



## Review Article

## Dietary nutrition, intestinal microbiota dysbiosis and post-weaning diarrhea in piglets

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## ABSTRACT

Weaning is a critical transitional point in the life cycle of piglets. Early weaning can lead to post-weaning syndrome, destroy the intestinal barrier function and microbiota homeostasis, cause diarrhea and threaten the health of piglets. The nutritional components of milk and solid foods consumed by newborn animals can affect the diversity and structure of their intestinal microbiota, and regulate post-weaning diarrhea in piglets. Therefore, this paper reviews the effects and mechanisms of different nutrients, including protein, dietary fiber, dietary fatty acids and dietary electrolyte balance, on diarrhea and health of piglets by regulating intestinal function. Protein is an essential nutrient for the growth of piglets; however, excessive intake will cause many harmful effects, such as allergic reactions, intestinal barrier dysfunction and pathogenic growth, eventually aggravating piglet diarrhea. Dietary fiber is a nutrient that alleviates post-weaning diarrhea in piglets, which is related to its promotion of intestinal epithelial integrity, microbial homeostasis and the production of short-chain fatty acids. In addition, dietary fatty acids and dietary electrolyte balance can also facilitate the growth, function and health of piglets by regulating intestinal epithelial function, immune system and microbiota. Thus, a targeted control of dietary components to promote the establishment of a healthy bacterial community is a significant method for preventing nutritional diarrhea in weaned piglets.

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## 1. Introduction

In modern pig industry, early weaning is an effective way to improve the productivity of sows, but it may also cause weaning stress in piglets (Upadhaya and Kim, 2021). Weaning is one of the most challenging periods in the life of piglets, which causes great pressure on piglets and is accompanied by damage to intestinal morphology, barrier function and microbial community (Jayaraman

and Nyachoti, 2017). Moreover, the diet of piglets is changed to a solid feed-based diet at weaning, resulting in reduction in feed intake. These changes caused by weaning can cause reduced growth performance and intestinal health damage in piglets (Upadhaya and Kim, 2021). The gut of piglets hosts a large number of microorganisms that have a symbiotic relationship with the host. They maintain the health and homeostasis of the body by producing a variety of metabolites, and play a major role in the development of disease (Ma et al., 2022; Wilson et al., 2020). Different types of nutrients, such as fiber, carbohydrate and protein, are fermented by different microorganisms in the intestine (Azad et al., 2020; Chen et al., 2020; Pilla and Suchodolski, 2021). Changes in diet can affect the availability of these substrates in the gut, leading to alterations in the microbiome and metabolome. Both the source and type of diet affect the composition and function of intestinal flora and have an impact on host-microbe interactions (Campaniello et al., 2022). Therefore, dietary composition is an important factor affecting the intestinal health of weaned piglets.

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Protein is a nutrient that is beneficial to the growth of animals, and is often added to the diet to promote the growth performance and development of various bodily functions of weaned piglets (Xia et al., 2022). In piglets, low-protein diets lead to poor production performance, but high-protein diets can cause allergic reactions and excessive fermentation, produce harmful substances in the intestine, and affect intestinal function and microbial structure, eventually leading to nutritional diarrhea (Bikker et al., 2006). Moreover, undigested nutrients can cause an increase in osmotic pressure between the plasma and intestinal cavity, then the water in the plasma enters the intestinal cavity through the intestinal mucosa to cause diarrhea. Some dietary substances added to the diet of piglets, such as dietary fiber, dietary fatty acids and dietary electrolyte balance, can enhance the barrier function and inhibit the growth of pathogenic bacteria, thereby alleviating nutritional diarrhea (Gao et al., 2019; Merchant et al., 2011; Mu et al., 2017). Therefore, this paper summarizes the causes of post-weaning diarrhea in piglets and the effects of dietary protein, fiber, fatty acids and electrolyte balance on nutritional diarrhea in piglets.

## 2. Impairment of gut barrier and dysbiosis of microbiota in piglets after weaning

The intestine is a dynamic organ that constantly interacts with external substances. By secreting digestive enzymes, mucins (MUC) and immunoglobulins, the gut selectively absorbs nutrients, and eliminates toxins and pathogens (Wijten et al., 2011a). The optimal functioning of this depends on the integrity of the intestinal barrier (Tang and Xiong, 2022a,b). The intestinal barrier is mainly composed of the intestinal mucus layer, immune cells, intestinal microorganisms and their metabolites (Pluske et al., 2018). Thus, it generally includes the mucosal barrier, immune barrier and microbial barrier, which effectively maintain intestinal homeostasis by cooperating with each other (Gou et al., 2022; Suzuki, 2020; Xu et al., 2022). Research has shown that weaning can lead to villus atrophy and increased crypt depth in piglets (Heo et al., 2013). These changes in intestinal structure and function are not conducive to the intestinal defenses of piglets, resulting in increased intestinal permeability, inflammation and barrier dysfunction (Fig. 1) (Hu et al., 2013; McLamb et al., 2013).

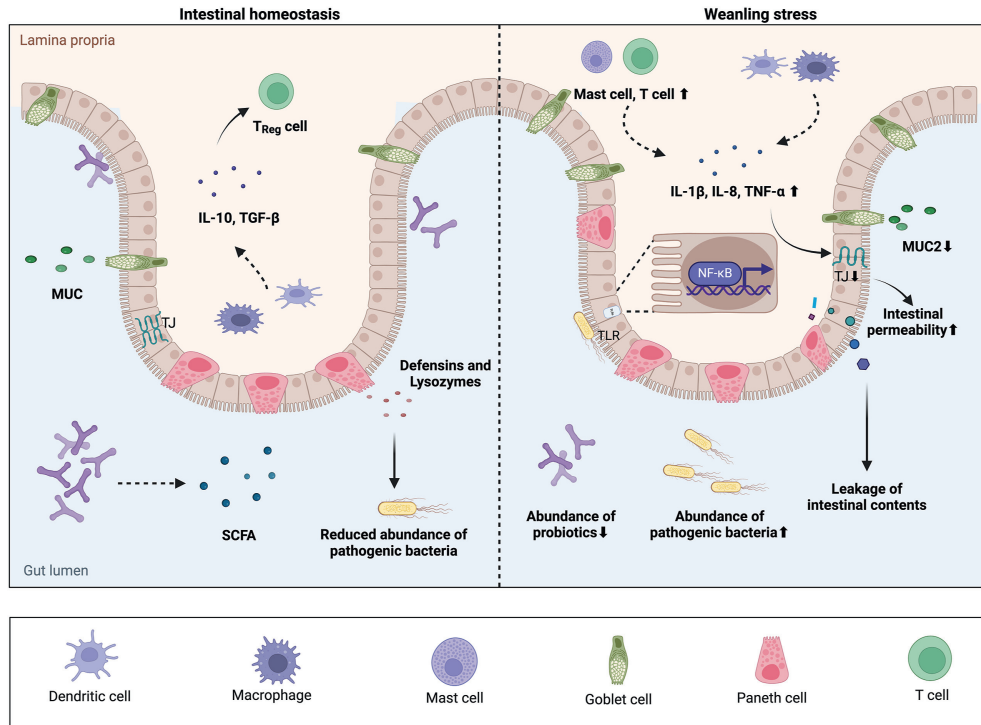
### 2.1. Weaning stress destroys the function of the intestinal mucosal barrier and immune barrier

The mechanical and chemical barriers of the intestine constitute the mucosal barrier (Zong et al., 2021). The intact intestinal epithelial cells (IEC) and tight junctions (TJ), which are an important functional component of IEC, ensure the function of the mechanical barrier (Balda and Matter, 2016; Zong et al., 2021). An important part of IEC is the apical junction complex, which is composed of TJ, adhesion junctions and desmosomes (Catalioto et al., 2011; Usuda et al., 2021). They block the transport of macromolecules between cells, maintain cell polarity, reduce intercellular space, screen for absorbed nutrients, and limit toxins and pathogens from entering mucosal tissue (Günzel and Yu, 2013). In the structure of the apical junction complex, TJ are the major determinant of regulating intestinal permeability and maintaining intestinal mechanical barriers (Turner, 2009). Tight junctions are mainly composed of transmembrane proteins, including junction adhesion molecules, occludin, and claudin (Vancamelbeke and Vermeire, 2017). Currently, occludin and claudin are considered to be the most important transmembrane proteins, especially claudin, which is the main protein to maintain TJ function (Fujiwara et al., 2022). Weaning stress has been shown to affect the expression of a variety of TJ proteins, such as claudin-1, occludin, zonula

occludens-1 (ZO-1), ZO-2 and ZO-3 (Cao et al., 2018; Wang et al., 2015), through the activation of transforming growth factor- $\beta$  and mitogen-activated protein kinase signaling pathways (Hu et al., 2013; Xiao et al., 2014). Increased intestinal permeability frequently facilitates the passage of intestinal contents into other tissues and organs across the intestinal mucosal barrier, leading to inflammation and infection (Wang and Ji, 2019; Wijten et al., 2011b). When using the Transwell model of porcine small intestinal epithelial cell line (IPEC-J2) cells to simulate the intestinal barrier, it was also found that intestinal permeability is an essential indicator for assessing intestinal mechanical barrier function (Xiao et al., 2018). Trans-epithelial resistance (TER) of the intestinal mucosa can reflect alterations in intestinal permeability and the intestinal barrier (Cao et al., 2018; Wijten et al., 2011a). The decrease in TER in piglets caused by early weaning indicates that weaning stress causes damage to the intestinal structure and function of piglets (Gresse et al., 2017).

The intestinal mucus layer has the function of protecting IEC from pathogenic bacteria, and it is capable of forming an intestinal chemical barrier that effectively prevents the colonization of pathogenic bacteria and maintains intestinal homeostasis (Paone and Cani, 2020; Pelaseyed et al., 2014). Mucus is mainly produced by intestinal Paneth cells and goblet cells, which can produce MUC, a glycosylated protein with high molecular weight (Kim and Ho, 2010; Yang et al., 2016). Normal secretion and expression of MUC is critical for maintaining the intestinal barrier function, as it is capable of forming a protective layer and preventing the binding of pathogenic bacteria to the IEC (Desai et al., 2016). Weaning stress leads to changes in the glycosylation pattern of MUC, increases the secretion of MUC3 by affecting the differentiation of goblet cells (Wang et al., 2020a), and causes intestinal infection and damage to intestinal chemical barrier function (Hedemann et al., 2007). MUC are divided into secretory MUC, including gel-forming MUC, non-gel-forming MUC, and transmembrane MUC (including a transmembrane domain and an intracellular domain) (Johansson and Hansson, 2016; Sharpe et al., 2018). Among them, MUC2 is a gel-forming MUC that forms most of the intestinal mucus and is involved in intercellular signal transduction and pathogen antagonism (Ma et al., 2018; Pelaseyed et al., 2014). The decreased expression of MUC2 in weaned piglets also indicates the destruction of the intestinal chemical barrier by weaning (Yang et al., 2016). In addition, Paneth cells also contribute to epithelial barrier function, as they disrupt the cell membrane or cell wall of bacteria by producing anti-microbial agents such as lysozyme and defensins, thereby inhibiting pathogenic bacteria and maintaining intestinal mucosal homeostasis (Bevins and Salzman, 2011; Sun et al., 2021a).

