



## Review Article

# Targeting mitochondria with antioxidant nutrients for the prevention and treatment of postweaning diarrhea in piglets

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## ABSTRACT

Post-weaning diarrhea (PWD) in piglets poses a significant challenge and presents a grave threat to the global swine industry, resulting in considerable financial losses and compromising the welfare of animals. PWD is commonly associated with gut homeostatic imbalance, including oxidative stress, excessive inflammation, and microbiota dysbiosis. Antibiotic use has historically been a common initiative to combat PWD, but concerns about the development of antibiotic resistance have led to increased interest in alternative strategies. Mitochondria are key players in maintaining cellular homeostasis, and their dysfunction is intricately linked to the onset and progression of PWD. Accumulating evidence suggests that targeting mitochondrial function using antioxidant nutrients, such as vitamins, minerals and polyphenolic compounds, may represent a promising approach for preventing and treating PWD. Moreover, nutrients based on antioxidant strategies have been shown to improve mitochondrial function, restore intestinal redox balance, and reduce oxidative damage, which is a key driver of PWD. The present review begins with an overview of the potential interplay between mitochondria and gut homeostasis in the pathogenesis of PWD in piglets. Subsequently, alternative strategies to prevent and treat PWD using antioxidant nutrients to target mitochondria are described and discussed. Ultimately, we delve into potential limitations and suggest future research directions in this field for further advancement. Overall, targeting mitochondria using antioxidant nutrients may be a promising approach to combat PWD and provides a potential nutrition intervention strategy for regulating gut homeostasis of weaned piglets.

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## 1. Introduction

The weaning period is widely recognized as one of the most pivotal phases in commercial pig husbandry. Although pigs naturally wean themselves between 12 and 17 weeks of age, large commercial farms usually wean piglets at 3 to 4 weeks old to

reduce physical stress on the sow, shorten the estrus interval, and improve reproductive performance (Zheng et al., 2021). However, for piglets, this stage is among the most taxing experiences they encounter during their lifetimes. During the weaning period, pigs undergo substantial physiological, environmental, and social changes that can heighten their susceptibility to diseases, including diarrhea, resulting in reduced production and economic losses (Campbell et al., 2013). Post-weaning diarrhea (PWD) is a severe symptom in piglets that results from multiple factors and occurs within the initial 14 days after weaning (Eriksen et al., 2021). The gut serves as a crucial location for digestion and nutrient uptake in the body, functioning as both the largest immune organ and a central hub for stress response. Research demonstrates that weaning stress usually impairs the intestinal barrier function in piglets, including increased permeability, accelerated ion transport, gut microbiota dysbiosis, and mucosal inflammation. Furthermore,

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oxidative stress represents the primary trigger for diarrhea in piglets, perturbing intestinal homeostasis and exacerbating the diarrhea condition (Guttman and Finlay, 2009).

Maintaining the normal function and balance of intestinal cell populations relies on the body's redox homeostasis, a process intricately linked to the proliferation, differentiation, barrier function, and mucosal defense of intestinal cells (Circu and Aw, 2011). Numerous studies have shown that early-weaned piglets are prone to elevated free radical levels in the intestine, which disrupts the balance of the redox state. This is mainly manifested by abnormally increased reactive oxygen species (ROS), destruction of redox balance, inflammation, and impaired mitochondria (Bomba et al., 2014; Ming et al., 2021; Tang et al., 2022).

Mitochondria play a crucial role in cellular energy metabolism and serve as an important hub for cells to carry out various physiological functions. Therefore, the function of mitochondria is essential for maintaining gut homeostasis (Rath et al., 2018). Previous research indicates that during the first week after weaning, there is a decline in the activities of mitochondrial respiratory complexes and the content of mitochondrial DNA (mtDNA) in the intestine (Cao et al., 2018b). Mitochondria constitute a crucial source of ROS, typically produced as byproducts of cellular respiration (Zorov et al., 2014). Excessive ROS production is recognized as a major cause of intestinal barrier dysfunction. Studies have demonstrated that weaning induces increased production of ROS in the serum and jejunum of piglets, while also inhibiting the development of the jejunum and cell cycle progression (Guo et al., 2019). Furthermore, dysfunction of mitochondrial and genomic variations in intestinal cells can disrupt the composition and function of the gut microbiome, while gut microbial metabolites can target mitochondria to regulate intestinal inflammation (Yardeni et al., 2019). To prevent mitochondrial damage, cells initiate an endogenous protective program known as mitochondrial quality control (MQC), which is crucial for maintaining mitochondrial homeostasis and function (Gan et al., 2018). MQC coordinates various processes such as mitochondrial biogenesis, fission and fusion, and mitophagy to collectively regulate the maintenance of mitochondrial homeostasis (Zhou et al., 2023). Considering the vital role of mitochondria in cellular energy metabolism and signal transduction, mitochondria

may represent an attractive therapeutic target for improving PWD in piglets.

