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

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Abstract

Three duplicate groups of individually tagged lumpfish (mean initial weight: 22.3 ± 2.5 g) were fed either daily (7DW); four days per week (4DW) or three days per week (3DW) at a feeding rate of 2% body weight^{-1} for a period of 126 days. There were significant differences in growth rates between the groups with the 7DW fish having the highest growth rates. Cataract prevalence was 53% lower in the 3DW group compared to the 7DW group. Histological examination showed that in some individuals in all three groups there was moderate expansion of the lamina propria in the mid and hind gut regions of the intestine with tissue most likely to represent fibrous tissue with scattered leucocytes. The severity of inflammation appeared to increase the more frequent the fish were fed with the 7DW fish having the highest inflammation score in these tissues. Feeding fish daily also resulted in higher levels of liver vacuolisation and chronic inflammation of the lamina propria in the mid and hind gut region of the intestines. Results from the present study show that restricted feeding regimes can be used to control growth and improve gut and eye health in lumpfish.

1. Introduction

The biological control of sea lice in Atlantic salmon farming using cleaner fish has recently become a feasible 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). Farmed cleaner fish are preferred due to better biosecurity through vaccination and screening programs, stocking at optimum times and sizes, and in reducing reliance on wild caught fish. Cleaner fish are now used as a biological control for sea lice on farmed salmon in Europe and Canada (Imsland et al. 2014ac-c; Powell et al. 2018). In Norway more than 70% of the fish farms in south and mid Norway used cleaner fish in 2016 and 25% of the farms in the north of Norway (Mortensen et al., 2017). The farming of cleaner fish is a growing and important aquaculture sector in Northern Europe, with 43 hatcheries engaged in production of cleaner fish in Norway and nine in the UK, and hatcheries have also opened in Iceland, Ireland, the Faroes, Canada and Chile. As a cold-water cleaner fish, the common lumpfish (*Cyclopterus lumpus*) has been suggested and initial results are very promising with up to 93-97% less sea lice infection (adult female lice) in sea pens with lumpfish (Imsland et al., 2014 a-c; 2015a-b; 2016a-b). The use of cleaner fish is an environmentally beneficial and efficient alternative for removal of sea lice and it reduces the stress for salmon due to less handling that is associated with medicinal bath or mechanical treatment. However, the cleaner fish need to be healthy to optimize lice removal and the welfare of the cleaner fish is of major importance.

Cataracts are opacities in the eye lens or the lens capsule that mediate an abnormal dispersion of light through the lens and hence cause reduced visual ability and, ultimately, blindness. The opacities result from a disruption of the normal arrangement of the lens fibers or from alterations in the conformation or water-binding capacity of the proteins of the lens (Benedek, 1997). Cataracts may be induced by a variety of factors of a nutritional, environmental, chemical or infectious nature (Bjerkås et al., 2006). For example, in salmonids several causes have been proposed for the development of cataracts, many of them nutritional (Waagbø et al., 2003, 2010). Other factors associated with cataract formation are rapid fluctuations in water temperature (Bjerkås and Bjørnestad 1999), rapid growth (Bjerkås et al., 1996), rapid change in water salinity (Bjerkås et al., 1998), UV radiation (Björnsson, 2004), and electrolytic imbalance (Rhodes et al., 2010). A recent study undertaken by Jonassen et al. (2017) showed cataract prevalence in both farmed and wild lumpfish varied between 20 and 100%. The study suggested that cataracts in lumpfish populations were possibly related to disturbed metabolism/ malnutrition, visualized as very high values of selected amino acids in different tissues from sampled fish. This can cause osmotic imbalance in fish tissues and cataract development, or is a consequence of osmotic imbalance (Jonassen et al., 2017). This illustrates a welfare issue and if cataracts are associated with sub-optimal nutrition, then further research in nutrition with lumpfish is therefore necessary. One way forward is to test different feeding frequencies to control growth and thereby indirectly cataract formation. 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 and is fundamental for learning more about their feeding physiology and habits especially for feed formulation prior to stocking in commercial salmon cages.

The aim of the current study was to compare growth rates, cataract development and gut health of juvenile lumpfish fed at different feeding frequencies i.e. fed 3 days per week (3DW), 4 days per week (4DW) and 7 days per week (7DW) in order to develop an optimal feeding strategy to maintain healthy lumpfish populations in the hatchery as well as in commercial salmon cages

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2. Materials and methods

2.1. Experimental fish and rearing conditions

The lumpfish were produced from fertilised eggs from Senja Akvakultursenter AS, Senja, Troms County, Norway. 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 25 September 2017. Health status was assessed by polymerase chain reaction (PCR) screening for Vibrio species, atypical furunculosis, pasteurella, moritella, pancreas disease (PD), infectious pancreatic necrosis (IPN), viral hemorrhagic septicaemia (VHS), Nodovirus and amoebic gill disease (AGD). From 25 September to 5 October 2017 the juveniles were fed a high protein low fat marine feed (Biomar lumpfish grower, 2 mm) using Van Gerven 7 L^{-1} feeding automats (The Netherlands). A 50% mixture of 1.5 mm and 2 mm pellets was used during this period.

One-week prior to the start of the trial on 5 October three duplicate groups of lumpfish with an initial mean (±SD) weight of 22.3 ± 2.5 g (n = 110; N = 330) were established from the original population and all fish 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 transferred to six 1.5 m^3 tanks (55 fish in each tank).