The intestine is considered the largest immune organ of the body, with 70% of immune cells located in the mucosa, which together with immune organs and immune molecules constitute the immune barrier of the intestine (Takiishi et al., 2017). The intestinal immune system recognizes exogenous antigens, prevents allergic reactions and maintains intestinal epithelial homeostasis through the secretion of immune molecules such as antimicrobial peptides, interferons (IFN), interleukins (IL), and immunoglobulins (Xiong et al., 2016). In general, the antigen entering the lamina propria is recognized by immune cells, including macrophages, dendritic cells, T cells, and B cells (Allaire et al., 2018; Chi et al., 2023). These immune cells recognize and interact with pathogen-associated molecular patterns on the surface of pathogenic organisms through pattern recognition receptors, such as Toll-like receptors (TLR) (Wang et al., 2019; Xiao et al., 2017), and then initiate nuclear factor  $\kappa$ B (NF- $\kappa$ B) and mitogen-activated protein kinase (MAPK) signaling pathways to regulate immune response and inflammation (O'Flaherty et al., 2010). The intestinal system of pigs



**Fig. 1.** The damage of weaning stress on the intestinal barrier of piglets. In intestinal homeostasis, Paneth and goblet cells secrete lysozyme, defensins and MUC respectively, which contribute to the maintenance of intestinal mucosal homeostasis and barrier function, and reduce the abundance of pathogens. Immune cells secrete IL-10 and TGF- $\beta$  to promote the differentiation of T<sub>Reg</sub>. At the same time, the abundant probiotic bacteria in the gut of normal piglets produce SCFA to provide energy to the host. Under weaning stress, immune cells produce excessive pro-inflammatory cytokines, including IL-1 $\beta$ , IL-8 and TNF- $\alpha$ , reduce the expression of tight junction (TJ) proteins and increase intestinal permeability. Moreover, the abundance of probiotics decreased and that of pathogenic bacteria increased when piglets were weaned. Toll-like receptors recognize pathogens and then initiate the NF- $\kappa$ B signaling pathway to initiate inflammatory response. MUC = mucins; T<sub>Reg</sub> = T regulatory cells; SCFA = short-chain fatty acids; TGF- $\beta$  = transforming growth factor- $\beta$ ; IL = interleukin; TNF- $\alpha$  = tumor necrosis factor- $\alpha$ ; NF- $\kappa$ B = nuclear factor  $\kappa$ B.

generally does not mature until the seventh week, so early weaning is a serious challenge for piglets aged 3 to 4 weeks (Zheng et al., 2021). Early weaning makes piglets more susceptible to diseases. Weaning causes abnormal expression of intestinal immune cells, which is manifested by a significant increase in the number of intestinal mast cells (Moesser et al., 2007), matrix metalloproteinases and intestinal inflammatory T cells (McCracken et al., 1999), and a decrease in the ratio of CD4<sup>+</sup>/CD8<sup>+</sup> T lymphocytes (Spreeuwenberg et al., 2001). Weaning also stimulates the intestinal immune system to produce excessive pro-inflammatory cytokines, including tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), IL-1 $\beta$ , IL-8, and IFN- $\gamma$  (Cao et al., 2022; de Groot et al., 2021; Deng et al., 2020), leading to intestinal damage and dysfunction (Al-Sadi et al., 2009). In addition, weaning disrupts intestinal integrity, which can be restored by immunoglobulin A (IgA) and defensins (de Groot et al., 2021).

## 2.2. After weaning, the gut microbiota barrier of piglets is disrupted, leading to intestinal infections and diarrhea

In mammals, there are billions of microorganisms inhabiting the intestinal tract, which are colonized on the surface of the mucosa, forming the microbial barrier and playing an indispensable role in the host's health (Beaumont et al., 2020; Zhou et al., 2020a). Intestinal microorganisms can not only promote the digestion and absorption of nutrients by regulating the gene expression of digestive enzymes in epithelial cells (Willing and Van Kessel, 2009), but also provide energy for the host by producing short-chain fatty acids (SCFA) (Duranti et al., 2015). Moreover, the gut microbiota is able to resist pathogens by competing for nutrients and adhesion sites or producing antimicrobial substances, thus regulating the

immune system and maintaining the normal function of the gut (Fanning et al., 2012). The composition and development of the intestinal microbial community of piglets are age-related (Karasova et al., 2021). The piglet's intestine is sterile at birth, and a few strains of bacteria, such as *Escherichia coli* and *Streptococcus*, begin to colonize the piglet's intestine once exposed to microorganisms and rapidly form a microbiota (Frese et al., 2015; Luo et al., 2022). In the following two days, obligate anaerobes, facultative anaerobes and aerobic bacteria are completely colonized in the gut of piglets (Beaumont et al., 2021). The colonization of the digestive tract is not changeless, and some microorganisms that initially colonize the gut are gradually replaced by other microorganisms more adapted to the intestinal microecosystem during development (Chen et al., 2018). Moreover, sows and milk also affect the further differentiation of the fecal microbiota of piglets during lactation, and the diversity of microorganisms increases (Bian et al., 2016). After a few weeks, the intestinal flora of piglets remains relatively stable until weaning, which is the most challenging event for piglets, and reshapes the intestinal flora.

The diet of early weaned piglets is a solid feed, which is not easily digested and utilized by the immature digestive system of piglets, and promotes the growth and reproduction of some pathogenic bacteria, resulting in dysbiosis of intestinal microflora and destruction of the microbial barrier (Shin et al., 2019). Studies have shown that the intestinal flora of both pre-weaned and weaned piglets was dominated by Firmicutes, Bacteroidetes and Proteobacteria, which account for 51%, 25% and 16%, respectively (Cremonesi et al., 2022). Weaning stress does not usually change the species of the phylum, but rather the level of the corresponding family and genus. For example, probiotics, such as *Lactobacillus*

*laurentii*, *Lactobacillus acidophilus*, *Alloprevotella* and *Oscillospira*, which produce SCFA and have intestinal barrier improvement and anti-inflammatory properties, were significantly reduced after weaning (Downes et al., 2013; Gophna et al., 2017; Konstantinov et al., 2006; Li et al., 2018). Since lactic acid bacteria are the main participants in disease prevention (Sugimoto et al., 2023), the sudden reduction of them during weaning may enhance the risk of disease, resulting in an increase in the abundance of pathogenic bacteria such as *Campylobacteraceae*, *Campylobacter* and *E. coli* (Li et al., 2018; Tanghe et al., 2023; Xie et al., 2011). A recent study has also shown that *Prevotella copri* is the most variable species in weaned piglets and plays a major role in the adaptation of piglets to weaning transition (Xiong et al., 2022). The decrease in the abundance of *Bacteroides* and its related metabolic dysfunction can also be regarded as a microbial marker of physiological diarrhea after weaning (Ren et al., 2022a). Moreover, the intestinal microbial diversity of piglets is reduced during weaning. At this time, symbiotic bacteria are more likely to degrade the glycans on the mucus layer and produce mannose, galactose or fucose that can promote the growth of pathogenic bacteria (Bäumler and Sperandio, 2016). Fucose can be used by enterohemorrhagic *E. coli* to activate gene expression of the type III secretion system, which can be used by some pathogenic bacteria to sense and adhere to host IEC (Pacheco et al., 2012; Tran et al., 2018). In addition, the increased permeability caused by weaning transition facilitates the passage of toxins and pathogens through the epithelium, exacerbates intestinal infections, and further provides favorable conditions for the growth and reproduction of Enterobacteriaceae (Zeng et al., 2017). In fact, the intestinal inflammatory response also produces nitric oxide, which is released into the intestinal cavity and converted into nitrate, resulting in the growth advantage of *E. coli* (Spees et al., 2013; Zeng et al., 2017). Additionally, the oxygen concentration in the inflammatory intestine increases due to higher blood flow, which may be beneficial to the reproduction of facultative anaerobic bacteria and reduce obligate anaerobic bacteria, resulting in the loss of bacterial diversity (Wei et al., 2017). Thus, weaning of piglets creates a vicious cycle of intestinal infections.

Diarrhea is a common symptom of weaning stress. Compared with healthy piglets after weaning, the intestinal microflora of diarrhea piglets changes significantly, including the decrease of *Ruminococcus*, *Bacteroides*, and *Treponema*, which are vital in nutritional metabolism (Sun et al., 2019; Yang et al., 2019). The disorder of gut microbiome aggravates diarrhea in piglets. After early weaning, enterotoxigenic *E. coli* (ETEC) easily colonizes the intestinal tract of piglets and leads to post-weaning diarrhea, with the serotype of  $\beta$ -hemolytic ETEC significantly increased in the small intestine of weaned piglets (Gresse et al., 2017; Schierack et al., 2006). Therefore, post-weaning diarrhea is sometimes also known as post-weaning colibacillosis, which is associated with the production of adhesin that helps bacteria adhere to the gut and enterotoxin that causes diarrhea (Fairbrother et al., 2005; Le Bouguéneq, 2005). After attachment to the epithelium, ETEC releases heat-labile (LT) and/or heat-stable (ST) enterotoxin, disrupting the electrolyte homeostasis of epithelial cells, leading to fluid loss and ultimately diarrhea. LT and ST can stimulate the accumulation of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), respectively. The increase of cGMP activates cGMP dependent protein kinase II and leads to the phosphorylation of cystic fibrosis transmembrane conductance regulator (CFTR) located on the apical membrane of epithelial cells, while cAMP also activates CFTR by targeting protein kinase A (Thiagarajah and Verkman, 2003). Then, the secretion of  $\text{Cl}^-$  and  $\text{HCO}_3^-$  is induced from the cells to the intestinal cavity. At this time, the ion electrolytic balance of the entire epithelial cells is affected, and the increase in intestinal salt concentration and osmotic

pressure force a large amount of water to flow out of the intestinal cells. Moreover, heat-stable enterotoxin b (STb) can also increase the level of intracellular  $\text{Ca}^{2+}$  by stimulating guanosine triphosphate-binding regulatory proteins (Dreyfus et al., 1993), thereby affecting the activity of phospholipase A2 and C, with arachidonic acid released from membrane phospholipids then to form prostaglandin E2 and 5-hydroxytryptamine, which can mediate the transport of water and electrolytes from intestinal cells (Harville and Dreyfus, 1995).

### 3. The immature immune system and imbalanced nutritional levels lead to nutritional diarrhea in weaned piglets

Immature immune system that is caused by gastrointestinal physiological function defects in weaned piglets is one of the main factors leading to nutritional diarrhea. Due to the immature digestive organs, the digestion, absorption and immune response functions of piglets are weak (Lallès et al., 2007). At this time, the small intestinal villi in the growth and differentiation period shrink after weaning due to feed consumption. During lactation, piglets can maintain a low pH environment in the intestine by ingesting abundant lactose and fermenting it into lactic acid under the action of intestinal microorganisms, which can maintain digestion ability of piglets. However, the decrease in lactose intake of weaned piglets and the strong acid-binding ability of the feed lead to the increase in pH of the gastrointestinal tract, the reduction of brush border and pancreatic enzyme activity, the low digestibility of proteins and abnormal fermentation (Dong and Pluske, 2007). In addition, the elevated pH reduces the abundance of beneficial bacteria and accelerates the reproduction of harmful bacteria, then the bacterial imbalance causes further deterioration of intestinal epithelial damage, leading to impaired nutrient absorption and aggravation of diarrhea symptoms (Heo et al., 2013). Diet is the main exogenous factor affecting the pH of the digestive tract, with high pH in the diet leading to an increase in acidity of the stomach, causing inflammation and edema of the intestinal wall and a large amount of water being discharged from the intestinal cavity, eventually causing diarrhea, dehydration, and even death in piglets. Moreover, the abundance and diversity of microbes living in the intestine are affected by diet. The maintenance of intestinal health depends on the balance between diet, symbiotic microorganisms and the mucosa. Because protein has a strong acid binding capacity in the stomach, ETEC will adhere to the intestinal tract to release enterotoxin and cause hypersecretory diarrhea if the protein content in the diet is high (Rist et al., 2014). Moreover, undigested nutrients cause an increase in osmotic pressure between plasma and the intestinal cavity, so the water in plasma enters the intestinal cavity through the intestinal mucosa and causes diarrhea. Studies have shown that specific dietary interventions, such as controlling the content of protein, fiber, fatty acids and electrolyte balance in the daily diet, can help establish and maintain intestinal barrier function and reduce nutritional diarrhea in weaned piglets (Gao et al., 2019).

