Does Dietary Supplementation With an Organic Acid Improve Gut Health and Growth Performance in Atlantic Salmon, *Salmo salar*, in the Seawater Production Phase

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Abstract

In this study Atlantic salmon were fed 7 experimental feeds of which 6 (the OA series) were formulated to include relatively less refined plant proteins (field beans, corn gluten and guar protein) supplemented with different inclusions of an organic acid whilst the 7th feed (LAR, low anti-nutrient risk) was made using only refined vegetable proteins i.e wheat gluten and soy protein concentrate. The feeding trial was carried out at The Mowi Field Trials Station on Averøy in Norway using 21 marine net pens (5x5x5m; 125 m^3) between August and November 2020 with each feed being fed to triplicated pens. The salmon grew from approximately 220g to ≥ 900g with slightly increased growth arising from increased inclusion of the additive in the organic acid-containing feeds. Histological survey of key gut tissues indicated no significant correlation between organic acid dose and morphological changes though, the lowest level of morphological changes was observed amongst salmon fed the LAR feed and the feed with the highest level of organic acid supplementation. The results suggest that the addition of organic acid at inclusions between 490-773 mg/kg could benefit performance and reduce gut pathology if less refined (potentially, more antinutrient rich) raw materials are to be used in salmon feed.

1. Introduction

Aquaculture is the fastest growing food production sector of recent years but, infectious diseases and the development of new raw materials are important factors determining its advance and further development. Under some circumstances, e.g. when unrefined or very crude feed materials are used, substitution of fish meal (FM) and fish oil (FO) with plant based alternatives may cause structural and functional disorders with, the intestine and liver representing examples of target organs. Short chain volatile fatty acids are end products of the bacterial fermentation of carbohydrate in the gut and stimulation of organic acid production in the gut is desirable in terrestrial animals being associated with improved gut health. It is also possible to add organic acids to the feed with a number of organic acid products being made available for application in feeds for terrestrial and aquatic animals.

Objective

The primary objective of the present study was to establish potential salmon performance and gut health improvements associated with organic acid supplementation using a dose response design under semi-commercial conditions. In the knowledge that the adverse effects of anti-nutrient rich plant ingredients e.g. HiPro soya can be ameliorated by organic acid supplementation, a second objective was to establish whether organic acid supplementation of formulations containing guar meal and dehulled faba beans could raise its efficacy to that of a feed containing a mix of more refined plant proteins.

2. Materials and Methods

2.1 Experimental feed formulation

The feeds (5mm diameter) were formulated by Mowi Feed and produced at the Nofima Feed Technology Centre in Bergen (Norway). Seven experimental feeds were formulated to secure equal outcomes for digestible protein (DP) and digestible energy (DE) and to be matched with regards the profile of digestible amino acids, essential fatty acids and key micronutrients. All seven feeds were formulated with a fishmeal (NE Atlantic) inclusion of 10% using NE Atlantic fish oil as the main source of marine fats. Feeds $OA_{0.0}$ to $OA_{0.24}$ represented a subset of 6 feeds with identical formulation except the variable inclusion of a proprietary organic acid product. This subset of feeds included guar meal, dehulled field beans and corn gluten as representatives of feed materials with higher anti-nutrient risk. These 6 feeds were subsequently identified by a name indicating the presence of organic acid "OA" and the dose of the organic acid product added to the feed (% of feed). Feed LAR (low anti-nutrient risk) was formulated without guar meal, corn gluten and dehulled beans which were largely replaced by raised levels of soy protein concentrate, wheat gluten and whole wheat with additives including amino acids, vitamins and minerals facilitating parity with the organic acid-containing feeds in terms of digestible nutrient values and balance (Table 1).

Analysis of the feeds was carried out by NOFIMA Biolab in Bergen, Norway with the actual content of the organic acid in feed being confirmed by the supplier of the proprietary product (Table 2)

Table 1. Formulation of the seven experimental feeds fed to Atlantic salmon (*Salmo salar*).

