Colitis-Complex Diarrhea

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Colitis-complex diarrhea (CCD) in pigs can be defined as a type of diarrhea, which is associated with colonic inflammation and disrupted colonic gut barrier functionality as well as infiltration of neutrophils at the inflamed colon in growing pigs (8–16 weeks of age) and can bear large costs on the pig farms. The exact etiology of CCD is still unclear; however, pathogens including Brachyspira (B.) hyodysenteriae, B. pilosicoli, and swine whipworms such as Trichuris (T.) suis have been involved in specific colitis (SC). In the absence of specific pathogens, dietary factors, such as high levels of protein, pelleted feedstuffs, and lack of sufficient antioxidants, can result in non-specific colitis (NSC). On the other hand, supplement of polyunsaturated fatty acids (PUFA) and polyphenols, sufficient supply of essential amino acids (e.g., threonine, cysteine, and proline), short-chain fatty acids (SCFA; especially butyrate), and resistant starch have shown to confer preventing/ameliorating effects on CCD.

colonic inflammation   adult pigs   biomarkers   dietary strategies   specific colitis

non-specific colitis

1. Colonic Epithelium Function

1.1. Colonic Ion Exchange

The pathogenesis of diarrhea related to inflammation in the colon is a multifactorial event [1]. Understanding the ion-absorption/secretion mechanisms in the colon and the effects of inflammatory mediators on epithelial transport function is therefore of great importance. **Figure 1** depicts the major ion transportation pathways in the colon. Generally, normal stool is low in Cl^- and Na^+ and high in K^+ since reabsorption of Na^+ and secretion of K^+ (both active and passive) takes place in the colon [2]. The reason for the low level of Cl^- in normal stool is that short-chain fatty acids (SCFA) produced in the colon can replace Cl^-, while luminal concentration of HCO_3^- is similar to its concentration in plasma [2]. In the colon, Na^+ gets absorbed through the stimulatory effect of SCFA, through an aldosterone-sensitive sodium absorption by the epithelial Na^+ channel (ENaC) in the distal colon [3], and through an Na-H exchange parallel to a Cl-HCO_3^- exchange (in the proximal colon) which is responsible for an electro-neutral Na-Cl absorption [4]. SCFA are the primary anions in the lumen, which also contribute to Na absorption through apical Na-H, Cl-SCFA, and $SCFA$-HCO_3^- exchanges; this type of Na^+ absorption is not hampered by cyclic adenosine monophosphate (cAMP) [4]. Secondary messengers, such as cAMP, cyclic guanosine monophosphate (cGMP), intracellular calcium (Ca^+), and neurohumoral substances, can activate Na-H exchange (NHE) genes such as NHE3, which is involved in neutral absorption of sodium [3]. However, increased mucosal cAMP and intracellular Ca^{2+} concentration in the colon can inhibit apical Na-H and Cl-HCO_3^- exchanges and reduce

