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Bacteria Grown on Natural Gas Prevent Soybean Meal-Induced Enteritis in Atlantic Salmon[1–3]

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Abstract

Dietary inclusion of solvent extracted soybean meal (SBM) is associated with inflammation in the distal intestine of salmonid fish, commonly referred to as SBM-induced enteritis. The enteritis is linked to alcohol soluble components in SBM, but the mechanisms have not been established. Previous studies show that bacterial meal (BM) containing mainly *Methylococcus capsulatus* grown on natural gas is a suitable protein source for salmonids. The BM is rich in nucleotides, phospholipids, and small peptides that might be beneficial for intestinal homeostasis. In this study, a fish meal (FM)-based control diet (FM diet) and diets with 200 g/kg SBM (SBM diet), 300 g/kg BM (BM diet), and 300 g/kg BM and 200 g/kg SBM (BM-SBM diet) were fed to juvenile Atlantic salmon (*Salmo salar*) for 80 d. Dietary inclusion of SBM reduced growth ($P = 0.007$). Inclusion of BM reduced digestibility of protein ($P = 0.002$) and lipids ($P = 0.011$) and increased ($P < 0.01$) the relative weights (g/kg whole body) of total gut, liver, and stomach, and mid and distal intestine. Fish fed the SBM diet developed enteritis, lacked carbonic anhydrase 12 in the brush border of epithelial cells in distal intestine, and had more epithelial cells reacting for proliferating cell nuclear antigen compared with fish fed the other diets. Fish fed the same amount of SBM combined with BM showed no signs of inflammation in the distal intestine. Our results demonstrate that BM grown on natural gas can be used to prevent SBM-induced enteritis in Atlantic salmon. J. Nutr. 141: 124–130, 2011.

Introduction

The rapid global growth in fish farming and limited supply of fish meal (FM)[6] have generated reduced inclusion of FM in compound feed for salmonids and increased substitution with plant protein sources (1). Conventional solvent extracted soybean meal (SBM) is a suitable protein source for farmed salmonids with a favorable amino acid composition and acceptable digestibility (2). However, SBM is known to cause a serious enteropathy in the distal part of the intestine (3,4), commonly referred to as SBM-induced enteritis, that limits its use in diets for salmonids. Morphological changes have been found in Atlantic salmon fed diets containing as low as 7.6% SBM (5).

A complex set of mechanisms ensures that the gastrointestinal (GI) tract provides absorption of nutrients and simultaneously keeps potential deleterious immune reactions against feed ingredients under control. In salmonids, this homeostasis seems to be disrupted by alcohol soluble components in SBM (6), such as saponins (7,8), resulting in an apparent immunological dysregulation accompanied by an impaired immune tolerance. The condition is maintained as long as the fish are fed SBM, but the intestinal tissue will be rebuilt to normal after 3 wk if given a SBM-free diet (3). Intestinal histopathological changes typical for the soy enteropathy have also been found in other fish species fed SBM, such as Asian sea bass (9), channel catfish (10), gilthead sea bream (11), and common carp (12). Even terrestrial animals such as young goats (13) and mink (14) have been shown to develop intestinal enteropathy when fed soy products. The intestinal response to SBM in salmonids is possibly a T-cell–mediated and lymphokine-driven inflammation (15,16) caused by a disruption of the intestinal barrier, with subsequent exposure of otherwise shielded layers of the mucosa to luminal ingredients, including food-derived and microbial antigens. However, the precise mechanisms remain to be established.

A bacterial meal (BM) BioProtein grown by aerobic fermentation of natural gas by the obligate methanotroph *Methylococcus capsulatus* together with minor parts of the heterogenic bacteria *Ralstonia* sp., *Brevibacillus agri*, and *Aneurinibacillus* sp., has been shown to be a suitable protein source for farmed salmonids and other monogastric animals (17). The bacterial biomass is sterilized by short-time heat treatment and spray-

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[3] Supplemental Figure 1 is available with the online posting of this paper at jn.nutrition.org.
[6] Abbrevations used: BM, bacterial meal; CA12, carbonic anhydrase isoenzyme 12; FCR, feed conversion ratio; FM, fish meal; GI, gastrointestinal; PCNA, proliferating cell nuclear antigen; SBM, soybean meal; TBS, Tris buffered saline; TGC, thermal growth coefficient; TLR, toll-like receptor; TSA, tyramide signal amplification.
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dried to a meal with a proximate chemical composition of ~96% dry matter, 70% crude protein, and 10% crude lipids, which is similar to FM, and ~10% nucleic acids (17). The lipids in BM are mainly phospholipids that together with the nucleic acids might affect immune reactions and stimulate intestinal growth and GI differentiation (18,19). Thus, BM may trigger immune responses in the poikilothermic salmonids, as shown in mice after oral ingestion (20), and thereby prevent development of enteropathy in Atlantic salmon fed SBM.