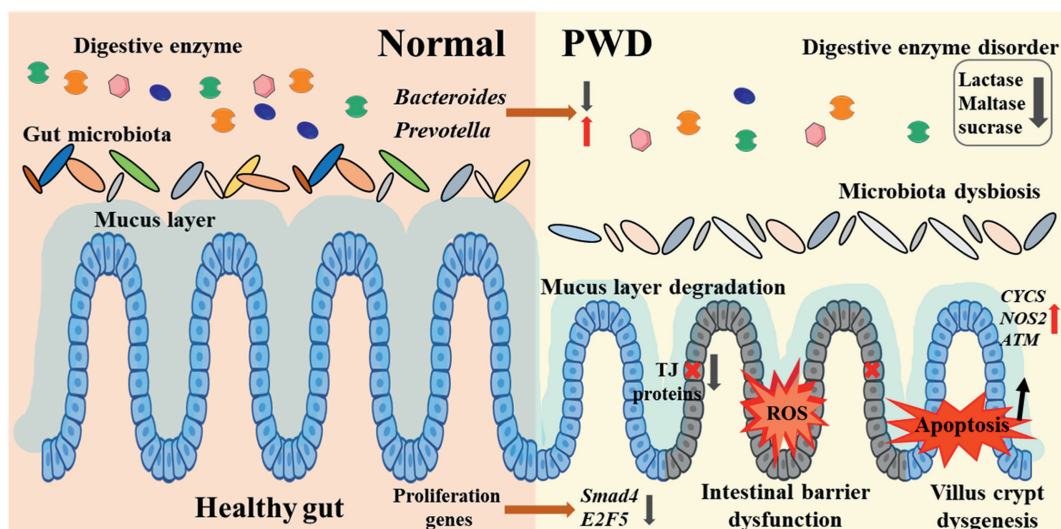
Here, the impact of weaning stress on gut homeostasis of piglets was discussed from the perspective of mitochondrial function, thereby providing a theoretical basis for developing an effective nutrition intervention strategy to alleviate PWD in piglets in future studies. To the best of our knowledge, there is no comprehensive review that explores the potential interplay between mitochondria and gut homeostasis in the pathogenesis of PWD and the use of antioxidant nutrients as a nutrition intervention strategy to combat PWD in piglets. Therefore, this review aims to fill this knowledge gap by providing an up-to-date overview of the current understanding of the role of mitochondrial function in PWD and alternative strategies to prevent and treat PWD using antioxidant nutrients.

## 2. Imbalances in gut homeostasis after weaning lead to diarrhea in piglets

The gut serves as a crucial organ for piglets to respond to stress, functioning not only as the primary site for nutrient digestion and absorption, but also as a vital defense mechanism against bacterial and external antigen invasion into the intestinal lumen (Tang et al., 2022). When piglets are suddenly switched to a solid diet after weaning, carbohydrates replace fat as the main energy source. Moreover, piglets are also subjected to stress from nutritional, psychological, environmental, physiological, and social factors. Due to these stressors, weaning often leads to slow growth and a higher frequency of diarrhea in piglets (Gao et al., 2019). Fig. 1 shows the intestinal pathological changes of PWD piglets.

### 2.1. Small intestine morphological disruption

The rapid changes in feeding methods and environment (separation from sow and new pen mates) are crucial for the immature digestive system of weaned piglets, making them highly vulnerable to negative impacts. During this period, the morphology and histology of the small intestine in piglets undergo alterations, such as a decrease in villus height and an increase in crypt depth (Bomba



**Fig. 1.** Intestinal homeostasis imbalance in postweaning diarrhea (PWD) piglets. The gut is a crucial organ for piglets to cope with stress. After weaning, the height of the villi in the small intestine of piglets decreases significantly and the crypt depth increases, which may be associated with the disruption of the balance between intestinal epithelial cell proliferation and apoptosis induced by weaning stress. Additionally, the activity and content of disaccharidases (key enzymes for piglet digestion and absorption of carbohydrates) decrease noticeably. Moreover, the disruption of the intestinal barrier and dysbiosis of gut microbiota are the leading causes of PWD. TJ = tight junction; *E2F5* = E2F transcription factor 5; *Smad4* = SMAD family member 4; *CYCS* = cytochrome-c; *NOS2* = nitric oxide synthase 2; *ATM* = ataxia telangiectasia mutated; ROS = reactive oxygen species.

et al., 2014). Furthermore, weaning stress can lead to a reduction in the proportional weight of the small intestine, with the overall weight of the intestine reaching only half of its pre-weaning weight 15 days post-weaning (Montagne et al., 2007). The above phenomenon may be associated with the disruption of the balance between the proliferation of intestinal epithelial cells (IECs) and apoptosis induced by weaning stress. Research has indicated that in the jejunum of post-weaned piglets, the expression of genes associated with proliferation and differentiation decreases, while those related to cell apoptosis increase (Zhu et al., 2014). Additionally, under conditions of oxidative stress, the length of the cell cycle in IECs is prolonged, leading to villus crypt dysgenesis that impairs nutrient absorption (da Silva et al., 2019).

## 2.2. Digestive enzyme disorder

PWD is characterized by digestive enzyme (pepsin, trypsin, chymotrypsin, and amylase) disorder and overgrowth of pathogenic bacteria, and is considered an intestinal disease following weaning (Bomba et al., 2014; Jensen et al., 1997; Yue et al., 2020). Disaccharidases in the small intestine, such as lactase, maltase, and sucrase, are key enzymes for piglets to digest and absorb carbohydrates (Tang et al., 2022). The activity and content of disaccharidase, however, significantly decreases after weaning, which is considered a key factor leading to the occurrence of PWD in piglets (Marion et al., 2005).