### 4. High-protein diets are the main cause of nutritional diarrhea in weaned piglets

To improve the growth of weaned piglets, they are usually fed diets containing protein after weaning. Although a high-protein diet (CP > 20%) can support weaned piglets' growth more effectively than a low-protein diet (CP < 20%), too much protein is difficult to be completely digested and absorbed due to the immature piglet digestive tract (Table 1). In addition to triggering allergic reactions in weaned piglets, high-protein diets can cause damage to the intestine and induce diarrhea by producing a large

**Table 1**  
Effects of protein on the gut health and performance of weaned piglets.

Protein sources	Dietary protein level	Gut health	Performance/Other observations	References
SBM	13.05%, 18.83%	Low-protein diets increased the relative abundance of beneficial bacteria and decreased that of potential pathogens	Low-protein diet increased the gene expression of <i>Pept-1</i> and the fecal apparent digestibility of crude protein, but decreased the fecal nitrogen content	Wang et al. (2022a)
SBM	14.14%, 17.32%, 20.27%	Jejunum and colon microbiota were not affected at either the phyla or genera level in any of the diets.	Low-protein diet tended to decrease nitrogen emissions and impair digestive capacity	Yu et al. (2019b)
SBM	15.5%, 17.5%	Low-protein diet increased the abundance of Fibrobacteres, Proteobacteria, and <i>Spirochaetes</i> , and decreased diarrhea scores	High-protein diet increased average daily gain and feed conversion ratio	Marchetti et al. (2023)
SBM	16%, 20%	Low-protein diet increased relative abundance of <i>Parabacteroides</i> , <i>Butyricoccus</i> , <i>Olsenella</i> , <i>Succinivibrio</i> and <i>Pseudoramibacter</i> , decreased that of <i>Alloprevotella</i> and <i>Faecalicoccus</i>	High-protein diet increased the average daily gain and gain-to-feed ratio	Hou et al. (2021)
SBM	17%, 21%	Low-protein diet tended to increase crypt depth and reduce villus height to crypt depth	High-protein diet increased the average daily gain and gain-to-feed ratio	Almeida et al. (2017)
SBM	18.8%, 22.2%	Low-protein diet reduced crypt depth and <i>Lactobacillus</i> counts in the colon, but increased <i>Lactobacillus</i> counts in the ileum	High-protein diet tended to improve the gain-to-feed ratio	Sadurní et al. (2023)
SBM	18%, 20%, 22%, 24%	Under 20% CP feeding, AQP2, AQP4 and AQP9 protein expression in the colons of piglets peaked at certain time points	–	Ren et al. (2023)
SBM	18.54%, 20.19%, 21.77%, 23.84%	As the CP level increased, the number of pathogens, such as <i>Clostridium difficile</i> and <i>Escherichia coli</i> , increased, while the number of beneficial bacteria, such as <i>Lactobacillus</i> , <i>Bifidobacterium</i> , and <i>Roseburia</i> , decreased	When 20.19% CP was used, structures of the jejunum and the ileum of piglets were intact, and the expressions of occludin mRNA and protein in the small intestine were significantly higher	Ren et al. (2022b)
Casein	17%, 30%	High-protein diet group had persistent diarrhea, and decreased aquaporin and tight junction protein levels	High-protein diet group had highly digestibility and reduced growth performance	Gao et al. (2020)
Casein	17%, 30%	High-protein diet increased diarrhea rate, but decreased ileal microbiota diversity	High-protein diet decreased the concentrations of short-chain fatty acids and branch-chain fatty acids	Gao et al. (2022)
HWP, FSBM, ESBM	18%	ESBM enhanced the relative abundance of Bacteroidetes, Oscillospiraceae and Christensenellaceae, reduced that of Clostridiaceae	ESBM increased the fecal butyrate level, tended to decrease the cadaverine level	Zhang and Piao (2022)
Wheat, SBM, ESBM, HRSM, casein	18%	Protein source did not affect the small intestinal morphology response parameters	Casein decreased the standardized ileal digestibility of CP	Engelsmann et al. (2022)

SBM = soybean meal; CP = crude protein; HWP = wheat protein; FSBM = fermented soybean meal; ESBM = enzyme-treated soybean meal; HRSM = hydrothermally treated rapeseed meal; *Pept-1* = oligopeptide transporter 1; AQP = aquaporin.

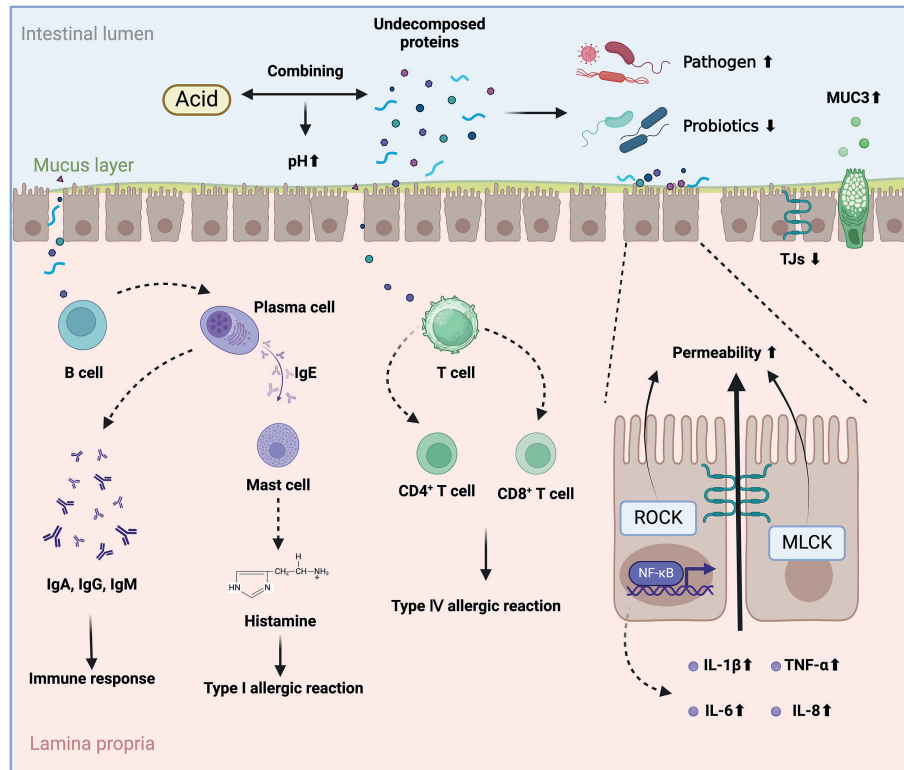
amount of biogenic amines and ammonia in the colon (Fig. 2). In addition to protein levels, the source of dietary protein also affects the intestinal health of piglets (Table 1).

#### 4.1. Different levels and sources of dietary protein have different effects on the growth performance, intestinal health, and diarrhea of piglets

Dietary change from breast milk to a solid feed-based diet at weaning causes piglets to be unaccustomed to the feed and reduce intake, resulting in decreased growth performance and watery feces, triggering high morbidity and mortality (Klues et al., 2010; Rist et al., 2013; Wellock et al., 2008a). When piglets are fed dietary protein, protein enters the gastrointestinal tract where it is degraded into peptone by gastric acid and proteases. It enters the gut with the undigested protein and is decomposed into oligopeptides and amino acids, which are absorbed into the bloodstream and transported to the liver. Long-term feeding of low-protein diets in piglets can not only lead to growth retardation, but also cause hypoproteinemia, resulting in edema of the gastrointestinal mucosa. Although high-protein diets can provide more nutrients for early-weaned piglets, the high concentration of protein will

increase the microbial fermentation of undigested protein and reproduction of pathogens, resulting in the aggravation of diarrhea symptoms (Gresse et al., 2017; Pieper et al., 2016). The appropriate level of protein for weaned piglet diets is currently considered to be about 20%; more protein can promote the growth performance although it is more likely to cause diarrhea in piglets. It is gratifying to note that a low-protein diet can also promote the growth of piglets by cooperating with other nutritional interventions. Supplementation of piglets on a low-protein diet with essential amino acids, including isoleucine, valine, tryptophan, methionine, lysine and threonine, can reduce piglet diarrhea and increase piglet growth at the same time (Bhandari et al., 2010; Heo et al., 2013; Nørgaard and Fernández, 2009).

In addition to the growth performance of piglets, the protein content in the diet also affects the intestinal health of piglets. Feeding a high-protein diet immediately after weaning may lead to the accumulation of incompletely digested protein in the large intestine, which can be fermented by *Clostridium*, *Propionibacterium*, *Bacteroides* and *Streptococcus* to produce toxic by-products such as biogenic amines, hydrogen sulfide, ammonia, branched-chain fatty acids, indole and phenol (Rist et al., 2014; Zhao et al., 2019). Most of these products compromise epithelial integrity and promote



**Fig. 2.** The mechanism of high protein-induced diarrhea in weaned piglets. High-protein diets not only reduce the expression of tight junction (TJ), but also up-regulate the expression of genes related to MLCK and ROCK signaling pathways, thereby increasing the permeability of intestinal mucosa. The NF-κB pathway is also activated to increase the expression of cytokines such as IL-1β, TNF-α, IL-6 and IL-8. When excessive protein enters the intestine, B cells differentiate into plasma cells. On one hand, IgA, IgG and IgM are secreted to mediate the intestinal immune response. On the other hand, IgE is secreted to promote mast cells to release active mediators such as histamine, and induce type I allergic reactions. Moreover, T cells differentiate into CD4<sup>+</sup> T cells and CD8<sup>+</sup> T cells after recognizing foreign antigen proteins, inducing type IV allergic reactions. For the regulation of microorganisms, the combination of protein and acid increases the pH value of the intestine, increasing the abundance of pathogenic bacteria and decreasing that of probiotics. MLCK = myosin light chain kinase; ROCK = Rho-associated coiled-coil containing kinase; Ig = immunoglobulin; IL = interleukins; TNF-α = tumor necrosis factor-α; NF-κB = nuclear factor κB.

inflammatory responses, as well as reducing fluid reabsorption capacity and triggering diarrhea (Pieper et al., 2016; Rist et al., 2013). Studies have also shown that the increase in the concentration of biogenic amines and hydrogen sulfide in the intestine caused by high-protein diets can affect intestinal health, increase the risk of disease and incidence of diarrhea by reducing the supply of energy in colon cells and increasing the permeability of the mucus barrier, respectively (Heo et al., 2015; Ijssennagger et al., 2015; Pieper et al., 2012).

