Aquaculture 555 (2022) 738214

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Review

A meta-analysis to determine factors associated with the severity of enteritis in Atlantic salmon (*Salmo salar*) fed soybean meal-based diets

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ARTICLE INFO

Keywords:
Meta-regression
Ordinal logistic regression
Mucosal fold height
Supranuclear vacuole
Lamina propria
Submucosal cellularity

ABSTRACT

A meta-analytic approach was used to determine factors associated with the severity of enteritis in distal intestine of Atlantic salmon fed soybean meal (SBM)-based diets. Dataset from 26 fish studies were extracted and standardized for use in the meta-analysis. After standardization, the data were analyzed with ordinal logistic regression model by comparing the SBM treatment(s) in each study with the neutral-reference treatment. The log-odds ratio of the proportional odds model and its standard error were extracted and analyzed using the random effects model to estimate the effect size of dietary SBM on enteritis using four semi-quantitative histological variables: reduction in mucosal fold height; disappearance of supranuclear vacuolization; inflammatory cell infiltration of lamina propria, and of submucosa. Both univariate and multivariate meta-regression were used to identify study factors with significant association to the severity of enteritis in Atlantic salmon. The results showed that fish production phase, feed type, SBM inclusion level, year of study and water temperature are significantly associated with the severity of enteritis in Atlantic salmon. Further meta-analysis of sub datasets according to production phase, revealed that fish reared in seawater were more prone to develop enteritis compared with fish reared in freshwater. The absence of positive relationship between SBM inclusion level and the severity of enteritis was probably due to difference in source, batch, processing, and level of anti-nutritional factors in the SBM used in the different studies combined in the meta-analysis. Subgroup analysis based on year of study revealed that the severity of enteritis in fish fed SBM-based diets has decreased over the years. Additional results revealed that fish fed SBM-based diet at low water temperature showed increased severity of enteritis, compared with fish raised in high water temperature. Linear and quadratic regressions conducted to explore possible impact of enteritis on fish performance, showed that the specific growth rate and thermal growth coefficient of fish decreased with increased severity of enteritis. However, this relationship depends on the fish production phase and the histological variables used for the regression analysis. The current study concluded that the severity of enteritis in Atlantic salmon fed SBM-based diets are significantly associated with fish production phase, feed type, SBM inclusion level, year of study and water temperature, but not the exposure time. Also, the study showed that increased severity of enteritis reduced specific growth rate and thermal growth coefficient of fish fed SBM-based diets.

1. Introduction

Over the years, concerted efforts have been dedicated to finding alternative protein sources for use in formulated feeds for farmed salmon as fishmeal availability is limited and market prices have increased (Tacon et al., 2011). In recent time, the dependency on marine ingredients has reduced as more plant ingredients are used in salmon feeds (Ytrestøyl et al., 2015; Aas et al., 2019). Among several protein alternatives, soybean meal (SBM) is an attractive plant ingredient for fish feeds due to its high protein content, its availability and price competitiveness (Gatlin III et al., 2007). A major reason for the low/no inclusion of SBM in Atlantic salmon feeds is that several studies have shown that its dietary inclusion can induce enteritis in the distal intestine of Atlantic salmon (Baeverfjord and Krogdahl, 1996; Marjara et al.,

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https://doi.org/10.1016/j.aquaculture.2022.738214
Received 11 January 2022; Received in revised form 4 March 2022; Accepted 29 March 2022
Available online 3 April 2022
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2012; Urán et al., 2008b; Urán et al., 2009a; Van den Ingh et al., 1996; Van den Ingh et al., 1991). The cause of SBM-induced enteritis in Atlantic salmon is linked to various anti-nutritional factors (ANFs) present in SBM (Baeverfjord and Krogdahl, 1996; Bakke et al., 2014; Krogdahl et al., 2015; Krogdahl et al., 2020; Refstie et al., 1998). Alcohol-soluble components of SBM (especially saponins) have been implicated as the causative agents of SBM-induced enteritis, but there are indications that the severity might be amplified by presence of other ANFs (such as protease inhibitors, trypsin inhibitors and lectins) (Chikwati et al., 2012; Knudsen et al., 2007; Knudsen et al., 2008; Van den Ingh et al., 1996). Typical signs of SBM-induced enteritis, firstly described by Van Den Ingh and Krogdahl (1990), are reduction in mucosal fold height; disappearance of supranuclear vacuolization; thickening of both lamina propria and sub-epithelia mucosa with a severe infiltration of inflammatory cells (Baeverfjord and Krogdahl, 1996; Krogdahl et al., 2003; Urán et al., 2008b; Van den Ingh et al., 1996). In several studies, SBM-induced enteritis has been qualitatively assessed by describing histological alterations of the distal intestine. However, Urán et al. (2008b) realized that such method cannot be used to compare the impact of different study factors (e.g., variety of SBM, SBM inclusion level, environmental factors) on the development of SBM-induced enteritis between different fish studies. The authors, therefore, used a "semi-quantitative" method, that quantifies the degree of enteritis by scoring system using five scales for different parts of the distal intestine (Knudsen et al., 2008; Urán et al., 2008b). The scoring system was based on an ordinal scale comprising of normal, mild, moderate, marked, and severe depending on the degree of enteritis, where the lowest degree was regarded as normal, and the highest scale was regarded as severe. Although, several studies have since adopted this scoring system to assess the degree of SBM-induced enteritis in Atlantic salmon, to the best of our knowledge, no study has quantitatively summarized the available information in a systematic literature review.

Furthermore, several studies have investigated different ways to ameliorate SBM-induced in fish. The most common approach is the use of single-cell ingredients (SCI) as possible functional ingredients to counteract enteritis in fish (Agboola et al., 2022; Agboola et al., 2021; Grammes et al., 2013; Reveco-Urzua et al., 2019; Romarheim et al., 2013). The SCI are a relatively broad class of resources including bacteria, yeast and microalgal derived products or their combination and have potential to be used in aquafeeds (Glencross et al., 2020). The cell wall of SCI, particularly the yeasts and bacteria meal, contain bioactive components such as β-glucan, mannan and chitin, which has immunomodulating properties that reduced inflammation caused by SBM in fish (Grammes et al., 2013; Romarheim et al., 2013). Despite the number of available studies on the possible ameliorating effects of SCI on SBM-induced enteritis in fish, no study has quantitatively evaluated this effect using a meta-analytic approach.