absorption of Na^+ and Cl^-, consequently reducing the water absorption [4]. For apical exchange of Cl-HCO_3^- in the colon, downregulation in adenoma (DRA or SLC26A3) is the major exchanger, which is a chloride-sulfate anion transporter in the upper crypt and surface epithelium of the colon [1][5]. DRA activity was shown to be inhibited by increased cellular cAMP and Ca^{2+} [6]. Moreover, the gene expression of DRA was reported to be heavily diminished in colonic inflammation as an effect of the interleukin-1β (IL-1β) cytokine, hence hampering Cl^- absorption [1].
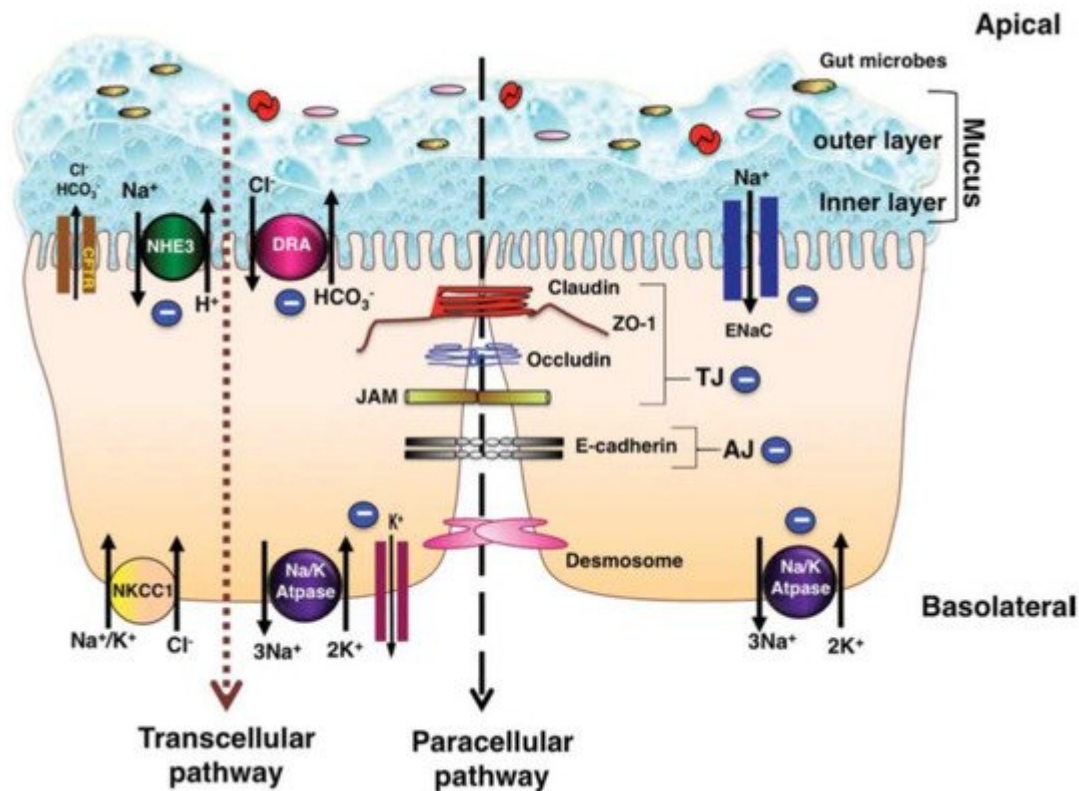
Figure 1. Schematic pathways of colonic ion transportation, adopted from Anbazhagan et al. [5].

In animal studies, it has been shown that some bacterial pathogens, such as *Salmonella typhimurium* [7], *Shigella dysenteriae* toxin type 1 [8], and *Campylobacter jejuni,* increase intracellular concentrations of Ca^{2+}, resulting in inhibition of NHE3 and stimulation of an excessive secretion of Na^+ and Cl^- [3]. Therefore, impaired Na^+ absorption and stimulation of Ca^{2+} secretion can result in diarrhea [3]. Excessive intracellular secretion of Ca^{2+} could be a tertiary effect of microbial pathogenesis since pathogens first stimulate the enteric nervous system, then increase the release of neurotransmitters and, ultimately, enhance the secretion of Ca^{2+} [8]. Increased levels of luminal ions can be expected as a result of inhibition of Na^+ and Cl^- absorption along with stimulation of excessive Cl^- secretion after disruption of the colonic mucus layer by either pathogen- and/or feed-induced inflammatory mediators [9]. Quantification of recovered Cl^- from fecal samples [10] of affected pigs can potentially be an indicator of an inflamed colonic epithelium of pigs with colitis.

1.2. Cytokines and Luminal Function

Cytokines are small peptide molecules acting as important mediators in the regulation of the immune and inflammatory responses produced by epithelial cells, endothelial cells, and fibroblasts [11]. Cytokines such as interferon-γ (IFN-γ) can directly alter the epithelial tight junctions and increase the transepithelial permeability [11]. In an inflamed colon, cytokines, such as IL-1β [1], IFN-γ, and tumor necrosis factor-α (TNF-α) [12], are culprit keys involved in perturbing the absorption of Na^+ and Cl^- and consequently the water absorption [5], giving rise to stool water content. Overexpression of these cytokines in the colonic mucosa can also cause mucosal damage and dysfunction, which may lead to diarrhea [13]. The increased ionic level in the lumen can reciprocally disturb the absorptive/secretive balance and cause diarrhea in two distinct ways: (1) by increasing the further secretion of ions upon the induced imbalanced electrical charge, and (2) by increasing the extracellular osmotic pressure, resulting in more water diffusion from the enterocytes.