Excessive microbial fermentation of protein causes nutritional diarrhea in weaned piglets (Klues et al., 2010), which is characterized by increased microbial fermentation of undigested proteins, resulting in the formation of watery feces, accompanied by increased growth disorders, morbidity and even mortality (Riaz Rajoka et al., 2017). Feeding low-protein diets to piglets reduced the concentration of bacterial fermentable protein, increased the consistency of feces, and reduced nutritional diarrhea (Qiu et al., 2018; Yin et al., 2018). In contrast, high-protein diets lead to a higher incidence of diarrhea, with the concentration of plasma urea and ammonia also elevated (Bikker et al., 2006; Heo et al., 2008). Elevated ammonia concentration not only hinders the growth and differentiation of IEC (Ni Lochlainn et al., 2018), but also disrupts intestinal microbial homeostasis and causes excessive growth of pathogenic bacteria, especially ETEC, which attach to the small intestine and release enterotoxins that cause diarrhea in piglets (Rist et al., 2014). Reduced abundance of *Lactobacillus* caused by high-protein diets also affects intestinal immune function and SCFA production, which in turn further increases the proliferation of

ETEC (Gao et al., 2019). In addition, the increase of pH value in the small intestine caused by high-protein diets is beneficial to the proliferation of pathogenic bacteria (Htoo et al., 2007). Conversely, low-protein diets promote the production of SCFA (Ma et al., 2017) and increase the abundance of beneficial microorganisms that rapidly proliferate and occupy the binding sites on the intestinal mucosa (Anthony et al., 2009), thus reducing the abundance of pathogenic bacteria, as well as the inflammatory response (Opapeju et al., 2010). However, the influence of gut microbiota composition by dietary protein levels is not definitive. In the study of Pieper et al. (2012), although high-protein diets increased ammonia concentrations in the gut, there were no significant differences in the abundance of *Bacteroides*, *Enterobacteriaceae* and *Lactobacillus*. This indicates that the increase in dietary protein content leads to the increase in harmful by-products of protein fermentation in the gut, but not necessarily to changes in the intestinal bacterial community, presumably because the microbiota is able to adapt to changes in nutrients to some extent (Heo et al., 2013). According to the above studies, feeding a high-protein diet is more inclined to, but does not necessarily, change the composition of intestinal bacteria, but almost always increases the concentration of harmful protein fermentation products, such as ammonia. Therefore, reducing the protein content effectively decreases the incidence of nutritional diarrhea in piglets.

Different protein sources also have different effects on the intestinal health and diarrhea of weaned piglets. In general, plant-derived proteins are more difficult to digest than animal-derived proteins due to the presence of anti-nutritional factors (Heo et al.,

2013). Trypsin inhibitor is a common protein-based anti-nutritional factor in soybean meal, which not only affects protein digestion by reducing the activity of trypsin and chymotrypsin (Park et al., 2020), but also causes the loss of endogenous sulfur-containing amino acids and pancreatic swelling, resulting in digestive disorders and slow growth in piglets (Chi and Cho, 2016). Glycine and  $\beta$ -glycine, the other two anti-nutritional factors in soybean meal, are known food allergens and are considered to be the main causes of abnormal small intestinal morphology and diarrhea in newborn animals such as piglets (Wang et al., 2014), negatively affecting the growth and health of post-weaning piglets. The study of Genova et al. (2019) has also shown that weaned piglets fed soybean meal showed shorter intestinal villi, lower chymotrypsin activity and weight gain, higher plasma urea level, and diarrhea rate than those fed skim milk powder. On the contrary, compared with plant protein, the concentrations of TNF- $\alpha$  and IL-8 were decreased in piglets fed with an animal protein source, such as skimmed milk powder, fish meal, and whey protein, and the growth performance and nutrient digestibility increased (Li et al., 2022a, 2021). In addition, the results of studies on the effects of different protein sources on the composition of intestinal microbiota are different. Some studies have found that piglets fed with plant proteins have lower abundance of *Staphylococcus* and *E. coli*, as well as higher microbial diversity in the intestine, which helps to increase the stability of the microbial community to resist disease (Yang et al., 2022). However, some of the early studies suggested that no significant differences in piglet fecal scores and the ratio of *Lactobacillus* to *E. coli* were observed in either plant-based diet or animal-based diet (Wellock et al., 2008a; Wellock et al., 2006).

#### 4.2. High-protein diets cause nutritional diarrhea in weaned piglets by affecting intestinal function and microbial community structure

In general, high protein levels in the diet can cause intestinal barrier dysfunction. As an important part of TJ, claudin, occludin and ZO-1 are essential for the mechanical barrier function of the intestine. The expression of TJ in the gut of piglets fed with a high-protein diet was decreased with a higher incidence of diarrhea, compared with piglets fed with a low-protein diet (Buckley and Turner, 2018). This may be caused by a large number of antigenic proteins that enter the intestinal tract with dietary proteins. They can not only reduce the gene and protein expression of TJ, but also up-regulate the gene expression related to the myosin light chain kinase (MLCK) and Rho-associated coiled-coil containing kinase (ROCK) signaling pathway. MLCK-induced phosphorylation of the regulatory light chain is significant for regulating the function of the actin-based cytoskeleton, and ROCK also plays a central role in regulating the actin-myosin cytoskeleton, which regulates the permeability and barrier function of the gut (Du et al., 2016; Martínez-Sánchez et al., 2023). Moreover, TJ proteins change their distribution in cells through endocytosis, resulting in increased permeability (Du et al., 2016), which can result in damage to the mechanical barrier function. In addition, when a high concentration of protein enters the intestine, the integrity of the intestinal epithelium is destroyed, causing deepening of the intestinal crypts and atrophy of the villi, thereby impairing barrier function. At this time, the intestinal tract reduces the absorption of inorganic salt ions and water, while the water in the feces increases, thus causing diarrhea in weaned piglets. Bile, gastric acid, lysozyme and digestive enzymes are important components of the intestinal chemical barrier. When fed a high-protein diet, piglets showed decreased digestive enzyme activity and increased intestinal pH (Yu et al., 2019a), and the expression of goblet cells and their secreted MUC increased significantly (Lan et al., 2015), causing damage to the chemical barrier. The immune response of the intestine is mainly

mediated by T lymphocytes and B lymphocytes, in which B lymphocytes differentiate into plasma cells and secrete IgM, IgG and IgA after being stimulated by antigens, mediating the immune response of the intestine (Engels and Wienands, 2018; MacIver et al., 2013). When the diet contains excessive protein, a large number of antigenic proteins enter the body of piglets to promote the proliferation and differentiation of lymphocytes and the secretion of immunoglobulin, the body's metabolic load increases, and the immune system is affected (Xia et al., 2022). The studies have also found that the MAPK and NF- $\kappa$ B signaling pathway was up-regulated in the gut of weaned piglets fed a high-protein diet, with the expression of cytokines such as IL-6 and IL-8, IL-1 $\beta$  and TNF- $\alpha$  also increased (Peng et al., 2019; Zhang et al., 2017). High concentrations of cytokines exacerbate allergic reactions and induce intestinal mucosal edema, leading to poor absorption of liquid and electrolytes, eventually leading to diarrhea in piglets.

Anaerobic bacteria, such as *Bifidobacterium*, attach to the intestinal mucosa to form a microbial barrier by binding to the intestinal epithelium to prevent the invasion of pathogenic bacteria and produce SCFA to provide nutrition, reduce pH, and maintain barrier function (Duranti et al., 2015; Kumar et al., 2014). Due to the high acid-binding capacity of high protein, the intestinal pH value of piglets fed with high-protein diets increases, and the undigested protein provides a nutritional source for the proliferation and fermentation of intestinal microorganisms, resulting in an increase in the abundance of pathogenic bacteria such as *Streptococcus* and ETEC, whereas the abundance of probiotics such as *Lactobacillus* and *Bifidobacterium* decreases (Hooda et al., 2013). However, other researchers have found that changing dietary protein levels has no significant effects on the intestinal microbial diversity and abundance of microbial community (Bikker et al., 2006; Nyachoti et al., 2006). Therefore, it is difficult to determine the exact effect of dietary protein levels on the intestinal microbiota of pigs, which may be further understood through the study of protein catabolic activity. Changes in intestinal microbial composition caused by elevated protein levels affect the community's catabolic activity of proteins, including decarboxylation, deamination and production of carboxylic acid and ammonia, producing ammonia and SCFA as the main final products (Diether and Willing, 2019; Fan et al., 2015). Different strains can produce different metabolites by using different amino acids. For example, methyl mercaptan and hydrogen sulfide are produced by the catabolism of sulfur-containing amino acids such as methionine and cysteine. Histidine, tyrosine and ornithine are metabolized to produce biogenic amines such as histamine, tyramine and putrescine, respectively. Aromatic amino acids are metabolized to produce phenols and indoles (Yao et al., 2016; Zhang et al., 2020a). The accumulation of toxic protein fermentation products is also one of the causes of protein-induced nutritional diarrhea in piglets. High-protein diets increase the concentrations of hydrogen sulfide, ammonia, and putrescine, which exert a toxic effect on the IEC (Pieper et al., 2016; Wen et al., 2018). They can not only destroy the structure and function of the IEC and reduce energy supply, but also promote the occurrence of inflammatory response and increase the permeability of the mucus barrier (Hamer et al., 2012). The colon is the major area for the absorption of water and diarrhea occurs when its ability to absorb water is diminished.

In addition, abundant undigested protein, such as glycine and soybean protein, may cause a variety of allergic reactions. Glycine, the main antigen that causes allergies, increases intestinal mucosal permeability after entering the digestive tract, leading to diarrhea in piglets (Sun et al., 2008). Soybean protein can cause type I and type IV allergic reactions, which are mediated by specific immunoglobulins and specific T lymphocytes, respectively (Xia et al., 2022). When excess soybean protein enters the intestine,

immunoglobulin E is secreted and releases crystallized fragments that bind to its receptor, degranulating mast cells and releasing active mediators, such as cytokines and histamine, and inducing a type I allergic reaction (González-de-Olano and Álvarez-Twose, 2018). As for type IV allergic reactions, T cells recognize abundant soybean proteins and differentiate into CD4<sup>+</sup> T cells, a helper T lymphocyte that exerts immune function by releasing cytokines (Panagoulas et al., 2016), and CD8<sup>+</sup> T cells, a cytotoxic T lymphocyte that destroys target cells through the release of granzymes and perforin (Farhood et al., 2019), resulting in type IV allergic reaction, and eventually causing diarrhea (Hooda et al., 2013).

## 5. Dietary fiber helps alleviate diarrhea in weaned piglets

Dietary fiber is usually defined as an indigestible carbohydrate with more than three monomer units, which is the main energy source for intestinal microbial fermentation and affects the composition of bacterial communities and microbial metabolic activity (Zhang et al., 2016). Adding dietary fiber to the diet is beneficial to the regulation of intestinal morphology, immune system and host gene expression in weaned piglets, and reduces the incidence of diarrhea (Molist et al., 2014). Notably, different dietary fibers showed different effects on microbial composition and diversity (Table 2).

### 5.1. Positive effects of dietary fiber on growth performance, intestinal health, and diarrhea in piglets

Dietary fiber maintains the health of weaned piglets by positively affecting intestinal function, which is related to the integrity of the intestine, altered microbial communities, SCFA generation, and decreased toxic metabolites (Molist et al., 2014). The addition of dietary fiber can improve the adaptability, nutritional metabolism and gastrointestinal development of weaned piglets, and the performance of piglets varies with fiber composition (Molist et al., 2014; Superchi et al., 2017). Adding wheat bran to daily feed can increase the height of ileal villi, thereby improving the integrity of the intestinal mucosa and protecting it from pathogens (Tang et al., 2022). Additionally, the supplementation of dietary fiber also promotes the increase in goblet cells (Vancamelbeke and Vermeire, 2017), which are vital to gut health because they synthesize and secrete several mediators (Wijten et al., 2011b). The integrity of the intestinal barrier reflects the gap between epithelial cells and may prevent the spread of pathogens (Pelaseyed et al., 2014). Dietary fiber maintains the integrity of the intestinal barrier by regulating the expression of TJ, which is related to the proliferation of probiotics such as *Bifidobacterium* and *Lactobacillus* (Sharpe et al., 2018). Besides, dietary fiber also promotes intestinal immune barrier function by regulating the mRNA levels of pro-inflammatory cytokines (Tang et al., 2022).