Meta-analysis is a type of systematic review that can be used to draw important conclusions from different, but related studies. Unlike narrative reviews, meta-analyses use rigorous statistical procedure to provide objective and unbiased interpretation of findings from multiple dataset (Higgins et al., 2019; Sauvant et al., 2008). Several studies have used meta-analysis to provide quantitative evidence on different continuous variables (e.g., growth and nutrient digestibility indices) (Hua and Bureau, 2012; Prabhu et al., 2013; Sales and Glencross, 2011) in aquaculture, but there is no published meta-analysis on variables measured with ordinal data, such as the effect of dietary SBM on enteritis in Atlantic salmon. Therefore, the objective of the current study was to quantitatively evaluate factors associated with the severity of enteritis in Atlantic salmon fed SBM-based diets. Additionally, in majority of studies on development of enteritis in fish fed SBM, no clear effect on performance was shown, thus the secondary objective of the present study was to evaluate the relationships between severity of SBM-induced enteritis and growth performance of Atlantic salmon.

2. Materials and methods

2.1. Literature search strategy and study selection

A literature search based on Oria, Web of Science, Scopus, and Google Scholar after duplicate removal identified a total of 356 references related to the use of SBM using the following search terms; "soybean meal", "induced", "enteritis", "enteropathy", "intestinal/gut health", "Atlantic salmon" and "*Salmo salar*". The search strategy and literature selection process used in the meta-analysis dataset is presented in Fig. 1. References were removed if the titles and abstracts indicated use of a fish species other than Atlantic salmon, presented no histological scores, were specifically related to subfractions of SBM (such as saponin and molasses), focused on other plant protein sources (such as pea protein), presented only morphometric scores of fish intestine or were review papers. This resulted in a list of 46 potentially relevant articles after being assessed for the following eligibility criteria: (1) the trial was conducted with Atlantic salmon; (2) the studies contained at least one SBM and a neutral-reference diet (NRD); (3) random allocation of fish during the trial; (4) random selection of fish for histological evaluation and the histology was conducted on the fish distal intestine (5) blind evaluation of histological variables; and (6) the studies contained semi-quantitative scoring for the histological variables. The NRD are fish feeds (without SBM) containing ingredients such as fishmeal, soy-protein concentrate, wheat gluten meal among others which are known not to induce enteritis in Atlantic salmon.

After literature review, the semi-quantitative scoring was presented as mean values or as graphical representation of the actual fish number within each scoring category (i.e., normal to severe enteritis). However, the histological scores were ordinal categorical variables and the actual

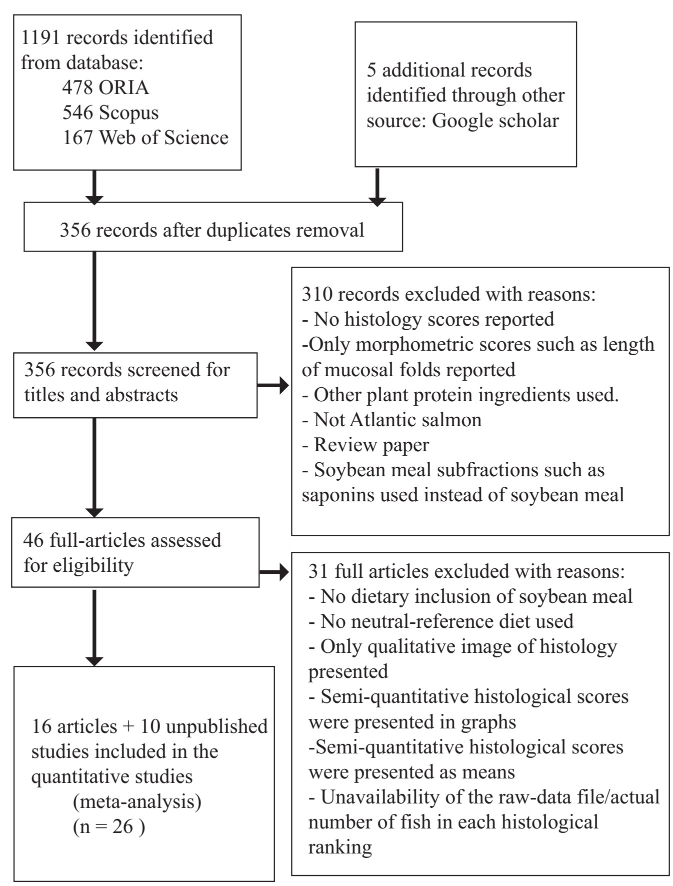

Fig. 1. A flow diagram showing the search strategy and literature selection for the meta-analysis.

fish number in each scoring category were needed for this meta-analysis, thus the original histological data were solicited from the authors of the relevant articles. These solicitations yielded data for 16 peer-reviewed articles, which were included in the meta-analysis database. The reference list of trials which failed to meet the inclusion criteria and/or unavailability of raw dataset are presented in Table S1. Additionally, data from 10 unpublished trials that met the inclusion criteria were also included in the meta-analysis, making a total of 26 experimental studies that were included in the final analysis. The description of studies included in the meta-analysis database is presented in Table S2.

2.2. Data extraction and standardization

An Excel spreadsheet was created for data extraction. Data were extracted for variables on inclusion level (%) of SBM, SBM types, feed type (i.e., whether the feed contained SBM with or without SCI), the scoring system used and fish production data. The fish production variables collected were fish production phase, initial and final body weight of fish, water parameters (temperature and salinity), and the exposure time. In addition, specific growth rate (SGR) and thermal growth coefficient (TGC) were calculated from the extracted data using equations (a) and (b), respectively.

$$SGR = \frac{ln(FBW) - ln(IBW)}{d} \times 100 \tag{a}$$

$$TGC = FBW - \frac{IBW}{t \times d \times 1000}\sqrt[3]{}\sqrt{} \tag{b}$$

Where FBW and IBW are final and initial body weight of fish; d is the exposure time in days; and t is the average water temperature recorded during the experiment.

After reviewing the literature, different scoring system: 0–4, 0–2, 1–5, 0–10 and 1–10 generally denoting normal, mild, moderate, marked, and severe enteritis were used to evaluate the degree of enteritis in Atlantic salmon. Regardless of the scoring system used, the data were extracted based on the number of fish recorded for each score category (normal to severe enteritis). Data from each study were extracted for the four histological variables normally used to evaluate enteritis for estimation of effect size. The four histological variables observed to be associated with inflammatory changes were: (1) reduction in mucosal fold height (MFH); (2) disappearance of supranuclear vacuolization (SNV); (3) increases in cellularity of lamina propria (LP); and (4) of submucosa (SC) due to infiltration by inflammatory cells.