## 2.3. Intestinal barrier dysfunction

The premature weaning of piglets poses a challenge to their immature intestinal immune system, as it needs to adapt to the colonization of gut microbiota and feed antigens (Su et al., 2022). In addition, the intestinal tract is highly susceptible to oxidative stress due to its prolonged exposure to exogenous substances. ROS can phosphorylate ZO-1, leading to its degradation, ultimately disrupting intestinal barrier function (Kawauchiya et al., 2011). Moreover, free radicals can also cause abnormal DNA structures in IECs, affecting protein expression in epithelial cells, damaging mitochondria and endoplasmic reticulum, and ultimately leading to apoptosis of IECs (Phaniendra et al., 2015). Therefore, the intestinal tract of piglets is particularly susceptible to oxidative stress, which is considered the primary cause of damage to intestinal barrier function during the weaning process. Zhu et al. (2012) have shown that weaning results in an increase in levels of nitric oxide (NO) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) in piglet serum, a significant decrease of antioxidant enzyme expression in jejunum, and damage to intestinal villi. Our study also found similar results, that early weaning led to a decrease in antioxidant capacity of piglets, an increase in malondialdehyde (MDA) and ROS levels in jejunum, and a significant decrease in antioxidant enzyme activities. Additionally, the structure and function of mitochondria were significantly disrupted (Qiao et al., 2023). During weaning, piglets are exposed to stressful stimuli which cause an imbalance in the intestinal redox state, leading to increased permeability of the intestinal mucosal barrier (Hu et al., 2013). This makes it easy for harmful substances and pathogens to cross the intestinal wall and enter the body, further exacerbating intestinal inflammation and diarrhea symptoms.

## 2.4. Gut microbiota dysbiosis

Early weaning in piglets can lead to a reduction in microbial diversity and dysbiosis of the gut microbiota, thereby increasing susceptibility to diarrhea (Guevarra et al., 2018). Due to the immaturity of the digestive system in piglets, they are unable to

fully digest and utilize nutrients, which provides a favorable nutritional environment for the growth of pathogenic bacteria (Shin et al., 2019). Alain et al. (2014) found that Firmicutes and Bacteroidetes were the most predominant phyla before and after weaning, respectively. Moreover, there was a noticeable shift in the composition of gut microbiota during the weaning transition, with a decrease in *Bacteroides* and an increase in *Prevotella*. *Bacteroides* are generally 'friendly' commensals while residing in the gut. They possess multiple polysaccharide utilization loci, allowing them to digest carbohydrates and hydrolyze starch, as well as break down host-derived glycan and glycosaminoglycan to produce nutrients and energy for the body, and provide nutrition for other microorganisms in the gut (Wexler and Goodman, 2017). *Bacteroides* can also promote the growth and proliferation of intestinal mucosal cells and enhance the integrity of the intestinal mucosal barrier, thereby boosting gut immunity (Zafar et al., 2021). The increased abundance of *Prevotella* after weaning in piglets may be related to changes in the diet. *Prevotella* has the ability to degrade plant-based feed, such as hemicellulose and xylans. Additionally, it possesses unique mucin glycoprotein degradation capabilities, which could allow *Prevotella* to thrive while posing a threat to the intestinal mucosal barrier (Alain et al., 2014). In addition, the relative abundances of *Bacteroides*, *Ruminococcus*, *Bulleidia*, and *Treponema*, which play important roles in nutrient metabolism, are decreased in piglets (age consistent with healthy piglets) with PWD when compared to healthy piglets (Yang et al., 2019).

The mucus layer, which is comprised of O-glycosylated mucins produced by goblet cells, forms a polymer network that acts as the first line of defense against harmful antigens and helps to regulate inflammation by interacting with the microbial community and maintaining intestinal homeostasis (Schroeder, 2019). In addition to preventing bacterial invasion, the resulting O-glycans can also serve as a nutrient source and provide attachment sites for specific bacteria (Xia et al., 2022). However, during the weaning-related period of feed deprivation, the depletion of nutrients in the body leads to excessive degradation of O-glycans by intestinal bacteria, which in turn causes erosion of the mucus layer and an increase in the degradation of mucins (Desai et al., 2016; Gresse et al., 2021).