All tanks were supplied with full salinity sea water pumped from 70 m depth at a temperature of between 6.7 and 12.2°C during the trial period and oxygen saturation was maintained above 80% during the whole experimental period. Water temperature and oxygen concentration was recorded daily in each tank using a Handy Polaris 2 probe (OxyGuard International A/S). The tanks were illuminated by fluorescent daylight tubes (Aura Light 124 International AB, Karlskrona, Sweden) with a computer program and electronic regulators to simulate the natural photoperiod for Bodø. Norway (67°N, 14°E), including twilight periods. Light intensity was monitored regularly with a Traceable® Dual-display Lux meter (Texas, USA). The study period was from 5 October 2017 to 8 February 2018 (126 days).

The present 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.

2.2. Experimental set-up

At trial start, each duplicate group were fed a different feeding regime. The first group were fed daily (group 7DW), The second group fed four days per week (group 4DW, Monday, Wednesday, Thursday, Saturday) and the third group fed three days per week (group 3DW, Monday, Wednesday, Saturday). All three groups were fed a feeding rate of 2% body weight (BW) day^{-1} during the study period. The fish were weighed every two weeks to regulate the feeding rate. Feed was delivered over a 12-h period (6 am to 6 pm) using Van Gerven 7 L^{-1} feeding automats (The Netherlands) controlled via Cotech digital outdoor timers (IP44; Class Ohlson. Norway). The fish were fed with a standard

commercial lumpfish diet (Biomar grower 2.0 mm, Biomar, Norway). The composition of the pelleted feed was: 56.5% protein; 15.8% lipid; 11% carbohydrate and 6.6% moisture. The energy content of the feed pellets was 20.7 MJ kg^{-1}.

2.3. Growth and feed consumption

All lumpfish from each tank were individually weighed and fork-length recorded along with their individual PIT-tag ID at two week intervals during the trial period. Specific growth rate (SGR) of individual lumpfish was calculated according to the formula of Houde and Schekter (1981):

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

where $g = (\ln (W_2)-\ln (W_1) / (t_2-t_1)$ and W_2 and W_1 are weights on days t_2 and t_1, respectively.

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. All tanks were checked for mortalities every day. Any mortalities present were removed and their weight, length and pit-tag number registered.

2.4. 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.

2.5. Histopathology

For histological evaluation, 10 fish were sampled immediately prior to the start of the study and 10 fish from each dietary treatment (5 from each replicate) were samples 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.

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 1. Epithelium was evaluated for degeneration/necrosis and vacuolisation. Goblet cells stained

positive with PAS and Alcian blue in the mid-gut were also assessed semi-quantitatively according to criteria in Table 1.

Intestinal fold length was measured in the mid-gut using Aperio eSlide Manager by measuring the height of all intact folds in the cross section of the loop with the most optimal orientation. Measurements were taken from the tip of the fold immediately under the epithelium until the start of the muscularis.

2.7. Statistics

All statistical analyses were conducted using Statistica™ 13.3 software. Possible differences in mortality between dietary groups was tested with a χ^2 tests (Zar, 1984). 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 frequencies on growth performance, cataract scores and histological data. 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 frequency 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 $\varepsilon_{ijk} \sim$ 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 feed conversion ratio

Mortality rates for feeding regimes 3DW and 7DW was 0.9% whilst no mortalities occurred in treatment 4DW. No difference was seen in mortality between the three feeding groups (χ^2 test, $P > 0.65$). Mean weight and specific growth rates (SGR) varied between the dietary regimes from day 15 onwards (SNK post hoc test, $P < 0.05$, Fig. 1). Fish fed daily (7DW), had the highest mean weight and SGR throughout the project period compared to the 4DW and 3DW treatment groups. At the end of the study these fish achieved a mean (± SD) weight of 294.0 ± 64.0 g while the 4DW and 3DW dietary treatments had mean (± SD) weights of 256.7 ± 73.9 g and 206.2 ± 72.0 g respectively. SGR decreased in all three feeding groups during the trial period.

The dietary group fed three days per week had a significantly lower biological FCR (0.56) compared to fish fed four days per week (0.72) and fish fed daily (1.19) (SNK post hoc test, $P < 0.01$) at the end of the study period.

3.2. Cataracts

At the start of the study period all three groups had a cataract prevalence of 4.2% (Fig. 2), and the prevalence increased for all groups throughout the experiment. From day 43 onwards there was significantly higher increasing prevalence in fish fed daily compared to fish fed three days per week (two-way nested ANOVA, $P < 0.001$). The 7DW and 4DW treatment groups had similar increasing prevalence of cataracts during the study period.

As the prevalence of cataract increased over time, so did the severity. There was little differences in the predominantly mild (score 1-2) cataracts in the beginning (Fig. 3B), but the differences increased significantly as cataract severity developed over time. The medium score (3-4) was higher for 7DW and 4DW compared to the 3DW group at days 28 and 61 (SNK post hoc test, $P < 0.05$), and sever cataracts (score 5-8) were dominating from day 28 onwards for all three feeding groups. Sever cataract was significantly highest for 7DW compared to the 3DW group from day 61 onwards and at days 61 and 89 compared to the 4DW group (Fig. 3D, SNK post hoc test, $P < 0.05$). The majority of cataracts were bilateral, while incidences of unilateral cataract was low and found only until day 43 (varying from 4% at start to 1% at day 43). The fish in the 7DW group had frequently significantly more bilateral cataracts compared to fish fed three days per week (Fig. 4, SNK post hoc test, $P < 0.05$).

3.3. Histopathology

For baseline samples, there was mild evidence of inflammation in tissue sampled from the pyloric caeca, midgut and hindgut and mild epithelial vacuolisation in the pyloric caeca (Table 2). Midgut tissue stained with either PAS or Alcian blue was scored as 2.0 (2-7 positive cells per 20 epithelial cells) and liver vacuolisation was assessed as moderate (score 2).

