Fish and Shellfish Immunology 107 (2020) 172–186

Contents lists available at ScienceDirect

Fish and Shellfish Immunology

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

Full length article

Anatomy, immunology, digestive physiology and microbiota of the salmonid intestine: Knowns and unknowns under the impact of an expanding industrialized production

Håvard Bjørgen [a,1], Yanxian Li [b,1], Trond M. Kortner [b], Åshild Krogdahl [b], Erling Olaf Koppang [a,*]

[a] Section of Anatomy, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Oslo, Norway
[b] Nutrition and Health Unit, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Oslo, Norway

ARTICLE INFO

Keywords:
Atlantic salmon
Digestive physiology
Feed
Immune system
Microbiota
Mucosal immunity

ABSTRACT

Increased industrialized production of salmonids challenges aspects concerning available feed resources and animal welfare. The immune system plays a key component in this respect. Novel feed ingredients may trigger unwarranted immune responses again affecting the well-being of the fish. Here we review our current knowledge concerning salmon intestinal anatomy, immunity, digestive physiology and microbiota in the context of industrialized feeding regimes. We point out knowledge gaps and indicate promising novel technologies to improve salmonid intestinal health.

1. Introduction

In their natural environment, salmon species are carnivores with a lifecycle comprising a pre-smoltification period in rivers followed by migration to sea and subsequent return to the river to spawn. This free and challenging life is in stark contrast to the confined and more crowded environment farmed fish experience. Here, they are also fed a diet very different from the catch obtained by their free-living relatives. Taken together, these life-altering changes may impact intestinal health and integrity in farmed salmon. Crowded environments facilitate the spread of pathogens, many of which are introduced to the host through the gastrointestinal tract as recently reviewed in fish [1]. Breeding programs focusing on disease resistance may alter the host's responsiveness to pathogens but also to non-pathogenic commensals, which again may lead to unwarranted intestinal inflammatory responses. Last but not least, the dietary impact on gastrointestinal health and function is well-established and well-studied also in salmonid fish. All these factors may alone or together impact the intestinal microbiota [2–6].

The on-going debate regarding pain reception in fish has prompted increasing concern regarding fish welfare [7]. Intestinal health is a major issue in all animal productions, also with respect to welfare [8]. If we induce unwarranted effects through husbandry, it is our responsibility to identify such effects and seek to avoid them. From own experience, we know that intestinal inflammatory conditions may be highly troubling. If the fish experiences anything similar, it is imperative to avoid intestinal inflammatory conditions. As clinical observations of such in fish are difficult (for instance registration of diarrhea), we rely on other methods, primarily histological examination from selected individuals in given populations. Here we review our current knowledge of salmonid fish intestinal anatomy, immunology, digestive physiology and reactions to feed in the context of unwarranted farming-induced conditions with emphasis on immune reactions.

2. Anatomy

2.1. Gross anatomy

As recently reviewed by Hellberg (2019) [9], detailed information on

Abbreviations: **BBM**, brush border membrane; **CA**, cardia; **CD**, cluster of differentiation; **FAs**, fatty acids; **FSMI**, first segment of the mid-intestine; **GI**, gastro intestinal; **GPCRs**, G-protein coupled receptors; **HDAC**, histone deacetylates; **IBD**, inflammatory bowel disease; **IEL**, intra-epithelial lymphocyte; **Ig**, immunoglobulin; **IL**, interleukine; **LPS**, lipopolysaccharide; **MHC**, major histocompatibility complex; **NF**, nuclear factor; **PAR**, proteinase-activated receptor; **PAS**, periodic acid Schiff; **PC**, pyloric caeca; pH, *pondus Hydrogenii*; **RAG**, recombination-activating gene; **SBMIE**, soy bean meal induced enteritis; **SCFAs**, single chain fatty acids; **SSMI**, second segment of the mid-intestine; **TNF**, tumor necrosis factor.

* Corresponding author.
E-mail address: erling.o.koppang@nmbu.no (E.O. Koppang).
[1] HB and YL: Equal contributions to the paper.

https://doi.org/10.1016/j.fsi.2020.09.032
Received 9 June 2020; Received in revised form 18 September 2020; Accepted 21 September 2020
Available online 23 September 2020

the embryology of the gastrointestinal tract of fishes is available from studies of the zebrafish (*Danio rerio*) and the medaka (*Oryzias latipes*) [10–12]. There is also one study in the Atlantic salmon that addresses different developmental stages and the impact of soybean meal [13] and one study addressing the impact of climate and mass-specific feeding of salmon [12]. For information on the general framework of fish ontogeny, the reader is referred to other reviews dedicated to the topic [14,15]. Nevertheless, in all vertebrates, the alimentary canal is formed as a tube between the mouth and the anus with an embryology that seems well conserved between vertebrates [16]. Different portions of this canal have specialized functions which is reflected in its construction. From the mouth, this canal is divided into the oral cavity, the pharynx, the esophagus, the stomach, the intestine and the anus. The intestine is the focus of this review, and its gross anatomy has been confusing as different authors have used different anatomical terminology to describe it. Especially confusing are the terms fore-gut, mid-gut and hind-gut, as the criteria for these distinctions have not been published. In zebrafish, Wallace et al. (2005) [17] proposed a nomenclature dividing the intestine into three segments, namely anterior,- mid,- and posterior intestine. The anterior intestine comprises the esophagus and stomach when present. The mid-intestine is divided into a first and second segment, where the second segment was proposed to resemble the mammalian ileum. Finally, the posterior intestine, which is very short in fish, was proposed to correspond to the mammalian colon [17,18]. Based on this nomenclature, Løkka et al. (2013) [19] addressed the anatomy of the gastrointestinal tract of salmon. Here, literature addressing the intestinal tract has frequently applied the term "hind--gut". This term seems in most cases to correspond to the zebrafish second segment of the mid intestine. "Fore-gut" seems to have been applied to the zebrafish corresponding segment termed "first segment of the mid intestine". Studies in salmonids addressing the segment corresponding to the posterior intestine in zebrafish seem missing, and thus no special terminology has been used. To establish an anatomical nomenclature in salmonids which both reflected that of the zebrafish and reflected the actual functions of the different segments, on both gross anatomical differences and histological characterizations, Løkka et al. (2013) [19] proposed a nomenclature which provided an exact and referable reference for the salmon intestinal anatomy (Fig. 1). From the pyloric part of the stomach, this system divided the intestine into the first segment of the mid-intestine (with apertures to the pyloric caeca); the first segment of the mid-intestine posterior to the apertures of the pyloric caeca; the second segment of the mid-intestine and finally the short posterior segment. This segment corresponds to the mammalian colon. Confusingly, the term "hindgut" is often regarded as an equivalent to the mammalian colon, but this is thus not the case.

Several studies in salmon have shown that the second segment of the mid intestine is immunologically more active than the other segments of the gastrointestinal tract. Important immune gene transcripts are significantly higher expressed in this portion [20–23]. In an investigation by Løkka et al. (2014) [24] addressing transcript levels of several gene products of the immunoglobulin superfamily and RAG 2 in wild,- and in farmed un-vaccinated and vaccinated salmon, the authors noted that "in all fish groups, there was a trend of higher transcript levels in the second segment of the mid-intestine and the posterior segment compared with the pyloric caeca and the first segments of the mid-intestine for most of the investigated immune-related genes". Adverse immune reactions also seem more prominent in this portion compared with other segments of the intestine. For example, soybean meal induced enteritis appears much more frequently in the second segment of the mid intestine compared with the other segments [25,26].

The suggested corresponding mammalian ileum is also immunologically very active. The ileum is rich in immune cells and possesses extensive lymphoid tissues organized in Peyer's patches. Here, organizations of B cells in follicles are found surrounded by T cells. Towards the intestinal lumen, Peyer's patches are covered by epithelial cells with many specialized antigen-sampling cells termed microfold cells or M cells. Cells with some M-cell like functions have also been identified in the salmonid second segment of the mid intestine, but not in the segment corresponding to the first segment of the mid intestine [27]. Further, in this segment, macrophage-like cells were found to extent cytoplasmic protrusions between epithelial cells, seemingly sampling material from the intestinal lumen [27]. This finding also supports the assumption that the second segment of the mid intestine corresponds to the immunologically active mammalian ileum.

2.2. Microanatomy

For purpose of the readability of the following section, the general histological construction of the Atlantic salmon intestine is presented in Fig. 2 where important structures are marked. In contrast to fish, the intestinal epithelium of mammals forms crypts (crypts of Lieberkühn) and villi in the small intestines and crypts but no villi in the colon. Epithelial cell proliferation occurs in the crypts, and from this stem cell area, there is continuous proliferation and differentiation of the main cell phenotypes in the intestinal epithelium, namely columnar cells, enteroendocrine cells, goblet cells and Paneth cells [28]. In salmonids, no crypts have been identified [19], but interestingly, similar structures have been identified in the intestine of the common wolfish (*Anarhichas lupus* L.) [29]. Stem cell regions, as identified as areas of proliferation in the salmonid gut, are located at the base of primary and secondary intestinal folds [19]. Columnar cells are most abundant, and goblet cells may be identified using PAS staining [19]. Enteroendocrine cells have also been identified in the salmonid gut [30]. Paneth cells (named after the Viennese physiologist Joseph Paneth who first described them) are present in a number of species but have not been reported in fish. Paneth (1888) [31] identified these cells in the fundus pars of the crypts of Lieberkühn and initially termed them "Körnchenzellen" – or "cells with small granula". These cells produce defensins, which are thought to be vital for keeping the crypts of Lieberkühn germ-free and thus protecting the stem cell region. We have tried to identify Paneth cells in salmon using staining methods to identify granula, but so far, these efforts have been negative (E.O. Koppang, unpublished results). However, it is worth noting that in salmonid intestine transcriptional data show production of β-defensins but not α-defensins [32]. In mammals, a variety of epithelial cells may produce β-defensins, whereas α-defensins are produced by Paneth cells. Nevertheless, future studies should address the possible existence of Paneth cells or Paneth-like cells in fish as this information would be essential in our understanding of intestinal immunology in lower vertebrates.

As in mammals, enterocytes are polarized cells, attached to the basal membrane and forming microvilli towards the intestinal lumen. It is thought that these cells may develop into microfold cells or M cells. In mammals, such cells are typically found covering Peyer's patches, and they lack microvilli. However, they may also be found in villi [33]. M cells are specialized in sampling intestinal antigen. Cells with certain M cell properties have been identified in salmonids [27], but in contrast to M cells in mammals, they possess microvilli, and it has not been demonstrated that they are capable of sampling particles as large as bacteria or yeast cells. In experiments aiming at revealing such properties, Løkka and co-workers rather observed yeast uptake in macrophage-like cells embedded within the epithelium but also in the intestinal lumen [34]. Immune cells, commonly referred to as intraepithelial lymphoid cells (IELs), are present in the salmonid intestinal epithelium. In mammals, most intraepithelial lymphocytes are T cells. Both αβ- and ϒδ T cells are present. Dendritic ϒδ T cells surveil the epithelium and may be directly activated and respond either to ϒδ ligands or epithelial stress signals [35,36]. These cells are placed functionally between classical innate and adaptive immune cells [36]. In salmonids, intraepithelial MHC class II-expressing cells were identified by Koppang et al. (1998) [20] and CD3 positive cells were described in 2010 [37]. It has not been established if the MHC-class II positive cells were T cells, but some of them might have been. In addition, some of

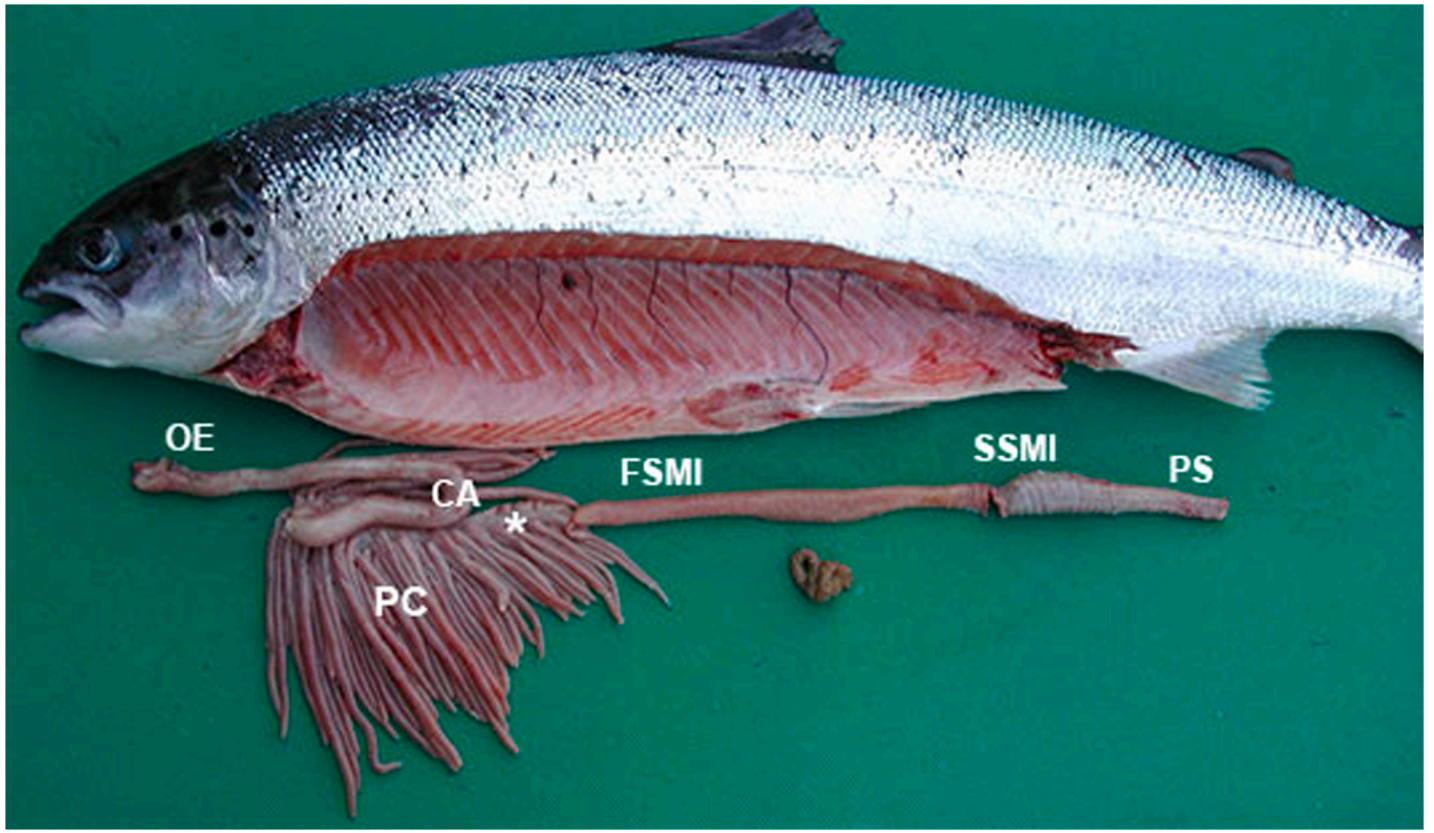

Fig. 1. Macroscopic image of the gastrointestinal tract of the Atlantic salmon. OE - oesophagus, CA - cardia, PC - pyloric caeca, FSMI - first segment of the mid-intestine, SSMI - second segment of the mid-intestine, PS - posterior segment. Modified after Løkka et al., 2013 [19].