Ingredients (%)	LAR	$OA_{0.0}$	$OA_{0.015}$	$OA_{0.03}$	$OA_{0.06}$	$OA_{0.12}$	$OA_{0.24}$
Fishmeal	10,00	10,00	10,00	10,00	10,00	10,00	10,00
Fish oil	8,47	8,46	8,46	8,46	8,46	8,46	8,46
Soy protein concentrate	29,38	22,03	22,03	22,03	22,03	22,03	22,03
Corn gluten meal	0,00	5,00	5,00	5,00	5,00	5,00	5,00
Guar meal	0,00	5,00	5,00	5,00	5,00	5,00	5,00
Wheat	19,64	17,01	17,01	17,01	17,01	17,01	17,01
Dehulled beans	0,00	7,00	7,00	7,00	7,00	7,00	7,00
Wheat gluten meal	10,80	4,09	4,08	4,06	4,03	3,97	3,85
Vegetative oils	15,73	15,72	15,72	15,72	15,72	15,72	15,72
Minerals	2,30	2,29	2,29	2,29	2,29	2,29	2,29
Vitamins & carotenoids	0,70	0,70	0,70	0,70	0,70	0,70	0,70
Yeast derivatives	0,43	0,42	0,42	0,42	0,42	0,42	0,42
Synthetic amino acids	2,26	2,34	2,34	2,34	2,34	2,34	2,34
Organic acid product	0,00	0,00	0,02	0,03	0,06	0,12	0,24

2.2 Fish and husbandry

The feeding trial was conducted at Mowi's Averøy Field Trials Station in Norway between August and November 2020. Atlantic salmon (mean weight 220 g) were randomly distributed amongst 21 marine net pens (5x5x5m; 125 m^3) at an abundance of 100 fish per pen. The fish were given a period of 7 days acclimation to environmental conditions whilst fed a commercial feed (Mowi Jupiter 200) before the study started. Upon commencement of the 80-day feeding period, the fish were fed one of 7 feeds with 3 pens allocated to each feed. The salmon were fed in excess using a combination of automatic feeders and a waste feed collection system. A daily over-feed of 15% was targeted and the excess feed amount was confirmed gravimetrically on dry weight corrected, recovered waste feed before feed intake and feed conversion factor were calculated. Fish health and welfare were monitored daily following Mowi's standard procedures. Water temperature spanned a range between 15 °C (August) and 11 °C (November) and a natural photoperiod was observed.

2.3 Fish sampling

At the end of the 80-day feeding period, all fish were individually weighed and counted with further post-mortem assessments being carried out on 10 salmon / pen (30 fish / feed type). Each fish was subjected to the following: visual health assessment and visual assessment of the internal organs. Tissue sampling was performed from 5 salmon / pen (15 fish / feed type) and were including liver, pyloric caeca, mid intestine and distal intestine for health evaluation through histology. Histological sections were prepared following standard histological methods with sectioning and staining being conducted at the Veterinary Institute histology laboratory in Oslo. Interpretation of the pathology and different morphological characteristics in the histological sections was carried out by Aquamedic. Aquamedic's assessment was carried out on a double-blind basis and reported using Code "A" for the LAR feed and "B to G" for fish fed the organic acid containing feeds in the order $OA_{0.0}$ to $OA_{0.24}$.