Aside from ion transportation, the intestinal epithelium performs a barrier function through enterocytes and their encircling tight junctions (zona occludens), which limits the passive flow of luminal contents into the blood and lymphatics and the other way around [2]. The restriction of tight junctions is higher in the colon vs. small intestine, and this restriction increases from the proximal to the distal colon [2]. In human patients with Crohn's disease (CD), the epithelium in inflamed intestinal segments is characterized by a reduction of tight junction strands, strand breaks, and alterations of tight junction protein content and composition [14]. In patients with ulcerative colitis (UC), micro-erosions caused by upregulated epithelial apoptosis as well as a remarkable increase in claudin-2 are the main reasons for early epithelial leaks [14]. The mucosal inflammation in UC increases the permeability of colonic epithelium by changing the tight junctions, which could be a contributing reason to diarrhea appearing due to colonic inflammation [4][15].

1.3. Mucins

In addition to the epithelial integrity and the presence of commensal bacteria, the non-immune intestinal barrier is due to mucus production [16]. Mucins are gel-forming high-molecular-weight glycoproteins, which are synthesized and secreted by goblet cells and also act as an important feature for gut barrier functionality [17]. In the intestinal tract, MUC2 is the predominant secretory mucin, and it is the main structural component of the colonic mucus layer [18]. Perturbed synthesis and secretion of mucin by the goblet cells, in response to pathophysiological alterations in the intestinal mucosa, result in changes in the mucus gel [19]. In the absence of inflammation, the mucus layer is about 700 μm thick [20], whereas exposure to specific bioactive factors, such as hormones, inflammatory mediators, and microbial factors, such as lipopolysaccharides (LPS), flagellin A, and lipoteichoic acids, can upregulate mucin production, in particular MUC2 [21]. Upregulation of mucins such as MUC5AC and MUC4 along with mucus hypersecretion of goblet cells were also shown to be associated with inflammatory diseases of the epithelium [22].

2. Colitis

Inflammation is a constant ongoing process of a normally functioning colon as the healthy colon is continuously offsetting inflammatory responses as a result of exposure to a wide variety of, for example, bacteria in the lumen, dietary antigens, and toxins [13]. If the inflammatory responses go beyond a point to jeopardize the luminal integrity,

it can result in diarrhea by increasing gut permeability [23] and impairing its absorptive functionality [9]. Colitis is referred to as inflammation in the colon, which is a consequence of a complex biological defense mechanism against harmful stimuli such as pathogenic bacteria and physical damages [24]. Diarrhea is a non-specific sign of colitis occurring in the acute phase of colitis [25] and for pigs commonly referred to as CCD. This type of diarrhea is typically non-hemorrhagic (in severe cases, hemorrhagic) mucoid diarrhea and infiltration of neutrophils (**Figure 2**) in the inflamed colon of growing pigs (4–16 weeks post-weaning) [26][27].
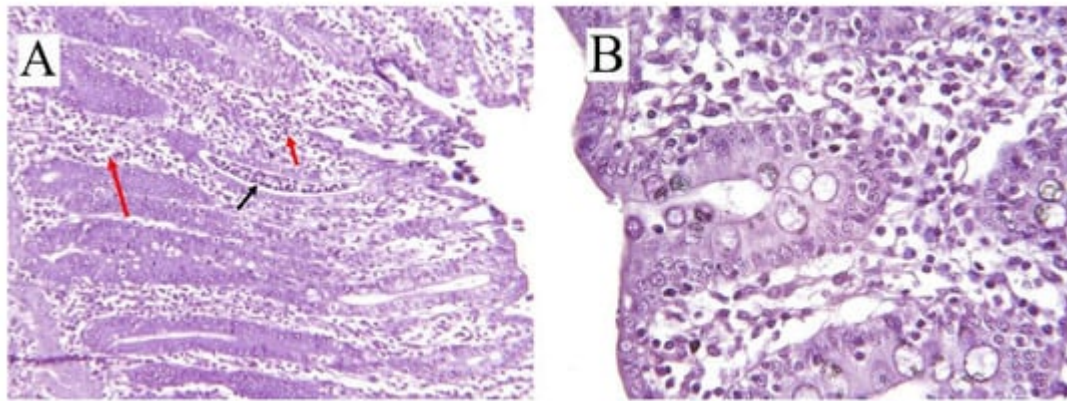
Figure 2. (**A**) Cross-sectional histology (hematoxylin/eosin-stained) of inflamed colon in pig (8 weeks old) with ×10 scale of magnification. Infiltration of neutrophils (black arrow) and mononuclear cells (red arrows) in the crypts can be seen. (**B**) Colonic cross-sectional histology of a healthy pig (11 week) with ×25 scale of magnification.

