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Lipoxygenase Enzymes, Oligosaccharides (Raffinose and Stachyose) and 11sA4 and A5 Globulins of Glycinin Present in Soybean Meal Are Not Drivers of Enteritis in Juvenile Atlantic Salmon (*Salmo salar*)

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Abstract: Soybean meal has been largely investigated and commercially used in fish nutrition. However, its inclusion levels have been carefully considered due to the presence of antinutritional factors, which depending on a series of factors might induce gut inflammation damaging the mucosal integrity and causing enteritis. Several strategies including genetic engineering have been applied attempting to reduce or eliminate some of the antinutritional factors. Accordingly, we assessed the intestinal health of juvenile Atlantic salmon fed high levels of speciality soybean genotypes with reduced-to-no content amounts of lipoxygenases, altered glycinin profile and reduced levels of oligosaccharides. No major signs of enteritis, only indication of enteritis progression, was noticed in the soybean meal-based diets illustrated by mild changes in distal intestine morphology. Whereas fish, fed fishmeal control feeds, displayed normal distal intestine integrity. Speciality soybean types did not improve intestinal health of juvenile Atlantic salmon suggesting these antinutrients are not drivers of the intestinal inflammatory process in this species. No additional benefits in terms of production performance or blood biochemistry were noticed in the speciality soybean types compared to the traditional soybean.

Keywords: antinutritional factors; soybean; gut health

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1. Introduction

Soybean meal (SBM) has been largely investigated and commercially used in fish nutrition. Although SBM offers several advantages to the aquafeed industry including worldwide availability, competitive pricing, consistent nutritional quality, and an acceptable amino acid profile it also displays some constraints, mainly associated with antinutritional factors [1,2]. There is a long list of antinutritional factors including saponins, lectins, phytic acid, oligosaccharides, isoflavones, and allergens, among others [1]. These antinutrients are known to impair feed intake, palatability, growth performance, digestive enzymes and in some instances induce gut inflammation damaging the mucosal integrity and causing enteritis [1,3–8]. The degree of physiological impairments induced by dietary SBM is linked to the SBM inclusion level, blends of raw materials, duration of feeding SBM-based feeds, and the species sensitivity to antinutritional factors [3–6,9,10]. Intestinal damage induced by SBM has been reported mostly in distal intestine and liver tissues across several fish species including Atlantic salmon *Salmo salar* [3,4,9,10], Totoaba *Totoaba macdonaldi* [5,6], Seriola spp *Seriola lalandi, Seriola dorsalis* [7,11–13], common Carp *Cyprinus carpio* [14,15], and Largemouth Bass *Micropterus salmoides* [8]. Distal intestine

histology shows changes in the length of the mucosal fold, reduction in the number of supranuclear vacuoles of the enterocytes and thickness of the lamina propria, among other pathohistological modifications [3,5,6,10,12,16,17].

Among the fish species, salmon appears to be one of the most sensitive to the antinutritional factors presented in SBM developing a condition known as soybean meal-induced enteropathy, which exhibits similar changes as those described above [3,4,9,17]. Some salmon species such as pink salmon *Oncorhynchus gorbuscha* appears to be more resistant to antinutritional factors present in dietary SBM than chinook *O. tshawytscha* and Atlantic salmon *S. salar* [18]. In the early 2000's, Buttle et al. [19], suggested the binding mechanism of soybean agglutinin (lectin) to Atlantic salmon intestinal epithelium as a primary contributor to pathological changes in this tissue. Saponins are other top candidates of key antinutritional factors present in soybean meal. A dose-response study reported increasing inflammatory process in Atlantic salmon distal intestine with greater dietary soybean saponins [20].

Several strategies have been applied attempting to reduce or eliminate some of the antinutritional factors in SBM including extrusion, fermentation, pre-processing techniques, and genetic engineering. For example, extrusion with shorter barrel retention times and higher temperatures improved utilization of SBM-rich diets (52% SBM) in salmonids [21]. Another commercial strategy is to increase the protein fraction of SBM through concentration (SPC) or isolation (SPI). A relatively small effort has been done in the genetic space focusing on selecting non-GM SBM for specific genotypes in aquafeeds. Recently, the removal of trypsin inhibitor, lectin and allergen P34/Gly m Bd 30 k from a soybean cultivar failed to alleviate inflammatory processes in Atlantic salmon [9]. Collectively, these studies suggest the challenge in identifying specific antinutritional factors present in soybean responsible for enteritis induction and highlight the complexity of interactions with compounds present in other plant ingredients largely used in aquafeed formulations. As a result, the salmon aquafeed industry has adopted ingredient inclusion limits and more processed soy protein products such as soy protein concentrate and isolate. However, from a cost-effective perspective, finding approaches to minimize or eliminate the soybean-induced enteritis in fish nutrition is worthwhile.

As part of the Australian Soybean improvement program, CSIRO has developed speciality SBM genotypes with reduced-to-no content amounts of lipoxygenases [22], altered glycinin profile [23], and reduced levels of oligosaccharides for human consumption. Accordingly, we assessed the intestinal health of juvenile Atlantic salmon fed high levels of these speciality SBM genotypes.

2. Materials and Methods

2.1. Formulations and Feed Manufacture

Dietary treatments are presented in Table 1. A fish meal-based diet (45%) was used as a control treatment, whereas the experimental diets contained 29% of fishmeal and 30% of SBM from three distinct genotypes of similar genetic background and matched as closely as possible for protein content: standard soybean meal (STD SBM); a soybean genotype homozygous for the gy4 allele conditioning null 11sA4 and 11sA5 globulins of Glycinin, homozygous for the l × 1, l × 2 and l × 3 alleles conditioning absence of seed lipoxygenases and homozygous for the rs2 allele conditioning near absence of seed raffinose and stachyose (TLP SBM); and a soybean genotype homozygous for the gy4 allele conditioning null 11sA4 and 11sA5 globulins of Glycinin (11sA4 null SBM).

All macro ingredients were milled to <750 µm, and well mixed with the remaining dry ingredients, and then extruded through a Baker-Perkins MPV24 twin-screw extruder. Each feed was manufactured using a 1.5 mm Ø die (~2.5 mm Ø pellets) using standard CSIRO Extrusion protocols (Table 2). The pellets were dried at 60 °C for 12 h, after which they were vacuum infused with their specific allocation of oil. All feeds were kept in frozen storage (−20 °C) throughout the feeding trial. All uneaten feed was removed from the collection tank of all treatments 1 h following feeding, and the collected waste feed was then dried to allow calculation of apparent feed intake and feed conversion ratio.