At the end of the study period there was a significant difference in inflammation of submucosa/lamina propria tissue from both the midgut and hindgut between the three dietary treatments (Fig. 5) with fish in the 7DW group having a higher score compared to the other two diet groups (SNK post hoc test, $P < 0.05$, Table 3). There were significant

reductions in pyloric caeca epithelial inflammation from the start of the study for all three groups with no evidence of inflammation observed at the end of the study period. Similarly, epithelial vacuolisation in the pyloric caeca was absent at the end of the study period. There was little or no change in the number of goblet cells from the start to the end of the study for all three experimental groups (Table 2).

4. Discussion

There were significant differences in growth rates between the groups with fish fed 7DW having the highest growth rates whilst fish fed 3DW achieved the lowest growth and was 35% smaller at termination of the trial. It should be noted that high growth is not an aim for lumpfish used as cleaner fish. Imsland et al. (2016a) found that small lumpfish (initial size approx. 20 g) have a higher overall preference for natural food items, including sea lice, compared to larger conspecifics (initial size 77 and 113 g). 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., 2014c) and adaptations in the feeding behaviour of the fish may be suppressed (Davenport et al., 1983; Davenport, 1985). Consequently, they may stop foraging for food sources which required an output of energy and go over to consume readily available salmon pellets which require much less energy expenditure. The environment of commercial salmon cages where food is relatively abundant both spatially and temporally may accelerate growth and thus maturation earlier than desired and therefore reduce sea lice grazing potential. Therefore, feeding regimes and diet types must be carefully considered prior to their deployment in salmon cages. The reduced growth observed in the most restricted group (3DW) suggests that more research is required to fully understand the lower limit to which lumpfish growth can be controlled without compromising health and ultimately lice grazing potential. The objective in any feed strategy for this species is to provide sufficient nutrition to maintain health and promote lice grazing potential. Moreover, overfeeding may result in lumpfish

becoming acclimated to the food source and thus reducing their need to seek out additional food sources such as sea lice.

Fish fed three days per week had 53% lower prevalence of cataracts compared to fish fed daily and 48% less prevalence compared to fish fed four days per week. A previous study has shown that the prevalence of cataracts can vary between 20% and 100% in lumpfish populations (Jonassen et al., 2017). Such high prevalence of severe cataract is only comparable with the highest incidences previously found in farmed Atlantic salmon caused by a histidine-deficient diet. It is known that high or rapid growth can increase the risk of cataracts in salmon (Ersdal et al., 2001) and in a previous study on lumpfish (Imsland et al., 2018) found that fish with high SGR also had the highest incidence of cataracts. Further, growth rates of small lumpfish is 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. Cataract formation may be affected not only by growth but also by the way the nutrients are utilized. Osmotic stress due to oversaturation of certain amino acids is hypothesized as an explaining for cataract formation. However, the way the nutrients are metabolized may also have significance. Hence optimizing feeding regimes for a sub-optimal diet may contribute to reduced cataract development. A reduction in cataract may enhance sea lice grazing efficiency, at least when sever cataract is present. Fish with severe cataracts have reduced ability to locate food items and as a result this affects feed intake, growth and weakened immunity and robustness of the fish (reduced stress tolerance) (Breck and Sveier, 2001).

The observed increase in cataract score with increasing prevalence and fish weight suggests a progressive development of cataract in farmed lumpfish as previously seen in

the study by Jonassen et al. (2017). However, Jonassen et al. (2017) reported that in the wild sexually matured lumpfish cataract prevalence and severity was significantly lower than in farmed matured broodstocks in the same size range, suggesting that cataract development in the farmed fish was related to a prolonged period of suboptimal rearing conditions. An array of environmental factors (Björnsson, 2004; Treasurer et al., 2007) and nutrient deficiencies (Breck et al., 2003; Bjerkås et al., 2006) has been associated with cataract formation. The progressive cataract development observed for lumpfish has also been observed for Atlantic salmon and was related to fast growth under suboptimal nutrition (Bjerkås et al., 1996; Waagbø et al., 1996, 1998; Ersdal et al., 2001). For Arctic char (*Salvelinus alpinus*), cataract prevalence and severity was higher in matured broodstock compared to immature fish (Peuhkuri et al., 2009) and was associated with prolonged exposure for eye parasites and eye damages in general. In Atlantic halibut (*Hippoglossus hippoglossus*) neither cataract prevalence nor severity were related to individual fish weight suggesting that growth per se was not an important influence (Treasurer et al., 2007). Physical damage largely affected the protruding non-migrated eye as a consequence of aggression in the hatchery and consequently physical damage of halibut in sea cages was well healed. Damage from UV light and nutritional deficiencies were regarded as possible factors in the formation of these anterior cataracts in ongrown halibut.

There was a higher incidence of lumpfish with bilateral cataracts compared to fish with unilateral cataracts in all groups throughout the study. Bilateral cataracts have been shown to have generally systemic causes, related to nutrition and other environmental factors, in Atlantic salmon (Breck et al., 2003), whilst unilateral cataracts are generally associated

with external mechanical stresses on fish, such as different types of handling that can create friction or damage to the eyes (Jonassen et al., 2017).

In some individuals in all three groups, there was mild to moderate expansion of the lamina propria in the mid and hind gut with tissue most likely to represent fibrous tissue with scattered leucocytes. Changes are most consistent with chronic inflammation. The severity of inflammation noted appeared to increase the more frequent the fish were fed with fish fed daily having the highest inflammation score in these tissues. There is no previous research into gut health based on diet performance for these fish and thus the changes are unspecific, and the cause is uncertain at this time.