In the present study, we show that the intestinal response to SBM is conspicuously modified by BM, suggesting that BM supports the intestinal homeostasis and thus protects against SBM-induced enteritis.

Materials and Methods

Diets. Four diets were formulated (**Table 1**): a control diet with high-quality FM as the main protein source (FM diet), a diet with 200 g/kg SBM, a diet with 300 g/kg BM (BM diet), and a diet with 300 g/kg BM and 200 g/kg SBM (BM-SBM diet). Soybean oil was added into the extruder barrel to lubricate and reduce expansion of the diets with BM. Yttrium oxide was included as an indigestible marker for determination of apparent digestibility of nutrients. The diets were produced by extrusion technology at the Center for Feed Technology, Ås, Norway. The extruded pellets were dried to ~7% moisture before vacuum coating with fish oil. Calculated gross energy and digestible energy contents of the diets were 23 and 19–20 MJ/kg diet, respectively.

Fish, conditions, and sampling. An 80-d experiment with Atlantic salmon (*Salmo salar*) was carried out at Nofima Marin's research station, Sunndalsøra, Norway. Salmon with a mean weight of 133 g were allocated to 12 fiberglass tanks with 75 fish/tank. Each diet was fed to 3 replicate fish tanks every 10 min using electrically driven disc feeders. Before receiving the experimental diets, all fish were fed the FM-based control diet for 4 wk to avoid possible interactions with the preceding commercial diet used at the research station. Uneaten feed was sieved from the outlet water to calculate feed intake according to the method of Helland et al. (21). The tanks were supplied with seawater (8.6°C; 32.5 g/L NaCl) and had light 24 h/d. The experiment was conducted in accordance with the regulations given by the National Animal Research Authority in Norway (Animal Protection Ordinance concerning experiments with animals of 15 January, 1996).

The fish were bulk-weighed at the start of the experiment. Fish used for sampling at the termination of the experiment were weighed individually. The fish were fed until sampling to avoid an empty GI tract in individuals intended for determination of digestibility and histological examination. All fish were anesthetized with MS 222 (60 mg/L) and killed by a sharp blow to the head prior to dissection. Three groups of 10 fish at the start of the experiment and 10 fish/tank at the termination of the experiment were used for whole body nitrogen analysis. Feces were stripped from 35 fish/tank as described by Austreng (22) and the fecal samples were pooled by tank and stored frozen at – 22°C prior to freeze drying and analysis. Ten fish per tank were selected at random for weight of GI sections and liver. Tissue samples from the distal intestine of 5 fish/tank (15 fish/diet) were sampled for histology and immunohistochemistry. The samples were fixed in 10% neutral phosphate buffered formalin, brought to 70% ethanol after 72 h, stored for 2–4 d at room temperature, and then further dehydrated in ethanol, equilibrated in xylene, and embedded in paraffin.

TABLE 1 Ingredients and chemical composition of the experimental diets[1]

	FM diet	SBM diet	BM diet	BM-SBM diet
Ingredients		*g/kg*		
FM	591.4	412.4	305.4	127.4
BM[2]	—	—	300.0	300.0
SBM[3]	—	200.0	—	200.0
Fish oil	233.0	224.0	206.9	198.8
Soybean oil	0	0	17.1	17.2
Wheat	170.0	158.0	165.0	151.0
Vitamin/mineral premix[4]	5.6	5.6	5.6	5.6
Analyzed chemical composition				
Dry matter	951	954	948	949
Crude protein	433	418	448	423
Crude lipid	270	254	258	245
Starch	105	108	95	116
Total ash	79	74	64	53
Gross energy, *MJ/kg*	23.0	23.4	23.5	23.3