It is reported that dietary fiber can reduce the incidence of diarrhea, and the therapeutic effect depends on the type of dietary fiber, which is divided into soluble fiber and insoluble fiber according to the solubility in water. Soluble dietary fiber typically includes hemicellulose, pectin, gum, and soluble oligosaccharides and is found primarily in legumes, fruits, vegetables, oats, wheat, and rice (Evans, 2020; Nakashima et al., 2018). Insoluble dietary fiber usually includes lignin, cellulose, resistant starch and part of hemicellulose, which is present in whole grains such as wheat bran and oat shell (Waddell and Orfila, 2022). When insoluble dietary fiber is added to the diet, such as oat shell, wheat bran, pure cellulose and lignocellulose preparations, the growth performance of piglets improves and the abundance of *E. coli* and diarrhea rate decrease (Canibe et al., 2022; Molist et al., 2011). The addition of wheat straw and oat husk to the diet of weaned piglets also

stimulates amylase activity and promotes the adaptation of the gastrointestinal tract of piglets to solid feed, thereby promoting intestinal health (Gerritsen et al., 2012). However, after adding cellulose to the weaning diet, although the concentration of plasma cyclooxygenase-2 (COX-2) was decreased and the ratio of villus to crypt was increased, there was no significant difference in diarrhea symptoms (Cho et al., 2021). On the contrary, soluble dietary fiber, such as sodium carboxymethyl cellulose and barley, can promote the growth of *E. coli* and aggravate the symptoms of diarrhea, which may be due to the prolonged transport time caused by the increased viscosity of the digesta, resulting in excessive growth of *E. coli* (Hopwood et al., 2004). Soluble dietary fibers that do not affect the viscosity of the digesta, such as inulin, can reduce intestinal pH, increase the ratio of *Lactobacillus* to *E. coli*, improve intestinal health, and reduce the incidence of diarrhea (Wellok et al., 2008b). Thus, the effect of soluble fiber on diarrhea in weaned piglets depends on whether it increases the viscosity of digesta.

### 5.2. Dietary fiber mainly alleviates diarrhea in weaned piglets by producing SCFA

On one hand, insoluble dietary fiber can reduce the residence time of the digesta in the proximal gastrointestinal tract, increase the fecal passage rate, regulate the microbial community, maintain intestinal health, and reduce diarrhea in piglets. On the other hand, fiber may inhibit the adhesion of pathogens to intestinal mucosa and reduce the proliferation of pathogens in the small intestine. Notably, SCFA are the main product of dietary fiber produced by microbial fermentation in the colon, such as acetic, propionic, butyric, valeric, and isovaleric acids, which is the main way for dietary fiber to provide benefits to the host. They are involved in regulating environmental stability, activating epithelial cell proliferation and differentiation, maintaining mucosal integrity, and attenuating inflammation (Fig. 3) (Martin-Gallausiaux et al., 2021).

SCFA can alleviate diarrhea in weaned piglets by affecting intestinal barrier function. Studies have found that SCFA can increase the expression of mucosal TJ proteins including occludin, claudin-1 and ZO-1, and maintain intestinal barrier in weaned piglets (Diao et al., 2019). Sodium butyrate induces store-operated calcium entry by regulating the calcium pool, and then the activation of AMP-activated protein kinase (AMPK) phosphorylation leads to the phosphorylation of Connexin43 protein at serine 368, resulting in a decrease in its binding to ZO-1, leading to a more stable TJ of ZO-1 on the cell membrane, which improves the structure and function of the intestinal epithelium (Miao et al., 2016). Synaptopodin is another intestinal epithelial TJ protein, which participates in intestinal epithelial barrier function by regulating actin filament assembly and maintaining intercellular interaction (Kremerskothen et al., 2005). Butyrate regulates synaptopodin by inhibiting histone deacetylase (HDAC), and then the expression of  $\alpha$ -actinin-4 and its accumulation at the adhesion junction are promoted, which contributes to the contraction of cells (Wang et al., 2020b). SCFA can promote the secretion of IL-18, antimicrobial peptides, and MUC by IEC through the G protein-coupled receptor 43 (GPR43) to maintain the integrity of the intestinal barrier. MUC2 is one of the most notable MUC. Its oligosaccharide chain structure can provide adhesion sites for probiotics, IgA and antimicrobial peptides in the intestinal flora to play a barrier role (Johansson et al., 2013). Butyrate and propionate can induce the expression of MUC2, which may be related to the activator protein-1 (AP1, c-Fos/c-Jun) cis-element at the MUC2 promoter, the acetylation of histone H3 and H4, and the methylation of MUC2 promoter H3 (Burger-van Paassen et al., 2009). In vitro experiments using LS174T cells (human colon adenocarcinoma cells), and sodium butyrate can also increase the

**Table 2**  
Effects of dietary fiber on the gut health and performance of weaned piglets.

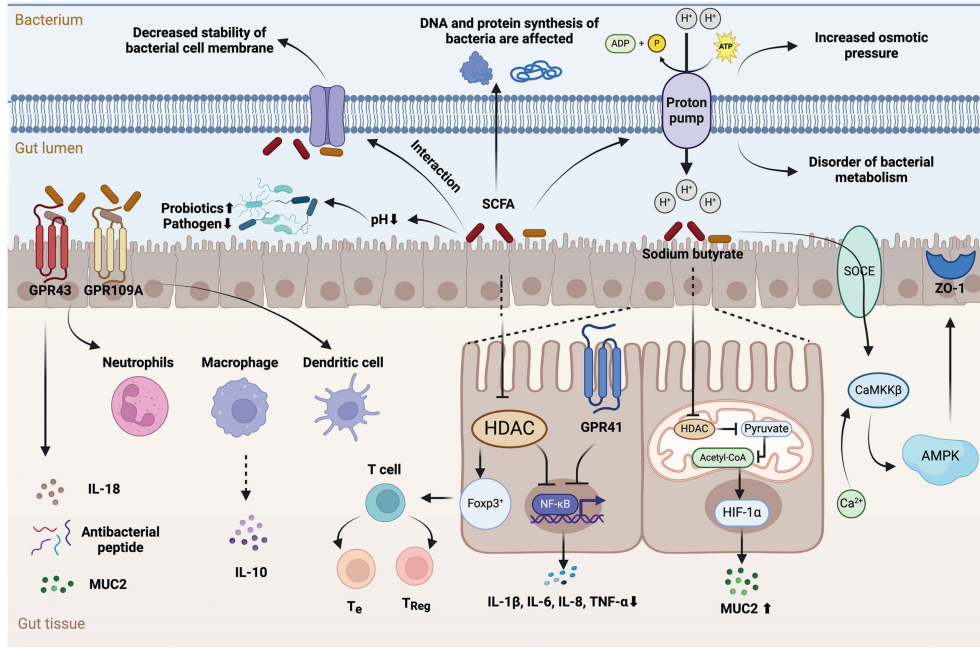
Protein sources	Dietary fiber level	Gut health	Performance/Other observations	References
Crude fiber concentrate	1%	Crude fiber concentrate decreased the diarrhea incidence	Crude fiber concentrate increased the average daily gain, average daily feed intake and feed efficiency	Superchi et al. (2017)
AF	5%	AF increased the relative abundances of cellulolytic bacteria and anti-inflammatory bacteria, concentrations of propionate, butyrate, and total SCFA	AF inhibited the expression of inflammatory signal pathways and proinflammatory cytokines, and upregulated the expression of barrier-related genes	Sun et al. (2021b)
Micro-fibrillated cellulose	1%	Micro-fibrillated cellulose increased the abundance of butyrate-producing bacteria, reduced that of pathogenic bacteria such as <i>Campylobacter</i> and <i>Escherichia</i> , and reduced diarrhea incidence	Micro-fibrillated cellulose increased the body weight, average daily growth and butyrate content	Uddin et al. (2023)
Araceae root and citrus	2 kg/tonne	The fiber mixture tended to decrease <i>E. coli</i> colonization	The fiber mixture tended to increase cecal butyric acid	Tanghe et al. (2023)
AF	6%, 12%, 18%	AF treatment decreased the Bacteroidetes phylum and increased the Firmicutes phylum, the Chao 1 richness and Shannon diversity increased with an increase in AF supplementation, 12% and 18% AF diets showed the lowest diarrhea incidence	AF decreased the feed conversion ratio, 18% AF diets showed the lowest average daily gain, 12% AF diets showed the highest average daily feed intake	Adams et al. (2019)
AMSLF	2.5%, 5%, 7.5%	AMSLF decreased diarrheal incidence, 2.5% AMSLF group showed the highest Shannon diversity, PD whole tree diversity indices and Chao value	Both 5% and 7.5% AMSLF increased growth performance; the digestibility of gross energy and dry matter increased with increasing AMSLF level; 7.5% AMSLF group showed the highest IL-2 and TNF- $\alpha$ levels	Che et al. (2019)
WB, SBP	6% WB, 4% SBP	WB increased $\alpha$ -diversity and the abundance of <i>Lachnospira</i> , and reduced diarrhea rate	WB increased G:F and ileal mRNA levels of occludin, decreased serum diamine oxidase activity and ileal IL-8 levels	Shang et al. (2021)
CB, WB, SB	5%	CB or WB increased the abundances of Actinobacteria, Firmicutes or Fibrobacteres	CB and WB groups increased weight gain and feed efficiency	Zhao et al. (2018)
MCC, WB, MOLP, SBP	5%	WB and MCC decreased relative levels of <i>Lactobacillus</i> and <i>Bifidobacterium</i> , but increased levels of <i>E. coli</i> and <i>Brachyspira hyodysenteriae</i>	SBP reduced diarrhea and increased SCFA production	Huang et al. (2022)
SI, MIX	3%	SI group showed the highest abundance of UCG-002 and Holdemanella, MIX group showed the highest abundance of Bacteroides	SI increased the level of taurohyocholic acid and $\alpha$ -muricholic acid, MIX increased the level of THCA and cholic acid, both SI and MIX decreased the glycodeoxycholic acid	Hu et al. (2023)
lc-AXOS, CELL	2% lc-AXOS, 5% CELL, 2% lc-AXOS + 5% CELL	CELL decreased the abundance of <i>Escherichia-Shigella</i>	CELL increased the concentration of volatile fatty acids, lc-AXOS increased the large intestinal fill, lc-AXOS + CELL increased the large intestinal weight	Van Hees et al. (2019)
lc-AXOS, CELL	2% lc-AXOS, 5% CELL, 2% lc-AXOS + 5% CELL	lc-AXOS increased the gut permeability, lc-AXOS + CELL increased the incidence of diarrhea	CELL decreased G:F	van Hees et al. (2021)
ARBOCEL, inulin	1% ARBOCEL, 1% inulin, 0.5% ARBOCEL + 0.5% inulin	Inulin showed the highest bacterial $\alpha$ -diversity and highest relative abundance of Proteobacteria, Actinobacteria, <i>Solobacterium</i> , <i>Succinivibrio</i> , <i>Blautia</i> and <i>Atopobium</i> , ARBOCEL showed the highest relative abundance of Bacteroidetes, Euryarchaeota, <i>Phascolarctobacterium</i> , <i>Coprococcus_1</i> , and <i>Prevotella_1</i>	Inulin increased the concentrations of SCFA, 0.5% ARBOCEL + 0.5% inulin upregulated the mRNA expressions of claudin-1, ARBOCEL and 1% inulin upregulated the mRNA expressions of mucin 2	Chen et al. (2019b)

AF = alfalfa fiber; MFC = micro-fibrillated cellulose; AMSLF = *Astragalus membranaceus*; WB = wheat bran; SBP = sugar beet pulp; CB = corn bran; SB = soybean hulls; MCC = microcrystalline cellulose; MOLP = *Moringa oleifera* leaf powder; SI = fructooligosaccharides; MIX = a mixture of fructooligosaccharides, long-chain inulin, and microcrystalline cellulose at the ratio 1:1:1; lc-AXOS = long-chain arabinoxylans from wheat; CELL = purified cellulose; SCFA = short-chain fatty acids; IL = interleukin; TNF = tumor necrosis factor; ARBOCEL = a crude fiber concentrate; G:F = gain-to-feed ratio.

transcription levels of *MUC3*, *MUC4* and *MUC12* genes, and activate the MAPK signal pathway, while enhancing the chemical barrier of the intestine and reducing the colonization of *E. coli* in the colon (Jung et al., 2015).