2.4 Calculations

Key performance indicators (KPIs) were calculated as followed:

1. Weight gain (Wg) = initial weight– final weight
2. The thermal growth co-efficient (TGC) = initial body weight – final body weight /(number of days x average temperature in °C) x 1 000
3. Biological feed conversion ratio (bFCR) = feed weight eaten/(finale weight – initial weight + weight mortality)
4. Economic feed conversion ratio (eFCR) = feed weight eaten/(final weight – initial weight)
5. Mortality = fish number input at start – fish number output at end

2.5 Statistical analysis

All performance data were statistically evaluated with a one-way ANOVA following a Tukey's multiple comparison test to find differences between each feed. A regression analysis was also performed to identify any correlation between the measured dietary organic acid content and fish performance for those fish fed Feeds $OA_{0.0}$ to $OA_{0.24}$. Differences in histological scores for the various evaluated morphological characteristics of the intestine were analysed for statistical significance using ordinal logistic regression run in the R statistical package (version 3.6.2; 2019) within the RStudio interphase (version 1.2.5033; 2019). All data are presented as means and standard error (SE), unless otherwise stated. Differences were regarded as significant when $p \leq 0.05$.

3 Results

3.1 Feed analysis

Table 2. Proximate composition, astaxanthin and organic acid content of the seven experimental feeds.

Composition	LAR	$OA_{0.0}$	$OA_{0.015}$	$OA_{0.03}$	$OA_{0.06}$	$OA_{0.12}$	$OA_{0.24}$
Moisture (%)	6,3	5,6	5,5	5,2	5,8	5	5,6
Crude protein (%)	45,5	47,6	47,8	46,9	46,7	46,7	46,8
Crude fat (%)	24,8	25,7	24,7	26,7	25,1	26,2	25,6
Astaxanthin (mg/kg)	49	50	57	54	54	60	58
Ash (%)	4,9	5					
Starch (%)	10,2	8,6		8			8,5
Crude fibre (%)	2,4	2,6		2,4			2,2
Organic acid (mg/kg)	Nf	Nf	34.9	93.1	111.5	404.7	772.9

* Nf = Not found

3.2 Performance

Growth performance

Overall, the growth rates, survival and FCRs achieved were very good. The highest weight gain was observed for fish fed the $OA_{0.24}$ feed although, according to the one-way ANOVA, there were no significant differences in growth across all 7 feeds. Linear regression analysis of the relationship between dietary organic acid content and growth (represented by final weight, weight gain and TGC) amongst the salmon fed the OA feed subset indicated a significant correlation between these parameters (Table 3 and Figures 1 & 2). A slight increase in FCR was seen between feeds, $OA_{0.0}$ and $OA_{0.06}$, though none of the FCR differences were significant according to ANOVA or linear regression. Low mortality was registered in all fish groups throughout the trial and no significant differences seen.

Table 3. Growth parameters for each dietary group

Growth parameters	LAR	$OA_{0.0}$	$OA_{0.015}$	$OA_{0.03}$	$OA_{0.06}$	$OA_{0.12}$	$OA_{0.24}$	Anova SEM	*Anova (p-value)*	*Linear regression (p-value)*
Initial weight (g)	222	223	224	224	226	224	223	2,18	0.547	
Final weight (g)	921	908	900	912	910	916	935	30,58	0.455	0.0226
Weight gain (g)	698	686	676	688	684	692	711	26,66	0.391	0.0208
TGC	3,61	3,57	3,53	3,57	3,54	3,58	3,66	0,08	0,285	0.0186
bFCR	0,83	0,83	0,86	0,85	0,85	0,84	0,84	0,92	0.860	0.7007
eFCR	0,84	0,84	0,86	0,85	0,85	0,84	0,85	0,92	0.830	0.6639
Mortality (%)	0,39	0,39	0,54	0,59	0,23	0,10	0,51	0,71	0.431	0.8569