[1] Ingredients are given as is and chemical composition on a dry matter basis.
[2] BioProtein, Norferm AS, Stavanger, Norway.
[3] Deno-Soy F, solvent extracted and toasted SBM, Denofa, Fredrikstad, Norway.
[4] Vitamin and mineral premix provided (per kg diet): all-*trans* retinyl acetate, 860 μg; cholecalciferol, 37.5 μg; *d,l*-α-tocopherol acetate, 200 mg; menadione, 10 mg; thiamin, 15 mg; riboflavin, 25 mg; nicotinic acid, 75 mg; pantothenic acid, 30 mg; pyridoxine, 15 mg; folic acid, 5 mg; cyanocobalamin, 20 μg; ascorbyl monophosphate, 125 mg; biotin, 0.25 mg; Ca, 1.1g; $ZnSO_4$, 296 mg; $MnSO_4$, 41 mg; $CuSO_4$, 13 mg; $CoSO_4$, 2.6 mg; CaI_2, 3.5 mg; astaxanthin, 175 mg; Y_2O_3, 100 mg.

Chemical analysis. Dry matter, crude protein (Kjeldahl N × 6.25), and ash were analyzed according to standard procedures (23). Crude lipid was determined after hydrolysis with petroleum ether on an Accelerated Solvent Extractor (ASE200) from Dionex. Starch was analyzed as total glucose after hydrolysis with glucosidase (Total Starch assay kit [AA/AMG], Megazyme International Ireland). The diets were defatted by acetone prior to the starch analysis. Yttrium was determined with an ICP-OES spectrometer (Perkin Elmer Optima 5300 DV).

Immunohistochemistry. Paraffin sections for proliferating cell nuclear antigen (PCNA) staining were cut ~4 μm thick, placed on polylysine-coated slides (Menzel-Gläser), and dried for at least 12 h at 37°C. The sections were deparaffinized in xylene and rehydrated in graded ethanol series. The sections were subjected to hydrated autoclaving in citrate buffer (10 mmol/L citric acid monohydrate, pH 6.0) for 15 min at 121°C. Endogenous peroxidase was inhibited by treatment with 0.05% phenylhydrazine (Sigma-Aldrich) and warmed in preheated PBS for 40 min at 37°C. To prevent nonspecific binding of antibodies, the sections were treated with normal horse serum containing 5% BSA in a Tris buffered saline (TBS) for 20 min at room temperature. Mouse monoclonal IgG2α-κ antibodies to PCNA (M0879; Dako Cytomation) were diluted 1:400 in 1% BSA/TBS buffer and sections were incubated overnight in a refrigerator at 4°C. Horse biotinylated secondary antibodies against Ig (BA 2000; Vector) were diluted 1:200 in 1% BSA/TBS for 30 min at room temperature. Incubation with the avidin/biotinylated enzyme-HRP complex (PK-4000; Vector Laboratories) diluted in 1% BSA/TBS was for 45 min at room temperature. The peroxidase reaction was developed with a 3-amino-9-ethyl carbazole kit (0027; Zymed) for 15 min. The sections were given 3 washes in PBS between each of the steps of the procedure. An exception was the treatment with horse serum where no washing was done; the serum was gently tapped off the sections. All incubations were done in a humid chamber placed on an orbital shaker. Sections were mounted in Faramount mounting medium (Dako Cytomation).

Carbonic anhydrase isoenzyme 12 (CA12) was stained by using a tyramide signal amplification (TSA)-indirect kit (NEN Life Science Products) according to the manufacturer's instructions but with some modifications. To block nonspecific binding, sections were incubated in avidin (Avidin/ Biotin Blocking kit; Vector) diluted 1:6 in a TNB buffer (0.1 mol/L Tris-HCl, pH 7.5, 0.15 mol/L NaCl, 0.5% blocking reagent supplied in the TSA kit) for 30 min. After gentle removal of the blocking solution, sections were incubated overnight at 4°C with the primary antibody diluted in a mixture of a TNB buffer with 1 part biotin (Vector Laboratories) to 6 parts TNB. The primary antibody was a mouse monoclonal IgG2α-κ antibody to CA12 (WH0000771M; Sigma). To

TABLE 2 Growth, feed conversion, apparent digestibility of nutrients, nitrogen retention efficiency, and fecal dry matter in Atlantic salmon fed diets with BM, SBM, both, or neither for 80 d[1]