The inhibition of HDAC and activation of GPR are the key pathways for SCFA to regulate immune responses. Paneth cells in the intestinal epithelium recognize intestinal bacteria by activating TLR, in which TLR-2, TLR-4 and TLR-5 recognize peptidoglycan, lipopolysaccharide and bacterial flagellin, respectively (Kayama et al., 2020). Then, the activation of the NF- $\kappa$ B pathway through myeloid differentiation factor 88 causes the overexpression of pro-

inflammatory factors including IL-1 $\beta$ , IL-6, IL-8 and TNF- $\alpha$ , leading to intestinal inflammation (Jang et al., 2013; Wang et al., 2017). Studies have found that SCFA can inhibit the activation of NF- $\kappa$ B by inhibiting HDAC and up-regulating the expression of GPR41 (Kumar et al., 2017; Liu et al., 2020). After HDAC is inhibited by butyrate, histone acetylation at the Foxp3<sup>+</sup> site is enhanced to promote T cell differentiation into effector T cells and regulatory T cells (Tanoue et al., 2016). In addition, SCFA also induce neutrophil chemotaxis (Vinolo et al., 2011) and anti-inflammatory properties of colonic macrophages and dendritic cells (Singh et al., 2014) by activating GPR43 and GPR109A, respectively. The differentiation of regulatory



**Fig. 3.** The mechanism of short-chain fatty acids (SCFA) in promoting intestinal health in weaned piglets. The inhibition of histone deacetylase (HDAC) and the activation of GPRs are the key pathways for SCFA to regulate immune responses. The activation of GPR43 and GPR109A by SCFA not only promotes the secretion of IL-18, antimicrobial peptides and MUC in intestinal epithelial cells, but also promotes the chemotaxis of neutrophils and the anti-inflammatory properties of colonic macrophages and dendritic cells, thereby regulating the production of IL-10. SCFA also inhibits the activation of NF- $\kappa$ B by inhibiting HDAC and up-regulating the expression of GPR41, thereby reducing the concentration of pro-inflammatory factors including IL-1 $\beta$ , IL-6, IL-8 and TNF- $\alpha$ . In addition, after the inhibition of HDAC is induced by butyrate, histone acetylation at the Foxp3<sup>+</sup> site is enhanced, which promotes the differentiation of T cells into T<sub>e</sub> cells and T<sub>Reg</sub> cells. In terms of the mucosal barrier, sodium butyrate activates the Ca<sup>2+</sup>/CaMKK $\beta$  pathway by regulating SOCE, thereby activating AMPK, leading to a more stable tight junction of ZO-1 on the cell membrane to improve the structure and function of the intestinal epithelium. Additionally, butyrate entering the mitochondria reduces the activity of the pyruvate dehydrogenase complex by inhibiting HDAC. At this time, colon cells cannot convert pyruvate to acetyl-CoA through  $\beta$ -oxidation, resulting in inhibition of oxidative respiration. This process helps maintain the stability of HIF-1 $\alpha$ , thereby promoting the secretion of MUC2 and increasing the thickness of the mucus layer. SCFA also maintains intestinal health by inhibiting pathogens. Bacteria need to consume huge amounts of energy to initiate H<sup>+</sup>-ATPase to pump out the excess H<sup>+</sup> produced by SCFA dissociation, which will lead to bacterial metabolic disorders and increase osmotic pressure in cells. The interaction of undissociated SCFA with phospholipids and lipoproteins in the cell membrane also destroys the stability of the bacterial cell membrane. SCFA can also change the intracellular protein and DNA synthesis to inhibit bacterial growth and metabolism. In addition, the acidic environment produced by SCFA is conducive to the reproduction of beneficial bacteria, thereby inhibiting the growth of harmful microorganisms. GPR = G protein-coupled receptors; IL = interleukin; TNF- $\alpha$  = tumor necrosis factor- $\alpha$ ; MUC = mucins; T<sub>Reg</sub> = T regulatory cells; T<sub>e</sub> = effector T cells; NF- $\kappa$ B = nuclear factor  $\kappa$ B; HIF-1 $\alpha$  = hypoxia-inducible factor-1 $\alpha$ ; SOCE = store-operated calcium entry; CaMKK $\beta$  = Ca<sup>2+</sup>/calmodulin-dependent protein kinase kinase- $\beta$ ; AMPK = AMP-activated protein kinase.

T cells and production of IL-10 are then regulated. Furthermore, butyrate enters the mitochondria and stimulates the promoter of pyruvate dehydrogenase kinase by inhibiting HDAC, and then the activity of pyruvate dehydrogenase complex is reduced (Blouin et al., 2011). At this time, colonic cells are unable to convert pyruvate to acetyl-CoA through  $\beta$ -oxidation, leading to the inhibition of oxidative respiration, a process that facilitates the maintenance of the stability of hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) (Kelly et al., 2015), thereby promoting the secretion of MUC and increasing the thickness of the mucus layer (Glover et al., 2016).

The inhibitory effect of SCFA on pathogens is mainly carried out in four aspects. Firstly, SCFA entering the bacterial cytoplasm will dissociate to release H<sup>+</sup> and reduce the pH in the cytoplasm (Jackman et al., 2020). In order to maintain the stability of the pH in the cytoplasm, bacteria are forced to consume huge amounts of energy to start H<sup>+</sup>-ATPase to pump out excess H<sup>+</sup>, which will cause bacterial metabolic disorders, and ultimately cause death of bacteria. Moreover, harmful microorganisms are suitable for growth in neutral and alkaline environments, whereas beneficial bacteria are suitable for growth in acidic environments. The acidic environment produced by SCFA is conducive to the reproduction of probiotics such as *Lactobacillus* and *Bifidobacterium*, thereby further reducing pH of gut and inhibiting the growth of harmful microorganisms such as *E. coli* and *Salmonella*. In addition, undissociated SCFA can interact with phospholipids and lipoproteins in the cell membrane

to destroy the stability of the cell membrane in bacteria (Alakomi et al., 2000). Further, when the protons produced by SCFA are pumped out of the cell membrane, the bacteria will pump out potassium ions, thereby increasing the osmotic pressure in the cell. Increased osmotic pressure leads to plasma membrane rupture and then leads to death of bacteria (Ricke, 2003). Fourthly, SCFA inhibit the growth and metabolism of bacteria by changing the intracellular protein and DNA synthesis of bacteria. For example, acetate and propionate reduced the synthesis of DNA, RNA, protein and lipid in *E. coli*, especially DNA synthesis (Cherrington et al., 1990). Additionally, according to the study of Xu et al. (2016), oral administration of sodium butyrate increased biological diversity in piglets.

## 6. Dietary fatty acids help alleviate diarrhea in weaned piglets

Dietary fatty acids have a positive effect on intestinal health and diarrhea in weaned piglets, which is related to the regulation of intestinal barrier function and microbial community (Li et al., 2022b; Yin et al., 2023). According to the carbon chain length, fatty acids are divided into SCFA, medium-chain fatty acids (MCFA), long-chain fatty acids and very long-chain fatty acids, which contain 1 to 5, 6 to 12, 13 to 21, and more than 22 carbon atoms, respectively (Upadhaya and Kim, 2021). In addition, fatty acids are also divided into saturated fatty acids, monounsaturated fatty acids,

and polyunsaturated fatty acids (PUFA) according to the degree of saturation. The effects of different fatty acid compositions on the digestion and absorption of fatty acids and intestinal inflammation vary, among which SCFA and MCFA have attracted more attention in alleviating piglet diarrhea due to their antibacterial effects and ability to reduce intestinal pH.

### 6.1. Positive effects of dietary fatty acids on growth performance, intestinal health, and diarrhea in piglets

MCFA is mainly present in the form of triglycerides in food. It is absorbed into portal vein blood and transported to the liver to provide energy for animals and improve intestinal integrity under inflammatory conditions (Lauridsen, 2020). In addition, MCFA can also break through a bacterium's semi-permeable membrane and destroy its structure, thereby exerting antibacterial activity (Hanczakowska et al., 2017). As for SCFA, mainly including propionate, acetate and butyrate, they are acidic substances that are easily neutralized by buffer substances in the upper digestive tract of animals. Therefore, coating techniques or SCFA salts are often used when adding SCFA to the diet (Lin et al., 2020). Moreover, SCFA can also be obtained by intestinal microbial fermentation of dietary fiber and provide 10% and 60% to 70% energy to human and colonic epithelial cells, respectively (LeBlanc et al., 2017). Most of the propionate is used for glucose synthesis, while acetate and butyrate are used for cholesterol synthesis in the liver (den Besten et al., 2013). SCFA can also regulate the composition of intestinal flora, optimize microbial flora, and improve production performance (Huang et al., 2015).

MCFA naturally exists in milk fat and various feeds in the form of medium-chain triglycerides, especially in coconut, palm oils and *Cuphea* seed oils. When MCFA is used in the pig industry, the direct addition of free MCFA often leads to a decrease in feed intake, and the combined addition of different lipases and medium-chain triglycerides in the diet can increase feed intake and daily gain (Zentek et al., 2011). This may be due to two reasons. Firstly, the strong pungent odor and poor palatability of non-esterified MCFA is difficult to accept by young animals (Decuyper and Dierick, 2003). Secondly, the secretion of cholecystokinin and other intestinal hormones induced by MCFA causes satiety, thereby affecting feed intake (Mabayo et al., 1992). However, piglet feed intake was not reduced by the addition of MCFA, and piglet growth performance was also improved (Gebhardt et al., 2020). Moreover, the mixture of MCFA and different organic acids also led to improvements in feed intake, daily gain and nutrient digestibility, which is generally considered to be related to its inhibitory effect on pathogenic bacteria and its effect on epithelial cell morphology (Upadhaya et al., 2018). The effects of MCFA on the morphology of IEC also affect the production performance of piglets. In piglets supplemented with MCFA, the crypt depth decreased, and the villus height and the intestinal absorption area increased, which was conducive to the absorption and utilization of nutrients (Jackman et al., 2020). In addition, medium-chain triglycerides can restore the villus morphology destroyed by lipopolysaccharides (De Keyser et al., 2019).