The data in this study were transformed into trichotomous outcomes and analyzed using ordinal logistic regression. In the newly created dataset, fish with normal score were grouped into the normal category, fish with mild/moderate scores were grouped into moderate category and fish with marked/severe scores were grouped into severe category for each study. These new categories were assigned a score of 1 for normal, 2 for moderate and 3 for severe enteritis. The data standardization used for the ordinal logistic regression analysis was done for both the SBM and the NRD groups.

2.3. Statistical analysis

Ordinal logistic regression was performed on the standardized trichotomous dataset using the *polr* command from MASS package in R. The proportional odds logistic regression model was performed by comparing the SBM treatment(s) with the NRD treatment in each study. The NRD was used as reference point (log-odds ratio (LOR) = 0) in each study. From this proportional odds regression model, the LOR and its standard error (Agresti, 2003; Higgins et al., 2019) for each SBM treatment were generated and extracted into a spreadsheet for the meta-analysis. The meta-analysis was performed using the Comprehensive Meta-analysis (CMA) software (version 3, Biostat Inc., USA). To determine factors associated with the severity of enteritis, a meta-regression analysis was performed (using the Comprehensive Meta-analysis v3) using the study factors in the database. Categorical variables including production phase, and feed type, as well as continuous variables including SBM inclusion level, year of study, water temperature, and exposure time were included in the meta-regression analysis. Meta-regression was conducted by screening individual variables using univariate meta-regression. Significant variables from the univariate meta-regression were subsequently combined in a multivariate meta-regression analysis to determine how much of these variations could be explained by the study factors combination. The meta-regression was conducted using a random-effects model which has the underlying assumption that the distribution of effects exists, resulting in heterogeneity among study results (Borenstein et al., 2009; Lean et al., 2009). Heterogeneity across studies were assessed using the chi-squared (Q) test and the I^2 statistics (Borenstein et al., 2009; Lean et al., 2009). Significant variation across studies were detected at $I^2 > 50\%$.

To control for variation across studies, the studies were stratified into different production phase (seawater vs. freshwater). Further subgroup meta-analysis was conducted with feed type (SBM with vs. without SCI) within each production phase to control for possible effect of feed type on study variations. Additional subgroup meta-analysis was conducted using study factors such as SBM inclusion level (< 20%, 20%, and > 20%), year of study (< 2014 vs. ≥ 2014) and rearing water temperature (≤ 10 °C vs. > 10 °C) to evaluate the impact of these factors on the heterogeneity observed across the studies in fish reared in seawater. There were not enough datapoints to conduct similar analysis with SBM inclusion level, year of study and water temperature in studies conducted in freshwater. The effect size for the analyzed variables was determined as LOR for the meta-regression and odds ratio (OR) for the sub-group meta-analysis at 95% level of confidence intervals. The significance of effect size was set at $P \leq 0.05$. The LOR and OR refers to the ratio of the probability that a particular event will occur to the probability that it will not occur and can assume any number from zero to infinity. In this study, publication bias was not examined due to presence of substantial heterogeneity with all outcomes, which may lead to false-positive claims for publication bias (Ioannidis and Trikalinos, 2007).

To determine the impact of enteritis on fish growth performance, linear and quadratic regression analyses were performed between LOR and fish production parameters, such as SGR, final weight and TGC of fish using the Excel 365 Software package (Microsoft Excel version 2102, Microsoft Corp., Redmond, WA). Unlike random-effect model used for the meta-regression analysis, the regression analyses between the fish production data and LOR were non-weighted and assumed equal variance across studies.

3. Results

3.1. Study characteristics

The characteristic of the studies included in the meta-analysis is presented in Table S2. Data from a total of 26 fish experiments (16 peer-reviewed studies and 10 unpublished data), representing 96 study datapoints were included in the meta-analysis. Fifty-eight percent of the studies were conducted in seawater, while the remaining were conducted in freshwater. The studies were conducted over a 19-year period (2001−2020) and included histological assessment data from a total of 1486 fish. Dietary inclusion of SBM ranged from 5 to 46%, with 20% inclusion level most commonly used. A vast majority of the study (60%) used solvent extracted SBM, whereas other SBM types such as HiPro SBM, full-fat SBM, genetically modified (GM) SBM, biotechnologically-processed SBM and triple-null SBM were also used.

3.2. Factors associated with the severity of enteritis

Consistent for the four histological variables, the results of the meta-regression analysis showed that the severity of enteritis in Atlantic salmon fed SBM-based diet was significantly ($P < 0.05$) associated with the fish production phase, feed type, SBM inclusion level, year of study and water temperature, but not exposure time (Table 1). The R^2 values (0.19–0.50) of the univariate analysis showed that water temperature had the highest effect on severity of enteritis for all the four histological variables (Table 1). The multivariate meta-regression analysis showed that ($P < 0.05$) production phase, feed type, SBM inclusion level, year of study and water temperature in combination explained 44%, 24%, 70% and 54% of the variations observed in enteritis associated with reduction in MFH, loss of SNV, infiltration of LP and SC, respectively (Table S3).

3.3. Effect of fish production phase on severity of enteritis

For the four histological analyses, the sub-group analysis showed that fish fed SBM-based diets in seawater (ORs = 33 to 50) were more sensitive ($P < 0.05$) to develop enteritis, compared with fish reared in freshwater (ORs = 4.3 to 18.7) (Table 2). Regardless of the production phase, the results also showed that loss of SNV (ORs of 50 in seawater and 18.7 in freshwater) was the most sensitive marker for evaluating SBM-induced enteritis, compared with reduction in MFH, infiltration of LP and SC (Table 2). The significant of Q-statistics and I^2 values (33–80.5%) showed there was significant heterogeneity across studies on the effect of production phase on severity of enteritis in fish fed SBM-based diets in both seawater and freshwater (Table 2).

3.4. Effect of feed type on severity of enteritis

There was an inconsistent effect of dietary inclusion of SCI on severity of enteritis in Atlantic salmon fed SBM-based diets (Tables S4a & S4b). Based on the four histological variables, there were no significant effects ($P < 0.05$) of feeding SCI on severity of enteritis in fish fed SBM-based diets in seawater, except for SC (Table S4a). Based on SC, dietary inclusion of SCI reduced ($P < 0.05$) the severity of enteritis (from OR of 79 to 22) in fish fed SBM-based diets in seawater (Table S4a). Similarly, based on reduction in MFH and loss of SNV, inclusion of SCI decreased the severity of enteritis in fish fed SBM-based diets in freshwater (Table S4b). The values of 95% confidence intervals, Q-statistic and I^2 showed large variability among studies on the effect of feed type on severity of enteritis in fish fed SBM-based diets (Table S4a & S4b).