						P-values		
Diet	FM diet	SBM diet	BM diet	BM-SBM diet	SEM	SBM	BM	SBM × BM
Start weight, *g/fish*	133[a]	132[a]	134[b]	135[c]	0.3	0.70	<0.001	0.007
End weight, *g/fish*	362[b]	319[a]	344[ab]	328[a]	8.0	0.007	0.60	0.12
TGC	2.90[b]	2.49[a]	2.70[ab]	2.53[a]	0.076	0.005	0.34	0.16
Feed conversion, *g gain:g feed*	1.39[b]	1.31[a]	1.45[c]	1.33[a]	0.014	<0.001	0.019	0.16
Apparent digestibility, %								
Crude protein	86.6[c]	86.1[bc]	83.3[a]	84.6[ab]	0.5	0.47	0.002	0.12
Crude lipid	96.1[b]	94.7[b]	92.2[a]	94.7[b]	0.6	0.39	0.011	0.011
Nitrogen retention efficiency, %	57.5	55.5	52.9	53.3	1.82	0.67	0.10	0.53
Fecal dry matter, %	15.3[b]	9.4[a]	16.6[c]	15.3[b]	0.28	<0.001	<0.001	<0.001

[1] Values are least square mean ± SEM, n = 3 tanks/treatment. Means in a row with superscripts without a common letter differ, $P < 0.05$.

detect binding of primary antibodies, the sections were incubated in biotinylated sheep anti-mouse Ig (RPN 1001; Amersham Biosciences) followed by a streptavidin-HRP complex (P0397; Dako). To improve specific labeling, sections were treated with biotinyl-tyramide followed by streptavidin-HRP; both reagents were supplied in the TSA-kit. Finally, bound antibody was visualized with 3-amino-9-ethyl carbazole (Sigma-Aldrich) as substrate. Otherwise, the technical aspects of the incubations were similar to those for PCNA.

Calculations and statistical analysis. Feed intake was calculated as dry matter in feed (dry $matter_{feed}$)/initial body weight (BW_0). Thermal growth coefficient (TGC) was calculated as 1000 × [final body weight $(BW_1)^{1/3} - BW_0{}^{1/3}$]/(mean daily temperature × days in the experiment). The feed conversion ratio (FCR) was calculated as ($BW_1 - BW_0$)/dry $matter_{feed}$. Apparent digestibility was calculated as $100 \times [1 - (D_i/F_i \times F_n/D_n)]$, where D_i and F_i represent the concentration of inert marker in diet and feces, and D_n and F_n represent the concentration of nutrients in diet and feces, respectively. Nitrogen retention efficiency was calculated as $100 \times FCR \times [(BW_1 \times N_1) - (BW_0 \times N_0)]/[(BW_1 - BW_0) \times N_{diet}]$, where N_{diet} is the content of nitrogen in the diet, and N_0 and N_1 represent the initial and final N concentration in whole minced fish.

Morphometry was done on sections stained for PCNA. Stretches of PCNA reactive epithelial cells were measured by ImageJ software (ImageJ, 1.42q version) and expressed as the length of stretches stained for PCNA in relation to the length of a baseline drawn through the base of the folds.

The experiment was designed as a completely randomized factorial experiment. Data were analyzed by 2-way ANOVA to determine the main effects of SBM and BM and their interaction. Statistical analysis was carried out using ANOVA procedures in SAS from SAS Institute. Differences among means were ranked by Duncan's Multiple Range Test ($P < 0.05$). The results are presented as least square means ± pooled SEM.

Results

Feed intake and growth. Total feed intake was 1.24 kg dry matter/kg initial biomass for fish fed the FM diet and 1.08 for those fed the other diets, but there were no significant differences among the groups ($P = 0.083$). The cumulative feed intake after 3 d was greater ($P < 0.05$) in fish fed the FM and SBM diets than in fish fed the diets with BM and greater in fish fed the FM diet than in fish fed the SBM diet after ~2 wk (**Supplemental Fig. 1**). Cumulative feed intake did not differ between fish fed the SBM diet compared with those fed the BM and BM-SBM diets beginning on d 53, and none of the groups differed from d 73 to the end of the experiment. Overall, 26 fish died during the first 17 d of the experiment. These fish were omitted when feed intake was calculated, because there were no indications that the mortality was related to the dietary treatments. Final weight ($P = 0.007$) and TGC ($P = 0.005$) were reduced by inclusion of SBM (**Table 2**). Diets with SBM reduced FCR ($P < 0.001$), whereas the BM diet increased FCR ($P = 0.019$).