Table 1. Dietary formulation and proximate composition.

Ingredients g kg^{-1}	FM Control	STD SBM	TLP SBM	11sA4 Null SBM
Fishmeal [a]	450.0	290.0	290.0	290.0
Wheat flour	223.0	110.0	93.0	106.0
Wheat gluten	120.0	89.0	106.0	93.0
Blood meal [a]	60.0	60.0	60.0	60.0
Fish oil [a]	70.0	70.0	70.0	70.0
Poultry oil [a]	70.0	70.0	70.0	70.0
Stay-C 35% [b]	1.0	1.0	1.0	1.0
Vitamin mineral premix [c]	6.0	6.0	6.0	6.0
Standard soybean meal (STD SBM) [d]	0.0	300.0	0.0	0.0
Soybean meal triple lipoxygenase plus (TLP SBM) [d]	0.0	0.0	300.0	0.0
Soybean meal 11sA4 null (11sA4 null SBM) [d]	0.0	0.0	0.0	300.0
Methionine [e]	0.0	3.0	3.0	3.0
Taurine [f]	0.0	1.0	1.0	1.0
Proximate composition (g kg^{-1})				
Dry matter	959	960	954	954
Protein	506	492	494	505
Lipid	166	171	179	170
Ash	68	63	62	62
Gross energy (kJ g^{-1})	23.7	24.2	24.3	24.3
Amino acid (g kg^{-1})				
ASP	39	43	45	43
SER	20	22	23	21
GLU	88	87	93	89
GLY	26	24	26	23
HIS	16	15	15	15
ARG	22	25	25	26
THR	19	19	20	19
ALA	25	24	24	24
PRO	29	28	29	28
CYS	5	5	5	5
TYR	14	19	20	19
VAL	23	23	24	23
MET	11	11	11	10
LYS	26	28	28	28
ILE	17	18	19	18
LEU	38	38	39	38
PHE	22	23	25	23
TAU	3	3	3	3

[a] Ridley, Aquafeeds, Queensland, Australia. [b] DSM, Heerlen, Netherlands. [c] Rabar Pty Ltd., Queensland, Australia. [d] CSIRO, Australia. [e] Redox, Queensland, Australia. [f] Bulk Nutrients, Tasmania, Australia.

Table 2. Dietary extrusion parameters.

Parameter	FM Control	STD SBM	TLP SBM	11sA4 Null SBM
RPM	220	220	220	220
Feed (g min^{-1})	72	62	59	53
H2O (g min^{-1})	16	22	20	20
Torque (%)	8	6	6	6
SME (kJ kg^{-1})	99	69	73	80
Zone 1 (Cone)	120	120	120	120
Zone 2	95	95	95	95
Zone 3	85	85	85	85
Zone 4 (Inlet)	70	70	70	70
Die Diameter (mm)	1.5	1.5	1.5	1.5

RPM = revolutions per minute. SME = specific mechanical energy.

2.2. Experimental System and Feeding Trial

Sixteen tanks with 300 L at Bribie Island Research Centre (BIRC, Queensland, Australia) were used in this experiment with ~3 L/min flow of continuously aerated, recirculating freshwater at 15 °C ± 0.16 (mean ± SEM). Photoperiod was set at 12L:12D. Twenty-five salmon of 37.3 g ± 0.42 (mean ± SD) were randomly allocated to each tank. Fish were fed to apparent satiation twice daily for 56 days. This research was approved by the CSIRO Queensland Animal Ethics Committee—AEC Number: 2018-44.

2.3. Analytical Methods

Chemical analyses were carried out to confirm proximate composition of dietary treatments, and main ingredients (Tables 1 and 3) [24]. Samples were dried at 105 °C for 12 h to determine gravimetrically dry matter and followed by ashing at 550 °C for 12 h. Total nitrogen was measured by combustion (CHNS auto-analyzer, Leco Corp., St. Joseph, MI, USA) and crude protein was calculated by nitrogen conversion (%N × 6.25). Total lipid was gravimetrically determined via Folch extraction [25]. Finally, gross energy was measured via an adiabatic bomb calorimeter (Parr 6200, Par Instrument Company, Moline, IL, USA).

Table 3. Key ingredients proximate and amino acid composition (g kg^{-1}).

	Fishmeal	STD SBM	TLP SBM	11sA4 null SBM
Proximate composition (g kg^{-1})				
Dry matter	911	949	918	926
Protein	719	495	521	462
Lipid	117	119	116	118
Ash	126	54	52	58
Amino acid (g kg^{-1})				
ASP	67.7	46.0	48.5	48.7
SER	29.8	19.6	20.8	21.6
GLU	97.4	69.5	73.4	69.1
GLY	45.2	16.0	16.3	16.7
HIS	16.2	9.1	10.2	9.3
ARG	39.7	24.6	26.0	25.0
THR	30.8	14.3	15.2	16.3
ALA	40.7	16.3	17.5	17.6
PRO	31.6	19.3	20.5	19.7
CYS	6.5	5.5	5.2	5.3
TYR	24.4	11.7	13.9	13.2
VAL	34.4	16.7	17.5	16.7
MET	22.8	2.8	3.0	3.1
LYS	58.9	21.1	23.7	23.4
ILE	29.0	15.8	16.7	17.5
LEU	52.6	27.6	30.4	29.8
PHE	30.1	18.2	18.8	20.4
TAU	7.8	ND	ND	ND

ND = not detected.

Total amino acid (TAA) quantification was performed by mass detection following high performance reverse-phase liquid chromatography with pre-column derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl (AQC). Analyses were undertaken on a Shimadzu Nexera X2 series UHPLC (Shimadzu Corporation, Kyoto, Japan), coupled with a Shimadzu 8030 Mass Spectrometer using a modification of the Waters AccQ-tag system (Waters Corporation, Milford, MA). Bovine serum albumin (BSA) (ICN), milk powder (NIST SRM 1549a) and a well characterized aquafeed were used as reference materials. Samples or reference materials were hydrolyzed using phenolic 6N HCl at 112 °C according to the protocol for complex feed samples outlined by Waters Corp. (1996).

