM feeding and the severity escalated with extended

exposure time (van den Ingh et al., 1991; Baeverfjord & Krogdahl, 1996).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, Izquierdo, Kjørsvik, Fernández & Rosenlund, 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 & Jan, 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.

5 | CONCLUSION

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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Data Availability Statement

Data sharing is not applicable to this article due to commercial restrictions.

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REFERENCES

Adedeji, A.A., Zhou. Y.G., Fang. X.Y., Davis. D.A., Fahrenholz. A. & Alavil. S. (2017). Utilization of sorghum distillers dried grains in extruded and steam pelleted shrimp diets. *Aquaculture Research*, 48, 883-898.

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. *Journal of Fish Diseases*, 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. *Journal of Fish Diseases*, 24, 281–291.

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. *Bulletin of the European Association of Fish Pathologists*, 21, 91–103.

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. *Journal of Fish Diseases*, 27, 531–541.

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). *Journal of Animal Science*, 10, 3226-3234.

Ersdal, C., Midtlyng, P.J., & Jarp, J. (2001). An epidemiological study of cataracts in seawater farmed Atlantic salmon *Salmo salar*. *Diseases of Aquatic Organisms,* 45, 229-236.

Houde, E.D. & Schekter, R.C. (1981). Growth rates, rations and cohort consumption of marine fish larvae in relation to prey concentrations. *Conseil International pour l'exploration de la Mer. Rapports et Procès Verbaux*, 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. *Journal of Fish Biology*, 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. *Aquaculture International*, 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 sala*r L.). *Aquaculture*, 425-426, 18-23.

Imsland, A.K., Reynolds, P., Eliassen, G., Hangstad, T.A., Nytrø, A.V., Foss, A., … Elvegård, T.A. (2014b). Notes on behaviour of lumpfish in sea pens with and without Atlantic salmon. *Journal of Ethology*, 32, 117-122.

Imsland, A.K., Reynolds, P., Eliassen, G., Hangstad, T.A., Nytrø, A.V., Foss, A., … 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., … 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., … Elvegård, T.A. (2015b). Assessment of suitable substrates for lumpfish in sea pens. *Aquaculture International*, 23, 639-645.

Imsland, A.K., Reynolds, P., Eliassen, G., Mortensen, A., Hangstad, T.A., Jónsdóttir, Ó.D.B., … Jonassen, T.M. (2016). 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., Jonassen, T.M., Hangstad, T.A., Jónsdóttir, Ó.D.B., Stefansson, S.O., …… Mikalsen, B. (2018a). Feeding behaviour and growth of lumpfish (*Cyclopterus lumpus* L.) fed with feed blocks. *Aquaculture Research*, 49, 2006-2012.

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.). Aquaculture Research 49, 3131-3141.

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. *Journal of Fish Diseases*, 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 Management Science,* 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 (*Salmon salar*) net-pens. *Animal Feed Science Technology*, 208, 98-106.

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 Scientiarum. Biological Sciences*, 35, 467–474

Moldal, T., Løkka, G., Wiik-Nielsen, J., Austbø, L., Torstensen, B. E., Rosenlund, G., … 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 Veterinary Research,* 10, 60.

Nytrø, A.V., Vikingstad, E., Foss, A., Hangstad, T.A., Reynolds, P., Eliassen, G., …… Imsland, A.K. (2014). The effect of temperature and fish size on growth of juvenile lumpfish (*Cyclopterus lumpus* L.). *Aquaculture,* 434, 296-302.

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. *Reviews in Aquaculture,* 10, 683-702.

Purushothaman, K., Lau, D., Saju, J. M., Mustaq, S. K., Lunny, D. P., …… Orbán, L. (2016). Morpho-histological characterisation of the alimentary canal of an important food fish, Asian seabass (*Lates calcarifer*). PeerJ 4:e2377; DOI 10.7717/peerj.2377.

Shiau, S.Y., & Jan, F.I. (1992). Dietary ascorbic acid requirement of juvenile tilapia *Oreochromis niloticus* × *O. aureus*. *Bulletin of the Japanese Society of Scientific Fisheries*, 58, 671–675.

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.

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. *British Journal of Nutrition,* 104, 1460–1470.

Wall, T., & Bjerkås, E. (1999). A simplified method of scoring cataracts in fish. *Bulletin of the European Association of Fish Pathologists*, 19, 162–165.

Zar, J.H. (1984). Biostatistical Analysis, 2nd edition, Prentice-Hall, Inc., Englewood Cliffs, N.J.

TABLE 1 Frequency distribution of recorded feeding response used during the study period. There were 60 fish in each experimental tank.

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 10 % of the fish
3	Regular grazing by 10 - 19 % of the fish
4	Regular grazing by 20 - 29 % of the fish
5	Regular grazing by 30 - 39 % of the fish
6	Regular grazing by 40 - 49 % of the fish
7	Regular grazing by over 50 % of the fish

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

TABLE 3 Analysed diet composition of feed blocks and pellets used in the study

Composition	Pellets	Blocks
Fat (%)	15.8	10.3
Protein (%)	56.5	50.1
Moisture content (%)	6.6	23.2
Starch and simple sugars (%)	6.3	8.2
Astaxanthin (mg kg^{-1})	-	133.0
Astaxanthin esters (mg kg^{-1})	11.9	-
Vitamin C (L-ascorbyl-2-phosphate) (mg kg^{-1})	1020.0	613.0
Calculated gross energy (GE, MJ kg^{-1})*	20.7	17.3
Calc. DP	50.3	44.6
Calculated dietary energy (DE, MJ kg^{-1})§	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.).

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.*
Σ 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
Σ NEAA	28.1	19.9

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

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 (SNK post hoc test, $p < 0.05$)

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 ± 0.1	2.20 ± 0.1	2.40 ± 0.1	0.64	n.s.
Midgut	**Goblet cells: PAS**	2.0 ± 0.1	2.0 ± 0.1	2.0 ± 0.1		n.s.
	Goblet cells: Alcian blue	2.0 ± 0.1	2.0 ± 0.1	2.1 ± 0.3	1.00	n.s.

Figure legends

FIGURE 1 Feed blocks deployed in the experimental tanks (A) and feed blocks prior to deployment (B).

FIGURE 2 (A) Mean weight (g); and (B) Specific growth rates (SGR) of lumpfish fed either feed blocks or extruded pelleted feed. Values represent means ± SD. Different letters indicate significant differences (SNK test, $p < 0.05$); n.s., not significant.

FIGURE 3 Percentage weight loss of feed blocks after immersion at different time intervals.

FIGURE 4 Occurrence of lumpfish with cataracts (% prevalence) calculated at each of the sampling days. Values represent means ± SD from duplicate treatment groups. Different letters indicate significant differences (SNK test, $p < 0.05$); n.s., not significant.

FIGURE 5 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.

FIGURE 6 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).