## 3. The role of mitochondria in gut homeostasis

Mitochondria are organelles responsible for producing energy in the form of ATP (Chakrabarty and Chandel, 2022). Currently, mitochondria are a subject of intense interest due to their function as signaling organelles that play a pivotal role in regulating physiology and disease. Mitochondria have emerged as essential regulators of cellular fate by coordinating processes such as metabolism, immunity, stress responses, and apoptosis (Rath et al., 2018). In addition, the oxidative phosphorylation (OXPHOS) of the mitochondrial inner membrane respiratory chain is the primary source of ROS. Any conditions that disrupt mitochondrial structure and function can trigger an increase in ROS production. The accumulation of excessive ROS can initiate oxidative damage reactions in the cell. Redox homeostasis is a critical factor for the normal operation of mitochondria. Oxidative stress induced by ROS from mitochondria can further attack the mitochondria themselves, exacerbating structural damage and functional disorder, thereby disrupting cellular homeostasis (Zorov et al., 2014). Thus, controlling optimal mitochondrial quantity, quality, and function plays a critical role in maintaining normal cellular physiology. Due to constant renewal and prolonged exposure to external substances, IECs are highly vulnerable to ROS attacks, which can cause mitochondrial structure and function damage, ultimately affecting gut

homeostasis (Qiao et al., 2022). Mitochondria are critical in regulating gut homeostasis (Fig. 2), and could serve as a potential target for preventing gut dysbiosis (Guerbette et al., 2022).

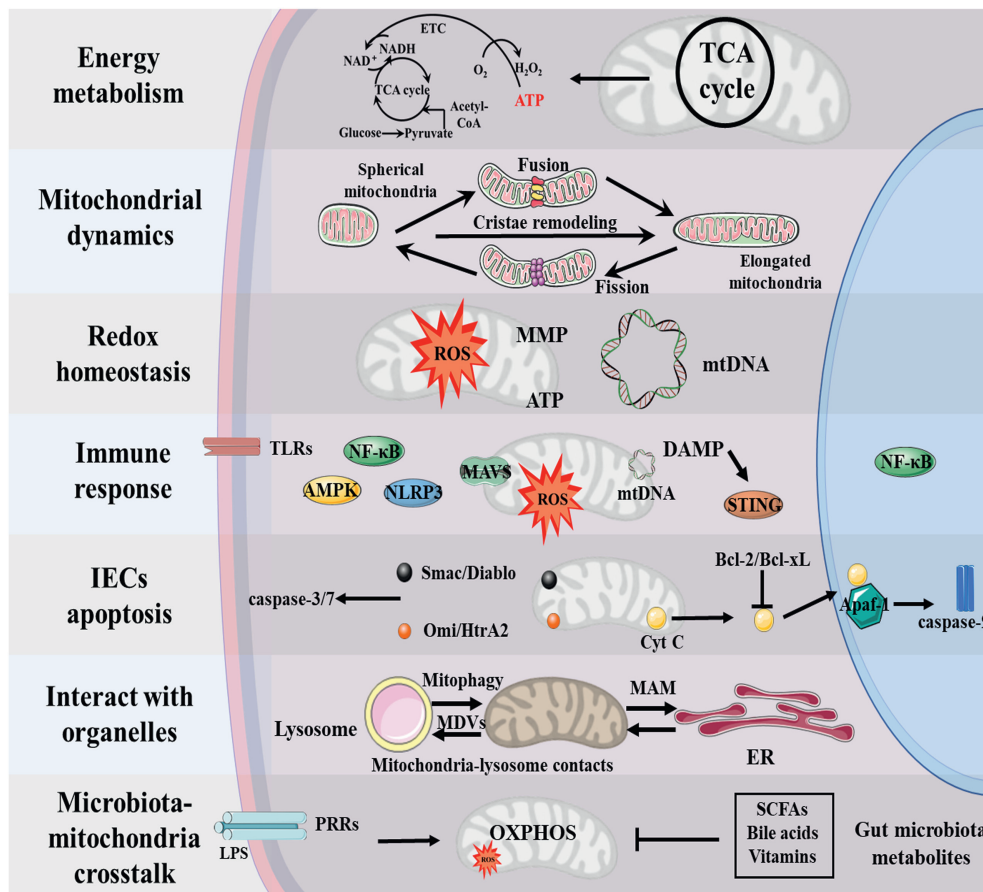
### 3.1. Energy metabolism

While the gastrointestinal tract constitutes just 5 % of the body's weight, the constant regeneration of the intestinal epithelium, the ATP-dependent transport proteins, and the synthesis of various proteins like mucin glycoproteins, tight junction (TJ) proteins, and antimicrobial peptides necessitate a substantial amount of energy. These processes account for as much as 20 % of the body's oxygen consumption (Blachier et al., 2009). The primary pathways involved in cellular mitochondrial energy metabolism include glycolysis, the tricarboxylic acid (TCA) cycle, and OXPHOS (Yan et al., 2021). Under normal oxygen levels, glucose undergoes cytoplasmic glycolysis to produce pyruvic acid, which is then converted to acetyl coenzyme A, ultimately generating the electron donors NADH and FADH<sub>2</sub> for OXPHOS. This creates an electrochemical gradient called the mitochondrial membrane potential (MMP,  $\Delta\Psi_m$ ), which drives proton transport through the ATP synthase complex to phosphorylate ADP and produce ATP (Wang et al., 2021). These energy metabolic pathways are carried out in IECs, goblet cells, immune cells and stem cells in the gut and provide a sufficient energy supply for their survival and function.