2.4. Production Parameters

The following production parameters were calculated for the 28 and 56 days of the feeding trial; feed conversion ratio (FCR), hepatosomatic index (HSI), and condition factor (K). No differences in production performance parameters were observed among the dietary treatments throughout the feeding trial.

$$FCR = \frac{FI}{WG} \quad (1)$$

$$HSI = \frac{W_{Liver}}{W_{Fish}} * 100 \quad (2)$$

$$K = 100 * \frac{W_{Fish}}{{L_{Fork}}^3} \quad (3)$$

where FCR = feed conversion ratio, FI = apparent feed intake (g), WG = weight gain (g), HSI = hepatosomatic index, W_{Liver} = wet weight of liver (g), W_{Fish} = fish whole weight (g), K = condition factor, L_{Fork} = fish fork length (mm)

2.5. Histology

Upon completion of the feeding trial, three fish per tank were randomly selected and euthanized via AQIS overdose (~70 ppm). The distal intestine, i.e., last 2 cm section of the intestine, of each fish was sampled and stored in Davidson's solution for 24 h, and then transferred to 70% ethanol until further analysis. For each fish sample, the distal intestine was divided into three sections and gradually dehydrated in ethanol, clarified in benzene and embedded in paraffin. As a result, a complete intestinal annular ring from each fish (nine per treatment) was cut into three sections (n = 36) and mounted onto individual glass slides for histological assessment.

Transversal sections of 3 µm were cut using a rotary microtome (Leica RM2245), stained with hematoxylin and eosin (H&E). The slides were blind examined after randomization, using the Zeiss Axiocam light microscope. The pictures were taken using the camera function on the Zeiss Axiocam microscope and then processed and analyzed using Zeiss Zen Light (Version 3.1) image analysis software.

To assess the degree of intestinal damage, a semiquantitative scoring system was used. In this scoring system, three parameters were quantified independently based on [10]: (1) the appearance and length of mucosal folds (MF); (2) the degree of widening of the lamina propria (LP); (3) the abundance of goblet cells (GC). For each of these parameters a score was given on a scale of 1 to 5. An increasing score value represents a greater degree of intestinal damage.

2.6. Statistical Analysis

All data were analyzed for normality and equality by Levene's tests, respectively, and then subjected to one-way ANOVA (analysis of variance, NCSS 12.0). When significant effects were identified, the post-hoc Tukey's HSD pairwise comparison test was used to determine difference among means with a significance level of 0.05.

3. Results

Juvenile Atlantic salmon fed the fishmeal control dietary treatment displayed normal distal intestine integrity (Figure 1A). There were no major signs of enteritis, only an indication of enteritis progression was noticed in the SBM-based diets illustrated by mild changes in distal intestine morphology, including reduced number and length of mucosal folds, enlargement of the apical zone of mucosal folds, thickening of lamina propria, and changes in abundance of goblet cells (Figure 1B–D). The removal of lipoxygenases, 11sA4 and A5 globulins of glycinin, and oligosaccharides from SBM failed to prevent morphological changes linked to the inflammatory process. Histology scoring demonstrated statistically higher scores of goblet cells and lamina propria in the SBM treatments than the fishmeal control

(Table 4). Scoring of mucosal folds was higher in the speciality SBM groups compared to the control group.

Blood biochemistry was largely unaffected by the dietary treatments. Out of the sixteen parameters analyzed, only albumin and protein were statistically higher in TLP SBM than STD SBM (Table 5).