There were no significant differences in liver vacuolisation between the dietary groups and baseline samples, but the more frequent the fish were fed, there was an increase in vacuolisation with fish fed daily having the highest level of vacuolisation (score 2.8). These results may indicate that fish fed daily were storing excess fat in the liver. It is known that excess fat is stored in the liver (Caballero et al., 2004) and this can be manifested as increased vacuolisation.

There was no increase in the number of goblet cells present in the hindgut between the three dietary treatment 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).

5. Conclusions

There were clear differences in growth between the three dietary groups with fish fed diet daily attaining the best growth and final mean weight was 35% lower in the 3DW treatment group. The most restricted feeding regime (3DW) resulted in a significantly lower prevalence of cataracts. Prevalence was 53% lower in this group to fish fed daily. Feeding fish daily also resulted in higher levels of liver vacuolisation and chronic inflammation of the lamina propria in the mid and hind gut region of the intestines. The results indicate that cataract development and gut health may be compromised at high levels of feeding whilst restricting feeding may also help to lower cataract prevalence and maintain good gut health. More research is required to fully elucidate the potential of reducing feeding frequency in combination with reduced ration size.

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References

Benedek, G.B., 1997. Cataract as a protein condensation disease. (The Proctor Lecture). Inv. Ophthal. Vis. Sci. 38, 1911–1921.

Bjerkås, E., Bjerkås, I., Moxness, E., 1998. An outbreak of cataract with lens rupture and nuclear extrusion in wolf-fish (Anarhicas spp.). Vet. Ophthalmol. 1, 9-15

Bjerkås, E., Bjørnestad, E., 1999. Is there a connection between rapid fluctuation in water temperature and cataract development in the Atlantic salmon (*Salmo salar* L.). Bull. Europ. Ass. Fish Pathol. 19, 166–169.

Bjerkås, E., Waagbø, R., Sveier, H., Breck, O., Bjerkås, I., Bjørnestad, E., Maage, A., 1996. Cataract development in Atlantic salmon (*Salmo salar* L) in fresh water. Acta Vet. Scan. 37, 351–360.

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: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources, 1, 1–16.

Björnsson, B., 2004. Can UV-treated seawater cause cataract in juvenile cod (*Gadus morhua* L.)? Aquaculture, 240, 187–199.

Boxaspen, K., 2006. A review of the biology and genetics of sea lice. ICES J. Sea Res. 63, 1304-1316.

1 Breck, O., Sveier, H., 2001. Growth and cataract development in two groups of Atlantic
2 salmon (*Salmo salar* L) post smolts transferred to sea with a four-week interval. Bull.
3 Europ. Ass. Fish Pathol. 21, 91–103.
4 Breck, O., Bjerkås, E., Campbell, P., Arnesen, P., Haldorsen, P., Waagbø, R., 2003.
5 Cataract preventative role of mammalian blood meal, histidine, iron and zinc in diets for
6 Atlantic salmon (*Salmo salar* L.) of different strains. Aquac. Nutr. 9, 341–350.
7 Boxaspen, K., 2006. A review of the biology and genetics of sea lice. ICES J. Sea Res. 63,
8 1304-1316.
9 Caballero, M.J., Izquierdo, M.S., Kjørsvik, E., Fernández, A.J., Rosenlund, G., 2004.
10 Histological alterations in the liver of seabream, *Sparus aurata* L., caused by short- or
11 long-term feeding with vegetable oils. Recovery of normal morphology after feeding
12 fish oil as the sole lipid source. J. Fish Dis. 27, 531–541.
13 Davenport, J., 1985. Synopsis of biological data of the lumpsucker *Cyclopterus lumpus* (L
14 1758). FAO Fisheries synopsis No. 147.31 pp.
15 Davenport, J., Lønning, S., Kjørsvik, E., 1983. Ammonia output by eggs and larvae of the
16 lumpsucker, *Cyclopterus lumpus*, the cod, *Gadus morhua* and the plaice, *Pleuronectes*
17 *platessa*. J. Mar. Biol. Assoc. U. K. 63, 713–723.
18 Denholm, I., Devine, G.J., Horsberg, T.E., Sevatdal, S., Fallang, A., Nolan, D.V., Powell,
19 R., 2002. Analysis and management of resistance to chemotherapeutants in salmon lice
20 *Lepeophtheirus salmonis* (Krøyer) (Copepoda: Caligidae), Pest Manag. Sci. 58, 528–
21 536.
22 Ersdal, C., Midtlyng, P.J., Jarp, J., 2001. An epidemiological study of cataracts in seawater
23 farmed Atlantic salmon *Salmo salar*. Dis. Aquat. Org. 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. Rapp. P.-v. Réun. Cons. Int. Explor. Mer. 178, 441-453.

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., Vikingstad, E., 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., 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. Aquaculture International 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 (*Cyclopterus lumpus*) parentally controlled? Aquaculture 459, 156-165.

Imsland, A.K., Reynolds, P., Jonassen, T.M., Hangstad, T.A., Elvegård, T.A., Urskog, T.C., Mikalsen, B., 2018. Effects of three commercially available diets on growth, cataract development and health of lumpfish (*Cyclopterus lumpus* L.). Aquac. Res. 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. J. Fish Dis. 40, 1903-1914.

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.* 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.

Mortensen, S., Skiftesvik, A.B., Bjelland, R., Karlsbakk, E., Durif, C., Sandlund, N., 2017. Bruk av rensefisk i lakseoppdrett ("Use of cleanerfish in Norwegain salmon farming").