3.3 Linear regression

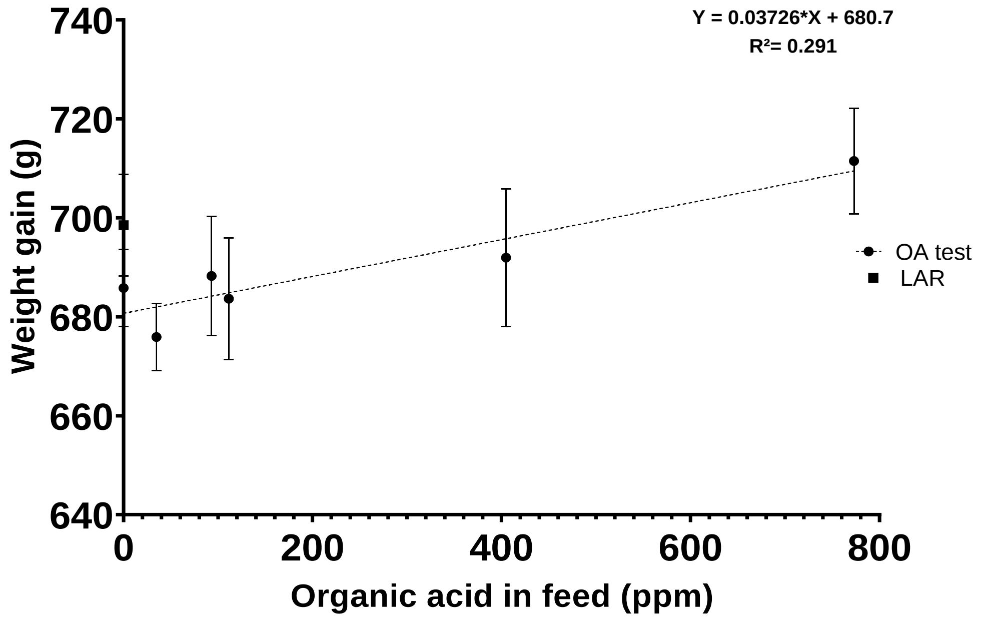

Figure 1. Linear regression evaluating the impact of increasing dietary organic acid content on weight gain of salmon for Feeds $OA_{0.0}$ - $OA_{0.24}$ with growth of salmon fed the LAR (low anti-nutrient feed) superimposed on the plot

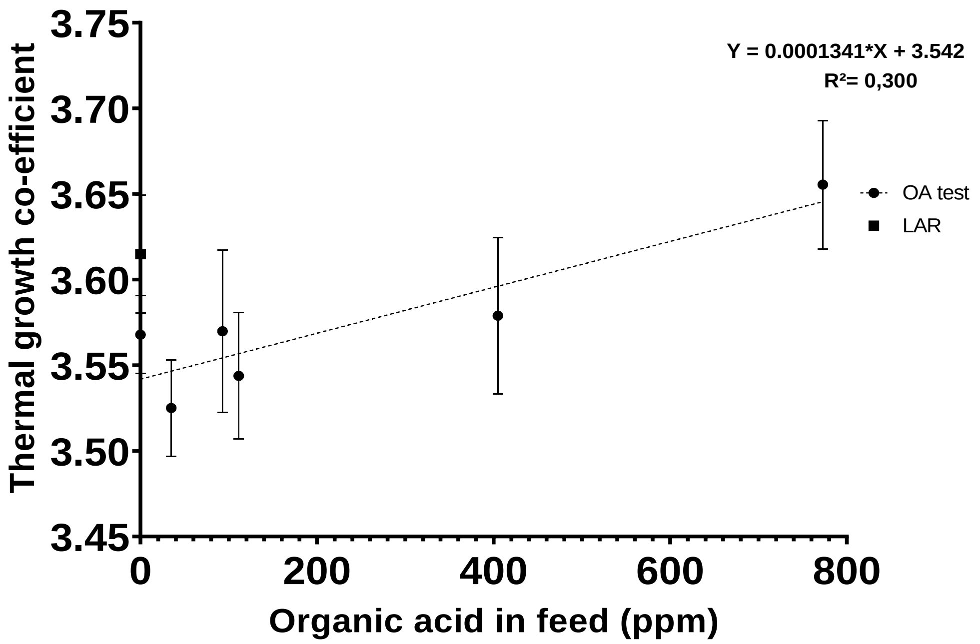

Figure 2. Linear regression evaluating the impact of increasing dietary organic acid content on specific growth rate of salmon for Feeds $OA_{0.0}$ - $OA_{0.24}$ with TGC of salmon fed the LAR (low anti-nutrient feed) superimposed on the plot.