3.5. Effect of SBM inclusion level on severity of enteritis

Based on the four histological variables, there were no definite trends on the impact of SBM inclusion level on the severity of enteritis in fish fed SBM-based diets in seawater (Table S5). It was consistently shown that increasing the level of SBM (< 20%, 20% or > 20%) in the diets did not necessarily increase the severity of enteritis (Table S5). The 95% confidence intervals and I^2 statistic showed substantial variation across studies combined to evaluate the effect of SBM inclusion level on severity of enteritis in fish fed SBM-based diets in seawater.

3.6. Effect of year of study on severity of enteritis

Subgroup analysis based on year of study showed that the severity of enteritis was higher ($P < 0.05$) in fish fed SBM-based diets before 2014, compared with study conducted after 2014 (Table 3). These results were consistent for changes associated with reduction of MFH (ORs of 54.3 before and 17.7 after 2014), infiltration of LP (ORs of 82.4 before and 10.2 after 2014) and submucosal cellularity (ORs of 99.3 before and 9.9 after 2014), but not for the loss of SNV (Table 3). There was no significant difference ($P > 0.05$) in severity of enteritis associated with loss of SNV in studies conducted before (OR = 54.1) and after (OR = 43.7) 2014.

Table 1
Univariate meta-regression to determine possible study factors associated with the severity of enteritis in fish fed soybean meal (SBM)-based diets. Log odds ratio is the dependent variable (effect size) of the equation generated with this meta-regression.

Study factors	Intercept	Coefficients	95% CI		R^2	Number of studies	Q	I^2 (%)	P-value
			Lower	Upper					
Reduction in mucosal fold height									
Production phase: seawater	1.99	1.52	0.80	2.24	0.25	92	238.6	61.9	< 0.0001
Feed type: with SCI[1]	3.34	−0.94	−1.69	−0.19	0.09	92	238.6	61.9	0.014
SBM Inclusion level (%)	4.42	−0.06	−0.10	−0.02	0.22	92	238.6	61.9	0.0013
Year of study	250.8	−0.12	−0.19	−0.06	0.22	92	238.6	61.9	0.0001
Temperature (°C)	6.71	−0.33	−0.46	−0.20	0.42	82	201.5	59.8	< 0.0001
Exposure time (day)	3.19	−0.01	−0.02	0.01	0.00	87	225.2	61.8	0.305
Loss of supranuclear vacuolization									
Production phase: seawater	2.90	1.05	0.13	1.96	0.11	94	343.7	72.9	0.025
Feed type: with SCI	3.95	−0.94	−1.87	−0.02	0.06	94	343.7	72.9	0.044
SBM Inclusion level (%)	5.16	−0.07	−0.12	−0.02	0.15	94	343.7	72.9	0.007
Year of study	103.8	−0.05	−0.13	0.03	0.03	94	343.7	72.9	0.214
Temperature (°C)	6.68	−0.28	−0.45	−0.11	0.19	84	289.0	71.3	0.001
Exposure time (day)	3.66	−0.005	−0.02	0.01	0.0	89	311.1	71.7	0.531
Infiltration of lamina propria									
Production phase: seawater	1.44	2.16	1.46	2.86	0.43	85	235.2	64.3	<0.0001
Feed type: with SCI	3.10	−0.67	−1.48	0.14	0.02	85	235.2	64.3	0.104
SBM Inclusion level (%)	4.14	−0.06	−0.10	−0.01	0.08	85	235.2	64.3	0.010
Year of study	365.9	−0.18	−0.24	−0.13	0.46	85	235.2	64.3	<0.0001
Temperature (°C)	7.47	−0.40	−0.53	−0.28	0.50	77	199.5	61.9	<0.0001
Exposure time (day)	3.23	−0.01	−0.02	0.004	0.01	82	226.3	64.2	0.188
Submucosal cellularity									
Production phase: seawater	1.97	1.78	0.96	2.60	0.20	85	226.9	63.0	<0.0001
Feed type: with SCI	3.59	−1.15	−1.99	−0.32	0.11	85	226.9	63.0	0.007
SBM Inclusion level (%)	4.65	−0.07	−0.12	−0.02	0.09	85	226.9	63.0	0.007
Year of study	344.8	−0.17	−0.25	−0.11	0.34	85	226.9	63.0	<0.0001
Temperature (°C)	7.49	−0.39	−0.54	−0.24	0.36	77	202.3	62.4	<0.0001
Exposure time (day)	3.49	−0.01	−0.02	0.004	0.01	82	226.3	64.2	0.170

[1] SCI – single cell ingredient.

Table 2
The effect of production phase on the severity of enteritis in Atlantic salmon fed soybean meal (SBM)-based diets.

Group/ Sub-Group[1]	Number of comparisons	Effect Size Estimates			Heterogeneity tests		
		Odds ratio[2]	95% CI	P-value[3]	Q	P-value[4]	I^2 (%)
Reduction in mucosal fold height							
Seawater	56	33.4[a]	21.02–52.9	***	110.2	***	50.1
Freshwater	36	7.4[b]	4.2–12.9	***	89.7	***	61.0
Loss of supranuclear vacuolization							
Seawater	56	50.0[a]	30.8–81.3	***	123.6	***	55.5
Freshwater	38	18.7[b]	8.1–43.1	***	189.3	***	80.5
Infiltration of lamina propria							
Seawater	51	39.8[a]	24.0–65.9	***	117.6	***	57.5
Freshwater	34	4.3[b]	2.8–6.7	***	49.9	*	33.8
Submucosal cellularity							
Seawater	52	43.7[a]	24.7–77.5	***	134.4	***	62.1
Freshwater	33	7.2[b]	4.2–12.4	***	61.0	***	47.6

[1] Subgroups analysis with production phase (seawater vs. freshwater).
[2] Odds ratio within the same subgroup, but with different superscript ([a, b]) are significantly different ($P < 0.05$).
[3] P-values of analysis comparing the odds ratio of the SBM treatments with the neutral reference group (Odds ratio = 1). Asterisks denote level of significance (NS: not significant, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).
[4] *P*-values of chi-square (Q) statistic used to detect heterogeneity across the studies. Significance level of the heterogeneity was denoted with asterisks (NS: not significant, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

Table 3
The effect of year of study on the severity of enteritis in Atlantic salmon fed soybean meal (SBM)-based diets in seawater.