However, in younger pigs (e.g., during nursery and post-weaning stage), the main reason for diarrhea is enterotoxigenic *Escerichia* (*E.*) *coli* (ETEC) with fimbriae types F4 and F18 [28][29]. CCD occurs if the epithelia's barrier function is compromised by loss of epithelial cells or disruption of tight junctions (**Figure 1**); in that scenario, hydrostatic pressure in blood vessels and lymphatics will cause water and electrolytes, mucus, protein, and in some cases, red and white cells to accumulate luminally [2]. The bottleneck of colitis diagnosis is the examination of postmortem lesions and histological test (hematoxylin and eosin staining) on the colonic epithelium (**Figure 2**) [29] [30]. In the case of acute or chronic inflammation or necrosis of the colonic mucosa, exudative diarrhea occurs, which is characterized by an increase in fluid production, excretion of the inflammatory products such as serum proteins, and a reduction in absorption of fluids and electrolytes [28]. Unlike the small intestine, the colonic absorptive function is related to the epithelial intercrypt surface instead of villi, while the immature epithelial cells of the crypts perform the secretory function as in the small intestine [9].

2.1. Specific Colitis

The most important etiology and epidemiology of common forms of SC (i.e., when one or more pathogens are involved) are briefly presented in **Table 1**, which will be discussed in the following sections.

Table 1. Etiology and epidemiology of CCD in growing pigs according to the causative factors and mechanisms of action.

Type of Colitis	Causative Factor	Affected Site	Pigs Age, Week	Mechanism of Action	Clinical Signs	Gross Lesion	References
SC							
Swine dysentery	*B. hyodysenteriae*	Cecum and colon	6–18	Absorptive dysfunctionality, hemolysins, and degradative enzymes	Loose stool, mucoid, hemorrhagic diarrhea, dehydration, and retarded growth rate	Inflamed epithelium, mucosal damage, hyperplasia of the crypts, and spirochetal attachment	[31][32][33][34]
Spirochetal colitis	*B. pilosicoli*	Cecum and colon	4–20	Absorptive dysfunctionality	Mild non-hemorrhagic, mucoid diarrhea, retarded growth rate	Inflamed epithelium, moderate catarrhal colitis, flaccid and thin luminal wall, appearance of small adherent nodules of digesta	[9][35][33][34][36]
Parasitic colitis	*T. suis*	Cecum and spiral colon	4–10	Stimulation of the epithelium and cascading inflammatory responses by hatched eggs and adult worms	Dark loose stool, mucoid to hemorrhagic diarrhea, dehydration, anorexia, and increased feed conversion ratio	Crypt hyperplasia, goblet cell hyperplasia, a general hypertrophy of mucosa, and presence of bipolar eggs	[37][38][39][40][41]
NSC	Dietary factors	Cecum and colon	4–12	Absorptive dysfunctionality and increased epithelial permeability	Loose and mucoid, non-hemorrhagic diarrhea	Mucosal hyperplasia, mononuclear cell infiltration, multifocal mucosal erosions,	[33][23][30][42]

Type of Colitis	Causative Factor	Affected Site	Pigs Age, Week	Mechanism of Action	Clinical Signs	Gross Lesion	References
						increased crypt depth	

2.1.1. Swine Dysentery

Swine dysentery (SD) is a severe mucohemorrhagic diarrheal colitis caused by *B. hyodysenteriae,* formerly known as *Treponema hyodysenteriae* and *Serpulina hyodysenteriae*, a Gram-negative and flagellated anaerobic spirochete [32][43]. Together, the abundance of goblet cells (as a matrix and source of nutrients) and the anaerobic environment in the large bowel contribute to a favorable environment for *B. hyodysenteriae*, confided to the colon [43]. Colonization of the large intestine (cecum and colon) by *B. hyodysenteriae* results in lysis of mucosal cells by bacterial virulence factors, such as hemolysins and proteases, degradative enzymes, and inflammation [31]. Bacterial proteases were reported to be involved in malabsorption of carbohydrates by inactivating the oligosaccharidases of the brush border [2], and the damages to the intestinal wall could aid proliferation of other pathogens [33]. The incidence of SD occurs in pigs during 6–18 weeks of age [33], and the first clinical symptom is excretion of watery or semisolid feces, which progresses as diarrheal feces with large amounts of mucus and variable amounts of blood [44]. Physiologically, SD is associated with dehydration, acidosis, hyperkalemia and, in severe cases, death [44]. Nonetheless, the apparent prevalence of *B. hyodysenteriae* in Danish herds was shown to be relatively low: 2.5% [45].