Digestibility, nitrogen retention efficiency, and fecal dry matter. Digestibility of crude protein ($P = 0.002$) and crude lipids ($P = 0.011$) was reduced by dietary inclusion of BM. There was an interaction between SBM and BM ($P = 0.011$) on lipid digestibility; it was significantly lower for fish fed the BM diet than in those fed the other 3 diets, which did not differ from one another. Nitrogen retention efficiency was not affected by diets ($P = 0.32$). Fecal dry matter was affected by SBM and BM as well as by their interaction ($P < 0.001$). It was greater in fish fed the BM diet than in those fed the FM and BM-SBM diets and it was greater in these groups than in fish fed the SBM diet.

TABLE 3 Relative weights of organs in Atlantic salmon fed diets with BM, SBM, both, or neither for 80 d[1]

						P-values		
Diet	FM diet	SBM diet	BM diet	BM-SBM diet	SEM	SBM	BM	SBM × BM
			g/kg body weight					
Total gut	98[a]	95[a]	100[ab]	104[b]	1.7	0.67	0.008	0.054
Liver	16.1[a]	15.3[a]	17.2[a]	19.3[b]	0.56	0.27	0.002	0.032
Stomach	7.7[a]	8.1[ab]	9.3[b]	9.2[b]	0.38	0.68	0.007	0.62
Pyloric region	48.9	47.4	48.2	49.3	0.94	0.86	0.54	0.21
Mid intestine	3.1[a]	3.0[a]	4.1[b]	4.3[b]	0.23	0.83	0.001	0.59
Distal intestine	5.8[a]	5.6[a]	7.3[b]	7.3[b]	0.32	0.69	0.001	0.74

[1] Values are least square mean ± SEM, n = 3 tanks/treatment. Means in a row with superscripts without a common letter differ, $P < 0.05$.

TABLE 4 Microscopy of distal intestine in Atlantic salmon fed diets with BM, SBM, both, or neither for 80 d

Diet	FM diet	SBM diet	BM diet	BM-SBM diet
			n[1]	
Fish with normal intestine, n	15	0	15	15
Fish with intestinal inflammation,[2] n	0	15	0	0

[1] n = 15 fish/treatment.
[2] Inflammation comprised atrophy of simple and complex folds, loss of the epithelial vacuolation, decreased heights of epithelial cells, and infiltration of inflammatory cells in intestinal mucosa.

Liver and intestinal weight. The relative weights (g/kg whole body) of total gut, liver, stomach, mid intestine, and distal intestine were increased by dietary inclusion of BM ($P < 0.01$), whereas SBM inclusion did not affect intestinal weights ($P > 0.1$) (**Table 3**). There was a SBM × BM interaction on liver weight ($P = 0.032$), and fish fed the BM-SBM diet obtained significantly higher liver weights than fish fed other diets. The relative weight of stomach, mid intestine, and distal intestine was from 19 to 39% higher in fish fed the BM diet than in fish fed the FM diet. The weight of the pyloric region did not differ among the groups ($P = 0.54$).

Intestinal morphology, immunohistochemistry, and morphometry. The intestine of all sampled fish fed the FM, BM, and BM-SBM diets was characterized as normal, whereas all studied fish fed the SBM diet had histopathological features typical of SBM-induced enteritis in the distal intestine (**Table 4**; **Fig. 1**), as reported by Baeverfjord and Krogdahl (3). Briefly, the changes comprised atrophy of simple and complex folds, loss of the epithelial vacuolation that is typical for mature epithelial cells in this segment of the intestine, decreased heights of the individual epithelial cells, and prominent infiltration of the mucosa by inflammatory cells. Morphometry on PCNA-stained sections was affected by SBM and BM as well as by their interaction ($P < 0.001$) (**Figs. 2** and **3**). Fish fed the SBM diet had longer stretches of PCNA reactive cells than those fed the other diets and fish fed the BM-SBM diet had longer stretches than those fed the FM diet. The PCNA stained sections from fish fed the BM diet were intermediate compared with fish fed the BM-SBM and FM diets. A brush border reaction for CA12 was conspicuously missing in fish fed the SBM diet in contrast to the normal reactivity of fish fed the other diets (**Fig. 4***A–D*). In fish fed all diets, reactivity of CA12 was missing in stretches of epithelial cells at the base of the folds (Fig. 4*E*), corresponding to areas of dividing PCNA reactive epithelial cells (Fig. 2).