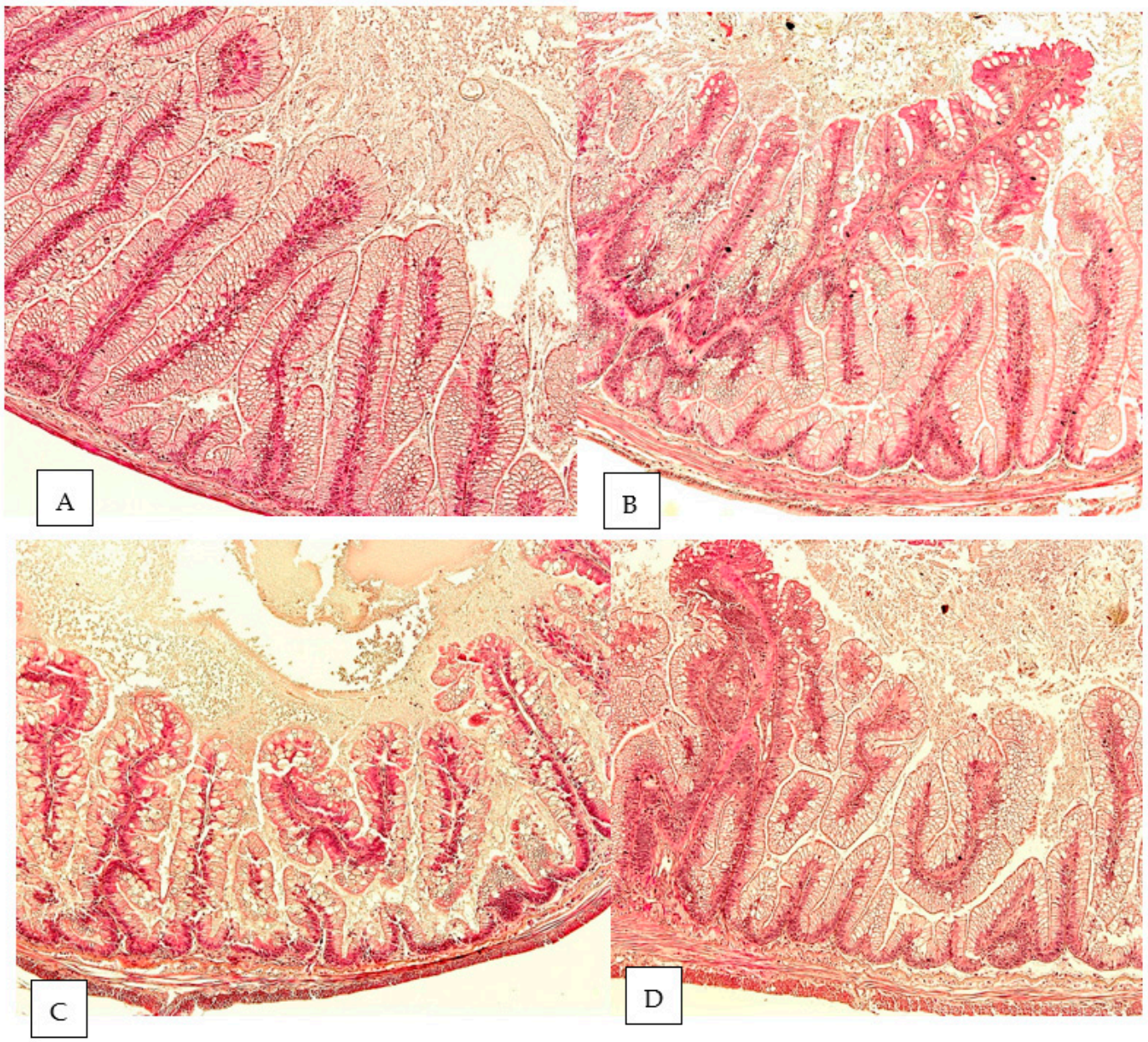

Figure 1. Light microscopic images illustrating morphological changes in the distal intestine associated with inflammatory process in Atlantic salmon fed different soybean meal types for 56 days ((**A**)—control fishmeal, (**B**)—STD SBM—standard soybean meal, (**C**)—TLP SBM – triple null soybean meal absent of seed lipoxygenases and homozygous for the rs2 allele conditioning and near absent of seed raffinose and stachyose, and (**D**)—11sA4 null SBM—soybean meal conditioning null 11sA4 and 11sA5 globulins of Glycinin).

Survival was high across the dietary treatments (97–100%; Table 6). No differences in production performance parameters, including CV, final weight, FCR, K, and HSI were noticed between the fishmeal control group and the SBM-based groups at day 28 and day of feeding trial.

Table 4. Semiquantitative scoring system (mean ± SD) of three parameters based on [10]: (1) the appearance and length of mucosal folds (MF); (2) the degree of widening of the lamina propria (LP); (3) the abundance of goblet cells (GC). For each of these parameters a score was given on a scale of 1 to 5. An increasing score value represents a greater degree of intestinal damage.

Scoring	FM Control	STD SBM	TLP SBM	11sA4 Null SBM	*p*-Value
Goblet cells	1.9 ± 0.3 b	3.2 ± 0.4 a	3.4 ± 0.5 a	3.7 ± 0.5 a	<0.001
Lamina propria	1.8 ± 0.4 b	3.2 ± 0.4 a	3.5 ± 0.5 a	3.6 ± 0.5 a	<0.001
Mucosal fold	1.9 ± 0.5 b	2.7 ± 0.7 ab	2.9 ± 0.6 a	3.3 ± 0.7 a	0.001

Table 5. Sixteen blood chemistry parameters of juvenile Atlantic salmon fed a fishmeal control diet (FM control) and different soybean meal types-based diets (STD SBM—standard soybean meal, C—TLP SBM—triple null soybean meal absent of seed lipoxygenases and homozygous for the rs2 allele conditioning and near absent of seed raffinose and stachyose, and D—11sA4 null SBM—soybean meal conditioning null 11sA4 and 11sA5 globulins of Glycinin) for 56 days.

Blood Chemistry Parameters	FM Control	STD SBM	TLP SBM	11sA4 Null SBM	*p*-Value
Albumin (g L^{-1})	20.3 ± 0.6 ab	20.0 ± 1.0 b	22.3 ± 1.1 a	20.7 ± 0.6 ab	0.044
Alkaline phosphatase (U L^{-1})	21.3 ± 6.1	18.7 ± 4.7	24.3 ± 2.1	15.7 ± 5.7	0.242
Anion gap (mmol L^{-1})	59.3 ± 3.5	56.0 1.0	53.0 ± 2.6	58.0 ± 8.7	0.463
AST (U L^{-1})	301.3 ± 53.6	301.7 ± 28.7	359.5 ± 2.1	315.7 ± 31.5	0.361
Bicarbonate (mmol L^{-1})	6.3 ± 0.6	5.3 ± 1.1	5.7 ± 0.6	6.7 ± 2.1	0.577
Chloride (mmol L^{-1})	123.3 ± 8.7	120.3 ± 1.5	125.3 ± 5.1	120.0 ± 1.0	0.561
Cholesterol (mmol L^{-1})	13.2 ± 2.9	9.8 ± 1.1	12.4 ± 0.9	11.9 ± 1.4	0.236
Creatine kinase (U L^{-1})	7286 ±1425	7992 ± 1198	7446 ± 1719	8089 ± 1674	0.890
Globulin (g L^{-1})	18.7 ± 2.1	18.7 ± 1.1	21.0 ± 1.0	20.3 ± 0.6	0.142
Glucose (mmol L^{-1})	5.2 ± 0.8	4.2 ± 0.3	4.2 ± 0.2	4.0 ± 0.4	0.055
Phosphate (mmol L^{-1})	3.1 ± 0.3	2.7 ± 0.3	2.6 ± 0.3	2.5 ± 0.3	0.103
Potassium (μm $mmol^{-1}$)	4.2 ± 0.6	4.1 ±0.6	4.1 ± 0.5	4.0 ± 0.4	0.963
Protein (g L^{-1})	39.0 ± 2.6 ab	38.7 ± 1.5 b	43.3 ± 1.5 a	41.0 ± 1.0 ab	0.042
Ratio	1.1 ± 0.1	1.1 ± 0.1	1.0 ± 0.1	1.0 ± 0.0	0.344
Sodium (mmol L^{-1})	184.7 ± 11.6	177.7 ± 0.6	179.7 ± 3.0	180.3 ± 5.1	0.683
Triglyceride (mmol L^{-1})	2.9 ± 0.4	3.5 ± 0.5	3.1 ± 0.8	3.5 ± 0.3	0.377

Table 6. Production performance at 28 and 56 days of juvenile Atlantic salmon fed a fishmeal control diet (FM control) and different soybean meal types-based diets (STD SBM—standard soybean meal, C—TLP SBM—triple null soybean meal absent of seed lipoxygenases and homozygous for the rs2 allele conditioning and near absent of seed raffinose and stachyose, and D—11sA4 null SBM—soybean meal conditioning null 11sA4 and 11sA5 globulins of Glycinin).