In: Svåsand, T., Grefsrud, E.S., Karlsen, Ø., Kvamme, B.O., Glover, K., Husa, V., Kristiansen, T.S. (Eds.), Risikorapport norsk fiskeoppdrett ("Risk report for Norwegian aquaculture"). Institue of Marine science, pp. 161-173 (in Norwegian).

Peuhkuri, N., Bjerkås, E., Brännäs, E., Piironen, J., Primmer, C., Taskinen, J., 2009. Looking fish in the eye—Cataract as a problem in fish farming. TemaNord 2009 Nordic Council of Ministers, Copenhagen, 515, 51 pp.

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.

Rhodes, J.D., Breck, O., Waagbø, R., Bjerkås, E., Sanderson, J., 2010. N-acetylhistidine, a novel osmolyte in the lens of Atlantic salmon (*Salmo salar* L.). Am. J. Physiol. - Reg. Integ. Comp. Physiol. 299, R1075-R1081.

Searle, S.R., Casella, G., McCulloch, C.E., 1992. Variance components. John Wiley & Sons, New York, 501 pp.

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.

Treasurer, J.W., Cox, D.I., Wall, T., 2007. Epidemiology of blindness and cataracts in cage reared ongrown Atlantic halibut *Hippoglossus hippoglossus*. Aquaculture 271, 77-84.

Waagbø, R., Bjerkås, E., Sveier, H., Breck, O., Bjørnestad, E., Maage, A., 1996. Nutritional status assessed in groups of smolting Atlantic salmon, *Salmo salar* L., developing cataracts. J. Fish Dis. 19, 365–373.

Waagbø, R., Sveier, H., Breck, O., Bjørnestad, E., Maage, A., Bjerkås, E., 1998. Cataract formation in smolting Atlantic salmon, *Salmo salar*, fed low and high energy diets. Bull.

1 Europ. Assc. Fish Pathol. 18, 201-205.
2 Waagbø R., Hamre K., Bjerkås E., Berge R., Wathne E., Lie O., Tortensen B., 2003.
3 Cataract formation in Atlantic salmon, *Salmo salar* L., smolt relative to dietary pro- and
4 antioxidants and lipid level. J. Fish Dis. 26, 213–229.
5 Waagbø, R., Trösse, C., Koppe, W., Fontanillas, R., Breck, O., 2010. Dietary histidine
6 supplementation prevents cataract development in adult Atlantic salmon, *Salmo salar*
7 L., in seawater. Brit. J. Nutr. 104, 1460–1470.
8 Wall, T. Bjerkås, E., 1999. A simplified method of scoring cataracts in fish. Bull. Europ.
9 Ass. Fish Pathol. 19, 162–165.
10 Zar, J.H., 1984. Biostatistical Analysis, 2nd edition, Prentice-Hall, Inc., Englewood Cliffs,
11 N.J., 718 pp.
12

Figure legends

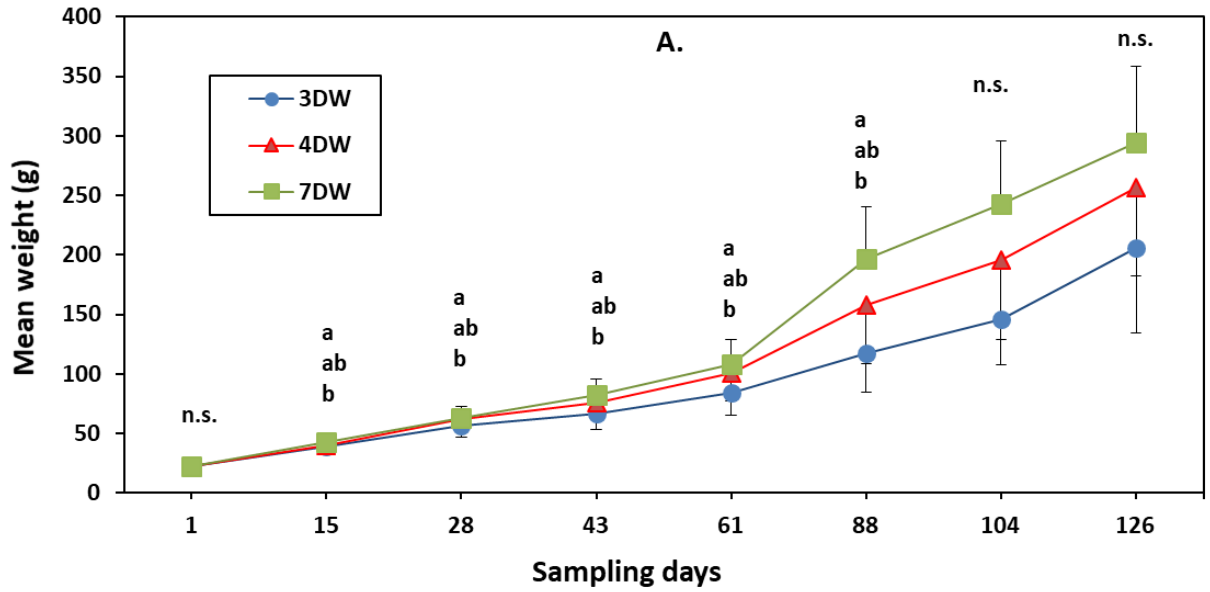
Fig.1 (A) Mean weight (g) and (B) Specific growth rates (% day $^{-1}$) of lumpfish fed three days per week (3DW); four days per week (4DW) and seven days per week (7DW). Values represent means ± SD. Different letters indicate significant differences (SNK test, $P < 0.05$); n.s., not significant.