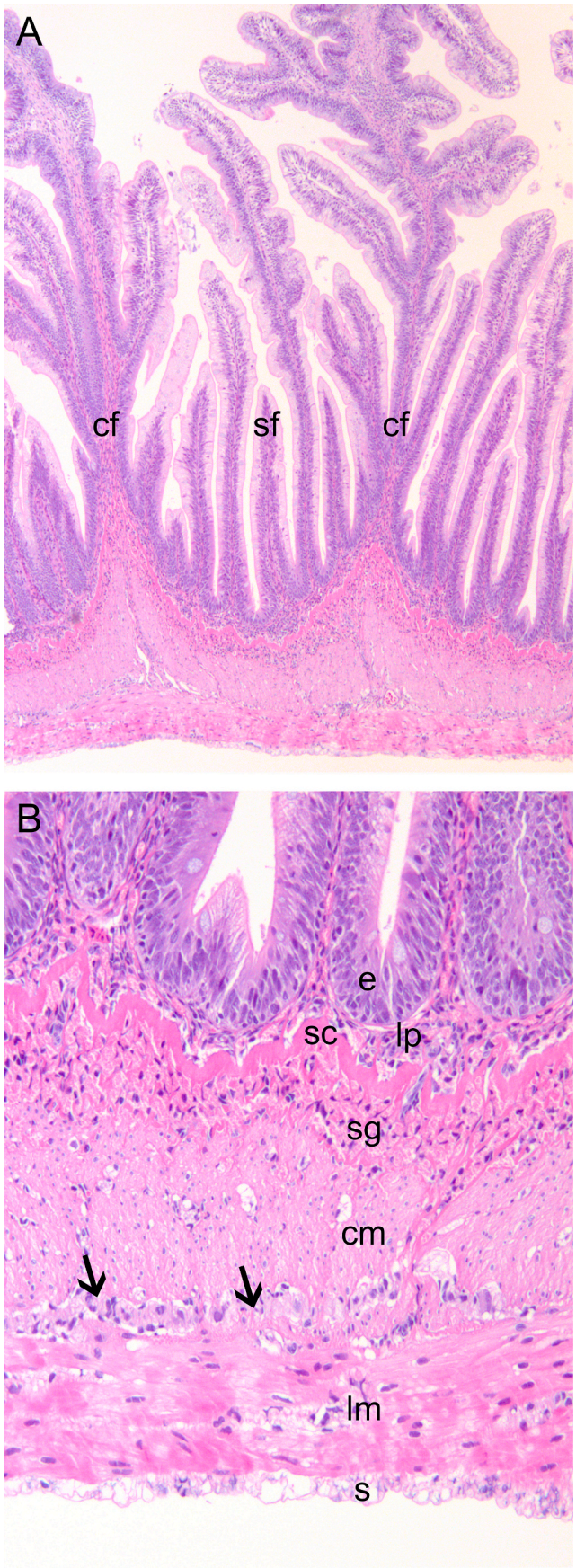

Fig. 2. Sections of second segment of the mid intestine, Atlantic salmon. A: Simple folds (sf) and complex folds (cf) are special for this portion of the intestine. B: The mucosa consists of the epithelium (e), the lamina propria (lp), the stratum compactum (sc) and the stratum granulosum (sg). The muscularis consists of an inner circular (cm) and an outer longitudinal (lm) layer. Between these layers, parasympathetic ganglion cells can be seen (arrows). The intestine is finally covered by the serosa (s). HE staining. (Modified from Løkka et al. [19]).

(caption on next column)

them resembled macrophage-like cells. Fuglem et al. (2010) [27] identified macrophage-like cells seemingly sampling luminal antigen, and Løkka et al. (2014) [34] described macrophage-like cells in context with yeast cells after exposure both within the epithelium and in the intestinal lumen. As for B cells, their majority consist of IgT positive cells, whereas IgM positive cells seem merely present in the subepithelial tissues [38]. Løkka et al. (2014) observed no IgM positive intraepithelial cells in the salmon but noted that Grove et al. (2006) [39] observed such cells in the epithelium of the Atlantic halibut (*Hippoglossus hippoglossus*). Also in the rainbow trout (*Oncorhynchus mykiss*), IgM positive cells were observed in the lamina propria, however, in the pyloric caeca, they could also be observed as intraepithelial lymphocytes [40]. IgT positive cells were primarily localized as intraepithelial lymphocytes [41,42]. In salmon, as in most other fishes, the knowledge of mucosal cell populations is primarily based on transcriptional analysis of intestinal wall containing both epithelium and underlying lamina propria. Interestingly, much more knowledge about the general composition of different intraepithelial immune cells is available with respect to the cloaca-based salmon bursa [43] compared with the intestines. So, when moving from transcription studies to morphology, there is still a large potential for exploring the diversity of IELs in fish intestine.

The epithelium rests on the basal membrane which defines the barrier between the mucosal epithelium and the underlying lamina propria. In mammals, studies have shown that this membrane is not solid but fenestrated, and the degree of fenestration varies between different intestinal segments and is especially prominent in relation to Peyer's patches [44]. It is believed that these disruptions facilitate the passage of leukocytes between the epithelium and the underlying lamina propria. Further, this fenestration has been demonstrated to be dynamic and responding to dietary conditions. In a study addressing fasting and non-fasting rats, the authors noted that the fenestration of the intestinal basal membrane responded to the dynamics of migrating leukocytes but also by regulating nutrient absorption, in particular lipids [45]. Similar studies have not been conducted in fish, but this information is highly warranted.

At its surface towards the intestinal lumen, the epithelium is covered by a glycocalyx layer. In addition to serving as an attachment layer for the covering mucus, it is also important in preventing bacterial entry into the epithelium [46]. To the best of our knowledge, studies addressing the intestinal glycocalyx in fish are missing, but this layer has been addressed in gills [47]. The glycocalyx is covered by a protective mucus layer which is formed by the activity of epithelial mucus cells [48]. Together, the mucus and the glycocalyx form an important and selective barrier between the enterocytes and the intestinal content (Fig. 3) [49]. Notably, the mucus layer is rich in immunologically active molecules such as complement proteins, lysozyme, proteases, antimicrobial peptides and secretory immunoglobulins [50], which are important for combatting pathogens while maintaining tolerance to commensal microbes. A recent study in rainbow trout showed that the secretory IgT at the gill mucosal surface is functionally analogous to mammalian IgA in terms of pathogen clearance and microbiota homeostasis [51]. It is unknown but likely that salmonid secretory IgT plays a similar role also in the intestinal mucosal immunity.

In general, for all intestinal segments, the lamina propria is located beneath the basal membrane and consists of connective tissue containing leukocytes. This layer is followed by a thick sheet of connective tissue called the stratum compactum. This layer is surrounded by the

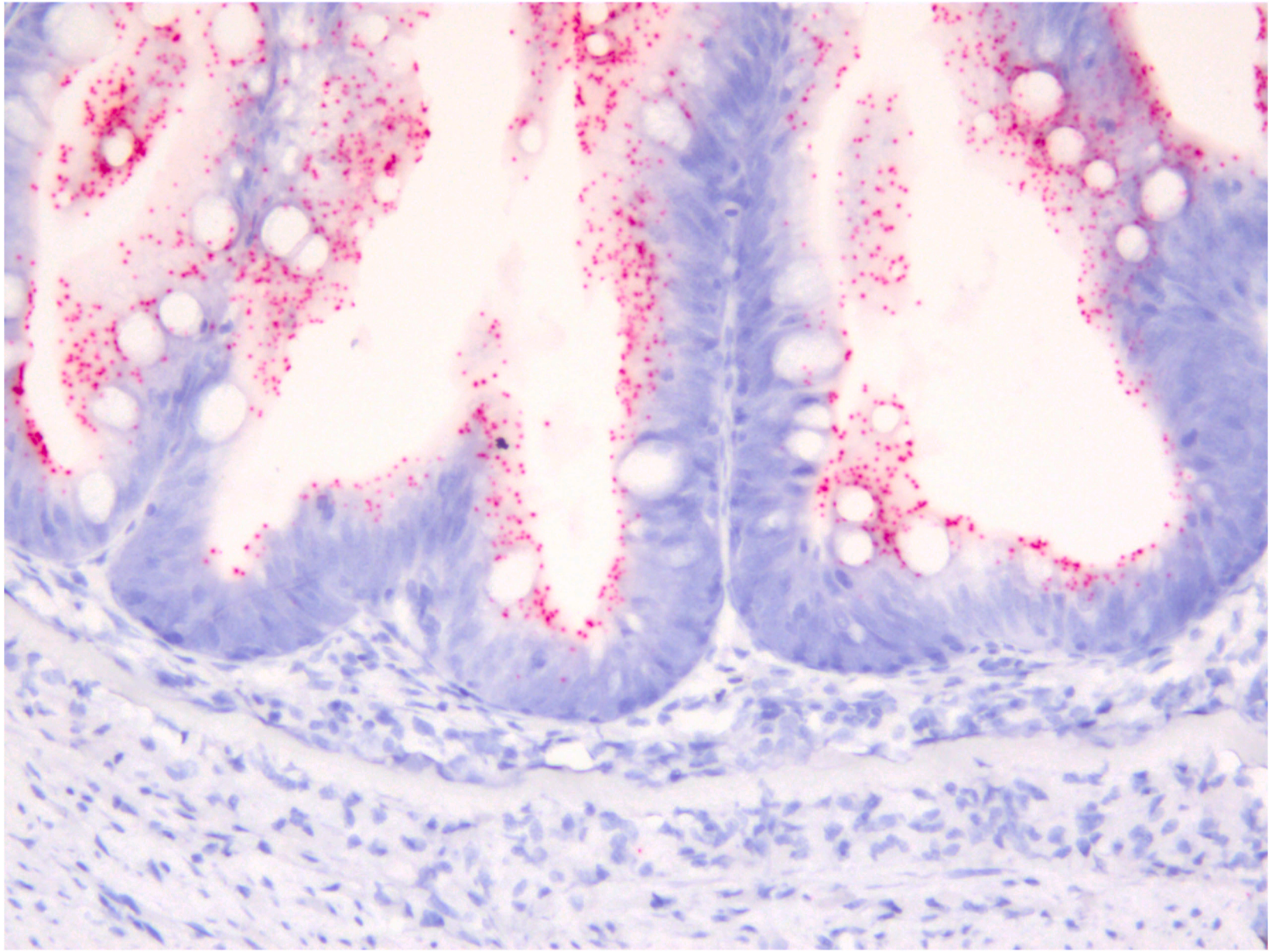

Fig. 3. Normal intestinal architecture, Atlantic salmon, second segment of the mid intestine. *In situ* hybridization for bacteria (16s rRNA) (red staining). Bacteria are confined to the intestinal lumen and the mucus and are rarely observed within epithelial cells. The mucus and the glycocalyx form effective barriers towards the external milieu. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

stratum granulosum which is rich in mast cells. The muscular layer is organized with an inner circular and outer longitudinal orientation of the muscle fibers. There are some minor variations with respect to the different intestinal segments [19] but these details are above the scope for this review. In the salmon lamina propria, IgM positive cells, T cells, antigen-presenting cells and mast cells may be found [24,26,38].