### 3.2. Mitochondrial dynamics

Mitochondria play a crucial role in maintaining cell homeostasis by continuously carrying out fission and fusion, a process known as mitochondrial dynamics. This dynamic balance of the mitochondrial network is essential in preserving the shape, distribution, and quantity of mitochondria within the cell (Yapa et al., 2021). The process of mitochondrial fission is reliant upon the crucial role played by dynamin-related protein 1 (Drp1) (Fröhlich et al., 2013). In response to the activation of mitochondrial fission factor (Mff), Drp1 is recruited to the mitochondria and undergoes oligomerization. Multiple Drp1 molecules then encircle the mitochondria tightly, forming a ring structure. Through its GTPase activity, Drp1 hydrolyzes GTP, resulting in the rupture of the inner and outer membranes of the mitochondria, facilitating mitochondrial fission (Ingerman et al., 2005; Ji et al., 2015). The proteins responsible for mitochondrial fusion comprise Mitofusin (Mfn) 1 and 2, and Optic Atrophy 1 protein (OPA1). While Mfn1 and Mfn2 primarily facilitate the fusion of the outer mitochondrial membrane, OPA1 is primarily involved in mediating the fusion of the inner mitochondrial membrane (Chan, 2006).

In addition, mitochondrial dynamics also includes mitochondrial biogenesis. To meet the differentiation and energy demands of IECs, extensive mitochondrial biogenesis is required in these cells (Sun et al., 2022). Mitochondrial biogenesis displays a high degree



**Fig. 2.** The role of mitochondria in gut homeostasis. Mitochondria are key determinants of cell fate and coordinate cellular metabolism, immunity, stress response, and apoptosis, and play a critical role in maintaining intestinal homeostasis and are potential targets for preventing imbalances in intestinal homeostasis. TCA cycle = tricarboxylic acid cycle; ETC = electron transport chain; ROS = reactive oxygen species; MMP = mitochondrial membrane potential; mtDNA = mitochondrial DNA; MAVS = mitochondrial antiviral signaling protein; DAMP = damage-associated molecular pattern; Cyt-c = cytochrome-c; Apaf-1 = apoptotic protease-activating factor 1; MDVs = mitochondria-derived vesicles; ER = endoplasmic reticulum; MAM = mitochondria-associated ER membrane; PRRs = pattern recognition receptors; LPS = lipopolysaccharide; OXPHOS = oxidative phosphorylation; SCFAs = short-chain fatty acids; IECs = intestinal epithelial cells.

of plasticity in response to cellular energy requirements, which are activated by developmental signals (Li et al., 2022c). Mammalian mitochondrial proteins are encoded by both the nuclear genome and the mitochondrial genome, indicating that mitochondrial biogenesis necessitates coordinated regulation between the mitochondrial and nuclear genomes. The increase of mitochondrial biogenesis is beneficial to the improvement of cellular OXPHOS ability, the reduction of pathological oxidative stress, and the repair of mitochondrial dysfunction (Cameron et al., 2017). In general, mitochondrial dynamics alter the mitochondrial size, shape, distribution, and number, which can regulate energy production, molecular synthesis, redox signaling, and metabolite signaling in response to cellular stress or nutrient supply (Chakrabarty and Chandel, 2022).

### 3.3. Redox homeostasis

As a consequence of normal OXPHOS, mitochondria generate ROS in a physiological range (Lambert and Brand, 2009). Oxidative stress can be described as a state in which there is an excess of ROS that surpasses catabolism or detoxification (Garza-Lombó et al., 2020). If oxidative stress surpasses the cell's capacity to repair biomolecule oxidation, there is a sharp increase in the level of ROS, which can severely compromise gut homeostasis. Nonetheless, mitochondria have developed antioxidant defense mechanisms to mitigate ROS levels (Mailloux, 2018). Superoxide dismutase (SOD) is the main ROS scavenging enzyme, among which SOD2 targets the mitochondrial matrix and can promote the conversion of superoxide to H<sub>2</sub>O<sub>2</sub>, which is subsequently converted by catalase (CAT), peroxiredoxin (PRX) and glutathione peroxidase (GPx) for water, thereby eliminating excess ROS (Dan Dunn et al., 2015). The two main antioxidants in mitochondria are glutathione (GSH) and thioredoxin (TRX), which are involved in the degradation of H<sub>2</sub>O<sub>2</sub> (Handy and Loscalzo, 2012). Unlike catalase, they rely on the reducing power of NADPH to eliminate ROS. GPx, a family of peroxidases containing selenium (Se), plays a critical role in oxidizing GSH to GSSG and decomposing H<sub>2</sub>O<sub>2</sub>. The intestine contains all four subtypes of GPx (GPx1, 2, 3, and 4), with GPx1 and GPx4 contributing to mitochondrial antioxidant defense (Chu et al., 2004; Wang et al., 2021). The TRX system utilizes the peroxidic cysteine (Cys<sub>P</sub>) of PRX3 or PRX5-activated sites to chelate H<sub>2</sub>O<sub>2</sub> (Wood et al., 2003). The oxidized Cys<sub>P</sub> is decomposed by the neighboring cysteine residue, forming a molecular disulfide bridge. In the mitochondrial matrix, the reduction force stored in TRX reactivates PRX3 or PRX5 through simple sulfide exchange reactions, and then, TRX2 is reactivated by thioredoxin reductase 2 (TrxR2) (Mailloux, 2018). Overall, eliminating high levels of ROS in mitochondria and their corresponding side effects is essential for maintaining gut homeostasis.