Fig. 2. Occurrence of lumpfish with cataracts (% prevalence) in lumpfish fed three days per week (3DW); four days per week (4DW) and seven days per week (7DW). Values represent means ± SD. Different letters indicate significant differences (SNK test, $P < 0.05$); n.s., not significant.

Fig. 3. 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.

Fig. 4. Percentage distribution of lumpfish with unilateral or bilateral cataracts fed three days per week (3DW); four days per week (4DW) and seven days per week (7DW). Values represent means ± SD. Different letters indicate significant differences (SNK test, $P < 0.05$); n.s., not significant.

Fig. 5. Micrographs of lamina propria from (A) midgut of fish fed 3 days per week; (B) from hindgut from fish fed 4 days per week and (C) from midgut from fish fed every day.



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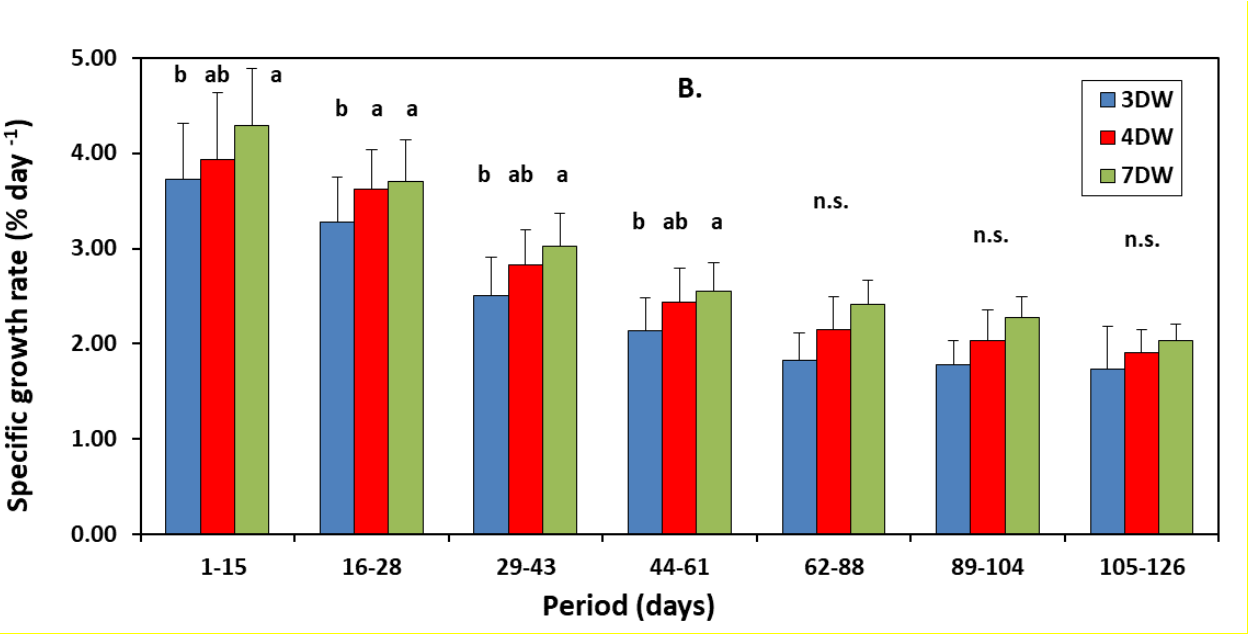

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3 Fig. 1. Imsland, Reynolds et al.

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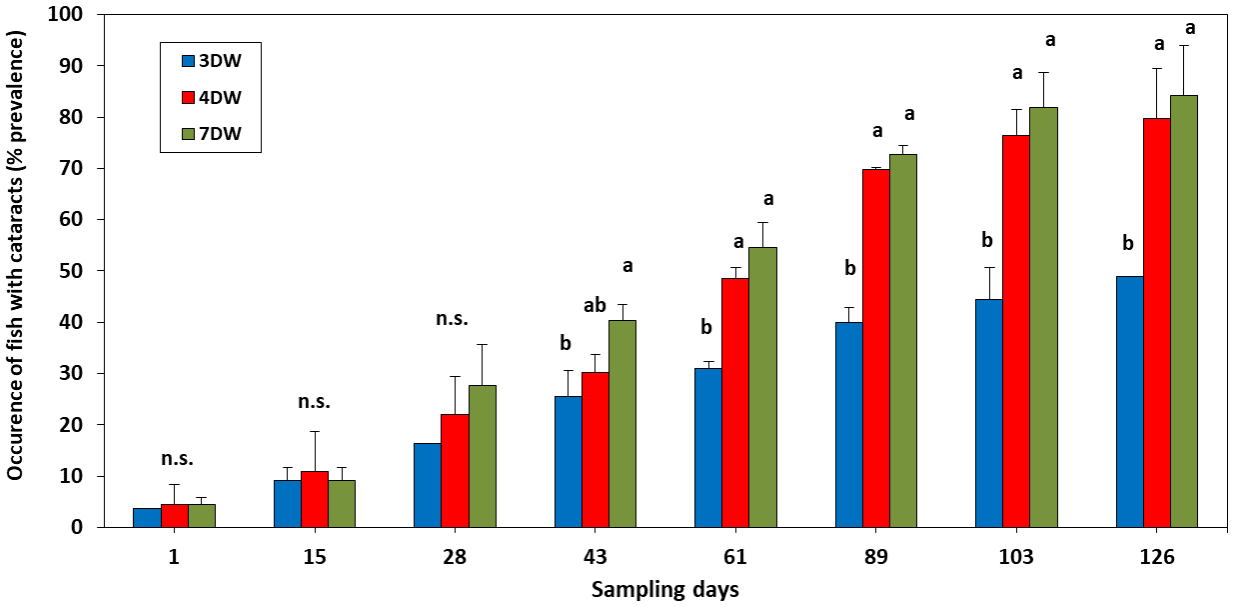

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4 Fig. 2. Imsland, Reynolds et al.