The uptake of antigens in the salmonid gut has been reviewed elsewhere [1]. Of note, it has not been established though which mechanisms bacteria may enter the organism through the mucosal surface. In mammals, an important part of the intestinal immune system is the lymphatic vessels. Lymphatic vessels drain the Payer's patches and the intestinal lymph nodes. Such structures are not present in the fish intestines. The existence of lymphatic vessels in fish has been disputed [52], but as referred to by Hellberg and co-workers, lymphatics have been described in the zebrafish, and these authors also identified them in the common wolfish [53]. Such vessels have so far not been described from salmonids. The clarification of their existence and function is warranted not only for the advancement of understanding of salmonid intestinal immunity but also for our understanding of lipid absorption though the gut where lymphatics play a central role in mammals.

3. Digestive function and responses to variation in diet composition

3.1. Digestion and absorption of nutrients

The physiological, chemical and enzymatic processes that collectively coordinate macro- and micronutrient digestion and absorption in fish have been reviewed extensively elsewhere [54–56]. A summary of the status of knowledge is provided here, with a specific focus on Atlantic salmon when detailed information is available.

After the digestive processes taking place in the stomach, the highly acidic digesta, also called chyme, is fed into the upper intestine at a controlled rate through the pyloric sphincter. Here, the digesta is mixed with secretions from the diffuse exocrine pancreas containing bicarbonate and digestive enzymes. As a result, the pH increases from about 4.8 in the stomach to about 8 in the first segment of the mid-intestine in salmon [57]. The digestive enzymes function to break down complex dietary nutrients into smaller components that can be absorbed across the intestinal wall. Many digestive enzymes, in particular the proteolytic, are synthesized and stored in inactive forms as proenzymes or zymogens. They become active after secretion into the digestive tract where trypsin becomes active through the action of enterokinase secreted by mucosal cells. The other proenzymes are activated by trypsin. There seems to be isozymes of most, if not all, enzymes [56,58]. The main digestive enzymes secreted by the pancreatic tissue are the proteases trypsin, chymotrypsin, elastase, collagenase, amino- and carboxy-peptidases, phospholipases, cholesterol and wax ester hydrolases, as well as ribo- and deoxyribonucleases [58]. Absence of a co-lipase dependent pancreatic lipase, similar to the one present in mammals and birds, is indicated for a number of fish species based on several studies [59,60]. Amylase, responsible for digestion of starch, is also a main pancreatic digestive enzyme, but has a lower activity in carnivorous fish species, particularly in Atlantic salmon [61,62]. This might be a result of evolutionary adaptation to diet, since starch is an uncommon dietary component for the strictly carnivorous salmon in the wild. Interestingly, the salmon amylase has a seven amino acid deletion that could impair substrate binding [62]. This might offer an explanation for the fact that salmon digest carbohydrates less efficient than many other fish species. As a result, commercial salmon feeds typically contain no more than 10% carbohydrates [63].

In addition to pancreatic secretions, the digesta is also mixed with bile transported from the gallbladder and entering the digestive tract via the common bile duct posterior to the pyloric sphincter. The majority of bile acids in salmon are taurine-conjugated, with taurocholic acid being the predominant individual bile salt [64]. Bile salt concentrations in salmon digesta can be extremely high in the proximal parts of the intestine, typically reaching levels up to 25% of the total dry matter content [65–69]. The concentration decreases gradually throughout the intestine, indicative of efficient reabsorption and recycling by yet unknown active and/or passive uptake mechanisms. Bile acids work as physico-chemical detergents and play a key role in emulsifying lipids, fat-soluble vitamins and other apolar components in the diet or from endogenous sources, thereby allowing for efficient hydrolysis by lipases. Bile salts also stabilize proteins, e.g. digestive enzymes, and thereby help the enzymes resist autodigestion in the proximal sections of the intestine [70]. After reabsorption of the bile salts in the distal intestine, digestion of endogenous proteins will accelerate.

Dietary nutrients, comprising proteins, polypeptides, amino acids, lipids, carbohydrates, vitamins, minerals and carotenoid pigments, are transported or otherwise absorbed from the intestinal lumen into the systemic circulation across the brush border membrane (BBM) of the enterocytes lining the post-gastric alimentary tract [54]. The enterocytes have both digestive and absorptive functions and are as such of vital importance for proper function of the digestive system. The folded nature of the BBM greatly increases the surface area and thereby the absorptive capacity of the intestine. The cell membranes of the microvilli contain important BBM digestive enzymes such as aminopeptidases, maltase, sucrases, trehalase, alkaline phosphatases and monoglyceride lipases. The BBM digestive enzymes are responsible for the final digestion of nutrients into small fragments ready for absorption. Nutrient absorption across the BBM into the enterocytes can occur by pinocytosis, simple diffusion following a concentration gradient, ion exchange or active transport by more or less specific protein transporters [54,56]. Simple diffusion may also occur via the paracellular route through the tight junctions. In salmon, the first segment of the mid-intestine with the pyloric caeca is the dominating region of secretory and nutrient absorptive functions and roughly accounts for 70% of the total nutrient absorption [71,72]. However, nearly the entire length of the salmon intestine has a functional BBM capable of nutrient transport [71]. Nutrient uptake may therefore be more prominent in posterior regions of the intestine in situations when the capacity of the proximal region is exceeded.

In general, mechanistic knowledge of nutrient absorption in fish is still rudimentary compared to that of mammals. Among the macronutrients, most dietary protein seems to be absorbed in the first segment of the mid-intestine as di- and tripeptides through the low-affinity/high-capacity H^+-dependent PetT1 and the high-affinity/low-capacity PetT2 peptide transporters located at the BBM [54]. The Atlantic salmon PepT1 transporter has been cloned and functionally characterized, and has a broad substrate specificity for both neutral and charged di- and tripeptides [73]. After absorption, most peptides are intracellularly hydrolyzed into free amino acids and exit the enterocytes across the basolateral membrane and enter the circulatory system. Some larger peptides or intact proteins may also be absorbed by pinocytosis in the distal intestine [74]. This absorption has been suggested to be involved in the recycling of digestive enzymes, or as part of the gut mucosal immune system and antigen sampling.

Lipid absorption in fish is in general not well understood but is presumed to occur as in mammals with some deviations [75]. Emulsification is initiated in the stomach and continues after being supplied with bile salts and phospholipids in the bile in region of the pyloric caeca. In Atlantic salmon, the emulsion droplets are acted upon by the lipases, producing free fatty acids (FAs) and glycerol. Short-chain FAs (2–10 carbons) and glycerol are probably absorbed directly through the brush border of the enterocytes, whereas medium and long-chain FAs must form micelles together with bile salts and phospholipids before they can be efficiently absorbed. The micelles, when in close vicinity of the BBM, disintegrate before the FAs are taken up by the enterocytes via active transport and/or passive diffusion [54]. Both membrane-bound and intracellular FA transporter proteins have been identified in salmon [68,76] but their relative contribution in quantitative aspects of

lipid uptake as well as their precise functions remain unknown. Inside the enterocyte, the FAs are re-esterified and packaged together with protein to form lipoproteins [75]. Similar to the other macronutrients, the primary site for lipid uptake in salmon is the proximal region with the pyloric caeca. However, chain length may affect where the FAs are absorbed, with the mid intestine contributing relatively more to the absorption of long-chain FAs than medium-chain FAs [72,77].

Most fish species can absorb a range of carbohydrate monomers, including glucose, galactose and fructose, all reaching the blood via specific transporters in the brush border and basolateral membrane, or by diffusion [54,58]. The mechanistic of glucose absorption has been most studied in fish to date, and gene sequences encoding the apical-located Na^+/glucose symporter SGLT1 have been identified in many fish species. In salmon, SGLT1 has been identified at both transcript and protein level [13,78], and carried-mediated glucose uptake was found to be highest in the pyloric caeca [71].

Present knowledge on the mechanisms of vitamin absorption in the GI tract of fishes is limited. Fat-soluble vitamins (A, D, E and K) and pigment carotenoids such as astaxanthin are thought to be incorporated into the micelles and absorbed when released as they disintegrate when touching the BBM surface. Minerals represent a particular case in fish, as they in addition to the alimentary tract, can also be absorbed through the gills and skin [79]. For example, metal uptake through the gills is highly interregulated with uptake in the alimentary tract [80].

3.2. Structural and functional responses to diet composition and fasting

The intestinal structure and function can respond rapidly and reversibly to changes in dietary load and composition. For example, feed restriction in salmon rapidly reduces the relative weight of the intestine, and also leads to changes in mucosal architecture that effectively reduce the absorptive area [81]. Starvation causes accumulation of digestive enzymes and bile in the pancreatic tissue and gallbladder, respectively, whereas feeding will promote emptying [82]. Enzyme secretion also appears to be regulated according to diet composition. For example, diets containing high protein levels, protein with low digestibility and/or antinutritional factors that inhibit proteases, can stimulate increased pancreatic secretion of trypsin [83–85]. The relationship between dietary lipid and carbohydrate levels and the corresponding enzymatic activity appears to be more complicated. In salmon, changes in dietary carbohydrate levels have little effect on pancreatic secretion of amylase [62,86]. Digestibility of individual fatty acids seems to decrease with increasing chain length and increase with increasing degree of desaturation [77].

4. Adverse reactions to feed

From nature's side, the salmon is a migrating carnivore. However, in an industrialized setting, salmon feed relies heavily on components obtained from terrestrial plant production. This dietary shift has not come without certain costs. The so far most severe adverse consequences have been the development of intestinal adenocarcinoma with metastasis to different organs [87]. Such findings represent however the exception. More commonly observed unwarranted feed effects are inflammatory changes. They have in particular been observed with the administration of standard soybean meal and have also been termed soybean meal induced enteritis (SBMIE). Substitution of dietary fish oil with plant oils does not seem to provoke inflammation but is rather associated with shortened mid intestinal folds in the Atlantic salmon [23].

Over the last thirty years, we have seen a steady, major change in nutrient sources and nutrient balance in salmon diets, from marine based and low lipid to high plant based and high fat [63]. The change has occurred without sufficient attention to the impact these changes might have on meeting the salmon's nutrient requirements and the impact of alien plant compounds. In parallel to diet changes, important gut health challenges have become apparent, emphasizing the need to investigate possible relationships between gut health and diet. An ongoing Norwegian research project, which was initiated with a field survey in salmon farms along the coast of Norway, revealed a high incidence of two pathological conditions which have clear links to dietary changes [88], i.e. inflammation in the second segment of the mid intestine (SSMI) (Fig. 4) and steatosis in the first (FSMI), including the pyloric caeca (Fig. 5). These conditions serve as examples of how diet may affect the structure, function and health of the intestine. Steatosis of the mid-intestine seems to be related to a dietary deficiency of choline [89,90]. Choline has until now not been considered an essential nutrient for larger Atlantic salmon. The underlying reason for this situation may be that biomarkers for capacity of lipid transport across the intestinal mucosa has not been endpoints in any of the few studies conducted to define choline requirement. Moreover, important aspects of choline and lipid metabolism, such as dependency on dietary lipid level and lipid quality, fish growth rate and feed intake, and environmental temperature, have not yet been investigated.

4.1. Intestinal inflammatory changes

The inflammation observed in the second segment of the mid intestine may be induced by one particular antinutrient, or a combination of antinutrients. Most plant feed ingredients contain several. Antinutrients are endogenous compounds in plant feedstuffs that, when fed to animals, may reduce nutrient digestibility and utilization, reduce feed intake and growth, alter the function of internal organs, and alter disease resistance. The functions of the antinutrients in the plants are, supposedly, to protect the plant from being eaten by animals, insects and microorganisms. Consequently, the antinutrients may impair functions and health of the intestine, as well as of other body organs and tissues. Legumes stand out amongst food plants, containing several of the more potent antinutrients. Table 1 lists the major, relevant antinutrients with potential to affect nutrition and health of fish. Standard varieties of soybeans contain more antinutrients than other legumes used for animal feed. Even though antinutrients got their name due to their effects on health, they may also have beneficial effects. They may act as antioxidants, stimulate immune functions, and have prebiotic effects, depending on the amount ingested.

Research on antinutritional effects in salmonids started in the late 1980s when a project was initiated to find whether soybean meal might serve as a protein source for salmon production. The results showed low nutritional value [93,94] for the standard soybean meals used for land production animals. Higher inclusion levels reduced growth and decreased both amino acid and fatty acid digestibility [95]. The most pronounced effect was, however, induction of severe inflammation in the second segment of the mid intestine even at inclusion levels as low as 5% [81,96,97]. The more proximal intestinal regions were not affected [97,98]. Later, also pea protein concentrates and other legume feed ingredients have been found to have the potential to induce similar symptoms of gut inflammation [2,99].