Brachyspira hyodysenteriae is associated with producing a cytotoxic hemolysin as a virulence determinant, which, concurrent with other anaerobic colonic bacteria such as *Fusobacterium necrophorum* or *Bacteroides vulgatus*, can synergize the development of SD in growing pigs [33][43]. The colonic goblet cells of pigs with severe clinical signs of SD may show a substantial increase in the levels of MUC2 mucin and de novo expression of MUC5AC mucin and increased *B. hyodysenteriae,* binding ability to the epithelial mucus [46]. This indicates that morphological changes such as increased mucin production in the colon induced by *B. hyodysenteriae* are mechanisms to facilitate the further infection by increasing the binding sites for *B. hyodysenteriae* attachment. Therefore, increased expression of MUC2 in the inflamed colon of pigs and subsequent higher levels in feces may potentially be considered as contributing biomarkers for the diagnosis of colonic inflammations (**Table 2**). The consequent increased turnover rate of intestinal mucosal cells could be a reason for increased crypt depth [47] observed in pigs with colitis [30]. However, the genes encoding MUC2 were remarkably downregulated in crypt cells of pigs infected with *Lawsonia (L.) intracellularis* [22], an obligate intracellular, Gram-negative, and microaerophilic pathogen, which infects cells of the ileal epithelium (small intestine) [31]. Therefore, characterization of the variations in mucin expression of the pig colon may be considered as a diagnostic tool for inflammatory diseases such as colitis and discriminating ileitis from colitis.

Table 2. Summary of non-invasive putative biomarkers associated with colonic inflammation.

Biomarkers	Type	Direction	Recovery Site	Causative Factor	Affected Site	Reference
MUC2 and MUC5AC	Mucin	Increased expression	Feces	Colitis, swine dysentry	Large intestine	[17][21][22]
LPS [1]	Saccharide	Increased expression	Serum	Gram-negative pathogens, e.g., *B. hyodysenteriae*	Small and large intestine	[48][49][50][51]
Calprotectin and lactoferrin	Protein	Increased expression	Feces and serum	Colitis and inflammatory factors	Large intestine	[52][53][54][55]
Na^+, Cl^-, HCO_{3-}, and K^+	Ion	Reduced absorption and increased luminal accumulation	Feces	*B. hyodysenteriae*	Large intestine	[9][5][12][44]
TNF-α [2], IFN-γ [3], IL-1β [4], IL-6 [5], and IL-10 [6]	Cytokines	Increased expression	Serum and mucus	Pathogens	Small and large intestine	[1][5][12][23][50][56]
NF-κB [7]	Protein	Increased expression in macrophages and in epithelial cells	Serum	IL-1β and TNF-α, LPS, and ROS [8]	Epithelial cells of inflamed colon	[51][56][57][58][59]
CRP [8], HP [9], and pig-MAP [10]	Protein	Increased concentration	Serum	LPS, IL-1β, and TNF-α	Epithelial cells of colon, and hepatic cells	[50]
FRAP [11], TBARS [12], and ROS [13]	TAC [14] assay	Increased expression	Serum	Oxidative stress	Epithelial cells of colon	[60][61]
TEAC [15], CUPRAC [16], AOPP [17], and H_2O_2	TAC assay	Increased expression	Saliva	Oxidative stress	Epithelial cells of colon	[61][62]

[1] Lipopolysaccharides; [2] Tumor necrosis factor-α; [3] Interferon-γ; [4] Interleukin-1β; [5] interleukin-6; [6] interleukin-10; [7] Nuclear factor kappa B; [8] C-reactive protein; [9] Haptoglobin; [10] Pig major acute phase protein; [11] Ferric reducing ability of plasma; [12] Thiobarbituric acid reactive substances; [13] Reactive oxygen species; [14] Total

antioxidant capacity; [15] Trolox equivalent antioxidant capacity; [16] Cupric reducing antioxidant capacity; [17] Advanced oxidation protein products.