### 3.4. Immune response and regulation

Mitochondrial ROS (mtROS) is a crucial component of host defense and immunity and a driving factor of inflammatory responses (West et al., 2011a). Ho et al. (2018) demonstrated that administering rotenone, a complex I inhibitor that increases mtROS levels, to the colons of mice resulted in an escalation of colonic inflammation. West et al. (2011a) showed that Toll-like receptor subsets facilitate the recruitment of mitochondria to macrophage phagosomes and enhance the production of mtROS, which is essential for the bactericidal function of macrophages. Furthermore, the increased levels of mtROS in macrophages trigger the activation of ROS-sensitive signaling pathways, such as nuclear factor kappa B (NF-κB) and AMP-activated protein kinase (AMPK), which in turn lead to heightened production of pro-inflammatory cytokines (West et al., 2011b).

Mitochondria also have a regulatory function in antiviral signaling. Upon recognition of viral RNA by cell membrane receptors, mitochondrial antiviral signaling protein (MAVS) is activated, which in turn recruits TNF receptor-associated factor 6 (TRAF6). The complex formed by TRAF6 and TNFR1-associated death domain protein (TRADD) subsequently activates the classical NF-κB signaling pathway (West et al., 2011b). The above studies reveal innate immune signaling and mitochondrial connections, suggesting that mitochondria are the hub of innate immune signaling.

### 3.5. Mitochondria-mediated intestinal epithelial cell apoptosis

Mitochondria are a major contributor to apoptosis signaling during oxidative stress (Baregamian et al., 2011). Excessive ROS production or impaired antioxidant defense can reduce MMP and trigger the release of pro-apoptotic proteins, resulting in mitochondrial-dependent apoptosis activation (Kim et al., 2012; Liu et al., 2022). Under physiological conditions, anti-apoptotic proteins (Bcl-2 and Bcl-xL) sequester pro-apoptotic proteins (Bax, Bad, Bak, Bim, and Bid) to maintain the integrity of the mitochondrial membrane, thereby preventing cell death (Jeong and Seol, 2008; Xiong et al., 2014). Specifically, under oxidative stress, including ROS, mitochondrial permeability increases, leading to a decrease in ΔΨ<sub>m</sub> and the induction of cytochrome-c (Cyt-c) release. Cyt-c interacts with apoptotic protease-activating factor 1 (Apaf-1) to activate caspase-9. Activated caspase-9 initiates pro-caspase-3 and pro-caspase-7, which in turn activate caspase-9, forming a positive feedback loop. At this point, activated caspase cleaves downstream substrates, resulting in DNA fragmentation and the formation of apoptotic bodies (Liu et al., 2022).

In addition, mitochondria can also release other proteins that are involved in cell apoptosis. Following mitochondrial stress, Smac/Diablo is released from mitochondria and competes with the inhibitor of apoptosis (IAP) proteins to bind with caspases. The binding of Smac/Diablo and IAP can relieve the inhibition of caspases by IAP on cysteine proteases, thereby enhancing the ability of Cyt-c-mediated activation of caspase-3 (Du et al., 2000). Omi/HtrA2 is a serine protease located in the intermembrane space of mitochondria that displays pro-apoptotic activity upon its release during the process of cell apoptosis (Estaquier et al., 2012). Furthermore, mitochondria also participate in caspase-independent cell death pathways. Apoptosis-inducing factor (AIF) is a mitochondrial intermembrane space protein that, with mitochondrial stress, is released and translocated to the nucleus to promote DNA fragmentation and chromatin condensation (Wang et al., 2021). Since apoptosis is the active death of cells under specific conditions, it is important to remove broken cells and balance cell proliferation, but excessive and sustained apoptosis under stressful conditions may damage the intestinal barrier.

### 3.6. Lysosome/endoplasmic reticulum-mitochondrion interaction

The interaction between organelles and mitochondria refers to the physical or functional connections between different organelles and mitochondria within cells, and this interaction plays an important role in maintaining intestinal homeostasis. A wealth of evidence indicates that mitochondria physically interact with other organelles, such as the endoplasmic reticulum (ER) and lysosomes, to regulate cellular fate and function (Chakrabarty and Chandel, 2022). Studies have also shown that mitochondria are in physical contact with the Golgi apparatus, peroxisomes, and lipid droplets, although the cellular processes regulated by these interactions have yet to be fully elucidated (Dolman et al., 2005; Fransen et al., 2017; Pu et al., 2011).