Lack of purified antinutrients has hampered efforts to identify which ones are responsible for the development of inflammation. Initially, several candidates were suspected. For some years a reasonably priced soy saponin concentrate of 95% purity was available, allowing use in salmon feeding studies. These studies identified saponins as the key antinutrient responsible for development of the inflammation [100]. Saponins are amphipathic molecules which compete with cholesterol for uptake. They also interfere with cell membrane structures weakening the mucosal barrier, and thereby allow influx of foreign compounds. As the inflammation induced by purified saponins seemed less severe than when the saponins were given as an integrated part of soybean meal, synergistic effects with other antinutrients were suggested [101,102]. Similar exposure studies with seabass (*Dicentrarchus labrax*) and seabream (*Sparus aurata*), at juvenile and on-growing stages, have indicated that these species are not responding with inflammation as the Atlantic

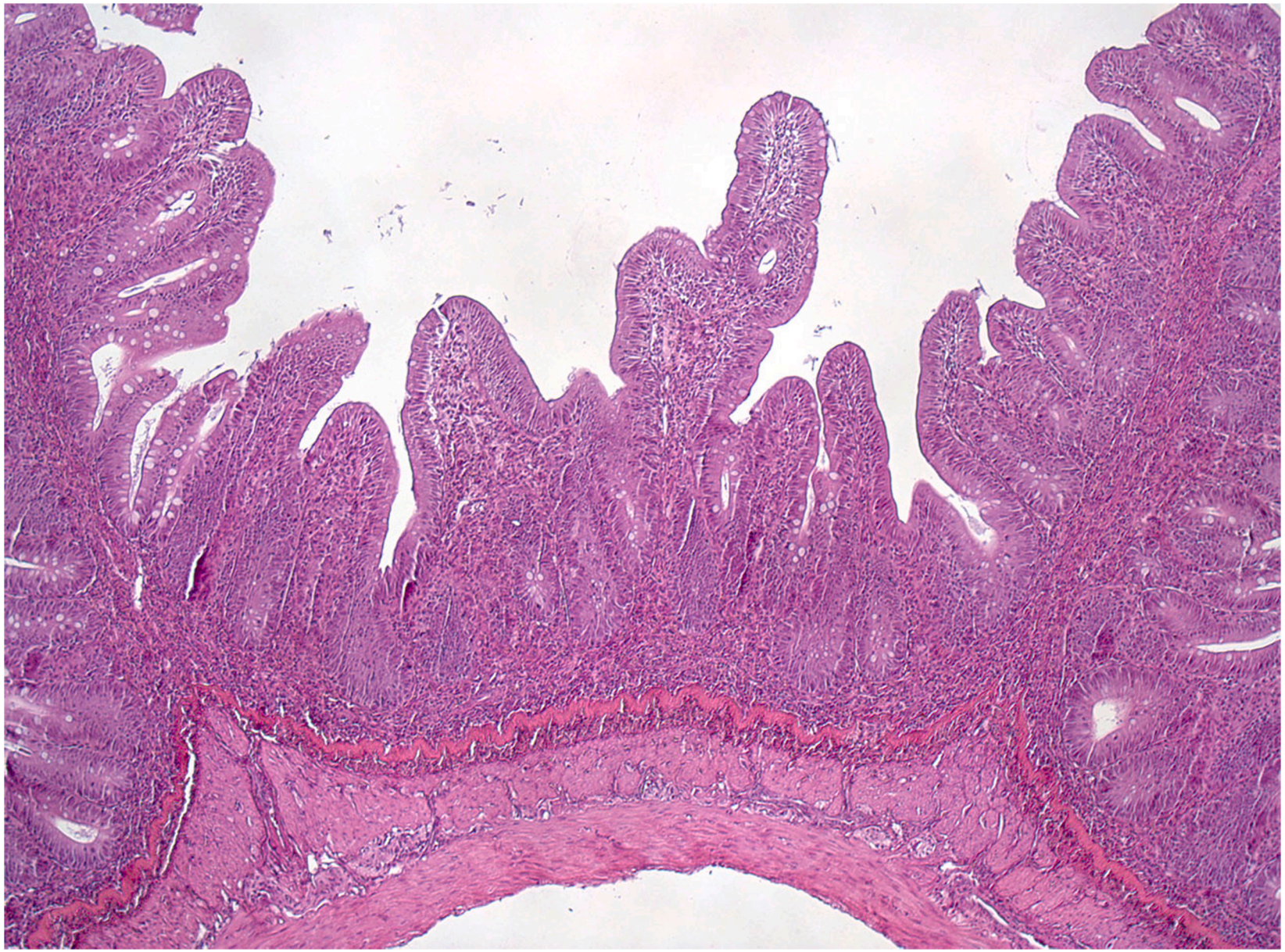

Fig. 4. Inflammatory changes in the gut. The image shows characteristics typical for soybean meal induced enteritis: Short mucosal folds, massive immune cell infiltration in lamina propria and absence of supranuclear vacuoles in the enterocytes.

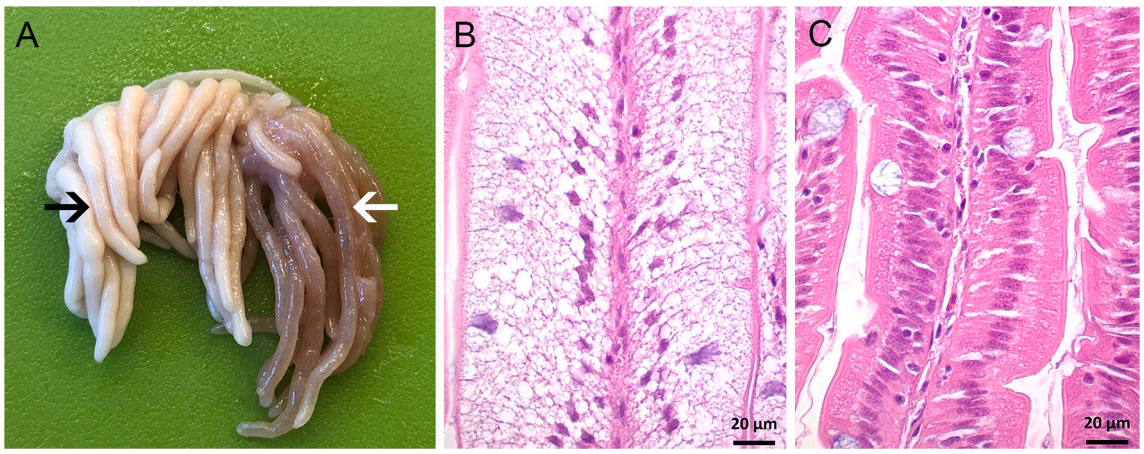

Fig. 5. Steatosis. A: Macroscopic appearance of steatosis in the pyloric caeca. Note both the swollen and pale caeca, a result of excessive lipid accumulation (black arrow), and the unaffected darker-appearing caeca (white arrow). B: Enterocytes of the pyloric caeca with high degree of hyper-vacuolization/steatosis and C: normal-appearing enterocytes.

Table 1
Current antinutrients which may affect digestive functions and gut health in salmon[a].

Antinutrient	Common mechanisms and effects
Enzyme inhibitors	Inhibit macronutrient digestion
Lectins	Bind to gut cell receptors and, depending on affected receptor, may accelerate gut growth, make the gut leakier and more open for increased influx of macromolecules and bacteria, stimulate insulin production and alter metabolism
Saponins	Interfere with lipid and protein digestion and which also may increase permeability of the gut mucosa
Phytosterols	Interfere with cholesterol absorption and metabolism
Phytic acid	Impairs mineral digestion and binds phosphorus in particular
Oligosaccharides	May cause diarrhea and alter the microbiota
Fibers	Interfere with digestion, absorption and utilization of macro as well as micronutrients

[a] Information extracted from reviews by Francis et al. [91] and Krogdahl et al. [92].

salmon, when fed purified saponins, although the sea bass juveniles showed some alterations in digestive and immune functions [103–106]. The authors suggested that these alterations might affect the fish at later stages, but this has not yet been investigated.

After the first observations of diet-induced enteritis, this condition has become a valuable, inducible condition for investigation of basic mechanisms including mucosal immune responses of the intestine, in particular the distal compartment, or the second segment of the mid intestine, which harbors the most complex conglomeration of barrier functions in the salmon. The results of the studies of soybean induced enteritis under varying dietary and other environmental conditions and at different life stages of the fish, have thrown light on the mechanisms, complexity and dynamics of the intestinal mucosa. The following paragraphs summarize the results of studies conducted over the last thirty years with a focus on understanding underlying mechanisms of this enteritis and possible dietary, preventive measures.

The symptoms of inflammation in the second segment of the mid intestine are characterized by shortening of mucosal folds, loss of normal vacuolization of enterocytes, widening of lamina propria with increased amounts of connective tissue and a profound infiltration of inflammatory cells. Electron microscope images reveal severe shortening and thinning of the brush border [97]. A reduction in tissue weight is also a clear symptom [100]. Similar symptoms have been observed in rainbow trout (*Oncorhynchus mykiss*) [107] and Arctic charr (*Salvelinus alpinus*) after feeding with soybean containing diets [108], whereas other fish species appear only temporary or unaffected by inclusion standard soybean meal qualities in the diets [109,110]. Atlantic cod (*Gadus morhua*) seem to tolerate soybean meal with saponins quite well showing no indications of intestinal inflammation [111].

In Atlantic salmon, the first pathological changes after initiation of feeding a diet with soybean meal, limited to the second segment of the mid intestine, may be observed as early as after two days. Within seven days, all mentioned symptoms are apparent, and they are increasing in severity at least until 21 days after initiation of soybean feeding [112,113]. The symptoms disappear gradually after termination of feeding with soybean meal, and the tissue appears normal again after about three weeks [81]. The inflammation causes severe functional losses of the brush border, indicated by loss of activity of 5' nucleotidase, Mg^{2+}-ATPase, alkaline phosphatase, leucine aminopeptidase, and several disaccharidases. Also the intracellular structures show impairment as indicated by loss of activity in alkaline and acid phosphatase, non-specific esterase and alanine aminopeptidase [98,114]. Moreover, presence of monocytes, including macrophages, as well as of neutrophilic granulocytes and IgM positive cells, increases in the lamina propria. In a more recent study [115], further details of the immune cells involved in the inflammation were revealed. Soybean meal in the diets increased expression of a complex polypeptide (CD3pp), CD4 and CD8b. Increased reactivity for extracellular IgM in the lamina propria and IgM positive material between the epithelial cells at the tips of the folds were also observed. The authors suggested that the observations could be due to leakage of IgM through an abrogated epithelial barrier and that this example of a food-sensitive enteropathy could involve T-cell-like responses. The observed up-regulation of genes and regulators related to production of cytokines, NFkB and TNFalpha, IL-17 and other regulators of T-cell function [102,116] supports this theory. The latter work also showed activation of Annexin-1, an important anti-inflammatory and gastroprotective compound [102]. The results of the work of De Santis et al. (2015) are in line with the results reviewed above [117].

The antinutrients in the soybean meal seem to reduce nutrient digestibilities by affecting epithelial cell differentiation in the second segment of the mid intestine and thereby impairing digestive functions by reducing presence of nutrient transporters and regulators of water balance (e.g. aquaporin, guanylin). Also expression of genes involved in a range of metabolic processes, e.g. in lipid, bile and steroid metabolism, are severely down-regulated [76,102,113,118].

Not only the digestive, metabolic and immune functions but also the many other elements of the mucosal barrier functions are affected in the inflamed intestine. The work of Kortner et al. (2012) showed induction of the complement and the respiratory burst complex which paralleled a down-regulation of genes for free radical scavengers and iron binding proteins. Marked down-regulation of xenobiotic metabolism was also

observed, possibly increasing vulnerability of the intestinal tissue to a wide range of organic compounds [102].

Many of the observed functional effects of legume antinutrients are supposedly closely linked to, and possibly a consequence of, the increase in cell division and migration of the cells towards the tip of the intestinal fold where shedding results in shorter lifetime of the cells and limited time for cell differentiation. Decreased migration time, with less time for differentiation, is well documented on both histological and molecular levels [102,119,120]. The estimated time to reach the tip of the mucosal fold in the second segment of the mid intestine was 112 and 36 days for fish fed a high fishmeal diet kept at 8 and 12 °C, respectively. In fish fed a diet with 25% soybean meal, the time was reduced to about 16 days, irrespective of environmental temperature, i.e. 8 and 12 °C [119]. Increased cell division increases demand for polyamines. Accordingly, up-regulation of arginase and ornithine decarboxylase has been shown.

A study by Krogdahl et al. [114], showed an increase in faecal trypsin-like activity with increasing soybean inclusion in the diet. This observation has later on been found to be linked to activation of trypsin-like enzymes in the mucosa which sloughs off at a high rate from the inflamed tissue [121]. Trypsin and other serine proteinases are known as key initiators of inflammation in animals through modulation of proteinase-activated receptor 2a (PAR-2). Upregulation was observed in the first days after the introduction of soybean meal in the diet [114], indicating a role in the initial stages of the inflammation, and down-regulation in the more chronic stages (after three weeks), suggesting desensitization of the receptor.

Most of the experiments done with Atlantic salmon to understand effects of soybean antinutrients and reveal effects on functional characteristics of the intestine have been conducted with fish in saltwater, or late in the freshwater phase. Very few have been conducted with fish at earlier stages. One exception is the study of Sahlman et al. with fish from hatching and 14 weeks onward [13]. The goal was to fill knowledge gaps regarding ontogeny of the structure and functions of the gastrointestinal tract, of utmost importance for successful introduction of alternative feed ingredients in salmon aquaculture. The fish were exposed to a high marine diet as well as a diet with 17% soybean meal level, well above the level causing enteritis in fish at later developmental stages. The digestive system of Atlantic salmon alevins was morphologically distinct with an early stomach, liver, pancreas, anterior and posterior intestine already seven days post hatch. About one week before start feeding, and before the yolk sac was empty, gastric glands and pyloric caeca were observed. At the same time expression of genes of digestive enzymes and nutrient transporters increased. In contrast to post-smolt Atlantic salmon, inclusion of SBM did not induce intestinal inflammation in the juveniles, nor or loss of function [13]. Similar observations were made when pure soya saponins were fed to juveniles [68,122]. Moreover, growth performance in these young fish responded positively to saponin supplementation [122], also this in contrast to salmon at later stages. The results suggest that the Atlantic salmon gut's immune apparatus is immature at the earlier life stages and does not respond to influx of alien compounds as the more mature intestine. Studies of the ontogeny of key immune molecules in the rainbow trout have shown fairly early expression post fertilization [123], but this does not imply that the immune system is competent.