Group/Sub-Group[1]	Number of comparisons	Effect Size Estimates			Heterogeneity tests		
		Odds ratio[2]	95% CI	*P*-value[3]	Q	*P*-value[4]	I^2 (%)
Reduction in mucosal fold height							
Before 2014	34	54.3[a]	28.6–103.0	***	67.9	***	51.5
After 2014	22	17.7[b]	9.7–32.3.	***	33.5	*	37.2
Loss of supranuclear vacuolization							
Before 2014	34	54.1	29.9–97.7	***	62.9	***	47.5
After 2014	22	43.7	18.9–101.1	***	60.5	***	65.3
Infiltration of lamina propria							
Before 2014	34	82.4[a]	48.3–140.8	***	51.0	*	35.3
After 2014	17	10.2[b]	5.2–19.9	***	28.2	*	43.2
Submucosal cellularity							
Before 2014	34	99.3[a]	51.1–193.0	***	68.9	***	52.1
After 2014	18	9.9[b]	5.0–19.6	***	27.0	NS	37.1

[1] Subgroups analysis with year of study (before vs. after 2014).
[2] Odds ratio within the same subgroup, but with different superscript ([a, b]) are significantly different ($P < 0.05$).
[3] P-values of analysis comparing the odds ratio of the SBM treatments with the neutral reference group (Odds ratio = 1). Asterisks denote level of significance (NS: not significant, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).
[4] P-values of chi-square (Q) statistic used to detect heterogeneity across the studies. Significance level of the heterogeneity was denoted with asterisks (NS: not significant, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

3.7. Effect of water temperature on severity of enteritis

The results of the sub-group analysis revealed that the severity of enteritis in fish fed SBM-based diets in seawater decreased ($P < 0.05$) with increasing water temperature (i.e., from <10 °C to >10 °C) (Table 4). These were consistent for changes determined for all four histological variables (Table 4). By increasing water temperature from <10 °C to >10 °C, the ORs decreased from 45.4 to 19.2, 54.8 to 33.0, 70.4 to 14.0 and 69.0 to 17.3 based on reduction in MFH, loss of SNV, infiltration of LP and SC; respectively (Table 4).

3.8. Relationships between enteritis and fish growth performance

Based on the four histological variables, there were both negative linear and quadratic relationships ($P < 0.05$) in the full dataset between LOR and SGR of fish fed SBM-based diets (Table 5). The SGR of fish decreased with increased severity of enteritis (higher LOR) in fish fed SBM-based diets (Table 5). In the freshwater dataset, there were no linear relationships ($P > 0.05$) between LOR and SGR of fish for all the histological variables, except for SC (Table 5). Conversely, there was negative quadratic relationships ($P < 0.05$) between LOR and SGR of fish fed SBM-based diets based on reduction in MFH, loss of SNV, and SC. Based on loss of SNV, there were both negative linear and quadratic relationships ($P < 0.05$) in the seawater dataset between LOR and SGR of fish fed SBM-based diets.

None of the histological variables showed significant linear or quadratic relationships ($P > 0.05$), neither in the full dataset nor the sub-dataset, between LOR and final weight of fish fed SBM-based diets

Table 4
The effect of water temperature on the severity of enteritis in Atlantic salmon fed soybean meal (SBM)-based diets in seawater.

Group/Sub-Group[1]	Number of comparisons	Effect Size Estimates			Heterogeneity tests		
		Odds ratio[2]	95% CI	*P*-value[3]	Q	*P*-value[4]	I^2 (%)
Reduction in mucosal fold height							
<10 °C	25	45.4[a]	25.4–81.2	***	32.9	NS	27.1
>10 °C	26	19.2[b]	9.8–37.6	***	52.8	***	52.7
Loss of supranuclear vacuolization							
<10 °C	25	54.8[a]	29.9–100.4	***	35.5	NS	32.4
>10 °C	26	33.0[b]	15.2–71.4	***	71.7	***	65.2
Infiltration of lamina propria							
<10 °C	25	70.4[a]	37.2–133.2	***	40.5	*	40.7
>10 °C	21	14.0[b]	7.3–27.0	***	38.4	**	48.0
Submucosal cellularity							
<10 °C	25	69.0[a]	37.2–127.8	***	34.0	NS	29.4
>10 °C	22	17.3[b]	7.0–42.4	***	62.7	***	66.5

[1] Subgroups analysis with water temperature (less than vs. greater than 10 °C).
[2] Odds ratio within the same subgroup, but with different superscript ([a, b]) are significantly different ($P < 0.05$).
[3] P-values of analysis comparing the odds ratio of the SBM treatments with the neutral reference group (Odds ratio = 1). Asterisks denote level of significance (NS: not significant, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).
[4] P-values of chi-square (Q) statistic used to detect heterogeneity across the studies. Significance level of the heterogeneity was denoted with asterisks (NS: not significant, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

(Table S6). Similarly, based on the four histological variables, there were no linear or quadratic relationships ($P > 0.05$) in the full dataset between LOR and TGC of fish fed SBM-based diets (Table S7). However, in the freshwater dataset, there were negative quadratic relationships ($P < 0.05$) between LOR and TGC of fish based on reduction in MFH, loss of SNV and SC (Table S7). In the seawater dataset, there were no quadratic relationships ($P > 0.05$) between LOR or TGC of fish for the histological variables, except for infiltration of LP (Table S7).