An indicator of infectious diarrhea is the presence of secretory and pro-inflammatory mediators, such as histamine, prostaglandins, and 5HT (5-hydroxytryptamine or serotonin) as well as proteases released from the mast cells [9] [63]. However, *B. hyodysenteriae* can be involved in diarrhea by causing mucosal inflammation in the large intestine upon its attachment, malabsorption by impairing Na^+, and water-absorptive functionality of the colon epithelium [9], resulting in an increased accumulation of water, Na^+, Cl^-, HCO_3^-, and K^+ in the luminal fluid [44]. As a consequence of disrupted absorptive functionality, acidosis and increased serum's K^+ could potentially be used as a diagnostic factor in SC [44]. In addition, Cl^- is a key-secreted ion that facilitates the transmucosal movement of both Na^+ and water, which is defused across the paracellular space and into the lumen under the effect of electrical and osmotic gradient [9]. Since glucose, galactose, and amino acid are co-transported with Na^+ into the colonocytes, their luminal accumulation as a result of SD is expected. Consequently, due to an osmotic gradient caused by undigested carbohydrates [9] in the lumen, more water diffusion could be anticipated; hence, exacerbated diarrhea in infected pigs can occur. This mode of action was attributed to *B. hyodysenteriae,* in addition to the fact that it has no pernicious effects on the intestinal secretory processes and solely disrupts the absorptive function of the epithelium [44].

According to Carr et al. [33], a noteworthy difference between the pathogenic etiologies of colitis is that *B. hyodysenteriae* is a strict anaerobe, occurring only in the large bowel (cecum and colon); therefore, the associated lesions of SD are not seen in the small intestine. In this regard, Argenzio [64] showed that the kinetics of glucose-stimulated water absorption were identical between infected and healthy pigs, indicating that SD had no disruptive effect on the small intestine and ileum. Therefore, oral glucose-electrolyte rehydration would be a pragmatic approach in restoring extracellular fluid losses associated with SD [64]. Glucose and amino acids are co-transported with Na^+, and the existing intracellular Na^+ gradient is a driving force for amino acids, oligopeptides, and sugar absorption with the following water absorption due to the osmotic pressure, hence rehydration [28][2].

2.1.2. Spirochetal Colitis

Spirochetal colitis or porcine colonic spirochetosis affects pigs aged 4–20 weeks, and the clinical signs commonly appear 10–14 days after mixing and changing the feed to a grower or pelleted diet [35][33] [36]. *Brachyspira pilosicoli* is the causative pathogen of spirochetal colitis [36] with the same mode of action as *B. hyodysenteriae* on the absorptive function of the colonocytes, albeit histopathologic lesions are less severe with milder non-hemorrhagic diarrhea [9]. *Brachyspira pilosicoli* causes inflammation and mucosal damage throughout the cecum and colon (typhlocolitis; [36]), which by reducing the surface area of the large intestine available for absorption causes diarrhea [33]. Gross lesions of spirochetal colitis are associated with diarrhea with loose to watery and in some cases mucoid feces, and mild mucosal reddening and flecks of pus, resulting in diarrhea and reduced growth rate [35][36]. Microscopic signs of this disease could be seen as mild to moderate catarrhal colitis with erosions of the surface epithelium and spirochetal colonization of the colon epithelium [35]. Trott et al. [36] characterized the postmortem signs of *B. pilosicoli* infection as increased size of the colon in infected animals with

flaccid and thin wall, filled with watery, slightly mucoid content, and the mucosal surface was covered with small adherent nodules of digesta.

The occurrence of this disease was characterized by the coinfection of the protozoa *Balantidium coli* associated with the mucosa and lamina propria along with *B. pilosicoli* attached by one end to the colonic epithelium, which triggers neutrophilic exocytosis, excess mucus and increased crypt cell mitotic index [36]. A study in 79 Danish pig herds showed an apparent prevalence of *B. pilosicoli* to be 19.0% [45]. In case of SC, the presence of microbially derived LPS in serum could be a reliable biomarker for microbial detections (**Table 2**) since LPS is a part of the outer membrane of Gram-negative bacteria and induces inflammatory responses [48]. *Brachyspira* LPS contain the lipid A-sugar core of approx. 10–16 kDa, which is the main difference from LPS of other species, and these lipopolysaccharides produced by *B. hyodysenteriae* are speculated to be involved in the colonic damage [49]. In an in vivo study, pigs administered with LPS developed acute phase response (APR) and hepatic production of acute phase proteins, e.g., C-reactive protein (CRP), haptoglobin (HP), and pig major acute phase protein (pig-MAP) as a result of increased stimulation of pro-inflammatory cytokines, such as TNF-α, IL-1β, and IL-6 [50]. Therefore, in case of SC, the seral measurement of LPS, CRP, HP, and pig-MAP could also be considered as putative biomarkers.