Another intriguing observation regarding development of soybean meal induced enteritis was made in a study with rainbow trout, a species showing very similar responses to soybean meal as the Atlantic salmon [120]. Two populations of fish were compared, one being a local unselected strain kept on a regular trout diet, and the other being a local strain selected for increased growth rate over four generations on an all plant diet. When the two strains were given a diet with 19% soybean meal, the unselected individuals grew slower than the selected and showed all signs of soybean induced enteritis. In the fish from the selection program, there were no indications of enteritis. The results indicate the ability of an animal species to adapt to dietary challenges over time.

4.2. Lipid malabsorption in Atlantic salmon

During the last 20 years, salmon farmers have reported symptoms indicating an intestinal problem, characterized by pale and foamy appearance of the enterocytes of the first segment of the mid-intestine (MI1), including the pyloric caeca (PC) [124,125]. The symptoms, also called steatosis, are a result of intracellular accumulation of lipid (triacylglycerol) droplets [90]. Very recently, the steatosis, was shown to be due to a deficiency of dietary choline [89,90,126,127]. The symptoms increase with increasing levels of plant ingredients in the diet, strongly suggesting that they are related to the high plant content of today's salmon feeds. In practical terms, diets with < 5–10% fish meal will be severely deficient in choline if not supplemented. The choline requirement will most likely vary with production conditions such as dietary lipid level and quality, growth rates and temperature, but such aspects have not been studied until now The recent results regarding choline requirement have also greatly accentuated the need to understand how lipids are transported from the intestine to the peripheral tissues in Atlantic salmon. It has long been a debate if lymphatic vessels in fish exist or not [128]. The work of Denstadli et al. [129] suggests that the portal vein is an important transport route for lipid in Atlantic salmon, but that also other routes are possible.

4.3. Inflammation and carcinogenesis

Chronic inflammation, as caused by for instance anti-nutrients, may over time induce additional side-effects. Dale et al. (2009) [87] described adenocarcinoma in broodstock salmon intestine following the inflammation – dysplasia – carcinoma sequence. Enterocytes are polarized cells with their nuclei located proximal towards the basal membrane. Following dysregulation of the cells, nuclei may change their location within the cells, and the term dysplasia is used to describe this phenomenon. Enterocyte dysplasia typically occurs in human patients suffering from inflammatory bowel disease (IBD). The next stage in an inflammation – dysplasia – carcinoma sequence will be dislocation of enterocytes below the basal membrane [130]. These dislocated epithelial cells may, or may not, develop into tumors. Recently, Bjørgen et al. (2018) [131] identified dislocated epithelial cells in fish fed commercial fish feed. Approximately at the same time, Mosberian-Tanha et al. (2018) [132] described similar findings but argued that seemingly dislocated epithelial cells were macrophages that had engulfed epithelial cells and migrated beneath the basal membrane. Anyhow, in the case of tumor development, the course of events was established by Dale et al. (2009) [87] who showed that solid tumors with metastasis developed in affected fish. In yet a recent study, Bjørgen et al. (2019) [26] demonstrated that the tumor microenvironment as defined by the presence of different leukocyte populations closely resembled that of human adenocarcinoma. The reactions to chronic intestinal inflammation and its consequences thus seem astonishingly similar between very distant species (fish and man).

5. Microbiota – new feed

It is well recognized in human medicine, that the gut microbiota may play pivoting roles for gut immune function and health in particular regarding inflammatory conditions [133,134]. However, present knowledge on gut microbiota in the fish intestine, and its role in for development of feed induced enteritis and other pathological conditions, is very limited. The following review of literature presenting relevant studies of gut microbiota in fish, with particular emphasis on Atlantic salmon, underlines this situation.

Intestinal microbiota, comprising dense populations of diverse microorganisms including bacteria, archaea, viruses and fungi, are located in two major compartments, the digesta and the mucus. It intimately interacts with the host in many ways, from food digestion and absorption [135] to lipid metabolism and energy balance [136,137]. The

intestinal microbiota is, in various aspects, closely connected to the intestinal function and health. It has become a therapeutic target for intestinal diseases in humans like inflammatory bowel disease [138,139] and *Clostridium difficile* infection [140]. Similar to the findings in germ-free mice [141,142], intestinal microbiota has also been demonstrated to be an essential element in the development of normal intestinal structure and function in zebrafish [143–145]. For instance, the intestinal epithelium of germ-free zebrafish, compared to normal fish, is arrested in its differentiation, as revealed by the lack of brush border intestinal alkaline phosphatase activity, the maintenance of immature patterns of glycan expression and a paucity of goblet and enteroendocrine cells [144]. Furthermore, intestinal microbiota interacts directly or indirectly with the intestinal immune system to induce pro- or anti-inflammatory responses, playing a fundamental role in the maintenance of homeostasis of intestinal immune responses. The interaction may take place via direct contact between microbes and intestinal epithelial cells [146] or immune cells [147], or via microbial-derived metabolites such as lipopolysaccharide (LPS) [143], polysaccharide A (PSA) [148] and short-chain fatty acids (SCFAs) [149,150]. The SCFAs, mainly acetate, propionate, and butyrate, are versatile microbial metabolites produced under anaerobic fermentation of dietary fiber and protein [151]. In mammals, the SCFAs, butyrate in particular, are well-known for the anti-inflammatory effects via inhibition of histone deacetylates (HDAC) and activation of G-protein coupled receptors (GPCRs) [152]. A recent study in zebrafish indicates that the anti-inflammatory effects of butyrate is most likely a conservated characteristic in vertebrates [153]. Besides dialoguing with the local immune system, the intestinal microbiota also interacts with the systemic immune system. Exposure to antibiotics in early life has been shown to impair antibody responses to vaccines in later life in mice. However, inoculation with the commensal microbiota following the antibiotic exposure restored the response [154]. In salmonids, sphingolipids produced by *Flectobacillus major*, a predominant symbiont at the gill and skin mucosal surfaces of rainbow trout, were able to increase the proportion of IgT positive to IgM positive B cells in the head kidney when administered intravenously [155].

Given the immunomodulatory effects of intestinal microbiota, dietary supplementation of microbial-derived products has been applied to mitigate intestinal inflammation in Atlantic salmon. For instance, dietary supplementation of two lactic acid bacteria (*Lactococcus lactis* and *Carnobacterium maltaromaticum*) was found to diminish the enteritis induced by diets containing 38% soybean meal [156], whereas the addition of Bactocell®, a commercial probiotic product containing *Pediococcus acidilactici* CNCM MA18/5 M, abated an intestinal inflammation chemically induced by anal intubation with oxazolone [157]. Bacterial meal and cell wall fractions produced from *Methylococcus capsulatus* grown on natural gas were also shown to prevent the enteritis induced by 20% soybean meal [158–160]. Besides bacteria, dietary inclusion of yeast (*Candida utilis*) was also reported to counteract the enteritis induced by 20% soybean meal [161]. However, later studies showed that the same dose of *Candida utilis* was unable to counteract the enteritis induced by 20% [162] or 40% [163] soybean meal. These results provide evidence that microbiota is a promising target that can be selectively manipulated to improve the fish gut health status. However, the mode of actions behind these microbial-derived products remains unexplored. A better understanding of factors influencing the dynamics of intestinal microbiota composition and function will allow for targeted engineering of microbiota to sustain a healthy gut. Thanks to the advances in the sequencing technologies in the last decade, there has been a great increase in the number of molecular-based studies of salmonid intestinal microbiota. Here we summarize important findings from recent studies and highlight knowledge gaps that need to be filled in.

Like in mammals [164,165], the salmon intestinal microbiota also shows a spatial heterogeneity in its composition [166]. Microbial communities are different not only along the intestinal tube, but also between digesta and mucosa within the same intestinal segment. Typically, the microbial richness and diversity are lower in the intestinal mucosa than digesta [2,167,168], suggestive of selection pressure from the host [169]. The salmon intestinal microbiota is influenced by many factors including, but not limited to, developmental stages [170,171], diets [2,4,172], rearing environments [3], antibiotics [173] and genetics [6]. In the early developmental stages in the freshwater, the salmon intestinal microbiota seems to be mostly dominated by Proteobacteria, Bacteroidetes, Firmicutes and Tenericutes. As the salmon enter the seawater and grow older, the abundance of Bacteroidetes and Firmicutes decreases while the abundance of Tenericutes and Spirochaetes increases [5,170,171]. The intestinal microbiota of salmon in the seawater, especially the adult salmon, is often predominated by a few phylotypes including *Allivibrio* (Proteobacteria), *Photobacterium* (Proteobacteria), *Mycoplasma* (Tenericutes) and *Brevinema* (Spirochaetes) [170,171,174–176], resulting in lower microbial richness in the later life stages. *Aliivibrio* and *Photobacterium*, both belonging to the Vibrionaceae family, are common bacterial inhabitants in the seawater. Their colonization in the salmon intestine may be facilitated by the seawater drinking behavior of post-smolt salmon to prevent dehydration in a hyperosmotic environment. In contrast, *Mycoplasma* tended to be rare [177] or absent [175,178] in the surrounding seawater where the salmon were sampled. *Mycoplasma* seems to be particularly well-adapted to the intestinal environment of Atlantic salmon [177, 179]. Notably, *Mycoplasma* also sporadically predominates intestinal microbial community of Chinook salmon (*Oncorhynchus tshawytscha*) [180] and rainbow trout [181–184]. Known for its small compact genome and limited biosynthesis capacities, *Mycoplasma* often forms obligate parasitic or commensal relationships with its host to obtain necessary nutrients [185]. *Mycoplasma* is likely a commensal microbe in the salmonid intestine whose ecological and functional significance remains to be revealed. *Brevinema* was recently reported to be selectively enriched in the intestinal mucosa of Atlantic salmon and associated with the immune gene expressions in the distal intestine [186]. Captive rearing of the salmon seems to favor the colonization of *Brevinema* in the intestine, which is impaired when salmon is translocated from hatchery to natural conditions [177].

Diet is a key factor in shaping the intestinal microbiota of fish. Different dietary components may selectively promote or suppress the growth of certain microbial clades, which in turn could produce profound effects on the host health and disease resistance [140,187]. The use of alternative feed ingredients for fishmeal and fish oil in salmon feeds can result in altered intestinal microbiota [2,4,172,188]. For instance, less-refined plant-based ingredients such as soybean meal seemed to selectively increase the abundance of lactic acid bacteria in the salmon intestine [2,4,188], whereas insect (*Hermetia illucens*) larvae meal was found to increase the abundance of specific microbial clades including *Actinomyces*, *Bacillus*, *Brevibacterium*, *Corynebacterium* and *Enterococcus* in the salmon [186] and rainbow trout intestine [189,190]. Notably, diet modulates digesta- and mucosa-associated intestinal microbiota to differing degrees. The mucosa-associated microbiota seems to more resilient to dietary changes [2,168,176,190–192]. It is believed that mucosa-associated microbiota may play a more crucial role in influencing the host physiological activities as these microbes can interact both directly and indirectly with the intestinal epithelial barrier, whereas the more transient digesta-associated microbiota can only interact indirectly [169]. As such, profiling digesta-associated microbiota alone, which is a common practice in microbiota studies, may obscure the response and importance of intestinal microbiota to dietary changes. Concurrent profiling of digesta- and mucosa-associated intestinal microbiota should be performed whenever feasible so that the response of intestinal microbiota to dietary changes can be fully disclosed.

While marker-gene sequencing has enabled reliable and affordable taxonomic profiling of intestinal microbiota, there is a knowledge gap on the functional implications of changes in the intestinal microbiota induced by dietary shifts. Collecting metadata related to host responses

and phenotypes of interests and identifying their associations with changes in the intestinal microbiota is the first step towards discovering keystone microbes that are pivotal to intestinal functions and health. Combining marker-gene surveys with other meta-omics approaches, such as shotgun metagenomics, metatranscriptomics and metabolomics, will add a new dimension to the microbial profiling in answering the question: what are the microbes doing. In particular, microbial metabolites play critical roles in bridging the dialogue, or the signaling pathways, between the intestinal microbiota and host. Coupling taxonomic profiling with metabolomics is a promising approach to gain functional insights and translational results, especially when the metabolites of interest can be extracted from natural products or synthesized. Establishing germ-free salmonid models will allow for testing hypotheses generated from the omics data and establishing causality between intestinal microbiota and host responses. However, germ-free fish models so far can only be maintained in the larval stage [193], which greatly limits their applications when it comes to studying the interactions between diet and microbiota.

6. Sum

In an increasing industrialized salmonid production, a key component to animal welfare, general health and growth, is a well-functioning gastrointestinal system. To understand its construction and function is thus of major importance for both the academic community and the industry. We still lack basic key knowledge regarding its construction and function, and our ability to solve the problems that we observe, and thus contributing to improved animal health and welfare, are still limited. In addition, the following knowledge gaps deserve attention in future studies:

1. Effects of vitamin and mineral deficiencies and excess on intestinal function and health are largely unknown.
2. Anatomical and physiological mechanisms involved in lipid transport have not been clarified.
3. The route of enzymes from the pancreatic tissue to the intestinal lumen has not been described.
4. The role of supranuclear vacuoles present in the distal most segments of a well-fed Atlantic salmon had not been described, i.e. whether they transport nutrients, intact proteins, endogenous enzymes, antigens, or have other purposes. They disappear when the tissue is inflamed, and when the fish is starved.
5. Most antinutrients in plant feedstuffs exert their main effect in the intestine, but present knowledge on their effects in the fish intestine is limited to a few of these
6. The immunological explanation for lack of saponin induced enteritis in young fish should be clarified
7. Present knowledge of gut microbiota in fish is still weak, but new tools and improved understanding of its importance for function and health stimulates efforts to characterize and find the important links. Together with improved knowledge concerning construction and function of the gastrointestinal system, this research may be of great benefit to sustainable aquaculture production.