Production Performance	FM Control	STD SBM	TLP SBM	11sA4 Null SBM
0 day				
Initial weight (g)	37.3 ± 0.0	37.4 ± 0.0	37.3 ± 0.1	37.3 ± 0.0
Initial CV (%)	12.1 ± 0.5	15.7 ± 3.4	11.1 ± 0.8	11.5 ± 0.5
28 days				
Survival (%)	99 ± 0.0	97 ± 0.0	99 ± 0.0	100
CV (%)	12.3 ± 1.3	13.0 ± 0.5	12.1 ± 0.7	13.1 ± 0.9
Mid weight (g)	49.6 ± 0.6	52.1 ± 0.7	49.8 ± 0.7	51.4 ± 0.6
FCR	1.0 ± 0.0	1.0 ± 0.0	1.1 ± 0.1	1.0 ± 0.0
K	1.3 ± 0.07	1.4 ± 0.09	1.5 ± 0.09	1.4 ± 0.01
HSI	1.8 ± 0.16	1.7 ± 0.17	1.6 ± 0.19	1.9 ± 0.40
56 days				
Survival (%)	98.5 ± 1.5	97.0 ± 1.5	98.5 ± 1.5	100
CV (%)	15.2 ± 1.5	15.5 ± 0.8	14.6 ± 1.0	15.2 ± 0.7
Final weight (g)	59.4 ± 1.5	66.1 ± 1.2	65.1 ± 2.1	64.4 ± 0.7
FCR	1.0 ± 0.0	0.9 ± 0.0	1.0 ± 0.0	0.9 ± 0.0
K	1.5 ± 0.17	1.4 ± 0.02	1.4 ± 0.04	1.3 ± 0.03
HSI	1.6 ± 0.19	1.4 ± 0.11	1.4 ± 0.07	1.6 ± 0.25

* CV = coefficient of variance. FCR = feed conversion ratio. K = condition factor. HSI = hepatosomatic index.

4. Discussion

Soybean meal-induced enteritis was not observed in juvenile Atlantic salmon, despite the high dietary SBM inclusion level of 30%. Nevertheless, intestinal health impairment was characterized by mild changes in distal intestine morphology (i.e., changes in length, shape, and number of mucosal folds, changes in thickness of lamina propria and changes in number of goblet cells), indicating enteritis progression. Speciality soybean types lacking 11sA4 and A5 globulins of Glycinin, lipoxygenases and oligosaccharides did not further reduce the intestinal inflammatory process compared to STD SBM, although inflammation was mild overall. Similarly, a recent thorough study removing three proteinaceous antinutrients, namely trypsin inhibitor, lectin and the allergen P34/Gly m Bd 30 k, from soybean meal did not mitigate enteritis in Atlantic salmon [9]. The authors suggested extrusion technology inactivated these compounds during feed manufacturing. Although soybean agglutinin, a type of lectin, has been reported to bind to Atlantic salmon intestinal epithelium contributing to pathological changes in gut health, the study did not describe the feed manufacturing procedure [19]. It is unlikely that extrusion technology inactivated the compounds investigated in the present study due to their chemical compositions and exposure to lower mechanical energy during extrusion conditions compared to those reported by [9] (specific mechanical energy69–80 vs. 1872–3060 kJ/kg, respectively).

Soybean-induced enteritis in Atlantic salmon has been widely reported. However, the main drivers of enteritis and their positive and negative interactions with other compounds present in feed formulations remain unclear. A summary of seventeen studies around soybean-induced enteritis in Atlantic salmon is provided in Tables 7–9. Due to the complexity of the matter, there is a wide breadth of experimental designs with different stocking densities, fish size, duration, water temperature, salinity, feeding ration and experimental systems. For example, most literature is focused on smaller fish ranging from 41–442 g and some on larger fish 500–927 g. Similarly, soybean type and pre-processing and dietary composition varies throughout the literature. Soybeans are from different parts of the globe and are under several pre-processing conditions such as dehulled, toasted, defatted, and solvent-extracted, included at various levels 8–34% in feeds containing crude protein of 35–47%, total lipids 23–31%, and gross energy 15–24 MJ/kg. Regardless of this variation in the experimental design, most histology analyses are focused on the distal intestine describing morphological changes of standardized parameters including mucosal folds, supranuclear vacuoles, lamina propria, eosinophilic granulocytes, sub-epithelial mucosa, and connective tissue, and continue to be one of the most reliable tools to detect this histopathological condition. Other parameters such as reduced feed intake and growth commonly used as indicators of enteritis are not as reliable with contradicting findings throughout the literature. Most studies highlight the inflammatory process in the intestine of Atlantic salmon fed soybean-based feeds leading to enteritis; however, there are exceptions where only minor to mild intestinal damage were reported, including the present findings. Collectively, these studies illustrate the challenge and high complexity in tackling this issue in fish nutrition. Understandably, the aquafeed industry does not prioritize this research topic and adopt a more conservative approach of using moderate inclusion levels of plant ingredients avoiding any potential intestinal health impairments caused by antinutritional factors. It is likely extremely difficult to identify the key antinutritional factors in the major plant ingredients and their interactions with compounds from other components of the formulations having the effect. Interestingly, the intestinal health research of fish fed soybean-based feeds has gone beyond the traditional highly carnivorous species reaching a wide range of species from different feed, salinity and water temperature preferences, including for example common carp [14,15], grass carp [26], kingfish [7,12], totoaba [5,6], and largemouth bass [8].

Table 7. Summary of the experimental design of soybean meal-induced enteritis studies with Atlantic salmon (*Salmo salar*).

Experimental Design											
IBW (g)	**SGR (% BW)**	**FCR**	**Tank Volume (L)**	**Stocking Density (Fish/Tank)**	**Duration (Days)**	**Salinity**	**Temp. (°C)**	**Feeding**	**System**	**Refeed to Control**	**Ref.**
927			2000	25	21	FW	12–13		Semi-RAS		[3]
280	0.94–1.05	0.81–0.94	450	54	60	SW	8.4				[4]
41		0.7	250	55	56	FW	14		RAS		[9]
300			400	50	20	SW	8 and 12	120%	RAS		[10]
550			27,000		120	3.2	5	120%	Net pens		[16]
550			27,000	80	42	SW	10.8 and 8.2	120%	Net pens	Yes	[17]
535 | 140 | 166 *			1900 | 650 | 850	50	21	SW	9	1% BW	Flow-through		[18]
54			1000	20	7	FW	15				[19]
442			1000	22		SW	12–9		RAS		[20]
900			125 000	300	300	SW	8.2		Net pens		[27]
213 | 202			400 | 100	30 | 20	62 | 44	SW	8.3 | 9	120%	RAS		[28]
396			400	25	28	SW	12	110%	RAS		[29]
500–600			1000	25	21	SW	9		RAS		[30]
500–600			1000	25–30	21	SW	9		RAS		[31]
80			400	70	53	SW	8.6	120%	Flow-through		[32]
60	1.36–1.47	0.71–0.74	500	75	93	SW	10.9	120%	Flow-through		[33]
207	0.7–0.9	0.92–1.17	600	50	84	FW	7	115%	Flow-through		[34]

IBW = initial body weight in g; SGR = specific growth rates in percentage of body weight; FCR = feed conversion ratio; Temp. = temperature; Ref. = references; RAS = recirculating aquaculture systems; SW = seawater; FW = freshwater; * numbers represent the following species Atlantic salmon, Chinook salmon and pink salmon.