3.4 Histology

3.4.1 Liver

Mild to marked hepatocyte steatosis was observed across all feed groups (Figure 3). Fish given Feed "B" ($OA_{0.0}$) showed the least number of fish affected (highest proportion of "normal" fish) and this significantly differed from the changes observed in the "A" group (LAR) ($p = 0.05$)

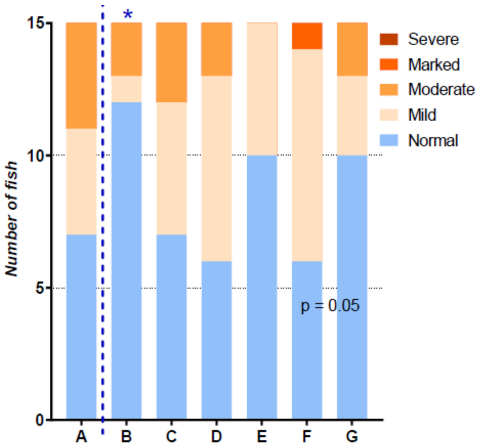

Figure 3. Number of liver tissue sections that were scored "normal", "mild", "moderate", "marked or "severe" for lipid vacuolization in hepatocytes of liver tissue sections evaluated during the current gut health assessment. *P-values* represent outcomes of an ordinal logistic regression for differences in the distribution of the histological scores in each diet group in comparison with A (LAR).

3.4.2 Pyloric caeca

Mild to marked enterocyte steatosis was observed in the pyloric caeca of 3 or 4 fish from the 15 assessed for each feed group (Figure 4a). Predominantly mild inflammation of the pyloric caeca mucosa was also observed in at least one fish from all the feed groups (Figure 4b). Fish from group A (LAR) were observed with numerically the most fish affected (5 individuals) and the group G ($OA_{0.24}$) had one fish affected. Statistically, no differences were detectable between fish fed the different feeds.

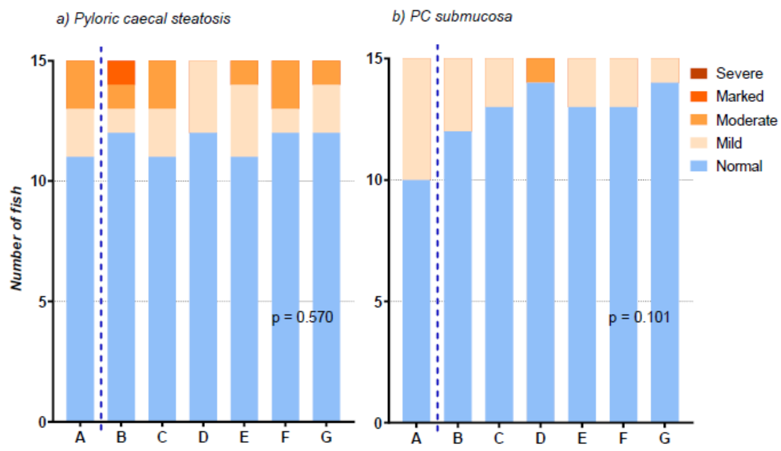

Figure 4. Number of pyloric caeca tissue sections that were scored “normal”, “mild”, “moderate”, “marked” or “severe” for enterocyte steatosis or hyper-vacuolization; and b) increases in the width and inflammatory cell infiltration in the submucosa. *P-values* represent outcomes of an ordinal logistic regression for differences in the distribution of the histological scores in each diet group in comparison with A (LAR).