### 3.6.1. Mitochondria-lysosome interaction

Lysosomes are the degradation centers and signaling hubs of cells and are involved in many forms of cell death (Yang and Wang, 2021). Mitochondria and lysosomes can interact through mitochondrial autophagy (mitophagy), mitochondria-derived vesicles (MDVs), and mitochondria-lysosome contacts, which are important for maintaining cellular homeostasis (Wong et al., 2019). Mitophagy is crucial for maintaining mitochondrial quality and cellular homeostasis through the autophagy of damaged mitochondria (Liu et al., 2017). Specifically, in response to stimuli like oxidative stress, dysfunctional mitochondria are recognized and selectively wrapped by the bilayer structure, which then fuses with lysosomes for degradation. This process, known as mitophagy, reduces the release of ROS and certain pro-apoptotic factors from the mitochondria (Wen et al., 2023). The microtubule-associated protein light chain 3 (LC3) family is the most critical protein for regulating mitophagy and is necessary for autophagosome closure. Mitochondrial receptors (such as PINK/Parkin, BNIP3, NIX, FUNDC) on the outer membrane of mitochondria interact with autophagosomes through the LC3-interacting region to mediate mitophagy (Fujita et al., 2009). MDVs can remove damaged mitochondrial components such as proteins and lipids caused by physiological stress. These components are then packaged into small MDVs and transported within the cell to lysosomes and peroxisomes for degradation (Popov, 2022). The production of these vesicles is an inherent property of mitochondria that has been inherited from their bacterial ancestors (Popov, 2022). In addition, in the absence of stress stimuli, healthy cells also establish contact (physical interaction) between lysosomes and mitochondria, known as the mitochondria-lysosome contacts, which is facilitated by the active GTP-bound lysosomal RAB7 (Wong et al., 2018).

### 3.6.2. Endoplasmic reticulum-mitochondrion interaction

The ER and mitochondria possess a unique tubular structure that allows for the formation of inter-organelle connections, known as mitochondria-associated ER membrane (MAM) (Marchi et al., 2014; Xia et al., 2019). The interaction between ER and mitochondria mainly participates in physiological processes such as lipid synthesis, regulation of calcium ( $\text{Ca}^{2+}$ ) signaling and its homeostasis, and mitochondrial biogenesis (Marchi et al., 2014).  $\text{Ca}^{2+}$ , as a crucial intracellular signaling molecule (second messenger), regulates multiple physiological and pathological processes. Mitochondria and ER are important cellular organelles for calcium storage.  $\text{Ca}^{2+}$  enters mitochondria from ER through the MAM, playing a critical role in controlling mitochondrial fission and apoptosis (Raturi and Simmen, 2013). Meanwhile, the transfer of calcium can also be terminated by increasing the distance of MAM (Csordás et al., 2010).

Overall, the communication between mitochondria and other organelles is crucial for intestinal homeostasis. Mitochondria play a pivotal role in maintaining cellular stability, and this requires not only self-regulation but also coordination with other organelles, forming a complex network. Any negative factor can disrupt this network, leading to mitochondrial dysfunction.

### 3.7. Bidirectional crosstalk between mitochondria and gut microbiota

Bidirectional crosstalk between the gut microbiota and host mitochondria has been demonstrated, particularly in the regulation of gut homeostasis (Zhang et al., 2022b). Metabolites produced by gut microbiota can regulate mitochondrial structure and function (Wang et al., 2019), while alterations in mitochondrial redox state can impact gut microbiota composition and abundance by inducing intestinal inflammation and oxidative stress (Yardeni et al., 2019).

The disruption of these two factors can exacerbate the imbalance in mitochondrial-microbiota crosstalk.

#### 3.7.1. Gut microbiota affects mitochondrial function

The gut microbiota produce various metabolites, including short-chain fatty acids (SCFAs), bile acids, vitamins, and gases. Among them, SCFAs are crucial for mitochondrial energy metabolism. Butyrate, a type of SCFA, can be used as an energy source by colonic epithelial cells and participate in fatty acid  $\beta$ -oxidation, producing acetyl-CoA for OXPHOS. Butyrate also upregulates mitochondrial uncoupling protein 2 (UCP2) expression, which increases inner membrane proton leak and reduces ROS production (Mafra et al., 2019). In addition, evidence indicates that propionate can mitigate mitochondrial dysfunction induced by free fatty acids in liver cells by increasing the expression of peroxisome proliferator-activated receptor-gamma coactivator (PGC)-1 $\alpha$  (PGC-1 $\alpha$ ). PGC-1 $\alpha$  regulates the expression of multiple genes essential for mitochondrial biogenesis and function by modulating the activity of different transcription factors (Wang et al., 2022a).

*Escherichia coli* and *Salmonella* can produce hydrogen sulfide ( $\text{H}_2\text{S}$ ) through the metabolism of sulfur-containing amino acids, which is then metabolized in mitochondria through sulfide quinone reductase (SQR), thiosulfate sulfurtransferase (TST), SDO/ETHE1, and sulfite oxidase (SUOX) (Di Meo et al., 2015). Moreover, low concentrations of  $\text{H}_2\text{S}$  positively affect the mitochondrial respiratory chain, significantly increasing cellular oxygen consumption, promoting the production of GSH, thus protecting mitochondria from oxidative stress damage (Kimura et al., 2010). However, high concentrations of  $\text{H}_2\text{S}$  can down-regulate the expression level of cytochrome oxidase (COX), thereby shifting oxidative metabolism towards glycolysis, increasing lactate production, reducing the generation of ATP-consuming cells, and up-regulating the expression of inducible NO synthase (iNOS) (Mimoun et al., 2012). Elevated iNOS results in elevated NO levels. NO, as an inhibitor of the mitochondrial respiratory chain, affects energy metabolism by reducing the production of acetyl-CoA. In addition, most gut bacteria can also produce NO through the use of nitrite (Wang et al., 2021). Furthermore, the secondary bile acids obtained by the degradation of primary bile acids by gut microbiota can interact with mitochondria through the regulation of transcription factors involved in lipid and carbohydrate metabolism (Nie et al., 2015). Lipopolysaccharide (LPS), the major component of Gram-negative bacteria membranes, can increase mtROS production by activating host cell pattern recognition receptors (PRRs) (Wang et al., 2021).