2.1.3. Parasitic Colitis

Swine whipworms can cause inflammation in the cecum and spiral colon, resulting in defecation of dark loose stools containing blood and diarrhea [37]. In Danish organic pig farms, with access to pastures, there is a high risk of the exposure of the animal to the parasite eggs, present mainly in the pasture [40]. Among swine parasitic whipworms, *Trichuris (T.) suis* can infect young and growing pigs in both indoor and outdoor production systems [40] that can cause severe mucoid to hemorrhagic diarrhea, dehydration, anorexia, reduced gain, and increased feed conversion ratio [39][65]. The eggs of the parasite are passed in feces from infected animals, and, once eaten, they can travel through the digestive tract, and in the small intestine and cecum, the larva hatches and penetrates the mucosa through the crypts of Lieberkühn in the distal ileum, cecum, and colon [39]. Crypt hyperplasia, goblet cell hyperplasia, and a general hypertrophy of mucosa in the proximal colon in addition to the increased level of parasite-specific antibodies in the serum of pigs infected with *T. suis* in week 10 of age were reported to be associated with *T. suis* infection [38]. Adult worms are highly potent to cascade an inflammatory response in addition to inducing immunosuppressive properties in infected pigs through high release of nitric oxide (NO) and upregulating arginase activity in macrophages [41]. The excessive production of NO in abnormal situations induces inflammation in the colon [66].

Conducting clinical serologic assays for the increased level of 20 kDa excretory/secretory glycoprotein antigen in the serum of infected pigs could be used for diagnosis of *T. suis* [67]. Hygienic practices to prevent the fecal-oral transmission of the eggs, including regular removal of feces and organic debris, could be considered as preventive strategies, and, once infected, pigs could be treated with anthelmintic [65].

2.2. Non-Specific Colitis and Role of Dietary Factors in Inducing Colitis

When no specific pathogens can be identified, the term NSC is designated to describe this form of colitis [68][69]. As shown in **Table 1**, NSC mainly affects growing pigs (12–40 kg, approx. 4–12 weeks post-weaning), and it has been estimated to affect 40–80% of pigs in the UK and other European countries [29][42]. Non-specific colitis is characterized by subacute colitis with mucosal hyperplasia, mononuclear cell infiltration, multifocal mucosal erosions, colonic lesions of increased crypt depth, and poor growth rate [29][30], mainly because of the impaired absorptive functionality and dehydration due to diarrhea. Hyperplasia in colon-associated lymphatic tissue, shining mucosa in colon, and mesenteric lymph node hyperplasia without any related pathogens detected in fecal samples were reported to be considered as the gross pathological signs of NSC [30]. As mentioned earlier, both SC and NSC induce morphological changes in the colon due to the cascaded inflammatory responses. Knowing the intermediate and possible end-products of these inflammatory mechanisms could be used as possible biomarkers for facilitating the diagnosis of colitis in diarrheal pigs. Calprotectin and lactoferrin are proteins derived from activated neutrophils, and since they are quite stable in feces and could be detected by quantitative ELISA, they are considered as inexpensive and non-invasive biomarkers for colitis in humans [53]. Calprotectin and lactoferrin have been reported to be closely correlated to the inflammations in patients with IBD when diagnosed by endoscopic measures [54]. Similarly, in case of colonic inflammation in the pigs, infiltration of neutrophils in the inflamed sites has been reported [30] for which fecal recovery of calprotectin and lactoferrin could be expected as a promising biomarker of colitis. However, only a few studies have been done on adopting this method for identifying colitis in pigs [52][55].