Table 8. Summary of soybean types and dietary treatments of soybean meal-induced enteritis studies with Atlantic salmon (*Salmo salar*).

SBM Information			Dietary Treatments							
Location	**Type**	**CP (%)**	**Pellet Type**	**CP (%)**	**TL (%)**	**GE (MJ /kg)**	**SBM Type/Inclusion (%)**	**SBM Type/Inclusion (%)**	**SBO Inclusion (%)**	**Ref.**
Norway	With hulls, toasted and extracted		Extruded	35	28		SBM 30			[3]
Norway	Solvent-extracted, toasted		Extruded	40–44	22–24	22–23	SBM—8, 12, 15, 19, 27			[4]
USA	Triple null and standard		Extruded	40	26	24	SBM 25	Triple null SBM 27		[9]
Netherlands	Extracted		Extruded	45	30		SBM 20			[10]
				43	20	15	FFSBM 30	SPC 28	0–10	[16]
							SBM 33		0–8.5	[17]
			Pelleted	37	23		SBM 20			[18]
	Soybean agglutinin						Soybean agglutinin 3.5			[19]
	Soya saponins			42–44	29–30	24	Soy saponins—0, 2, 4, 6, 10			[20]
	Dehulled solvent extracted SBM		Extruded	40	22		SBM 17 and 34			[27]
Norway	Defatted		Extruded				DSBM 20	molasses		[28]
	NA, EU, SA	44–49	Extruded	42	25	23	20			[29]
	Extracted			43	28	24	SBM 20			[30]
	Extracted			43	28	24	SBM 20			[31]
Norway	Defatted		Extruded	47	26	23	SBM 25 + soy saponins 0.17	Lupins + soy saponin concentrate 0.17 + soy saponins 0.11		[32]
Switzerland			Extruded	46	25	24	SBM 20	Pre-processed SBM 20		[33]
	Dehulled defatted SBM		Pelleted	40	31	24	SBM 30			[34]

Ref. = references; NA = North America, EU = Europe, SA = South America; FFSBM = full-fat soybean meal; SBM = soybean meal; DSBM—defatted soybean meal; SPC = soy protein concentrate; SBO = soybean oil.

Table 9. Summary of the main findings and parameters investigated of soybean meal-induced enteritis studies with Atlantic salmon (*Salmo salar*).

Growth Impairment	Feed Intake	Level of DI Inflammation	Enzymes	Time Sampling (Days)	Tissues Analyzed	Parameters	Ref.
SBM yes SPC no		SBM +++			PI and DI	MSA/LSC, ESA/LSC, LPSA/LSC, ESA/LPSA, GC/E, LM	[16]
Yes		+++		2, 7, 14 and 21	DI	MF	[17]
		++	5′ N, Mg-ATPase, ALP, ACP, NSE, LAP, AAP	21	MI and DI		[3]
Low SBM no High SBM yes	No changes	+, ++ and +++	ALP, LAP, maltase, isomaltase, lactase and sucrase		MI and DI	MF, SNV, LP, leycocyte	[4]
		+, ++ and +++			DI	MF, SNV, LP, CT	[28]
		++ and +++			DI	MF, GC, LP SNV, EG, SM	[29]
		++ and +++			DI	MF, GC, LP SNV, EG, SM	[10]
		+, ++, and +++	Pancreatic (trypsin, chymotrypsin, elastase, and lipase), chyme (LAP), brush border membrane (LAP and maltase)	0, 1, 2, 3, 5, 7, 10, 14, 17, and 21	PI, MI and DI		[30]
		+, ++, and +++		1, 2, 3, 5 and 7	DI		[31]
Low saponins no Mid-high saponins yes	Low saponins no Mid-high saponins yes	+, ++, and +++	Trypsin activity, bile acids, brush border membrane enzyme activity (LAP)		PI and DI	MF, LP, enterocyte vacuolization, GC, nucleos position within the enterocytes	[20]
		+++			DI	MF, SNV, LP, CT	[32]
		+, ++, and +++		7, 14 and 21	DI	Inflammation score, SM and microbiome	[18]
No	No changes				DI	MF, GC, LP, SNV, EG, SM	[33]
No	No changes	+ and ++	Brush border LAP, trypsin activity		DI	MF, SNV, SM, LP, microbiota, gene expression	[9]
		+ and ++			PI, MI and DI	MF, SNV, LP, CT,	[19]
SBM 17 no SBM 34 yes						Body composition and blood biochemistry	[27]
Yes	No changes	++ and +++			DI	MF, SNV, LP, CT,	[34]

Ref. = references; Level of enteritis: + mild, ++ moderate, and +++ high; 5′ N = 5′-nucleotidase; Mg-ATPase = Mg^{2+} dependent adenosine triphosphatase; ALP = alkaline phosphatase; ACP = acid phosphatase; NSE = non-specific esterase; LAP = leucine aminopeptidase, AAP = alanine aminopeptidase; PI = proximal intestine; MI = mid-intestine; DI = distal intestine; MF = mucosal fold; MSA = mucosal surface area; LSC = length mucosal stratum compactum; ESA = epithelial surface area; LPSA = lamina propria surface area; GC = goblet cells; E = 100 um epithelium; LM = length microvilli; SNV = supranuclear vacuoles; LP = lamina propria; EG = eosinophilic granulocytes; CT = connective tissue; SM = sub-epithelial mucosa.