Discussion

The main finding of the present study was that BM prevents the development of typical SBM-induced enteropathy in salmon. The intestinal homeostasis is a multifaceted mechanism preventing inappropriate and potentially harmful reactions to feed ingredients and intestinal microbiota. Typical of SBM-induced enteropathy is a breakdown of the normal intestinal homeostasis. The provoking mechanism has not been established, although it seems evident that alcohol soluble components are responsible for the intestinal inflammation in salmon (6), and saponins in combination with at least one more unidentified component are thought to initiate the reaction (7,8). The physicochemical surfactant properties of saponins include the ability to increase the permeability of intestinal cells and facilitate absorption of large molecules. The SBM-induced enteropathy increases the permeability of the distal intestine in salmon (24), and this may allow luminal antigens to permeate beyond the epithelial barrier. Inflammatory bowel diseases in humans have similarities to SBM-induced enteritis where microbes, and particularly the commensal flora, seem to play a central role (25). Nevertheless, the broad-spectrum antibiotic oxytetracycline added to diets with SBM showed no protective effect against the enteritis in Atlantic salmon (26). The inflammation is thus possibly caused by antigens from the feed or oxytetracycline-resistant microbial antigens that do not normally interact with the intestinal mucosa when the epithelial barrier is intact. Toll-like receptors (TLR) located on basolateral surfaces of the intestinal epithelial cells may be activated by ligands from the intestinal microflora if the intestinal barrier is disrupted, and activation of TLR may contribute to increased proliferation and migration of epithelial cells (27). The expression of TLR increases in patients with inflammatory bowel diseases such as ulcerative colitis and Crohn's disease (27).

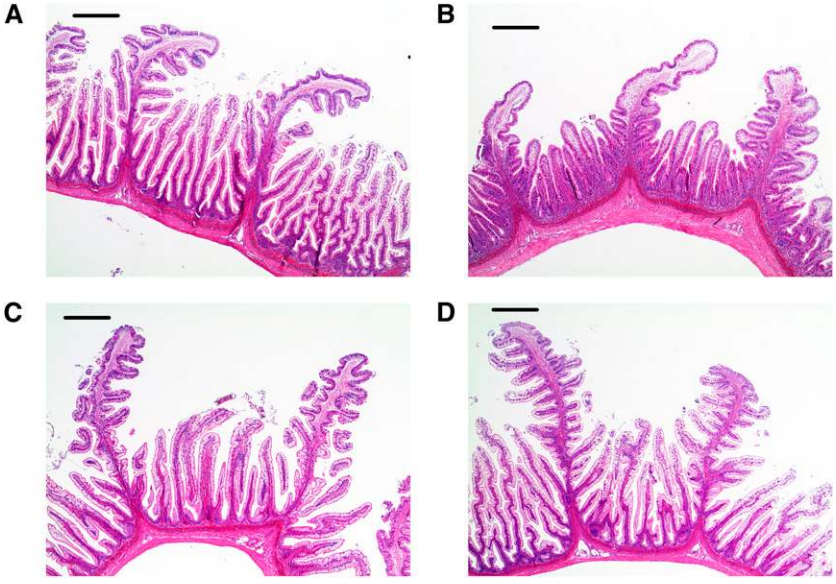

FIGURE 1 Microscopy of the distal intestine of Atlantic salmon fed diets with BM, SBM, both, or neither for 80 d: (*A*) FM diet, (*B*) SBM diet, (*C*) BM diet, and (*D*) BM-SBM diet. Fish fed the SBM diet had the features of SBM-induced enteritis, including atrophy of simple and complex folds and inflammatory leukocyte infiltration of the subepithelial layers, whereas fish fed the other 3 diets had normal features. The sections were stained with hematoxylin and eosin. Scale bar = 500 μm.