4. Discussion

To our knowledge, the current study was the first to apply meta-analytic approach to semi-quantitative histological (ordinal data) data in aquaculture. It was also the first study to quantitatively determine various factors associated with the severity of enteritis in Atlantic salmon fed SBM-based diets. In the current study, the four histological variables gave consistent results based on meta-regression analysis. The results of the meta-regression analysis consistently showed that the fish production phase, feed type, inclusion level of SBM, year of study and water temperature were significantly associated with the severity of enteritis in Atlantic salmon. The univariate meta-regression revealed a negative coefficient for SBM inclusion levels, suggesting the higher the SBM level, the less the severity of enteritis. It is likely that SBM inclusion level was confounded by other variables in the dataset. This could be explained by the reduced sensitivity of fish to SBM-induced enteritis over the years. To induce moderate to severe enteritis and uniformity in the sample group, the authors of the studies in the meta-analysis intentionally increased the level of SBM in the diet from 20% which was considered the most common dietary challenge before 2014 to 30 and 40%. Even at these high inclusion levels only mild to moderate enteritis with large individual variation among fish was observed e.g., Agboola et al. (2021); Hansen et al. (2019); Reveco-Urzua et al. (2019). The second reason for the observation may be linked to the variation in level of ANFs present in different commercial sources of SBM used in the studies combined for the meta-analysis. Different commercial sources of SBM contain different levels of ANFs (especially saponin), which does not necessarily correlate with SBM inclusion level in the diets. Thus, it is possible that the true effect of SBM inclusion level was masked by the level of ANFs in the diets and thus responsible for the negative coefficient observed with the univariate meta-regression. However, data on content of ANFs in the experimental diets used in the study combined were lacking, and thus we could not use level of ANFs in the diets as a covariate for the meta-regression analysis. The meta-regression analysis consistently showed that exposure time (from 20 to 224 days) had no association with the severity of enteritis in Atlantic salmon. This was in line with the results of previous studies where it had been documented that the development of full enteritis in Atlantic salmon occurs after 5–7 days of feeding diet containing 20–33% SBM (Baeverfjord and Krogdahl, 1996; Marjara et al., 2012; Urán et al., 2008b; Urán et al., 2009b). Therefore, fish developed full enteritis after few days of feeding, suggesting that there was no time related effect after 5–7 days of feeding SBM-based diet.

Sub-group analysis of studies based on production phase consistently showed for all the histological variables, that fish reared in seawater were more prone to develop enteritis than fish reared in freshwater. This finding was consistent with results of several studies conducted over the years. Typical intestinal changes associated with SBM-induced enteritis have been well documented in seawater-adapted Atlantic salmon (Baeverfjord and Krogdahl, 1996; Urán et al., 2008a; Urán et al., 2008b; Van den Ingh et al., 1991), but mild inflammatory responses have been reported in fish reared in freshwater (Agboola et al., 2021; Hansen et al., 2019; Sahlmann et al., 2015; Sanden et al., 2005). The enteritis has been linked to a T-cell-mediated hypersensitivity in seawater-adapted salmon (Bakke-McKellep et al., 2007; Marjara et al., 2012). However, in a study investigating the effect of SBM from start feeding on ontogeny of the digestive system of Atlantic salmon, Sahlmann et al. (2015) suggested that the Atlantic salmon juveniles' under-developed adaptive immune system may not have been equipped to provoke inflammatory responses, compared to post-smolt fish. This corroborated the findings of an earlier study that showed increased cell proliferation, but no inflammatory response in fish fed diet containing 12.5% SBM in freshwater (Sanden et al., 2005). In this, meta-analysis, the disparity in the maturation of the fish adaptive immune system, and their ability to mount inflammatory responses, may be responsible for the variation in the severity of enteritis between the seawater and freshwater phase. In the current study, loss of SNV showed the highest sensitivity among the typical variables of SBM-induced enteritis. This supported the results of previous findings which showed shrinkage of SNV (earliest noticeable signs after 3 days) in Atlantic salmon fed diets containing 20% SBM (Baeverfjord and Krogdahl, 1996; Urán et al., 2009b).

Table 5
Relationships (linear and quadratic) between the severity of enteritis and specific growth rate of fish fed SBM-based diets[a].

Dataset	Regression type	Number of datapoints	Equation	P-value[b]	R^2 (adjusted)
Reduction in mucosal fold height					
Full	Linear	78	$Y = -0.062x + 1.9$	*	0.04
	Quadratic		$Y = -0.013x^2 - 0.184x + 2.5$	***	0.17
Freshwater	Linear	35	$Y = 0.026x + 2.9$	NS	−0.01
	Quadratic		$Y = -0.015x^2 - 0.161x + 3.4$	*	0.19
Seawater	Linear	43	$Y = -0.009x + 1.0$	NS	−0.02
	Quadratic		$Y = 0.036x^2 - 0.262x + 1.3$	***	0.25
Loss of supranuclear vacuolization					
Full	Linear	78	$Y = -0.119x + 2.2$	***	0.14
	Quadratic		$Y = -0.011x^2 - 0.177x + 2.6$	***	0.26
Freshwater	Linear	35	$Y = -0.04x + 2.9$	NS	0.01
	Quadratic		$Y = -0.015x^2 - 0.161x + 3.5$	***	0.40
Seawater	Linear	43	$Y = -0.045x + 1.1$	*	0.07
	Quadratic		$Y = 0.024x^2 - 0.240x + 1.4$	***	0.21
Infiltration of lamina propria					
Full	Linear	73	$Y = -0.089x + 2.1$	**	0.08
	Quadratic		$Y = 0.0006x^2 - 0.088x + 2.1$	*	0.07
Freshwater	Linear	35	$Y = -0.038x + 2.9$	NS	0.01
	Quadratic		$Y = -0.002x^2 - 0.041x + 3.0$	NS	0.001
Seawater	Linear	38	$Y = 0.072x + 0.6$	***	0.32
	Quadratic		$Y = 0.005x^2 + 0.37x + 0.6$	***	0.31
Submucosal cellularity					
Full	Linear	78	$Y = -0.085x + 1.9$	***	0.18
	Quadratic		$Y = -0.003x^2 - 0.115x + 2.0$	***	0.18
Freshwater	Linear	35	$Y = -0.072x + 2.8$	**	0.21
	Quadratic		$Y = -0.006x^2 - 0.173x + 3.1$	***	0.22
Seawater	Linear	43	$Y = -0.013x + 1.0$	NS	0.02
	Quadratic		$Y = 0.001x^2 - 0.006x + 0.9$	NS	0.004

[a] Y = specific growth rate and X = log-odds ratio.
[b] Asterisks denote level of significance (NS: not significant, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

Other factors which may account for the observed disparity between the production phases are differences in water salinity, fish size, fish age, fish developmental stage, smoltification, drinking rate of fish and composition of the basal diets. During smoltification, Atlantic salmon maintain osmoregulation by ingesting water to compensate for water lost to the hyperosmotic environment. During this process, the osmoregulatory function of the gut increases active transport of ions across the intestinal mucosa (Usher et al., 1990). Active transport of ions increases the gut permeability (Hu et al., 2016), and possibly facilitates transfer of enteritis-inducing components of SBM into the underlying mucosa (Knudsen et al., 2007; Knudsen et al., 2008; Kortner et al., 2012). Thereby, the inflammatory response of fish fed SBM in seawater may be aggravated compared to the situation in freshwater. As possible impacts of water salinity, fish size, fish age, and feed composition on severity of SBM-induced enteritis were not tested in the current meta-analysis (due to lack of/insufficient number of datapoints), further studies elucidating the role of these factors are warranted.