This is not the first study where animal growth was not impaired by dietary SBM displaying equivalent performance as the fishmeal SBM-free feeds. As Krogdahl et al. [27] has demonstrated, no detrimental growth effects in feeding 20% SBM to juvenile Atlantic salmon are present; however, more aggressive inclusion levels of 40% reduced growth are evident as compared to the fishmeal control feeds. Indeed, this pattern has been described with other fish species, including California yellowtail *Seriola dorsalis* [7]. Removal of certain antinutritional factors from SBM also did not affect Atlantic salmon growth response compared to the standard SBM [9]. Conservative inclusion levels of standard SBM at the expense of fishmeal appears to be suitable as long as no intestinal health impairment is noticed.

High levels of dietary SBM resulted in mild intestinal inflammation indicating enteritis progression. Speciality soybean types lacking lipoxygenases, altered glycinin profile and oligosaccharides did not improve intestinal health of juvenile Atlantic salmon suggesting these antinutrients are not drivers of the intestinal inflammatory process in this species. No additional benefits in terms of production performance or blood biochemistry were noticed in the speciality soybean types compared to the traditional soybean. The present findings contribute to and summarize the growing literature in the antinutrient space, providing more insights to future research.

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References

1. Gatlin, D.M.; Barrows, F.T.; Brown, P.; Dabrowski, K.; Gaylord, T.G.; Hardy, R.W.; Herman, E.; Hu, G.; Krogdahl, A.; Nelson, R.; et al. Expanding the utilization of sustainable plant products in aquafeeds: A review. *Aquac. Res.* **2007**, *38*, 551–579. [CrossRef]
2. NRC (National Research Council). *Nutrient Requirements of Fish and Shrimp*; Natlional Academic Press: Cambridge, MA, USA, 2011. [CrossRef]
3. Bakke-McKellep, A.M.; Press, C.M.; Baeverfjord, G.; Krogdahl, A.; Landsverk, T. Changes in immune and enzyme histochemical phenotypes of cells in the intestinal mucosa of Atlantic salmon, *Salmo salar* L., with soybean meal-induced enteritis. *J. Fish Dis.* **2000**, *23*, 115–127. [CrossRef]
4. Krogdahl, A.; Bakke-McKellep, A.M.; Baeverfjord, G. Effects of graded levels of standard soybean meal on intestinal structure, mucosal enzyme activities, and pancreatic responses in Atlantic salmon (*Salmo salar* L.). *Aquac. Nutr.* **2003**, *9*, 361–371. [CrossRef]
5. Fuentes-Quesada, J.P.; Viana, M.T.; Rombenso, A.N.; Guerrero-Renteria, Y.; Nomura-Solis, M.; Gomez-Calle, V.; Lazo, J.P.; Mata-Sotres, J.A. Enteritis induction by soybean meal in *Totoaba macdonaldi* diets: Effects on growth performance, digestive capacity, immune response and distal intestine integrity. *Aquaculture* **2018**, *495*, 78–89. [CrossRef]
6. Fuentes-Quesada, J.P.; Cornejo-Granados, F.; Mata-Sotres, J.A.; Ochoa-Romo, J.P.; Rombenso, A.N.; Gurerrero-Renteria, Y.; Lazo, J.P.; Pohlenz, C.; Ochoa-Leyva, A.; Viana, M.T. Prebiotic agavin in juvenile tototaba, *Totoaba macdonaldi* diets, to relieve soybean meal-induced enteritis: Growth performance, gut histology and microbiota. *Aquac. Nutr.* **2020**. [CrossRef]
7. Viana, M.T.; Rombenso, A.N.; Del Rio-Zaragoza, O.B.; Nomura, M.; Diaz-Arguello, R.; Mata-Sotres, J.A. Intestinal impairment of the California yellowtail, *Seriola dorsalis*, using soybean meal in the diet. *Aquaculture* **2019**, *513*, 734443. [CrossRef]