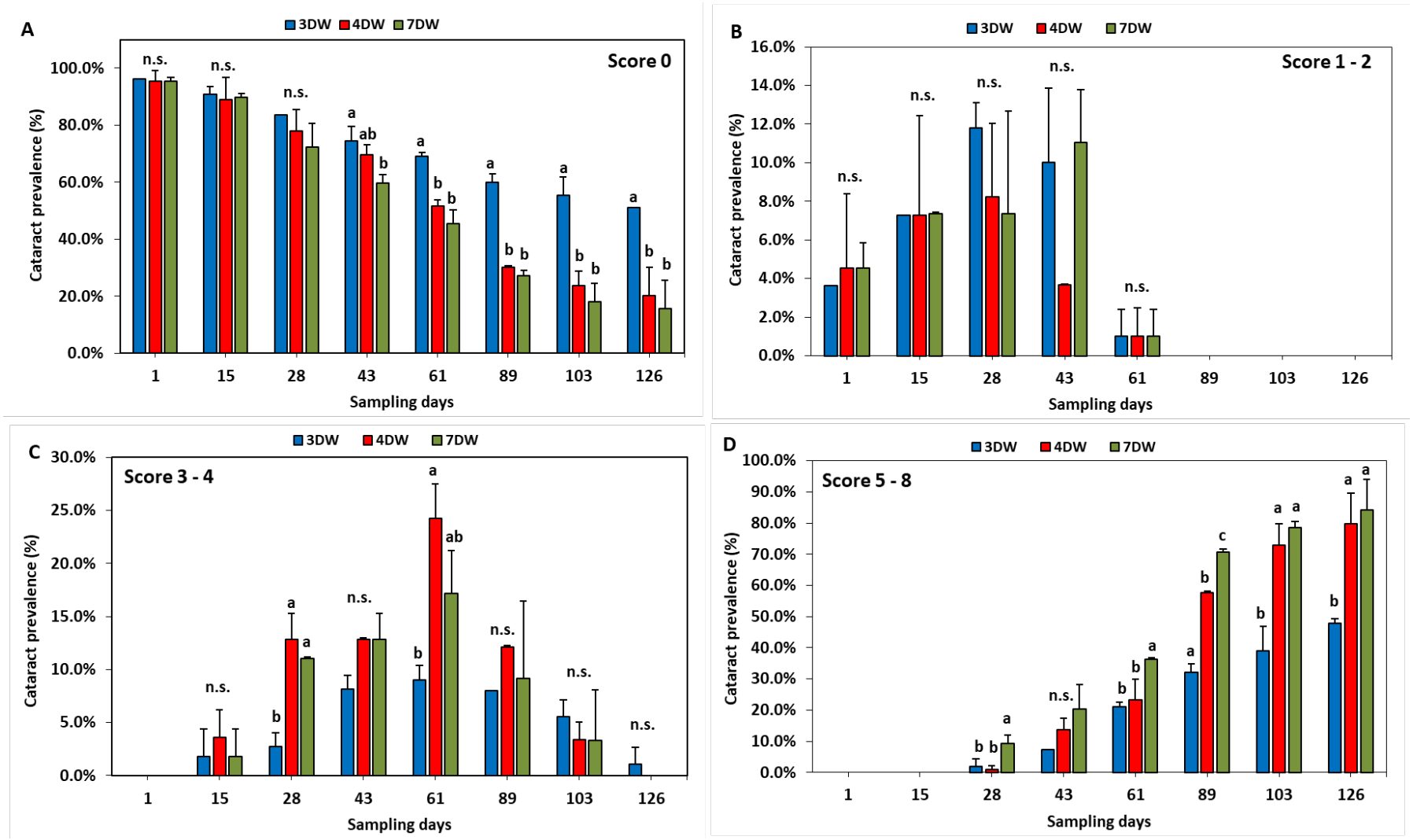

Fig. 3. Imsland, Reynolds et al.