Further subset analysis based on feed type showed that there was inconsistency regarding the ameliorating effect of single cell ingredient (SCI) on severity of enteritis between the two production phases and among the four histological variables. Dietary inclusion of SCI reduced the severity of enteritis as indicated by SC in fish raised in seawater, whereas MFH, loss of SNV and infiltration of LP did not show similar relationship. In freshwater reared fish, inclusion of SCI in fish diets reduced the severity of enteritis based on loss of SNV, but not on the other histology markers. The large variability observed on the impact of SCI in reducing the severity of enteritis could be linked to types of SCI, strain of SCI, bioactive components present in the SCI, batch-to-batch variation in the composition of SCI, inclusion level of SCI in the fish diet, and processing methods used after SCI harvest (Agboola et al., 2021; Grammes et al., 2013; Hansen et al., 2019). The SCI used in the studies included in this meta-analysis were bacteria meal (Romarheim et al., 2013), yeasts (Agboola et al., 2021; Grammes et al., 2013; Hansen et al., 2019; Reveco-Urzua et al., 2019) and microalgae (Grammes et al., 2013). These differ in content and physio-chemical properties of functional components. However, there are insufficient number of studies in this meta-analysis for further study stratification to account for the differences among the SCI.

Additional subgroup analysis based on SBM inclusion level showed that increasing dietary inclusion of SBM (from 8 to 46% in the diets) did not necessarily increase the severity of enteritis. This observation was consistent for the four histological variables. This observation is in contrast to the findings of previous studies, which reported dose-dependent increase in the severity of enteritis in fish fed diet containing increasing level of SBM (Krogdahl et al., 2003; Urán et al., 2009b).

Several factors such as sources of SBM, batch-to-batch variation, processing method used and level of ANFs remaining in the SBM products, could influence the severity of enteritis in Atlantic salmon (Urán et al., 2009a). The SBM of studies combined in this meta-analysis differed based on the aforementioned factors. Therefore, the discrepancy in our results and that of the previous studies (Krogdahl et al., 2003; Urán et al., 2009b), implies that other factors than SBM inclusion level may have a dominating effect on severity of enteritis. Thus, to study the impact of SBM inclusion level on severity of enteritis, source, batch, processing, and level of ANFs in SBM must be considered. The findings of the current meta-analysis showed that SBM-induced enteritis occur at dietary SBM inclusion level of <20% (average SBM inclusion level of 14%), which correspond with the previous studies (Krogdahl et al., 2003; Urán et al., 2009b).

Partitioning the study based on year of study showed that the severity of enteritis using the four histological variables, except for loss of SNV consistently declined over the years. The severity of enteritis was higher in fish fed SBM-based diets before year 2014, compared with studies conducted after 2014. The reduction in severity of enteritis over the years could be attributed to a number of factors such as increased tolerance of fish to SBM, the physico-chemical changes in SBM (such as breeding to reduce/eliminate ANFs), as well as improved feed formulation and processing in recent years. The tolerance of fish over the years could be the result of breeding and genetic selection of fish for improved growth performance and adaptability to plant-based diets. Previous studies have reported increased nutrient utilization, improved growth rate, and no signs of enteritis in strain of rainbow trout selected on a diet containing SBM, compared to non-selected strain (Abernathy et al., 2017; Callet et al., 2017; Overturf et al., 2013; Venold et al., 2012). However, similar studies in Atlantic salmon are scarce in scientific literature. Additionally, several factors such as genetics, breeding to reduce/eliminate ANFs, cultivation conditions, harvesting, processing and storage, may have considerable effect on nutritional, physical and chemical properties of SBM. These factors are constantly changing and might have altered the potency of SBM to induce enteritis in Atlantic salmon. There has been substantial improvement in the area of fish feed formulation and processing such as, the addition of premixes with claimed health beneficial components that may indirectly influence the response of fish to dietary SBM in recent years. Our results showed that there was no impact of year of study on severity of enteritis based on loss of SNV, implying that this parameter could still be used as an important marker in future studies to document the response of Atlantic salmon to dietary SBM and/or other plant ingredients as well as to evaluate the impact of functional feed on enteritis. Also, it supported earlier finding in this paper regarding the high sensitivity of this marker to enteritis, compared with the other variables. This finding calls for investigation of the role of the SNV in the distal intestine.

Subset analysis based on water temperature (≤10 °C or >10 °C) showed that the severity of enteritis decreased with increasing water temperature. This observation was similar for all the histological variables. However, this was not expected, and it was contrary to the findings of previous studies, which showed increased severity in enteritis with increasing water temperature (Chikwati et al., 2013; Urán et al., 2008b). Temperature drive feed intake in fish, thus, we expected that higher feed intake at higher water temperature would increase the amount of enteritis-inducing components of SBM exposed to the intestinal mucosa, thereby increasing the severity of enteritis. However, possible explanation for our findings could be attributed to the short period of no/low feed intake (possibly starvation) experienced by fish immediately after seawater transfer. Fish undergo various physiological changes after seawater transfer, as a consequent they can go through a period of starvation or depressed feed intake (Usher et al., 1991). Fish reared at low water temperature are possibly more prone to this starvation, which may trigger inflammatory responses that can be responsible for the increased severity of enteritis observed in fish in the present meta-analysis. This position is supported by the results of a previous trial, which demonstrated that fish can develop inflammatory response that resembles typical SBM-induced enteritis after 7 days of starvation (Baeverfjord and Krogdahl, 1996). Another possible reason is that water temperature was confounded by year of study in this meta-analysis. Majority of the study with water temperature of <10 °C were conducted before 2014, coinciding with the period of high sensitivity of fish to dietary SBM (already shown in this study). Confounding can complicate interpretation of a meta-analysis and can lead to misleading conclusions (Higgins et al., 2019). For this reason, the impact of water temperature on severity of enteritis reported in this study should be interpreted with caution.