The exact etiology and epidemiology of NSC is generally poorly understood [68][30]. However, several dietary factors seem to be implicated in the development of NSC (**Table 3**), and these dietary factors may also facilitate pathogenic infections in the large intestine and cause a synergized SC. For instance, NSC was reported to be more prevalent in fast-growing pigs fed high-density diets, i.e., diets with high-metabolizable energy [33] and the presence of trypsin inhibitors in peas, beans, and soya, and deficiency of vitamin E has also been associated with the occurrence of NSC [33]. Feeding diets poor in tryptophan, as a precursor of niacin (vitamin B3) biosynthesis, has also been related to the incidence of NSC [42]. For the biosynthesis of niacin out of tryptophan, the presence of riboflavin, vitamin B_6, and iron is required to be in adequate amounts. Urinary excretion of N-methylnicotinamide is a reliable indicator for nicotinic acid deficiency [70]. It was reported that sufficient dietary supplementation of niacin (≥15 μg/g feed) could prevent the incidence of NSC and also reverse the condition in pigs [27]. Niacin inhibits inflammation responses by downregulating nuclear transcription factors-κB (NTF-κB, **Table 2**) signaling pathway in guinea pigs [71] and was shown to reverse colitis in rats challenged by iodoacetamide [72]. Other dietary factors involved in the incidence of NSC could be dietary protein, dietary fiber, and pelleted feedstuff, which will be discussed below.

Table 3. Dietary factors involved in CCD.

Factor	Level	Effect	Reference
Trypsin inhibitor	High	Increased undegraded protein in large intestine and inflammation and causing NSC [1]	[33]

Factor	Level	Effect	Reference
Vitamin C and E, glutathione, ubiquinol, polyphenols, and β-carotene	Insufficient	Oxidative distress	[33][59][73][74]
Essential amino acids	Insufficient	Oxidative stress by reducing antioxidant enzymes, reduced mucin production	[16][17][42][75]
Dietary protein	≥23%	Increased undegraded protein in large intestine and inflammation due to NH_4^+, reducing gut barrier function and causing NSC	[23][29][42][76]
Soluble NSP [2] and RS [3]	Increased	Ameliorating/preventive effect on large intestinal inflammation, increased SCFA [4], and reduced luminal pH	[77][78][79][80][81][82]
Pelleted diet	-	Reduced endogenous enzymes in feedstuffs and causing NSC	[68][83]

[1] Non-specific colitis; [2] Non-starch polysaccharides; [3] Resistant starch; [4] Short-chain fatty acids.

2.2.1. Dietary Crude Protein and NSC

Histological examination of colonic tissue of pigs with NSC showed the loss of microvilli and apoptosis of surface epithelial cells, which could be due to the increased concentrations of NH_4^+, indoles, and phenols arising from increased bacterial fermentation of especially undegraded dietary protein [27][42]. The increased crypt depth related to NSC was also speculated to be a result of protein fermentation in the colon [30]. Moreover, increasing the dietary crude protein content from 17 to 23% (**Table 3**) in five-week-old pigs was shown to be associated with reduced tight junction genes (e.g., Zonula occludens-1 and Occludin) expression in the gut, resulting in increased gut permeability and diarrhea [23]. In the same study, the incidence of NSC was also related to the increased expression of pro-inflammatory cytokines IL-1β, IFN-γ, and cystic fibrosis transmembrane conductance regulators (CFTR) in the distal colon as a result of increased protein content of the diet [23]. Upregulation of gene expression of inflammatory cytokines such as IL-1β and IL-6 in the proximal colon of pigs can increase the intestinal epithelial permeability by altering tight junction proteins and disrupting the epithelial integrity [84][85]. This reflects the close correlation of these cytokines with the incidence of the colonic inflammation, which could be used as biomarkers for both NSC and SC in pigs. Wu et al. [23] reported an increased concentration of Cl^- and CFTR in the digesta of the terminal colon in piglets fed diets with 23% protein vs. 17%, indicating an increased epithelial permeability and excessive secretion of Cl^- due to the increased dietary protein. This can be an indication of the disrupted absorptive functionality of the colon epithelium since luminal accumulation of Cl^- ions and CFTR is tightly related to the incidence of diarrhea [86], and their quantification in the fecal samples could be used as possible biomarkers for protein-related NSC. An increased level of plasma urea nitrogen in the pigs with diarrhea induced by the increased level of dietary protein was also reported previously [23], which could be another indirect indicator of excessive protein fermentation in the colon. In addition, biogenic amines, NH_4^+, indoles, and phenols are byproducts of

protein fermentation in the large intestine, and with the recovery assays from the fecal samples, they may also be considered as potential biomarkers of colitis induced by protein fermentation.

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