8. Rossi, W., Jr.; Allen, K.M.; Habte-Tsion, H.-M.; Meesala, K.-M. Supplementation of glycine, prebiotic, and nucleotides in soybean meal-based diets for largemouth bass (*Micropterus salmoides*): Effects on production performance, whole-body nutrient composition and retention, and intestinal histopathology. *Aquaculture* **2021**, *532*, 736031. [CrossRef]
9. Krogdahl, A.; Kortner, T.M.; Jaramillo-Torres, A.; Gamil, A.A.A.; Chikwati, E.; Li, Y.; Schmidt, M.; Herman, E.; Hymowitz, T.; Teimouri, S.; et al. Removal of three proteinaceous antinutrients from soybean does not mitigate soybean-induced enteritis in Atlantic salmon (*Salmo salar*, L.). *Aquaculture* **2020**, *514*, 734495. [CrossRef]
10. Uran, P.A.; Schrama, J.W.; Rombout, J.H.W.M.; Obach, A.; Jensen, L.; Koppe, W.; Verreth, J.A.J. Soybean meal-induced enteritis in Atlantic salmon (*Salmo salar* L.) at different temperatures. *Aquac. Nutr.* **2008**, *14*, 324–330. [CrossRef]
11. Mata-Sotres, J.A.; Viana, M.T.; Tinajero, A.; Del Rio-Zaragoza, O.; Skrzynska, A. Estrés nutricional en peces y los nuevos desafíos de la nutrición. In *Revista Bio Ciencias–Coloquio de Nutrigenomica y Biotecnología Acuicola 2020*; Universidad Autónoma de Nayarit: Tepic, Mexico, 2021; p. e1132. [CrossRef]
12. Stone, D.A.J.; Bellgrove, E.J.; Forder, R.E.A.; Howarth, G.S.; Bansemer, M.A. Inducing subacute enteritis in yellowtail kingfish *Seriola lalandi*: The effect of dietary inclusion of soybean meal and grape seed extract on hindgut morphology and inflammation. *N. Am. J. Aquac.* **2018**, *80*, 59–68. [CrossRef]
13. Legrand, T.P.R.A.; Wynne, J.W.; Weyrich, L.S.; Oxley, A.P.A. Investigation both mucosal immunity and microbiota in response to gut enteritis in yellowtail kingfish. *Microorganisms* **2020**, *8*, 1267. [CrossRef]
14. Uran, P.A.; Goncalvez, A.A.; Taverne-Thiele, J.J.; Schrama, J.W.; Verreth, J.A.J.; Rombout, J.H.W.M. Soybean meal induces intestinal inflammation in common carp (*Cyprinus carpio* L.). *Fish Shellfish Immunol.* **2008**, *25*, 751–760. [CrossRef] [PubMed]
15. Van Der Marel, M.; Propsting, M.J.; Battermann, F.; Jung-Schroers, V.; Hubner, A.; Rombout, J.H.W.M.; Steinhagen, D. Differences between intestinal segments and soybean meal induced changes in intestinal mucus composition of common carp *Cyprinus carpio* L. *Aquac. Nutr.* **2014**, *20*, 12–24. [CrossRef]
16. Van Den Ingh, T.S.G.A.M.; Krogdahl, A.; Olli, J.J.; Hendriks, H.G.C.J.M.; Koninkx, J.G.J.F. Effects of soybean-containing diets on the proximal and distal intestine in Atlantic salmon (*Salmo salar*) L. a morphological study. *Aquaculture* **1991**, *94*, 297–305. [CrossRef]
17. Baeverfjord, G.; Krogdahl, A. Development and regression of soybean meal induced enteritis in Atlantic salmon, *Salmo salar* L.; distal intestine: A comparison with the intestines of fasted fish. *J. Fish Dis.* **1996**, *19*, 375–387. [CrossRef]
18. Booman, M.; Forster, I.; Vederas, J.C.; Groman, D.B.; Jones, S.R.M. Soybean meal-induced enteritis in Atlantic salmon (*Salmo salar*) and Chinook salmon (*Oncorhynchus tshawystscha*) but not in pink salmon (*O. gorbuscha*). *Aquaculture* **2018**, *483*, 238–243. [CrossRef]
19. Buttle, L.G.; Burrells, A.C.; Good, J.E.; Williams, P.D.; Southgate, P.J.; Burrells, C. The binding of soybean agglutinin (SBA) to the intestinal epithelium of Atlantic salmon, *Salmo salar* and Rainbow trout, *Oncorhynchus mykiss*, fed high levels of soybean meal. *Vet. Immunol. Immunopathol.* **2001**, *80*, 237–244. [CrossRef]
20. Krogdahl, A.; Gajardo, K.; Kortner, T.M.; Penn, M.; Gu, M.; Berge, G.M.; Bakke, A.M. Soya saponins induce enteritis in Atlantic salmon (*Salmo salar* L.). *J. Agric. Food Chem.* **2015**, *63*, 3887–3902. [CrossRef] [PubMed]
21. Barrows, F.T.; Stone, D.A.J.; Hardy, R.W. The effects of extrusion conditions on the nutritional value of soybean meal for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **2007**, *265*, 244–252. [CrossRef]
22. Yang, A.; Smyth, H.; Chalija, M.; James, A. Sensory quality of soymilk and tofu from soybeans lacking lipoxygenases. *Food Sci. Nutr.* **2016**, *4*, 207–215. [CrossRef]
23. James, A.T.; Yang, A. Interactions of protein content and globulin subunit composition of soybean proteins in relation to tofu gel properties. *Food Chem.* **2016**, *194*, 284–289. [CrossRef]
24. AOAC. *Official Methods of Analysis*; Association of Analytical Chemists: Arlington, VA, USA, 2005.
25. Folch, J.; Less, M.; Sloane-Stanley, G.H. A simple method for the isolation and purification of total lipid from animal tissues. *J. Biol. Chem.* **1957**, *276*, 497–507. [CrossRef]
26. Feng, L.; Ni, P.-J.; Jiang, W.-D.; Wu, P.; Liu, Y.; Jiang, J.; Kuang, S.-Y.; Tang, L.; Tang, W.-N.; Zhang, Y.-A.; et al. Decreased enteritis resistance ability by dietary low or excess levels of lipids through impairing the intestinal physical and immune barriers function of young grass carp (*Ctenopharyngodon Idella*). *Fish Shellfish Immunol.* **2017**, *67*, 493–512. [CrossRef] [PubMed]
27. Olli, J.J.; Krogdahl, A.; Vabano, A. Dehulled solvent-extracted soybean meal as a protein source in diets for Atlantic salmon, *Salmo salar* L. *Aquac. Res.* **1995**, *26*, 167–174. [CrossRef]
28. Knudsen, D.; Uran, P.; Arnous, A.; Koppe, W.; Frokler, H. Saponing-containing subfractions of soybean molasses induce enteritis in the distal intestine of Atlantic salmon. *J. Agric. Food Chem.* **2007**, *55*, 2261–2267. [CrossRef] [PubMed]
29. Uran, P.A.; Schrama, J.W.; Jaafari, S.; Baardsen, G.; Rombout, J.H.W.M.; Koppe, W.; Verreth, J.A.J. Variation in commercial sources of soybean meal influences the severity of enteritis in Atlantic salmon (*Salmo salar* L.). *Aquac. Nutr.* **2009**, *15*, 492–499. [CrossRef]
30. Chikwati, E.M.; Sahlmann, C.; Holm, H.; Penn, M.H.; Krogdahl, A.; Bakke, A.M. Alterations in digestive enzyme activities during the development of diet-induced enteritis in Atlantic salmon, *Salmo salar* L. *Aquaculture* **2013**, *402–403*, 28–37. [CrossRef]
31. Sahlmann, C.; Sutherland, B.J.G.; Kortner, T.M.; Koop, B.F.; Krogdahl, A.; Bakke, A.M. Early response of gene expression in the distal intestine of Atlantic salmon (*Salmo salar* L.) during the development of soybean meal induced enteritis. *Fish Shellfish Immunol.* **2013**, *34*, 599–609. [CrossRef]
32. Silva, P.F.; McGurk, C.; Knudsen, D.L.; Adams, A.; Thompson, K.D.; Bron, J.E. Histological evaluation of soya bean-induced enteritis in Atlantic salmon (*Salmo salar* L.): Quantitative image analysis vs. semi-quantitative visual scoring. *Aquaculture* **2015**, *445*, 42–56. [CrossRef]

33. Jacobsen, H.J.; Samuelsen, T.A.; Girons, A.; Kousoulaki, K. Different enzyme incorporation strategies in Atlantic salmon diet containing soybean meal: Effects on feed quality, fish performance, nutrient digestibility and distal intestinal morphology. *Aquaculture* **2018**, *491*, 302–309. [CrossRef]
34. Refstie, S.; Korsoen, O.J.; Storebakken, T.; Baeverfjord, G.; Lein, I.; Roem, A.J. Differing nutritional responses to dietary soybean meal in rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon *(Salmo salar*). *Aquaculture* **2000**, *190*, 49–63. [CrossRef]