The results of the linear and quadratic regression revealed negative relationship between LOR and fish performance indices such as SGR and TGC, indicating lower fish performance with increased severity of enteritis. However, this observation depends on the fish production phase and the histological variables considered for the regression analysis. Compared with freshwater, there were no clear relationship between severity of enteritis and growth performance of fish fed SBM-based diets in seawater. The discrepancy between the production phases may be linked to low feed intake and short exposure time in seawater-adapted fish. The feed intake and exposure time in seawater reared fish are possibly limiting the apparent effect of enteritis on growth performance of fish.

5. Conclusions

Based on the findings of studies included in this meta-analysis, the severity of enteritis in Atlantic salmon fed SBM-based diets was associated with fish production phase, feed type, SBM inclusion level, year of study and water temperature, but not the exposure time. Further regression analysis showed that increased severity of enteritis reduced SGR and TGC of fish fed SBM-based diets. However, this effect was more apparent in fish reared in freshwater, compared with seawater.

6. Limitations

The inclusion of unpublished data is a recurring debate when it comes to systemic review with meta-analysis. Experts seem to be divided on whether unpublished data should be included in a meta-analysis. A group of experts believed that studies derived from both published and unpublished studies can be used in a meta-analysis (Higgins et al., 2019; Kelley and Kelley, 2019; Liberati et al., 2009). Their position is based on the principle that publication bias is a major threat to the validity of systematic reviews, and obtaining and including data from unpublished trials appears to be one obvious way of avoiding this problem (Kelley and Kelley, 2019; Lean et al., 2009). There is convincing evidence that results that are statistically non-significant and unfavourable to the experimental intervention are less likely to be published than statistically significant results, and hence are less easily identified by the systemic review (Higgins et al., 2019). Van Driel et al. (2009) concluded that the difficulty in retrieving unpublished work could lead to selection bias. The group of authors that argued against the inclusion of unpublished data in a meta-analysis based their position on the following reasons. Firstly, the inclusion of unpublished data can itself introduce bias in that the unpublished studies may be an unrepresentative subset of the unpublished studies in existence. However, it has been stated that this bias is of less concern than the bias introduced by excluding all unpublished studies, based on what is known about the impact of reporting bias (Higgins et al., 2019). Secondly, a major concern regarding the inclusion of unpublished studies is the assumption that their methodological qualities are poorer than those already subjected to peer-review process. This is not the case for the unpublished data included in our meta-analysis. We can ascertain that the experimental procedure used in all the unpublished studies are of high-quality standard. Majority of this studies are bound by confidentiality agreement with the financing companies, therefore, they remained unpublished.

To understand the possible effect of literature source (published or unpublished) on the studies included in the meta-analysis, we conducted a univariate meta-regression using literature source (published vs. unpublished) as the co-variate. Based on MFH and SNV, the results showed that literature source did not associate with the severity of enteritis (Table S8). Conversely, based on LP and SC, the source of literature affected the severity of enteritis (Table S8). However, the R^2 revealed that the literature source contributes between 9 and 10% of the variation observed with these variables. Additionally, the Q and I^2 statistics showed other factors rather than literature source are responsible for this variation. Based on this meta-regression analysis, we recommend that the results on LP and SC in the current paper should be interpreted with caution.

Certain decisions could influence the outcome of a meta-analysis. While some of these decisions are clearly objective, some are very contentious and arbitrary (Higgins et al., 2019). In the current meta-analysis, certain decisions were made during the data standardization, which may or may not influence the observed results. For example, data standardization in this meta-analysis assumed that each score categories are similar between the studies. The standardization was done to avoid bias of choosing inappropriate cut-off points between the score categories. It was the reason for grouping the data into three, rather than two, which can be directly meta-analyzed. However, it is uncertain whether all the cut-off points were captured or whether there are some slightly missing cut-offs that were not addressed in this study. Sensitivity analysis could be used to determine if the findings of a meta-analysis are not dependent on such decision. Sensitivity analysis was not conducted in this meta-analysis because there was no baseline study with established cut-off points to be used in the analysis.

The conversion of a continuous variable to nominal/categorical variable is always questionable because it is assumed that in the absence of defensible cut-off point, such conversion may lead to omitting meaningful information that can result in over-estimation or under-estimation of intervention effect. However, continuous variable can be converted into categorical variable for sub-group analysis when it is conducted as a complementary analysis to a meta-regression (Higgins et al., 2019), like we did in this meta-analysis. However, such approach also has its limitation. Sub-group analyses are considered to be exploratory and their findings may need to be tested in original studies (Kelley and Kelley, 2019). Additionally, the conversion of continuous variable into categorical variable for sub-group analysis is prone to false-positive results, which may led to misleading conclusion (Higgins et al., 2019). Therefore, the results of the sub-group analysis conducted with continuous variables (SBM inclusion, water temperature and year of study) in the current study should be considered exploratory and the findings need to be further substantiated in original studies. In addition, the justification for partitioning the variables into different categories was based on the scatter plots obtained after the meta-regression analysis (Fig. S1). Partitioning the studies based on these graphs has its limitation, but that was the best possible approach we could think of when conducting the meta-analysis. For instance, our idea of stratifying the study based on year of study was to understand if the increase tolerance of fish is related to genetic strain of Atlantic salmon developed over the years. Information on strain of fish used are generally lacking in literature, and as such the best we could do was to stratify the studies based on year and develop speculation based on the results. This additional limitation strengthens why the results of the sub-group analysis with continuous variables should be considered exploratory. In addition, it should be noted that unlike the meta-regression analysis, the linear and quadratic analyses between fish production parameters and the LOR assumed equal variance across studies and did not correct for heteroscedasticity, as such, the obtained coefficients with the meta-regression may not be comparable with the regression analysis performed in Excel.

CRediT authorship contribution statement

Jeleel O. Agboola: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Elvis M. Chikwati:** Methodology, Investigation, Resources, Writing – review & editing. **Jon Ø. Hansen:** Methodology, Resources, Data curation, Writing – review & editing, Supervision. **Trond M. Kortner:** Methodology, Investigation, Resources, Writing – review & editing. **Liv T. Mydland:** Methodology, Resources, Data curation, Writing – review & editing, Supervision. **Åshild Krogdahl:** Resources, Writing – review & editing. **Brankica Djordjevic:** Methodology, Resources, Writing – review & editing, Supervision. **Johan W. Schrama:** Methodology, Resources, Writing – review & editing, Supervision. **Margareth Øverland:** Methodology, Resources, Data curation, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declared no competing interest.

Acknowledgement

The current experiment was supported by Foods of Norway, a Centre for Research-based Innovation (the Research Council of Norway; grant no. 237841/030).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2022.738214.

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