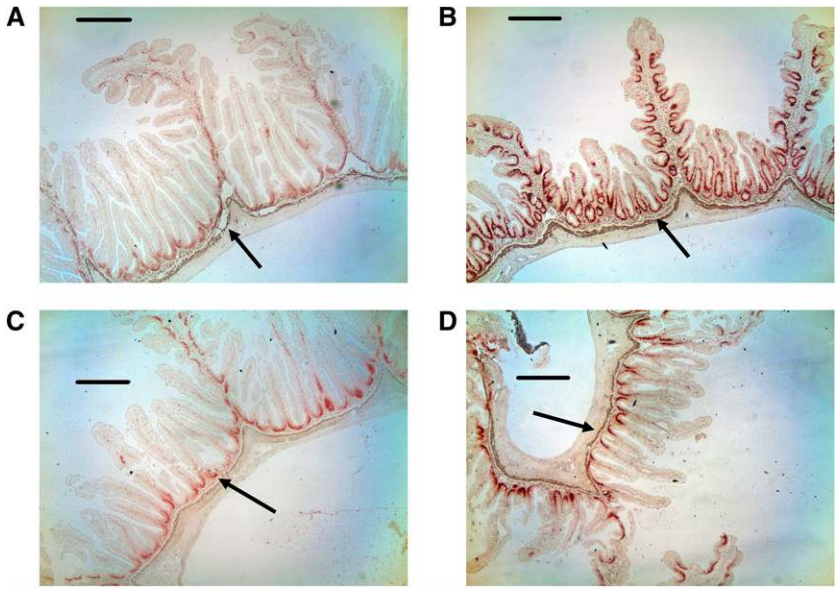

FIGURE 2 Areas at the base of the folds reacting for PCNA in fish fed diets with BM, SBM, both, or neither for 80 d: (*A*) FM diet, (*B*) SBM diet, (*C*) BM diet, and (*D*) BM-SBM diet. PCNA reactive cells were stained red as indicated by arrows. Scale bar = 500 μm.

Hence, a stimulation of TLR could possibly be the reason for the highly increased cell proliferation, as indicated by the high number of PCNA reactive cells in fish with SBM-induced enteritis.

The increased cell proliferation after the SBM diet shown in the present study may represent an attempt to compensate for an extensive loss of epithelial cells caused by SBM-induced enteritis. In such a situation, the pace of epithelial cell maturation or differentiation may not comply with the expected shorter life span of the epithelial cells. In the present study, CA12 was used to address the issue of epithelial cell differentiation. CA12 is typically located at the basolateral surfaces of the colon epithelial cells in humans and it catalyzes the conversion of CO_2 to HCO_3^- and H^+ (28). Thus, it contributes to transport of CO_2 out of tissues and available H^+ for active transport processes, but the exact physiological role of CA12 in salmon remains to be shown. The weak or absent reaction of CA12 at the base of the folds where cells are proliferating, although present in the luminal border of the epithelial cells, suggests that CA12 is a maturation feature of epithelial cells migrating along the folds and may be a useful indicator of epithelial cell differentiation in distal intestine of salmonids. The lack of CA12 in the enterocyte luminal border in fish with enteritis could be due to the rapid cell proliferation so that the epithelial cells may not be fully differentiated or mature, as suggested based on enzyme histochemical studies in salmon fed soy (29). Alternatively, components in SBM may restrict the differentiation of the epithelial cells.

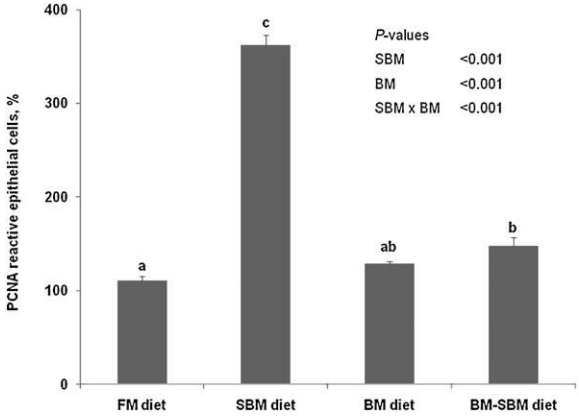

FIGURE 3 Stretches of PCNA reactive epithelial cells in the distal intestine of Atlantic salmon fed diets with BM, SBM, both, or neither for 80 d expressed as the length of such stretches stained for PCNA in percent of a baseline drawn through the base of the folds. Complex folds were not included in the measurements. Data are expressed as means ± SE, n = 3 tanks/treatment. Labeled bars without a common letter differ, $P < 0.05$.