3.4.3 Mid intestine

Mild to moderate steatosis was present in most of the feed groups, with one occurrence of marked changes observed in both groups A (LAR) and D ($OA_{0.03}$) (Figure 5a). Fish from group F ($OA_{0.12}$) had numerically the most fish showing steatosis changes (13 of the 15 fish assessed) while fish from group G ($OA_{0.24}$) showed changes in 4 individuals. None of the groups showed statistically significant differences in the occurrence or severity of the MI steatosis. As illustrated in Figure 5b, few individuals from groups B ($OA_{0.0}$), C ($OA_{0.015}$), E ($OA_{0.06}$) and F ($OA_{0.12}$) were observed with mild to moderate inflammation of the MI mucosa.

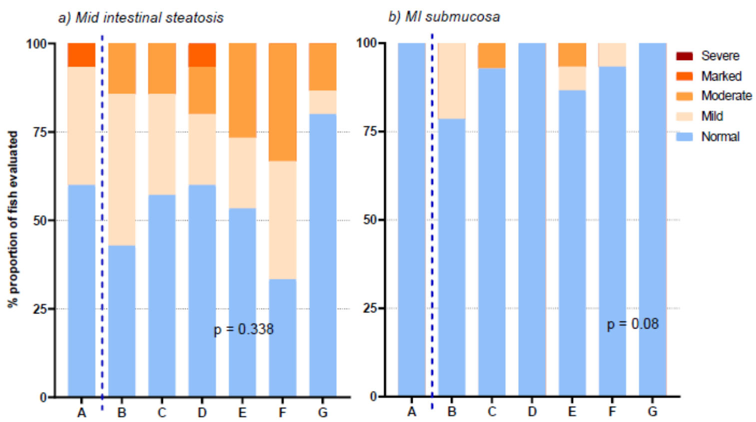

Figure 5. Number of mid intestine tissue sections that were scored “normal”, “mild”, “moderate”, “marked” or “severe” for a) enterocyte steatosis or hyper-vacuolization; and b) increases in the width and inflammatory cell infiltration in the submucosa. *P-values* represent outcomes of an ordinal logistic regression for differences in the distribution of the histological scores in each diet group in comparison with A (LAR).

3.4.4 Distal intestine

High proportions of inflammatory changes ranging mild to marked were observed in the submucosa of about 50% of all the fish assessed for each group (Figure 6a). A similar pattern was observed in the lamina propria with changes ranging between mild to moderate (Figure 6b). Changes to the fold height or vacuolization were observed in only two fish of all the 105 assessed (see Figures 6c and 6d). None of the groups showed statistically significant differences.

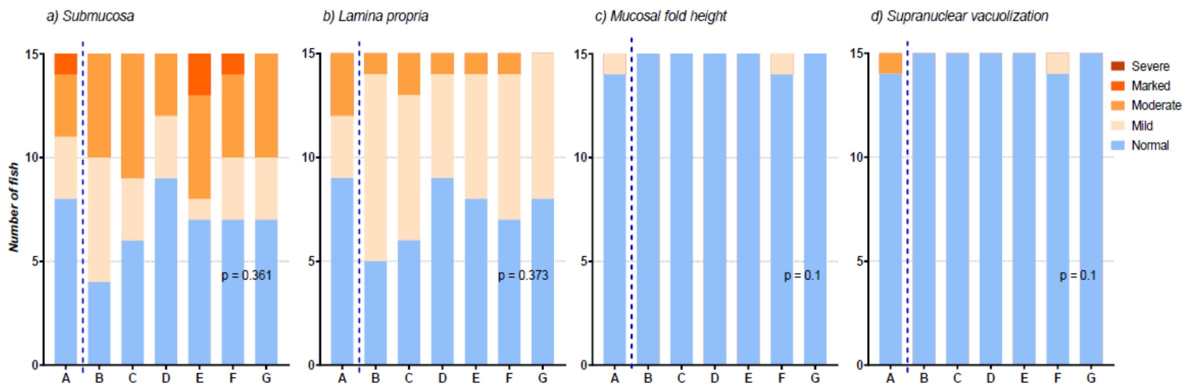

Figure 6. Number of DI tissue sections that were scored “normal”, “mild”, “moderate”, “marked or “severe” for the morphological characteristics of (a) mucosal fold height, increase in width and inflammatory cell infiltration of the submucosa (b) and lamina propria (c), and reduction in enterocyte supranuclear vacuolization (d). *P-values* represent outcomes of an ordinal logistic regression for differences in the distribution of the histological scores in each diet group in comparison with A (LAR).