Funding

We thank FHF – Norwegian Seafood Research Fund, project GutMatters, FHF project no. 901435, for economic support.

CRediT authorship contribution statement

Håvard Bjørgen: Literature collection, writing of the manuscript and editing of figures. **Yanxian Li:** Literature collection, writing of the manuscript and editing of figures. **Trond M. Kortner:** Literature collection, writing of the manuscript and editing of figures. **Åshild Krogdahl:** Literature collection, writing of the manuscript and editing of figures. **Erling Olaf Koppang:** Literature collection, writing of the manuscript and editing of figures.

References

[1] G. Løkka, E.O. Koppang, Antigen sampling in the fish intestine, Dev. Comp. Immunol. 64 (2016) 138–149.
[2] K. Gajardo, et al., Alternative protein sources in the diet modulate microbiota and functionality in the distal intestine of atlantic salmon (Salmo salar), Appl. Environ. Microbiol. 83 (5) (2017).
[3] C.E. Dehler, C.J. Secombes, S.A.M. Martin, Environmental and physiological factors shape the gut microbiota of Atlantic salmon parr (Salmo salar L.), Aquaculture 467 (2017) 149–157.
[4] V. Schmidt, et al., Influence of fishmeal-free diets on microbial communities in Atlantic salmon (Salmo salar) recirculation aquaculture systems, Appl. Environ. Microbiol. 82 (15) (2016) 4470–4481.
[5] T.M.U. Webster, et al., Interpopulation variation in the atlantic salmon microbiome reflects environmental and genetic diversity, Appl. Environ. Microbiol. 84 (16) (2018).
[6] X.P. He, S.R. Chaganti, D.D. Heath, Population-specific responses to interspecific competition in the gut microbiota of two atlantic salmon (Salmo salar) populations, Microb. Ecol. 75 (1) (2018) 140–151.
[7] J.A. Mather, Ethics and care: for animals, not just mammals, Animals 9 (12) (2019) 1018.
[8] N. Kraimi, et al., Influence of the microbiota-gut-brain axis on behavior and welfare in farm animals: a review, Physiol. Behav. (2019) 112658.
[9] Hellberg, H., Teleost gut morphology in sickness and health, with emphasis on mast cell ontogeny and alimentary canal development in common wolfish, Anarhichas lupus Linnaeus, 1758, In Department of Anatomy. 2019, Norwegian University of Life Sciences.
[10] K.N. Wallace, M. Pack, Unique and conserved aspects of gut development in zebrafish, Dev. Biol. 255 (1) (2003) 12–29.
[11] D. Kobayashi, et al., *Development of the endoderm and gut in medaka, Oryzias latipes.* Development, Growth & Differentiation 48 (5) (2006) 283–295.
[12] D.A. Beauchamp, Bioenergetic ontogeny: linking climate and mass-specific feeding to life-cycle growth and survival of salmon, American Fisheries Society Symposium, 2009.
[13] C. Sahlmann, et al., Ontogeny of the digestive system of Atlantic salmon (Salmo salar L.) and effects of soybean meal from start-feeding, PloS One 10 (4) (2015).
[14] M. Penaz, A general framework of fish ontogeny: a review of the ongoing debate, J Folia Zoologica 50 (4) (2001) 241–256.
[15] A. Zapata, et al., Ontogeny of the immune system of fish, J Fish shellfish immunology 20 (2) (2006) 126–136.
[16] P. De Santa Barbara, G.R. Van Den Brink, D.J. Roberts, Development and differentiation of the intestinal epithelium, Cell. Mol. Life Sci. 60 (7) (2003) 1322–1332.
[17] K.N. Wallace, et al., Intestinal growth and differentiation in zebrafish, Mech. Dev. 122 (2) (2005) 157–173.
[18] A.N. Ng, et al., Formation of the digestive system in zebrafish: III. Intestinal epithelium morphogenesis, Dev. Biol. 286 (1) (2005) 114–135.
[19] G. Løkka, et al., Intestinal morphology of the wild Atlantic salmon (Salmo salar), J. Morphol. 274 (8) (2013) 859–876.
[20] E. Koppang, et al., Differing levels of Mhc class II β chain expression in a range of tissues from vaccinated and non-vaccinated Atlantic salmon (Salmo salarL.), Fish Shellfish Immunol. 8 (3) (1998) 183–196.
[21] E. Koppang, et al., Expression of Mhc class I mRNA in tissues from vaccinated and non-vaccinated Atlantic salmon (Salmo salarL.), Fish Shellfish Immunol. 8 (8) (1998) 577–587.
[22] H. Harstad, et al., Multiple expressed MHC class II loci in salmonids; details of one non-classical region in Atlantic salmon (Salmo salar), BMC Genom. 9 (1) (2008) 193.
[23] T. Moldal, et al., Substitution of dietary fish oil with plant oils is associated with shortened mid intestinal folds in Atlantic salmon (Salmo salar), BMC Vet. Res. 10 (1) (2014) 60.
[24] G. Løkka, et al., Immune parameters in the intestine of wild and reared unvaccinated and vaccinated Atlantic salmon (Salmo salar L.), Dev. Comp. Immunol. 47 (1) (2014) 6–16.
[25] T. Van den Ingh, et al., Effects of soybean-containing diets on the proximal and distal intestine in Atlantic salmon (Salmo salar): a morphological study, Aquaculture 94 (4) (1991) 297–305.
[26] H. Bjørgen, et al., Tumor microenvironment and stroma in intestinal adenocarcinomas and associated metastases in Atlantic salmon broodfish (Salmo salar), Vet. Immunol. Immunopathol. 214 (2019) 109891.
[27] B. Fuglem, et al., Antigen-sampling cells in the salmonid intestinal epithelium, Dev. Comp. Immunol. 34 (7) (2010) 768–774.
[28] T. Ayabe, et al., The role of Paneth cells and their antimicrobial peptides in innate host defense 12 (8) (2004) 394–398.
[29] H. Hellberg, I. Bjerkas, The anatomy of the oesophagus, stomach and intestine in common wolffish (Anarhichas lupus L.): a basis for diagnostic work and research, Acta Vet. Scand. 41 (3) (2000) 283–298.
[30] E. Koppang, et al., Expression of insulin-like growth factor-I in the gastrointestinal tract of Atlantic salmon (Salmo salar L.), Fish Physiol. Biochem. 18 (2) (1998) 167–175.
[31] J. Paneth, Ueber die secernirenden Zellen des Dünndarm-Epithels, Arch. Mikrosk. Anat. 31 (1) (1887) 113.

[32] A. Harte, et al., Five subfamilies of β-defensin genes are present in salmonids: evolutionary insights and expression analysis in Atlantic salmon Salmo salar, Dev. Comp. Immunol. 104 (2020) 103560.
[33] M.H. Jang, et al., Intestinal villous M cells: an antigen entry site in the mucosal epithelium, Proc. Natl. Acad. Sci. Unit. States Am. 101 (16) (2004) 6110–6115.
[34] G. Løkka, et al., Uptake of yeast cells in the Atlantic salmon (Salmo salar L.) intestine, Dev. Comp. Immunol. 47 (1) (2014) 77–80.
[35] H. Ishikawa, et al., Curriculum vitae of intestinal intraepithelial T cells: their developmental and behavioral characteristics, Immunol. Rev. 215 (1) (2007) 154–165.
[36] D.P.H. van Konijnenburg, D. Mucida, Intraepithelial lymphocytes, J Current Biology 27 (15) (2017) R737–R739.
[37] E.O. Koppang, et al., Salmonid T cells assemble in the thymus, spleen and in novel interbranchial lymphoid tissue, J. Anat. 217 (6) (2010) 728–739.
[38] J.H. Rombout, G. Yang, V. Kiron, Adaptive immune responses at mucosal surfaces of teleost fish, Fish Shellfish Immunol. 40 (2) (2014) 634–643.
[39] S. Grove, et al., Immune-and enzyme histochemical characterisation of leukocyte populations within lymphoid and mucosal tissues of Atlantic halibut (Hippoglossus hippoglossus), Fish Shellfish Immunol. 20 (5) (2006) 693–708.
[40] N.A. Ballesteros, et al., The pyloric caeca area is a major site for IgM+ and IgT+ B cell recruitment in response to oral vaccination in rainbow trout, PloS One 8 (6) (2013).
[41] D. Parra, et al., B cells and their role in the teleost gut, Dev. Comp. Immunol. 64 (2016) 150–166.
[42] J.H. Rombout, et al., Teleost intestinal immunology, Fish Shellfish Immunol. 31 (5) (2011) 616–626.
[43] O.M. Løken, et al., A Teleost Structural Analogue to the Avian Bursa of Fabricius, J Anat, 2019.
[44] T. Takeuchi, T. Gonda, Distribution of the pores of epithelial basement membrane in the rat small intestine, J. Vet. Med. Sci. 66 (6) (2004) 695–700.
[45] R. Azumi, et al., Dynamics of basal lamina fenestrations in the rat intestinal villous epithelium in response to dietary conditions 39 (2) (2018) 65–74.
[46] A. Frey, et al., Role of the glycocalyx in regulating access of microparticles to apical plasma membranes of intestinal epithelial cells: implications for microbial attachment and oral vaccine targeting, J. Exp. Med. 184 (3) (1996) 1045–1059.
[47] M. Powell, D.J. Speare, G.M. Wright, Comparative ultrastructural morphology of lamellar epithelial, chloride and mucous cell glycocalyx of the rainbow trout (Oncorhynchus mykiss) gill, J. Fish. Biol. 44 (4) (1994) 725–730.
[48] C. Jin, et al., Atlantic salmon carries a range of novel O-glycan structures differentially localized on skin and intestinal mucins, J. Proteome Res. 14 (8) (2015) 3239–3251.
[49] I. Salinas, D. Parra, Fish mucosal immunity: intestine. Mucosal Health in Aquaculture, Elsevier, 2015, pp. 135–170.
[50] D. Gomez, J.O. Sunyer, I. Salinas, The mucosal immune system of fish: the evolution of tolerating commensals while fighting pathogens, Fish Shellfish Immunol. 35 (6) (2013) 1729–1739.
[51] Z. Xu, et al., Specialization of mucosal immunoglobulins in pathogen control and microbiota homeostasis occurred early in vertebrate evolution, Sci Immunol 5 (44) (2020).
[52] W.O. Vogel, Zebrafish and lymphangiogenesis: a reply, Anat. Sci. Int. 85 (2) (2010) 118–119.
[53] H. Hellberg, et al., Mast cells in common wolffish Anarhichas lupus L.: ontogeny, distribution and association with lymphatic vessels, Fish Shellfish Immunol. 35 (6) (2013) 1769–1778.
[54] A.M. Bakke, C. Glover, Å. Krogdahl, Feeding, digestion and absorption of nutrients, in: M. Grosell, A.P. Farrell, C.J. Brauner (Eds.), The Mutifunctional Gut of Fish, 2011.
[55] R.K. Buddington, Å. Krogdahl, A.M. BakkeMcKellep, The intestines of carnivorous fish: structure and functions and the relations with diet, Acta Physiol. Scand. 161 (1997) 67–80.
[56] M.B. Rust, in: J.E. Halver, R.W. Hardy (Eds.), Nutritional Physiology, 2002.
[57] A. Krogdahl, A. Sundby, H. Holm, Characteristics of digestive processes in Atlantic salmon (Salmo salar). Enzyme pH optima, chyme pH, and enzyme activities, Aquaculture 449 (2015) 27–36.
[58] A. Krogdahl, A. Sundby, Characteristics of pancreatic function in fish, Biology of the Pancreas in Growing Animals 28 (1999) 437–458.
[59] N. Smichi, et al., Efficient heterologous expression, functional characterization and molecular modeling of annular seabream digestive phospholipase A(2), Chem. Phys. Lipids 211 (2018) 16–29.
[60] N. Achouri, et al., Dissecting the interaction deficiency of a cartilaginous fish digestive lipase with pancreatic colipase: biochemical and structural insights, BioMed Res. Int. (2020) 2020.
[61] Å. Krogdahl, A. Sundby, J.J. Olli, Atlantic salmon (Salmo salar) and rainbow trout (Oncorhynchus mykiss) digest and metabolize nutrients differently. Effects of water salinity and dietary starch level, Aquaculture 229 (1–4) (2004) 335–360.
[62] M.K. Frøystad, et al., Cloning and characterization of alpha-amylase from Atlantic salmon (Salmo salar L.), Comparative Biochemistry and Physiology A-Molecular & Integrative Physiology 145 (4) (2006) 479–492.
[63] T.S. Aas, T. Ytrestoyl, T. Asgard, Utilization of feed resources in the production of Atlantic salmon (Salmo salar) in Norway: an update for 2016 15, Aquaculture Reports, 2019.
[64] T.M. Kortner, et al., Dietary cholesterol supplementation to a plant-based diet suppresses the complete pathway of cholesterol synthesis and induces bile acid production in Atlantic salmon (Salmo salar L.), Br. J. Nutr. 111 (2014) 2089–2103.
[65] O.H. Romarheim, et al., Comparison of white flakes and toasted soybean meal partly replacing fish meal as protein source in extruded feed for rainbow trout (Oncorhynchus mykiss), Aquaculture 256 (1–4) (2006) 354–364.
[66] O.H. Romarheim, et al., Lipid digestibility, bile drainage and development of morphological intestinal changes in rainbow trout (Oncorhynchus mykiss) fed diets containing defatted soybean meal, Aquaculture 274 (2–4) (2008) 329–338.
[67] T.M. Kortner, et al., Transcriptional regulation of cholesterol and bile acid metabolism after dietary soybean meal treatment in Atlantic salmon, Salmo salar L, Br. J. Nutr. 109 (2013) 593–604.
[68] M. Gu, et al., Effects of dietary plant meal and soya-saponin supplementation on intestinal and hepatic lipid droplet accumulation and lipoprotein and sterol metabolism in Atlantic salmon (Salmo salar L.), Br. J. Nutr. 111 (3) (2014) 432–444.
[69] T.M. Kortner, et al., Bile components and lecithin supplemented to plant based diets do not diminish diet related intestinal inflammation in Atlantic salmon, BMC Vet. Res. 12 (2016).
[70] G.M. Green, E.S. Nasset, Importance of bile in regulation of intraluminal proteolytic enzyme activities in the rat, Gastoenterology 79 (1980) 695–702.
[71] A.M. Bakke-McKellep, et al., Absorption of glucose, amino acids, and dipeptides by the intestines of Atlantic salmon (Salmo salar L.), Fish Physiol. Biochem. 22 (1) (2000) 33–44.
[72] V. Denstadli, et al., Lipid absorption in different segments of the gastrointestinal tract of Atlantic salmon (Salmo salar L.), Aquaculture 240 (1–4) (2004) 385–398.
[73] I. Ronnestad, et al., Molecular cloning and functional expression of atlantic salmon peptide transporter 1 in Xenopus oocytes reveals efficient intestinal uptake of lysine-containing and other bioactive di- and tripeptides in teleost fish, J. Nutr. 140 (5) (2010) 893–900.
[74] M.F. Sire, J.M. Vernier, Intestinal-absorption of protein in teleost fish, Comparative Biochemistry and Physiology A-Physiology 103 (4) (1992) 771–781.
[75] D.R. Tocher, Metabolism and functions of lipids and fatty acids in teleost fish, Rev. Fish. Sci. 11 (2) (2003) 107–184.
[76] F.F. Venold, et al., Intestinal fatty acid binding protein (fabp2) in Atlantic salmon (Salmo salar): localization and alteration of expression during development of diet induced enteritis, Comparative Biochemistry and Physiology A-Molecular & Integrative Physiology 164 (1) (2013) 229–240.
[77] C. Rosjo, et al., Lipid digestibility and metabolism in Atlantic salmon (Salmo salar) fed medium-chain triglycerides, Aquaculture 190 (1–2) (2000) 65–76.
[78] A.M. Bakke-McKellep, et al., Atlantic salmon (Salmo salar L.) parr fed genetically modified soybeans and maize: histological, digestive, metabolic, and immunological investigations, Res. Vet. Sci. 84 (3) (2008) 395–408.
[79] P.A.J. Prabhu, J.W. Schrama, S.J. Kaushik, Mineral requirements of fish: a systematic review, Rev. Aquacult. 8 (2) (2016) 172–219.
[80] N.R. Bury, P.A. Walker, C.N. Glover, Nutritive metal uptake in teleost fish, J. Exp. Biol. 206 (1) (2003) 11–23.
[81] G. Bæverfjord, Å. Krogdahl, 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. 19 (1996) 375–387.
[82] S. Einarsson, P.S. Davies, C. Talbot, The effect of feeding on the secretion of pepsin, trypsin and chymotrypsin in the Atlantic salmon, Salmo salar L, Fish Physiol. Biochem. 15 (5) (1996) 439–446.
[83] J.J. Olli, K. Hjelmeland, A. Krogdahl, Soybean trypsin-inhibitors in diets for atlantic salmon (Salmo-Salar, L) - effects on nutrient digestibilities and trypsin in pyloric ceca homogenate and intestinal content, Comparative Biochemistry and Physiology a-Physiology 109 (4) (1994) 923–928.
[84] Å. Krogdahl, et al., Effects of diet composition on apparent nutrient absorption along the intestinal tract and of subsequent fasting on mucosal disaccharidase activities and plasma nutrient concentration in Atlantic salmon Salmo salar L, Aquacult. Nutr. 5 (2) (1999) 121–133.
[85] Å. Krogdahl, A.M. Bakke-McKellep, G. Baeverfjord, Effects of graded levels of standard soybean meal on intestinal structure, mucosal enzyme activities, and pancreatic response in Atlantic salmon (Salmo salar L.), Aquacult. Nutr. 9 (6) (2003) 361–371.
[86] Å. Krogdahl, G.I. Hemre, T.P. Mommsen, Carbohydrates in fish nutrition: digestion and absorption in postlarval stages, Aquacult. Nutr. 11 (2) (2005) 103–122.
[87] O.B. Dale, et al., From chronic feed-induced intestinal inflammation to adenocarcinoma with metastases in salmonid fish, Canc. Res. 69 (10) (2009) 4355–4362.
[88] A. Krogdahl, et al., Tarmproblemer hos oppdrettslaks, i sør og i nord, sommer og vinter (Gut health problems in cultivated salmon, in the south and north, summer and winter), in: Kyst.no Norsk Fiskeoppdrett, Bergen, 2019, pp. 114–117.
[89] A.K.G. Hansen, et al., Dose-response relationship between dietary choline and lipid accumulation in pyloric enterocytes of Atlantic salmon (Salmo salar L.) in seawater, Br. J. Nutr. 123 (10) (2020) 1081–1093.
[90] A.K.G. Hansen, et al., Choline supplementation prevents diet induced gut mucosa lipid accumulation in post-smolt Atlantic salmon (Salmo salar L.), BMC Vet. Res. 16 (1) (2020).
[91] G. Francis, H.P.S. Makkar, K. Becker, Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish, Aquaculture 199 (3–4) (2001) 197–227.
[92] A. Krogdahl, et al., Important antinutrients in plant feedstuffs for aquaculture: an update on recent findings regarding responses in salmonids, Aquacult. Res. 41 (3) (2010) 333–344.
[93] J.J. Olli, et al., Nutritive value of four soybean products in diets for Atlantic salmon (Salmo salar, L.), Acta Agric. Scand. 44 (1994) 50–60.