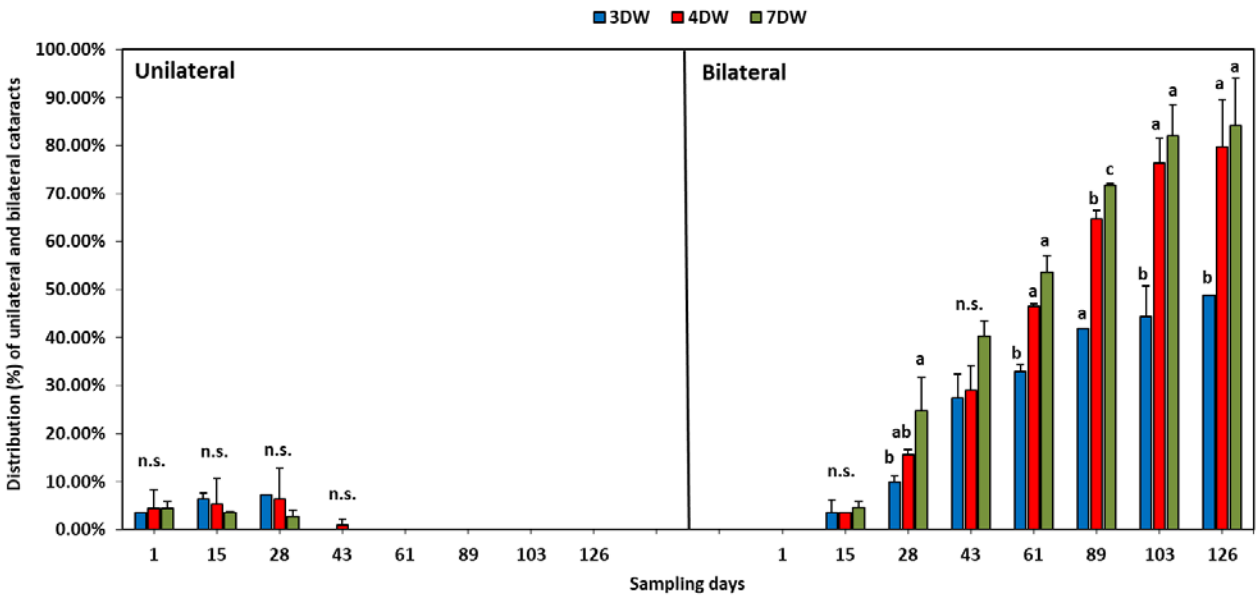

Fig. 4. Imsland, Reynolds et al.

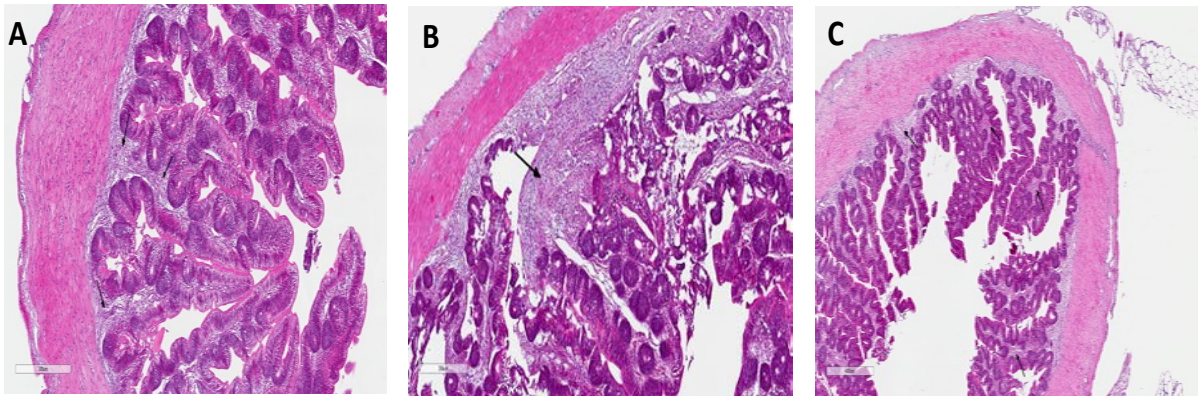

Fig. 5. Imsland, Reynolds et al.

1 Table 1. 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

1

Table 2. 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 three dietary treatments at the end of the study period. Mean values not sharing a letter were found to be significantly different by ANOVA and by Student-Newman-Keuls multiple range post hoc test.

Tissue	Analysis	Mean values				ANOVA	
		Baseline	3DW	4DW	5DW	*F*	*P*
Pyloric caeca	*Inflammation*						
	muscularis	0.0	0.0	0.0	0.0	-	-
	submucosa/lamina propria	0.1 ± 0.3	0.2 ± 0.6	0.00	0.3 ± 0.7	0.70	n.s.
	Epithelium	0.5 ± 0.5***a***	0.0***b***	0.0***b***	0.0***b***	9.00	0.001
Midgut	*Inflammation*						
	muscularis	0.0	0.0	0.0	0.0	-	-
	submucosa/lamina propria	0.2 ± 0.4***b***	0.8 ± 0.8***a***	0.2 ± 0.4***b***	1.1 ± 0.9***a***	4.64	0.008
	epithelium	0.2 ± 0.4	0.0	0.0	0.1 ± 0.3	1.32	n.s.
Hindgut	*Inflammation*						
	muscularis	0.0	0.0	0.1 ± 0.3	0.0	1.00	n.s.
	submucosa/lamina propria	0.3 ± 0.5***b***	0.4 ± 0.7***b***	0.6± 0.7***b***	1.4 ± 0.5***a***	6.74	0.001
	epithelium	0.3 ± 0 5***a***	0.0***b***	0.0***b***	0.0***b***	3.86	0.017
Pyloric caeca	Epithelium degeneration /necrosis	0.0	0.0	0.1 ± 0.3	0.0	1.00	n.s.
	Epithelium vacuolisation	0.6 ± 0 5***a***	0.0***b***	0.0***b***	0.0***b***	13.5	0.001
Midgut	Epithelium degeneration/necrosis	0.0	0.0	0.2 ± 0.4	0.0	2.25	n.s.
	Vacuolisation	0.0	0.0	0.0	0.0	-	-
Hindgut	Epithelium degeneration/necrosis	0.0	0.0	0.0	0.0	-	-
Liver	Vacuolisation	2.2 ± 0.4***b***	2.5 ± 0.5***ab***	2.4 ± 0.5***ab***	2.8 ± 0.4***a***	2.70	0.05
Midgut	Goblet cells: PAS	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.1 ± 0.3	1.00	n.s.
	Goblet cells: Alcian blue	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.2 ± 0.4	2.25	n.s.