All fish in our study had acceptable growth, FCR, nutrient digestibilities, and nitrogen retention efficiency compared with similar studies with Atlantic salmon fed diets containing BM or SBM (30–34). The daily feed intake curve in the Supplemental Figure 1 shows that fish fed the diets with BM had a feed intake progress that differed from fish fed other diets. Aas (35) reported low initial feed intake in rainbow trout fed diets with high inclusion of BM, but this was not observed in Atlantic salmon or Atlantic halibut. A likely explanation to a rather low initial feed intake of fish fed BM is that they need time to adapt to an unfamiliar diet. The somewhat harder texture of the extruded pellets with BM inclusion may also have suppressed the initial feed intake, because Atlantic salmon prefer a soft rather than a hard pellet (36).

The low fecal dry matter in fish fed the SBM diet is in agreement with other studies where salmonids have been fed SBM (34,37) and where intestinal inflammation has been initialized in Atlantic salmon by feeding soy saponins in combination with lupin kernel meal (7). The role of carbonic anhydrases in ionic regulation in the intestine is well established in fish (38). The lack of CA12, and possibly other carbonic anhydrases not measured in the present study, may have contributed to the low fecal dry matter observed in fish with distal intestine inflammation.

The observed increase in gut weight of salmon fed BM concurs with the finding of Storebakken et al. (31). The rapid turnover of the intestinal epithelium requires an exogenous supply of purine and pyrimidine bases for cell proliferation (39), and additional dietary nucleotides are known to stimulate the growth of the intestinal epithelium and increase intestinal volume (18,39). Nucleic acids comprise ~10% of the BM biomass on a dry matter basis (17), and thus higher nucleic acid content may explain the higher intestinal weight and cell

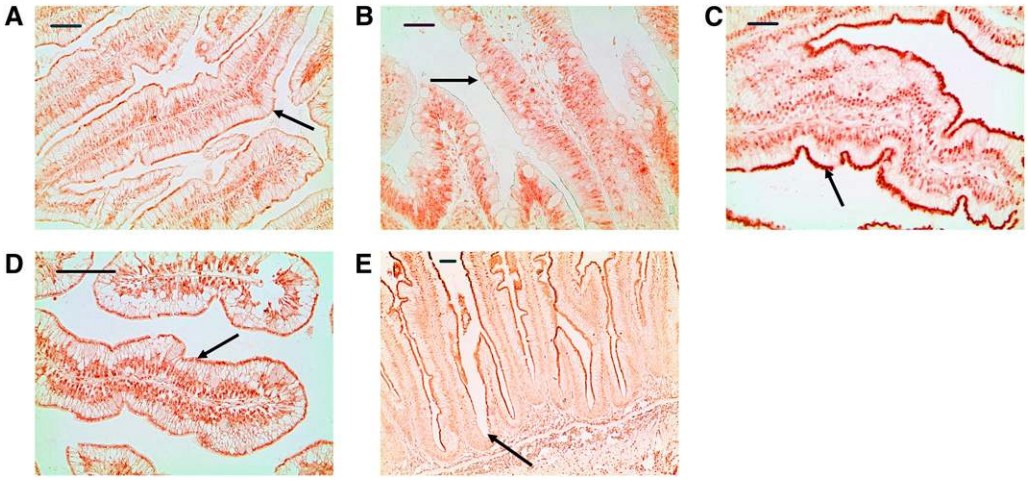

FIGURE 4 CA12 in the distal intestine of Atlantic salmon fed diets with BM, SBM, both, or neither for 80 d. Epithelial cells reactive for CA12 have a positive brush border reaction as indicated by arrows in fish fed the (*A*) FM diet, (*C*) BM diet, and (*D*) BM-SBM diet. No brush border CA12 reactivity was found in fish fed the SBM diet (*B*). Note that there is also reactivity in the nuclei of the epithelial cells. The reactivity of CA12 was absent or weak in brush border of epithelium at the base of the folds as shown in picture (*E*). Scale bar = 50 μm.

proliferation in fish fed the BM and BM-SBM diets compared with those fed the FM diet. Salmonids with SBM-induced enteropathy usually have reduced weight of the distal intestine (24,26,40). Thus, it was surprising that no significant difference was found between fish fed the SBM diet and the FM diet in the present study.

In conclusion, our studies showed that dietary inclusion of BM counteracts or neutralizes SBM-induced enteritis in Atlantic salmon. We suggest that BM supports the intestinal homeostasis and maintenance of an intact intestinal barrier in fish fed SBM. Further studies are needed to identify the underlying mechanisms of these effects.

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