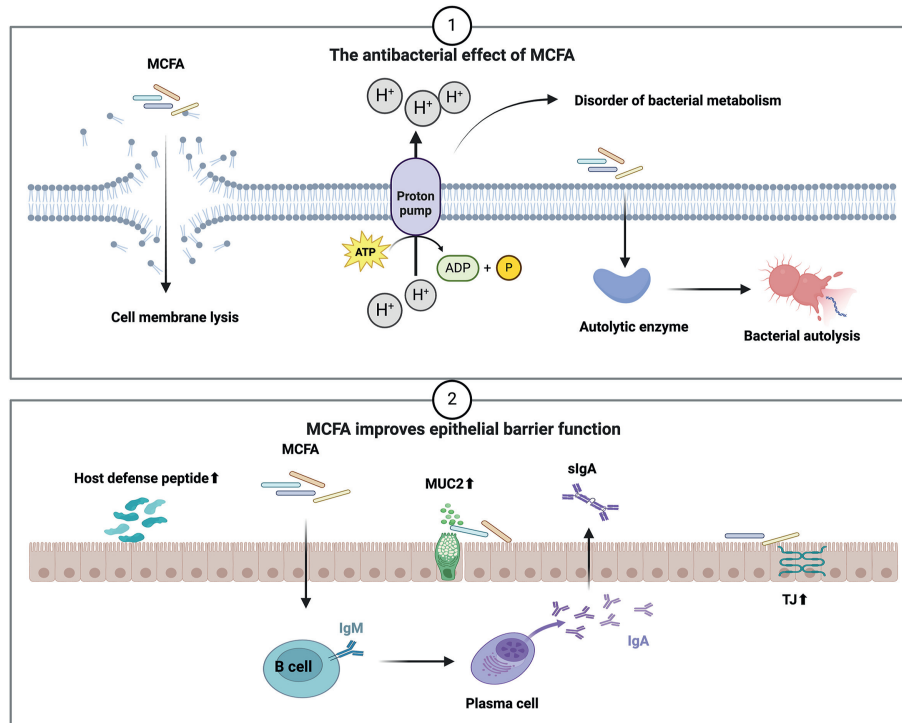
The role of SCFA in improving the production performance of weaned piglets has also been widely confirmed. For example, butyrate increased the average daily gain, increased villus height, and promoted intestinal health in weaned piglets (Upadhaya et al., 2020). After dietary supplementation with SCFA, the renewal rate of IEC in piglets was accelerated, and the villus height of the small intestine increased (Huang et al., 2015). Moreover, the villus height of sterile pigs fed with SCFA tended to increase, and the activities of lactase, sucrase, trypsin and lipase were enhanced, indicating that SCFA could promote intestinal development even in the absence of

microorganisms (Zhou et al., 2020b). In another study, a positive correlation was also found between colonic development and the concentration of SCFA in the intestine during weaning (Zhou et al., 2019b). When the intestinal barrier function is damaged by stress, the addition of SCFA can reduce intestinal permeability, prevent harmful substances from entering the body, regulate intestinal flora, alleviate inflammatory response, enhance intestinal barrier function, and reduce the incidence of diarrhea. Notably, in a recent study, the simultaneous addition of SCFA, MCFA and PUFA to the diet had a positive effect on the growth performance and intestinal health of piglets (You et al., 2023).

Studies on the alleviative effects of dietary fatty acids on piglet diarrhea have also yielded promising results. The rate of diarrhea caused by *E. coli* was significantly reduced in piglets fed a combination of MCFA and organic acids (Lei et al., 2017b). Meanwhile, the combination of SCFA and MCFA also reduced the diarrhea rate of piglets. For example, the combination of SCFA and MCFA prevented the occurrence of diarrhea in weaned piglets (Caprarulo et al., 2023), and the addition of a mixture of SCFA, MCFA and long-chain fatty acids to the diet of pregnant sows reduced the incidence of diarrhea in suckling pigs (Chen et al., 2019a). However, the therapeutic effect of single addition of MCFA esters on diarrhea is not significant (Braundmeier-Fleming et al., 2020). In addition, the source of dietary triglycerides can influence dietary fatty acid composition, which can influence the digestion, absorption and metabolism of dietary lipids. In general, unsaturated fatty acids are more easily digested due to their ability to promote the activity of lipase, while saturated fatty acids have the opposite effect (Ravindran et al., 2016). The digestion, absorption and metabolic activities of triglycerides with different chain lengths are also quite different. The hydrolysis of medium-chain triglycerides is faster and more complete than that of long-chain triglycerides, which may be due to the weaker inhibitory effect of medium-chain triglycerides on pancreatic lipase (Lauridsen, 2020). The long-chain triglycerides must be mixed with bile salts to form mixed micelles, which are absorbed through the unstirred water layer and enter the intestinal cells (Lauridsen, 2020). MCFA in the body form medium-chain triglycerides, which are hydrolyzed into fatty acids and glycerol under the action of tongue esterase and gastric esterase. Most of them are transported to the liver through the portal vein, and a small part is directly absorbed by the small intestine to release energy. In contrast, the metabolic process of long-chain fatty acids is more complex. Therefore, saturated MCFA present in the form of medium-chain triglycerides in milk and some plant fat sources, such as palm kernel oil and coconut, can provide instant energy for intestinal metabolic activity. Therefore, adjusting the source and composition of dietary fat may also control the intestinal health and reduce diarrhea in weaned piglets.

### 6.2. Dietary fatty acids mainly alleviate piglet diarrhea through antibacterial effects

The alleviating effect of MCFA on diarrhea is mainly related to their antimicrobial effect, which can be exerted by destroying the phospholipid membrane around the pathogen (Fig. 4) (Jackman et al., 2020). Firstly, MCFA is an anionic surfactant that can enter bacterial cells to increase the permeability of bacterial cell membranes and inhibit the activity of intracellular lipases, then the structure of cell membranes will be destroyed, with the bacterial contents being released, ultimately leading to bacterial death (Yoon et al., 2018). Secondly, MCFA releases  $H^+$ , just like SCFA, causing huge energy consumption and metabolic disorders in bacteria, eventually leading to death (Jackman et al., 2020). Thirdly, MCFA can also induce bacteria to produce autolytic enzymes, causing the death of bacteria (Jia et al., 2020). Compared with Gram-negative



**Fig. 4.** The protective effect of medium-chain fatty acids (MCFA) on the intestinal health of weaned piglets. The promoting effect of MCFA on intestinal health is mainly related to its antibacterial effect. MCFA destroys the structure of the cell membrane, leading to the death of the bacteria. The  $H^+$  released by MCFA can also lead to huge energy consumption and metabolic disorders in bacteria. Moreover, MCFA can induce bacteria to produce autolytic enzymes, leading to the death of bacteria. MCFA also regulates immune response and epithelial function by promoting the expression of endogenous host defense peptides, MUC2, slgA and TJ. MUC = mucins; slgA = secretory immunoglobulin A; TJ = tight junction.

bacteria, Gram-positive bacteria are more susceptible to MCFA inhibition, which is due to the simple single-lipid bilayer cell membrane structure of Gram-positive bacteria, and more complex inner and outer membrane structures of Gram-negative bacteria (Yoon et al., 2018). Studies have shown that different MCFA exert different antibacterial effects. Octanoic acid and decanoic acid can inhibit the activity of most Gram-positive bacteria, while lauric acid has a significant inhibitory effect both on Gram-positive bacteria and Gram-negative bacteria (Messens et al., 2010; Van Immerseel et al., 2004). In vitro studies have shown that MCFA are able to maintain an undissociated state and exert antimicrobial function in the range of pH 3 to 6, which will be gradually inhibited with the increase in pH value. When the environment reaches neutral pH, the MCFA will no longer exert obvious antibacterial effects because they are quickly absorbed and exist in the form of ions (Dierick et al., 2002). Therefore, it can be hypothesized that MCFA act mainly in the proximal small intestine and stomach. The antimicrobial effect can be improved if organic acids are added to increase the acidic environment in the stomach. Feeding piglets with a mixture of MCFA and organic acids will result in a better diarrhea treatment.

MCFA also affect intestinal morphology and epithelial barrier function through different mechanisms, and the energy produced by MCFA can be directly utilized by intestinal cells to maintain intestinal barrier function (Fig. 4). Medium-chain triglycerides can enhance mucosal function, the ratio of lipid phospholipids to protein in jejunal mucosal villi, membrane-bound enzyme activity, goblet cell proliferation and differentiation, and the content of IgA in the blood (De Keyser et al., 2019). Caproic acid and caprylic acid can also improve intestinal barrier function by reducing oxidative stress (Lee and Kang, 2017) and increasing the expression of endogenous host defense peptides such as  $\beta$ -defensins (Wang et al.,

2018a; Zhou et al., 2019a), respectively. In addition, the mixture of organic acids and MCFA increased the expression of MUC2, TJ proteins such as occludin and ZO-1, and host defense peptides including methyl-CpG binding domain 1 (mBD1), mBD2 and mBD3, which enhanced the intestinal barrier function (Wang et al., 2018b). Excessive production of pro-inflammatory cytokines during the activation of the intestinal immune system leads to intestinal damage and dysfunction. The effect of MCFA on the structure of IPEC-J2 cells showed that fatty acids provide energy for many metabolic pathways and signaling pathways, and also play a key role in improving the immune response of piglets (Martínez-Vallespín et al., 2016).

PUFA also have a protective effect on the intestinal health of piglets, among which n-6 PUFA and n-3 PUFA have received the most attention. An imbalanced proportion of n-6 PUFA to n-3 PUFA leads to the production of pro-inflammatory mediators (Xia et al., 2006). This is mainly because n-6 PUFA and n-3 PUFA compete for cyclooxygenase and lipoxygenase in the metabolic process, and produce pro-inflammatory factors such as thromboxane A2, leukotriene B4 and prostaglandin E1, and anti-inflammatory factors (including leukotriene B5 and prostaglandin E3) (Russo, 2009). PUFA can not only inhibit the immune response mediated by NF- $\kappa$ B and MAPK signaling pathways by reducing the protein level of nitric oxide synthase (Jin et al., 2018), but also play a significant role in maintaining redox homeostasis and resisting inflammation through the Nrf2-mediated signaling pathway (Mildenberger et al., 2017). The antibacterial effect of PUFA is not as strong as that of MCFA, and their effect on microorganisms is mainly produced by cell lysis, the uncoupling of oxidative phosphorylation, and the destruction of the electron transport chain (Shaikh et al., 2012). Zhang et al. (2020b) have shown that omega-3 PUFA are inversely correlated with the abundance of *E. coli* and positively correlated

with that of *Lactobacillus*. The mechanism by which SCFA alleviate diarrhea in weaned piglets has been described previously.

### 7. Appropriate dietary electrolyte balance promotes growth and intestinal function in weaned piglets

The intestinal cavity receives 8 to 10 L of liquid every day, and most of the water absorption occurs in the small intestine (Binder, 2009). The absorption and secretion of intestinal mucosa occur simultaneously, depending on the polarity of epithelial cells, and help to maintain membrane potential, cell viability, adequate nutrient absorption and regulation of microbial metabolism (Rao, 2019). The absorption of water in the intestine is a passive process, mainly through the osmotic gradient generated by the transcellular absorption of  $\text{Na}^+$ . Normally,  $\text{Na}^+$  can diffuse from the extracellular to the intracellular environment along the electrochemical gradient, and is then transported into the intercellular space or blood through the sodium-potassium pump on the cell bottom side membrane (Kato and Romero, 2011). In contrast, the  $\text{Na}^+$  absorption caused by the potential difference caused by the imbalance of ion entry and exit is called electrogenic absorption of  $\text{Na}^+$ . The potential difference can drive  $\text{Cl}^-$  to transport from the mucosa to the basement membrane.  $\text{Na}^+/\text{H}^+$  exchanger is a vital membrane protein that regulates intracellular pH and volume, and plays a significant role in initiating cell proliferation, differentiation and apoptosis (Kato and Romero, 2011). It is functionally coupled to the apical membrane  $\text{Cl}^-/\text{HCO}_3^-$  exchanger in the distal jejunum, ileum and proximal colon, which not only leads to the absorption of electrically neutral NaCl, but also leads to the production of  $\text{H}^+$ , an increase in pH and the transport of  $\text{HCO}_3^-$  out of cells (Gao et al., 2019).