[94] J.J. Olli, Å. Krogdahl, A. Våbenø, Dehulled, solvent-extracted soybean meal as protein source in diets for Atlantic salmon, Salmo salar L, Aquacult. Res. 26 (1995) 167–174.

[95] Å. Krogdahl, Effects of soybean antinutrients on intestinal environment, lipid hydrolysis and utilization on Atlantic salmon (Salmo salar, L), Effects of antinutrient on the nutritional value of legume diets 3 (1996). Luxembourgh: European Commission, Directorate-General XII, Science Research and Development.

[96] T.S.G.A. van den Ingh, Å. Krogdahl, Negative effects of antinutritional factors from soya beans in salmonids, Tijdsschr. Diergeneeskd 115 (1990) 935–938.

[97] T.S.G.A.M. Van den Ingh, et al., Effects of soybean-containing diets on the proximal and distal intestine in atlantic salmon (Salmo-Salar) - a morphological-study, Aquaculture 94 (4) (1991) 297–305.

[98] A.M. Bakke-McKellep, et al., 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. 23 (2) (2000) 115–127.

[99] M.H. Penn, et al., High level of dietary pea protein concentrate induces enteropathy in Atlantic salmon (Salmo salar L.), Aquaculture 310 (3–4) (2011) 267–273.

[100] A. Krogdahl, et al., Soya saponins induce enteritis in atlantic salmon (Salmo salar L.), J. Agric. Food Chem. 63 (15) (2015) 3887–3902.

[101] E.M. Chikwati, et al., Interaction of soyasaponins with plant ingredients in diets for Atlantic salmon, Salmo salar L, Br. J. Nutr. 107 (11) (2012) 1570–1590.

[102] T.M. Kortner, et al., Dietary soyasaponin supplementation to pea protein concentrate reveals nutrigenomic interactions underlying enteropathy in Atlantic salmon (Salmo salar), BMC Vet. Res. 8 (2012).

[103] A. Couto, et al., Effects of dietary phytosterols and soy saponins on growth, feed utilization efficiency and intestinal integrity of gilthead sea bream (Sparus aurata) juveniles, Aquaculture 432 (2014) 295–303.

[104] A. Couto, et al., Effects of dietary soy saponins and phytosterols on gilthead sea bream (Sparus aurata) during the on-growing period, Anim. Feed Sci. Technol. 198 (2014) 203–214.

[105] A. Couto, et al., Dietary saponins and phytosterols do not affect growth, intestinal morphology and immune response of on-growing European sea bass (Dicentrarchus labrax), Aquaculture Nutrition, 2015 n/a-n/a.

[106] A. Couto, et al., Saponins and phytosterols in diets for European sea bass (Dicentrarchus labrax) juveniles: effects on growth, intestinal morphology and physiology, Aquacult. Nutr. 21 (2) (2015) 180–193.

[107] O.H. Romarheim, et al., Comparison of white flakes and toasted soybean meal partly replacing fish meal as protein source in extruded feed for rainbow trout (Oncorhynchus mykiss), Aquaculture 256 (1–4) (2006) 354–364.

[108] A.A. Smith, et al., Effects of soybean meal and high-protein sunflower meal on growth performance, feed utilization, gut health and gene expression in Arctic charr (Salvelinus alpinus) at the grow-out stage, Aquacult. Nutr. 24 (5) (2018) 1540–1552.

[109] A. Bonaldo, et al., Influence of dietary soybean meal levels on growth, feed utilization and gut histology of Egyptian sole (Solea aegyptiaca) juveniles, Aquaculture 261 (2) (2006) 580–586.

[110] P.A. Uran, et al., Soybean meal induces intestinal inflammation in common carp (Cyprinus carpio L.), Fish Shellfish Immunol. 25 (6) (2008) 751–760.

[111] S. Refstie, et al., Digestive capacity, intestinal morphology, and microflora of 1-year and 2-year old Atlantic cod (Gadus morhua) fed standard or bioprocessed soybean meal, Aquaculture 261 (1) (2006) 269–284.

[112] E. Chikwati, et al., Alterations in digestive enzyme activities during the development of diet-induced enteritis in Atlantic salmon, *Salmo salar L.* Aquaculture 402 (2013) 28–37.

[113] C. Sahlmann, et al., 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. 34 (2) (2013) 599–609.

[114] A. Krogdahl, A.M. Bakke-McKellep, G. Baeverfjord, Effects of graded levels of standard soybean meal on intestinal structure, mucosal enzyme activities, and pancreatic response in Atlantic salmon (Salmo salar L.), Aquacult. Nutr. 9 (6) (2003) 361–371.

[115] A.M. Bakke-McKellep, et al., Response to soy: T-cell-like reactivity in the intestine of Atlantic salmon, Salmo salar L, J. Fish. Dis. 30 (1) (2007) 13–25.

[116] I.S. Marjara, et al., Transcriptional regulation of IL-17A and other inflammatory markers during the development of soybean meal-induced enteropathy in the distal intestine of Atlantic salmon (Salmo salar L.), Cytokine 60 (2012) 186–196.

[117] C. De Santis, et al., Nutrigenomic profiling of transcriptional processes affected in liver and distal intestine in response to a soybean meal-induced nutritional stress in Atlantic salmon (Salmo salar), Comparative Biochemistry and Physiology D-Genomics & Proteomics 15 (2015) 1–11.

[118] M. Gu, et al., Effects of dietary plant meal and soya-saponin supplementation on intestinal and hepatic lipid droplet accumulation and lipoprotein and sterol metabolism in Atlantic salmon, Br. J. Nutr. 111 (3) (2014) 432–444.

[119] E.M. Chikwati, et al., Intestinal epithelial cell proliferation and migration in Atlantic salmon, Salmo salar L.: effects of temperature and inflammation, Cell Tissue Res. 353 (1) (2013) 123–137.

[120] F.F. Venold, M.H. Penn, J. Thorsen, J. Gu, Å. Kortner, A.M. Bakke, Intestinal fatty acid binding protein (fabp2) in Atlantic salmon (Salmo salar): Localization and alteration of expression during development of diet induced enteritis, Comp. Biochem. Physiol. Mol. Integr. Physiol. 164 (2013) 229–240.

[121] E. Lilleeng, et al., Effects of diets containing soybean meal on trypsin mRNA expression and activity in Atlantic salmon (Salmo salar L), Comparative Biochemistry and Physiology A-Molecular & Integrative Physiology 147 (1) (2007) 25–36.

[122] M. Gu, et al., Effects of diet supplementation of soya-saponins, isoflavones and phytosterols on Atlantic salmon (Salmo salar, L) fry fed from start-feeding, Aquacult. Nutr. 21 (5) (2015) 604–613.

[123] U. Fischer, et al., The ontogeny of MHC class I expression in rainbow trout (Oncorhynchus mykiss), Fish Shellfish Immunol. 18 (1) (2005) 49–60.

[124] M. Penn, Lipid Malabsorption in Atlantic Salmon – the Recurring Problem of Floating Feces, Tekna fiskehelse, 2011, pp. 6–11.