3.4.5 Histology summary

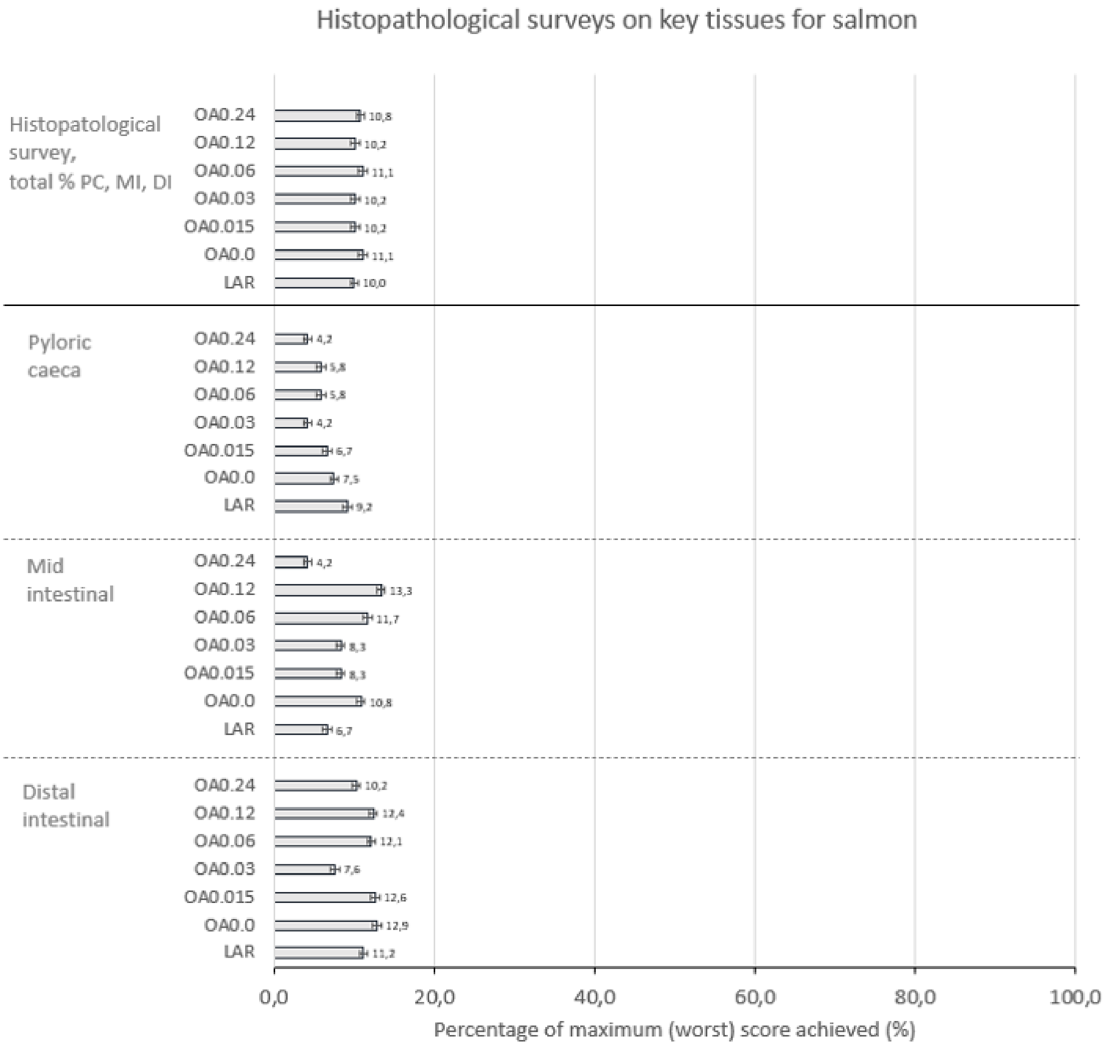

Figure 7. Data presented as total % for all gut tissues combined and for each of the organs; Pyloric caeca (PC), Mid intestine (MI) and Distal intestine (DI). Lowest % indicate best performing regarding histology with less pathology and morphological changes seen.

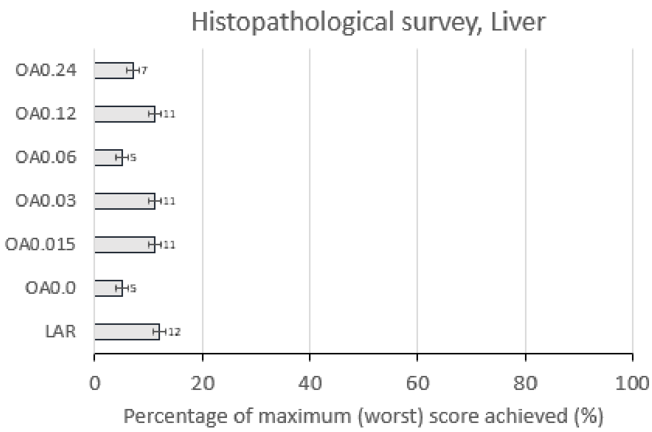

Figure 8. Data presented as total % for liver. Lowest % indicate best performing regarding histology with less hepatocyte steatosis seen.

4. Discussion

Overall, growth and FCR were very good and no differences in performance were observed across the 7 different feeds. However, in numerical terms, the highest growth rates were observed for fish fed the feed with the most refined vegetable proteins (LAR) and the less refined feed supplemented with the highest dose of organic acid ($OA_{0.24}$). Whilst regression analysis indicated a significant relationship between organic acid content in the feed and thermal growth coefficient, the regression line was strongly "anchored" at the lower end by the 3x feeds containing approximately 34, 93 and 111 mg organic / kg and not strongly influenced by the relatively high performance of the fish fed the equivalent feed without organic acid supplementation. If the average growth rates for the feeds individually are ignored and we focus on the regression line, it appears that feeds with a less refined vegetable protein content perform less well than the feed with more refined proteins unless the former are supplemented with the organic acid to a level somewhere between 490 and 773 mg/kg.

The histopathological survey of the gut tissues was characterized by a lack of significant effects to the extent that fish fed the feed with the low antinutrient risk (LAR) were indistinguishable from those fed the higher risk feeds (figure 3-6), with summarized results in figure 7. Indeed, the only significant difference in histopathology was observed in the liver where a higher proportion of the fish appeared "normal" when fed the higher risk feed ($OA_{0.0}$) than the low risk feed (LAR) (figure 3 & 8). Thus, the observations on gut and liver health do not corroborate other published findings in livestock and farmed salmon. Additionally, within the present case, the gut health data do not suggest a cause for the differences in growth rate observed. Assuming that the gut abnormalities observed are proportionate i.e. that the expectations of what constitutes a normal appearance for farmed salmon are not too high, there remains scope to improve the gut health of salmon. However, neither a more refined vegetable protein mix (devoid of guar protein, field beans and corn gluten) or supplementation with organic acid represent a way to improve the *status quo*.

Conclusions and recommendations

The data gathered in this study suggests, but does not strongly endorse, the addition of an organic acid supplement yielding active ingredient levels between 490 and 773 ppm to support salmon growth when feeding a feed with a relatively less refined vegetable protein content. The growth effects are not supported by the histopathological survey and so, the foundation for the observed effect remains to be established.