Electrolyte balance plays a key role in intestinal phenotype and function, and the electrolyte imbalance caused by weaning leads to excessive salt and water loss, which has a negative impact on the growth performance of weaned piglets (Patience et al., 1987). The electrolyte imbalance in the diet after weaning is manifested as changes in intestinal motility, absorption and secretion capacity, cell permeability, and electrolyte flux, ultimately leading to nutritional diarrhea after weaning. Minerals such as sodium, potassium, calcium, chlorine, sulfur, and phosphorus are the main substances that improve the electrolyte balance of the diet and are separated into cations and anions after dissolution in the body (Budde and Crenshaw, 2003; Liu et al., 2014). Dietary electrolyte balance represents the net acid-base load caused by diet. The increase in  $\text{Na}^+$  and  $\text{K}^+$  concentrations causes metabolic alkalosis, and the increase in  $\text{Cl}^-$  concentration promotes acidosis, which affects the nutritional metabolism and growth performance of animals (Guzmán-Pino et al., 2015). Therefore, maintaining an appropriate dietary electrolyte balance is key to promoting normal physiologic function of the piglet digestive tract system and increasing piglet growth performance. Compared with high electrolyte balance, piglets generally show a preference for a low electrolyte balance diet (Gao et al., 2019). However, different conclusions were obtained in Jones' study, with the growth performance of piglets significantly improved when the electrolyte balance was increased from 84 and 29 mEq/kg to 243 and 199 mEq/kg (Jones et al., 2019). Another study found that weaned piglets showed the best growth performance when fed 166 to 250 mEq/kg electrolyte balance diets (Lei et al., 2017a). The diarrhea promoting effect of electrolyte imbalance in weaned piglets may be due to two reasons. Firstly, newborn mammals have smaller reserves of water and electrolytes, which increase the incidence of diarrhea. Secondly, the imbalance of absorption and secretion of ions and solutes in the intestinal epithelium causes diarrhea. The large amount of water and electrolytes entering the intestinal cavity caused by the penetration of

unabsorbed nutrients in the diet (Buddington et al., 2012), as well as villus atrophy and crypt hypertrophy caused by weaning (Zhang et al., 2022), adversely changes the balance of absorption and secretion. In addition, the imbalance of electrolytes in the digestive tract of piglets may also be caused by the presence of bacteria. These bacteria cause intestinal infection while interfering with epithelial development by secreting toxins, which can lead to the formation of watery feces or nutritional diarrhea. Therefore, appropriate dietary electrolyte balance also has a certain alleviating effect on nutritional diarrhea in piglets.

### 8. The effects of other nutritional components on weaned piglets

Zinc is an essential micronutrient for piglet growth (Dong et al., 2023). It is a metal component of DNA and RNA synthetases, transferases and many digestive enzymes, and plays a vital role in lipid, protein and carbohydrate metabolism (Heo et al., 2013). Zinc deficiency can lead to growth retardation and loss of enzyme activity in tissues of piglets. In order to prevent the deterioration of diarrhea after weaning, many countries add a certain level of zinc oxide to the diet of piglets after weaning (Canibe et al., 2022). A previous study has shown that the supplementation of 2400 to 3000 mg/kg zinc can reduce diarrhea and mortality of piglets by exerting antibacterial effects (Holm and Poulsen, 1996). However, the effect of high levels of zinc oxide on the microorganisms of weaned piglets is controversial. One study found that the abundance of *E. coli* in piglets decreased after the use of zinc oxide (Starke et al., 2014), whereas other studies have obtained the opposite results (Højberg et al., 2005; Vahjen et al., 2011). Moreover, the addition of zinc oxide in the above experiments reduced the abundance of lactic acid bacteria that was beneficial for health. The accumulation of zinc in the liver, pancreas and kidneys also has a negative impact on animal health (Bednorz et al., 2013). In addition, high levels of zinc excreted by piglets pose a threat to the environment and may contribute to the development of antimicrobial resistance in bacteria (Canibe et al., 2022). Therefore, the beneficial effects of zinc oxide have not been widely recognized, and the EU has banned the use of medical levels of zinc in pig diets since June 2022.

Due to the stimulation of the immune system, amino acids are the most commonly affected nutrient, and piglets may have increased amino acid requirements during weaning (Jayaraman et al., 2015). The addition of some amino acids in diets can increase the level of anti-secretory factors in plasma and reduce the incidence of diarrhea in weaned piglets (Fairbrother et al., 2005). Studies have shown that, when the immune system is under attack, such as during weaning stress or lipopolysaccharide attack, piglets show an increased demand for tryptophan and threonine (Floc'h and Séve, 2007; McGilvray et al., 2019). Threonine can protect the submucosa from pathogens such as *E. coli*. One study demonstrated that adding threonine to the basal diet of weaned piglets elicited an improvement in intestinal mucin synthesis and an increase in the number of mucin-producing cells (Zhang et al., 2019). In contrast, tryptophan is not directly involved in the structure and function of the gastrointestinal tract, but it participates in the regulation of appetite and the control of stress response together with valine (Floc'h and Séve, 2007), and can also play an important role in the immune response by converting into kynurenine (Le Floc'h et al., 2011). Glutamine is a functional amino acid widely used in pig breeding, which is considered to be an important fuel for intestinal epithelial cell proliferation and integrity repair (He et al., 2016). Ala-glutamine (Ala-Gln) has been added to the diet of piglets as an affordable substitute for glutamine due to its low price and easy absorption by intestinal cells (Modina et al., 2019). It can maintain

the integrity and function of intestinal mucosa and prevent the development of intestinal diseases caused by sudden changes in the diet of weaned piglets. Moreover, Ala-Gln may also alleviate weaning stress in piglets by increasing the levels of TJ proteins and MUC in the intestine (Miguel et al., 2017). Another essential amino acid often used in piglet nutrition is arginine, which is involved in the synthesis of many important substances in the body such as protein, urea, and polyamines (Tong and Barbul, 2004). Supplementation of arginine in the diet can promote the repair of small intestinal mucosa by improving the DNA synthesis of intestinal epithelial cells (Tan et al., 2015). Therefore, when weaning stress causes morphological changes and pressure in the intestine, the supplementation of specific amino acids in the diet is beneficial to the intestine and may help prevent diarrhea after weaning.

When piglets are weaned, the transition from high-fat sow milk to a low-fat cereal diet causes a decrease in the dietary intake of piglets. Adding fat to the diet can improve the palatability and digestibility of feed (Wang et al., 2022b). Weng (2017) found that the milk fat of sows contains a large amount of palmitic acid and oleic acid, and piglets still prefer milky feed with high oleic acid content at weaning. Feeding complex milk replacers after weaning increased the energy intake of piglets and stimulated intestinal development and growth performance of piglets (de Greeff et al., 2016). Similarly, supplementation with whey permeates can impact gut health, activate immune responses, and improve intestinal cell proliferation (Jang et al., 2021). Therefore, the addition of appropriate lipids to the diet may contribute to the weaning transition of piglets. However, more studies are needed to explore the effect of fat on the prevention of post-weaning diarrhea.

## 9. The effects of different nutrients on the intestinal microbiota of piglets

In conclusion, different nutrients have different effects on the gut microbiota of weaned piglets. As far as dietary protein is concerned, both the level and source of protein affect the gut microbes of piglets. In general, a low-protein diet was more likely to promote the abundance of beneficial microorganisms and reduced that of pathogenic bacteria (Anthony et al., 2009). On the contrary, a high-protein diet increased the intestinal pH value due to its high acid-binding ability, and the undigested protein provided a nutrient source for the proliferation of pathogenic bacteria, resulting in an increase in the abundance of *Streptococcus* and ETEC, whereas the abundance of probiotics such as *Lactobacillus* and *Bifidobacterium* decreased (Hooda et al., 2013). However, the effect of dietary protein levels on the composition of the gut microbiota is not definitive. It has also been shown that there is no significant difference in the abundance of *Bacteroides*, Enterobacteriaceae and *Lactobacillus* across different levels of protein diet groups (Pieper et al., 2012), which may be due to the adaptability of the microbiota to nutrients (Heo et al., 2013). In addition, different results were obtained after the effects of different protein sources on the composition of intestinal microflora had been studied. Normally, piglets fed with plant protein showed lower abundance of *Staphylococcus* and *E. coli*, and higher microbial diversity in the gut than those fed with animal-derived protein (Yang et al., 2022). However, an early study has shown that no significant differences in the ratio of *Lactobacillus* to *E. coli* were observed in either plant-based or animal-based diets (Wellock et al., 2006). Therefore, the exact effect of dietary protein on the intestinal microbiota of piglets needs to be further explored through analyses such as proteolytic metabolism.

Studies on dietary fiber have shown that the solubility of dietary fiber has a great influence on the intestinal microflora of piglets. When insoluble dietary fiber was added to the diet, the abundance of *E. coli* in the intestine of piglets decreased (Canibe et al., 2022). In

contrast, soluble dietary fiber promoted the growth of *E. coli*, which may be due to the prolonged transport time caused by increased viscosity of the digesta (Hopwood et al., 2004). It is worth noting that soluble dietary fiber that does not change the viscosity of the digesta can increase the ratio of *Lactobacillus* to *E. coli* (Wellock et al., 2008b). SCFA are the main fermentation product of dietary fiber, which produces an acidic environment conducive to the reproduction of probiotics such as *Lactobacillus* and *Bifidobacterium*, thus further reducing the intestinal pH value, inhibiting the growth of harmful microorganisms such as *E. coli* and *Salmonella*, and increasing the microbial diversity of piglets (Xu et al., 2016). In addition to SCFA, the other two dietary fatty acids, MCFA (Hanczakowska et al., 2017) and PUFA (Zhang et al., 2020b), can prevent the excessive growth of pathogenic bacteria such as *E. coli* in the gastrointestinal tract and increase the abundance of probiotics such as *Lactobacillus* due to their antibacterial effects.

In addition, dietary electrolyte balance, trace elements and amino acids will also affect the diarrhea process by affecting the intestinal microbiota of piglets. Electrolyte imbalance can cause the excessive growth of intestinal pathogens (Guttman et al., 2007), and high levels of trace elements, such as zinc, can reduce the abundance of lactic acid bacteria (Starke et al., 2014), and the modest additions of amino acids can reduce the abundance of pathogenic bacteria such as *E. coli* (Zhang et al., 2019). It is worth noting that although the effects of the same nutrient on the gut microbiota of piglets generally trend in the same direction, there are still exceptions that need to be verified and confirmed by more experiments.

## 10. Conclusions

Weaning is one of the most stressful and complex events in the life of pigs. The separation of piglets from sows and the sudden change of piglet diets leads to growth retardation and intestinal health problems in piglets, which has caused significant economic losses to the pig industry. We have learned that dietary composition is a key factor affecting intestinal health, and some dietary nutrients can affect diarrhea in piglets after weaning as the digestive organs of weaned piglets have not yet matured. High dietary protein not only causes allergic reactions in weaned piglets, but also produces a large number of harmful substances, which damage the intestinal barrier function of piglets and induce nutritional diarrhea. Notably, dietary fiber, dietary fatty acids and dietary electrolyte balance can alleviate nutritional diarrhea in piglets by enhancing the mucosal barrier and immune barrier function and reducing the abundance of pathogenic bacteria. Thus, controlling dietary nutrition is a promising strategy for preventing nutritional diarrhea in weaned piglets. However, in the future, it is still necessary to further determine the interaction between different dietary nutrients and their effects on the host, and to develop the most appropriate diet for weaned piglets.

## Author contributions

**Xuebing Han:** Investigation, Writing – original draft, Writing – reviewing and editing. **Xiangdong Hu:** Investigation, Writing – reviewing & editing. **Wei Jin, Gang Liu:** Supervision.

## Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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