[125] R. Hanche-Olsen, et al., Sluttrapport: Nedsatt Tarmhelse Og Forekomst Av Flytefeces Hos Laks (In Norwegian), 2013. https://www.fhf.no/prosjektdetaljer/?projectNumber=900722.

[126] Å. Krogdahl, et al., Choline and phosphatidylcholine, but not methionine, cysteine, taurine and taurocholate, eliminate excessive gut mucosal lipid accumulation in Atlantic salmon *(Salmo salar L)*, Aquaculture 528 (2020) 735552.

[127] Hansen, A.K.G., Choline is an essential nutrient for post-smolt Atlantic salmon (Salmo salar L), in Department of Paraclinical Sciences|. 2020, Norwegian University of Life Sciences: Oslo. p. 194.

[128] W.O.P. Vogel, Zebrafish and lymphangiogenesis: a reply, Anat. Sci. Int. 85 (2) (2010) 118–119.

[129] V. Denstadli, et al., Medium-chain and long-chain fatty acids have different postabsorptive fates in atlantic salmon, J. Nutr. 141 (9) (2011) 1618–1625.

[130] R.D. Odze, Pathology of dysplasia and cancer in inflammatory bowel disease, Gastroenterol. Clin. N. Am. 35 (3) (2006) 533–552.

[131] H. Bjørgen, et al., Ectopic epithelial cell clusters in salmonid intestine are associated with inflammation, J. Fish. Dis. 41 (7) (2018) 1031–1040.

[132] P. Mosberian-Tanha, et al., Granulomatous enteritis in rainbow trout (Oncorhynchus mykiss) associated with soya bean meal regardless of water dissolved oxygen level, J. Fish. Dis. 41 (2) (2018) 269–280.

[133] D. Sterlin, et al., The antibody/microbiota interface in health and disease, Mucosal Immunol. 13 (1) (2020) 3–11.

[134] A. Nishida, et al., Gut microbiota in the pathogenesis of inflammatory bowel disease, Clinical Journal of Gastroenterology 11 (1) (2018) 1–10.

[135] A. Ray, K. Ghosh, E. Ringø, Enzyme-producing bacteria isolated from fish gut: a review, Aquacult. Nutr. 18 (5) (2012) 465–492.

[136] S. Falcinelli, et al., Lactobacillus rhamnosus lowers zebrafish lipid content by changing gut microbiota and host transcription of genes involved in lipid metabolism, Sci. Rep. 5 (2015) 9336.

[137] I. Semova, et al., Microbiota regulate intestinal absorption and metabolism of fatty acids in the zebrafish, Cell Host Microbe 12 (3) (2012) 277–288.

[138] S.R. Jeon, et al., Current evidence for the management of inflammatory bowel diseases using fecal microbiota transplantation, Curr. Infect. Dis. Rep. 20 (8) (2018) 21.

[139] N. Narula, et al., Systematic review and meta-analysis: fecal microbiota transplantation for treatment of active ulcerative colitis, Inflamm. Bowel Dis. 23 (10) (2017) 1702–1709.

[140] A.J. Hryckowian, et al., Microbiota-accessible carbohydrates suppress Clostridium difficile infection in a murine model, Nat Microbiol 3 (6) (2018) 662–669.

[141] L.V. Hooper, et al., Molecular analysis of commensal host-microbial relationships in the intestine 291 (5505) (2001) 881–884.

[142] T.S. Stappenbeck, L.V. Hooper, S. Gordon, Developmental regulation of intestinal angiogenesis by indigenous microbes via Paneth cells 99 (24) (2002) 15451–15455.

[143] J.M. Bates, et al., Intestinal alkaline phosphatase detoxifies lipopolysaccharide and prevents inflammation in zebrafish in response to the gut microbiota, Cell Host Microbe 2 (6) (2007) 371–382.

[144] J.M. Bates, et al., Distinct signals from the microbiota promote different aspects of zebrafish gut differentiation, Dev. Biol. 297 (2) (2006) 374–386.

[145] J.F. Rawls, B.S. Samuel, J.I. Gordon, Gnotobiotic zebrafish reveal evolutionarily conserved responses to the gut microbiota, Proc. Natl. Acad. Sci. U. S. A. 101 (13) (2004) 4596–4601.

[146] I.I. Ivanov, et al., Induction of intestinal Th17 cells by segmented filamentous bacteria, Cell 139 (3) (2009) 485–498.

[147] J.H. Niess, et al., CX(3)CR1-mediated dendritic cell access to the intestinal lumen and bacterial clearance, Science 307 (5707) (2005) 254–258.

[148] S.K. Mazmanian, J.L. Round, D.L.J.N. Kasper, A microbial symbiosis factor prevents intestinal inflammatory disease 453 (7195) (2008) 620–625.

[149] P.M. Smith, et al., The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis 341 (6145) (2013) 569–573.

[150] W. Liu, et al., Effects of dietary microencapsulated sodium butyrate on growth, intestinal mucosal morphology, immune response and adhesive bacteria in juvenile common carp (< i> Cyprinus carpio</i>) pre-fed with or without oxidised oil, Br. J. Nutr. (2014) 1–15.

[151] G.T. Macfarlane, S. Macfarlane, Bacteria, colonic fermentation, and gastrointestinal health, J. AOAC Int. 95 (1) (2012) 50–60.

[152] A. Koh, et al., From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites, Cell 165 (6) (2016) 1332–1345.

[153] P.M. Cholan, et al., Conserved Anti-inflammatory Effects and Sensing of Butyrate in Zebrafish, bioRxiv, 2020, p. 2020. .05.13.069997.

[154] M.A. Lynn, et al., Early-life antibiotic-driven dysbiosis leads to dysregulated vaccine immune responses in mice, Cell Host Microbe 23 (5) (2018) 653–660.

[155] A. Sepahi, et al., Symbiont-derived sphingolipids modulate mucosal homeostasis and B cells in teleost fish 6 (1) (2016) 1–13.

[156] P. Navarrete, et al., Short-term effects of dietary soybean meal and lactic acid bacteria on the intestinal morphology and microbiota of A tlantic salmon (Salmo salar), Aquacult. Nutr. 19 (5) (2013) 827–836.

[157] G. Vasanth, et al., A microbial feed additive abates intestinal inflammation in Atlantic salmon, Front. Immunol. 6 (2015).
[158] O.H. Romarheim, et al., Bacteria grown on natural gas prevent soybean meal-induced enteritis in Atlantic salmon, J. Nutr. 141 (1) (2011) 124–130.
[159] O.H. Romarheim, et al., Prevention of soya-induced enteritis in Atlantic salmon (Salmo salar) by bacteria grown on natural gas is dose dependent and related to epithelial MHC II reactivity and CD8α+ intraepithelial lymphocytes, Br. J. Nutr. 109 (6) (2013) 1062–1070.
[160] O.H. Romarheim, et al., Cell wall fractions from Methylococcus capsulatus prevent soybean meal-induced enteritis in Atlantic salmon (Salmo salar), Aquaculture 402 (2013) 13–18.
[161] F. Grammes, et al., Candida utilis and Chlorella vulgaris counteract intestinal inflammation in Atlantic salmon (Salmo salar L.), PloS One 8 (12) (2013).
[162] F.E. Reveco-Urzua, et al., Candida utilis yeast as a functional protein source for Atlantic salmon (Salmo salar L.): local intestinal tissue and plasma proteome responses, PloS One 14 (12) (2019).
[163] J.O. Hansen, et al., Effect of Candida Utilis on Growth and Intestinal Health of Atlantic Salmon (Salmo salar) Parr, Aquaculture, 2019, p. 511.
[164] K. Yasuda, et al., Biogeography of the intestinal mucosal and lumenal microbiome in the rhesus macaque 17 (3) (2015) 385–391.
[165] Z. Zhang, et al., Spatial heterogeneity and co-occurrence patterns of human mucosal-associated intestinal microbiota 8 (4) (2014) 881–893.
[166] K. Gajardo, et al., A high-resolution map of the gut microbiota in Atlantic salmon (Salmo salar): a basis for comparative gut microbial research, Sci. Rep. 6 (2016) 30893.
[167] K. Gajardo, et al., A high-resolution map of the gut microbiota in Atlantic salmon (Salmo salar): a basis for comparative gut microbial research, Sci. Rep. 6 (2016) 30893.
[168] D. Huyben, et al., Dietary live yeast and increased water temperature influence the gut microbiota of rainbow trout, J. Appl. Microbiol. 124 (6) (2018) 1377–1392.
[169] P. Van den Abbeele, et al., The host selects mucosal and luminal associations of coevolved gut microorganisms: a novel concept, FEMS Microbiol. Rev. 35 (4) (2011) 681–704.
[170] M.S. Llewellyn, et al., The biogeography of the Atlantic salmon (Salmo salar) gut microbiome, ISME J. 10 (5) (2015) 1280–1284.
[171] J. Lokesh, et al., Succession of embryonic and the intestinal bacterial communities of Atlantic salmon (Salmo salar) reveals stage-specific microbial signatures, MicrobiologyOpen 8 (4) (2019).
[172] Y. Jin, et al., Atlantic salmon raised with diets low in long-chain polyunsaturated n-3 fatty acids in freshwater have a Mycoplasma-dominated gut microbiota at sea, Aquac Environ Interact 11 (2019) 31–39.
[173] S. Gupta, J. Fernandes, V. Kiron, Antibiotic-induced perturbations are manifested in the dominant intestinal bacterial phyla of atlantic salmon, Microorganisms 7 (8) (2019).
[174] S. Gupta, et al., Macroalga-derived alginate oligosaccharide alters intestinal bacteria of Atlantic salmon, Front. Microbiol. 10 (2019).
[175] C. Karlsen, et al., The environmental and host-associated bacterial microbiota of Arctic seawater-farmed Atlantic salmon with ulcerative disorders, J. Fish. Dis. 40 (11) (2017) 1645–1663.
[176] S. Gupta, et al., Lactobacillus dominate in the intestine of Atlantic salmon fed dietary probiotics, Front. Microbiol. 9 (2019).
[177] T.M.U. Webster, et al., Environmental plasticity and colonisation history in the Atlantic salmon microbiome: a translocation experiment, Mol. Ecol. 29 (5) (2020) 886–898.
[178] T.M.U. Webster, et al., Interpopulation variation in the Atlantic salmon microbiome reflects environmental and genetic diversity, Appl. Environ. Microbiol. 84 (16) (2018) e00691-18.
[179] C. Heys, et al., Neutral processes dominate microbial community assembly in Atlantic salmon, Salmo salar, Appl. Environ. Microbiol. 86 (8) (2020).
[180] M. Ciric, et al., Characterization of mid-intestinal microbiota of farmed Chinook salmon using 16S rRNA gene metabarcoding, Arch. Biol. Sci. 71 (4) (2019) 577–587.
[181] P.P. Lyons, et al., Phylogenetic and functional characterization of the distal intestinal microbiome of rainbow trout Oncorhynchus mykiss from both farm and aquarium settings, J. Appl. Microbiol. 122 (2) (2017) 347–363.
[182] P.P. Lyons, et al., Effects of low-level dietary microalgae supplementation on the distal intestinal microbiome of farmed rainbow trout Oncorhynchus mykiss (Walbaum), Aquacult. Res. 48 (5) (2017) 2438–2452.
[183] S. Rimoldi, et al., The effects of dietary insect meal from hermetia illucens prepupae on autochthonous gut microbiota of rainbow trout (Oncorhynchus mykiss), Animals 9 (4) (2019).
[184] R.M. Brown, G.D. Wiens, I. Salinas, Analysis of the gut and gill microbiome of resistant and susceptible lines of rainbow trout (Oncorhynchus mykiss), Fish Shellfish Immunol. 86 (2019) 497–506.
[185] S. Razin, D. Yogev, Y. Naot, Molecular biology and pathogenicity of mycoplasmas, Microbiol. Mol. Biol. Rev. 62 (4) (1998) 1094–1156.
[186] Y. Li, et al., Differential Response of Digesta-And Mucosa-Associated Intestinal Microbiota to Dietary Black Soldier Fly (Hermetia Illucens) Larvae Meal in Seawater Phase Atlantic Salmon (Salmo salar), bioRxiv, 2020.
[187] Z. Wang, et al., Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease, Nature 472 (7341) (2011) 57–63.
[188] F.E. Reveco, et al., Intestinal bacterial community structure differs between healthy and inflamed intestines in Atlantic salmon (Salmo salar L.), Aquaculture 420 (2014) 262–269.
[189] D. Huyben, et al., High-throughput sequencing of gut microbiota in rainbow trout (Oncorhynchus mykiss) fed larval and pre-pupae stages of black soldier fly (Hermetia illucens), Aquaculture 500 (2019) 485–491.
[190] G. Terova, et al., Rainbow trout (Oncorhynchus mykiss) gut microbiota is modulated by insect meal from Hermetia illucens prepupae in the diet, Rev. Fish Biol. Fish. 29 (2) (2019) 465–486.
[191] S. Rimoldi, et al., The effects of dietary insect meal from hermetia illucens prepupae on autochthonous gut microbiota of rainbow trout (Oncorhynchus mykiss), Animals (Basel) 9 (4) (2019).
[192] A. Jaramillo-Torres, et al., Influence of dietary supplementation of probiotic Pediococcus acidilactici MA18/5M during the transition from freshwater to seawater on intestinal health and microbiota of Atlantic salmon (Salmo salar L.), Front. Microbiol. 10 (2019).
[193] M. Zhang, et al., Gnotobiotic models: powerful tools for deeply understanding intestinal microbiota-host interactions in aquaculture, Aquaculture 517 (2020) 734800.