#### 3.7.2. Regulation of mitochondria on gut microbiota

The regulation of gut microbiota by mitochondria has not been fully elucidated. However, changes in mitochondrial function can affect gut microbiota by influencing the gut environment. Studies have indicated that gut microbiota abundance and diversity in mice are influenced by ROS levels in IECs. Mice with a low Shannon diversity index have been found to have higher levels of ROS in their IECs, potentially due to ROS-mediated oxidation of mtDNA and activation of NLRP3, leading to intestinal inflammation and microbiota changes. Additionally, alterations in mitochondrial redox signaling may also disrupt the gut environment, leading to changes in microbiota structure (Yardeni et al., 2019; Zhang et al., 2022b). Changes in mitochondrial metabolism can also affect the structure of the gut microbiota. In the case of intestinal barrier damage or inflammation, the repeated repair process of the epithelial tissue can over-activate the mitochondrial unfolded protein response, inducing apoptosis of epithelial cells and increasing the risk of epithelial cell carcinogenesis. Additionally,

cancer cells increase glycolysis under non-hypoxic conditions, inhibiting the OXPHOS process causing oxygen diffusion, which disrupts the hypoxic environment of the intestinal lumen and thus affects the structure of the microbiota community (Zhang et al., 2022b). In addition, Hirose et al. (2017) investigated the impact of mtDNA mutations on the gut microbiome and found significant differences in its composition in mice with mtDNA mutations compared to the control group, which may contribute to the development of metabolic diseases. Moreover, variations in the mitochondrial genome such as polymorphisms in ND5, CYTB genes, or D-Loop region have been linked to specific gut microbiota compositions. Research by Ma et al. (2014) highlighted that A13434G, a synonymous single nucleotide polymorphism (SNP) found on the ND5 gene, and T15784C, a synonymous SNP on CYTB, were strongly correlated with the abundance of *Eubacterium* and *Roseburia* genera, which are highly oxygen-sensitive anaerobes and butyrate producers. These results suggest that host mitochondrial genome variants may inherently dictate the gut microbiome's composition and function, which ultimately shapes their community (Clark and Mach, 2017).

#### 4. Mitochondrial dysfunction in weaned piglets

Given the diverse and crucial functions exhibited by mitochondria in maintaining intestinal homeostasis, the mitochondria may be a key mediator in the pathogenesis and exacerbation of PWD. Cell culture and mice studies have identified mechanisms by which mitochondrial dysfunction leads to intestinal diseases, such as increased parietal permeability of IECs, internalization of commensal bacteria, and overproduction of ROS.

Compared to normal nursing piglets, early-weaned piglets have higher levels of lipid peroxidation, increased cortisol concentration, decreased activity of SOD and GPx, and excessive accumulation of free radicals, including NO and H<sub>2</sub>O<sub>2</sub>. In addition, an increase in intestinal cell apoptosis was observed in the mucosa of early-weaned piglets, along with increased expression of caspase-3, caspase-8, and caspase-9. The activation of caspase-8 and caspase-9 indicates that early weaning stress induces mitochondria-dependent and FAS-dependent apoptosis, leading to apoptosis of IECs (Zhu et al., 2013). Cao et al. (2018b) found that compared to before weaning, the transmembrane resistance of jejunal epithelial cells and the expression level of TJ proteins significantly decreased at 3 and 7 days after weaning. In addition, the activities of SOD and GPx in the jejunum were significantly reduced, and the MDA content in the jejunum significantly increased. The mtDNA content in the intestine and the activity of intestinal mitochondrial complexes (I, II, III, IV) were significantly reduced. Cao et al. (2018a) further demonstrated that oxidative stress led to significant swelling, vacuolization, marked matrix wrinkling of jejunal mitochondrial morphology, and reduction or breakage of mitochondrial cristae in piglets. In addition, oxidative stress increased intestinal mtROS production and decreased MMP and the expression levels of genes related to mitochondrial biogenesis and function. In addition, Novais et al. (2020) demonstrated that weaning induces mitochondrial dysfunction and oxidative stress in post-weaning piglets, leading to a decrease in ATP concentration in the liver and an increase in oxidative damage to DNA and proteins. Additionally, low birth-weight (LBW) piglets are more susceptible to mitochondrial energy deficits and oxidative stress during weaning than normal birth-weight (NBW) piglets. Further studies have revealed that the expression of mitochondrial respiratory chain subunit encoding genes is downregulated in LBW piglets. Interestingly, genes related to mitophagy and the antioxidant defense system are highly expressed in NBW piglets. These findings provided new

insights into the molecular origins of mitochondrial dysfunction and oxidative stress observed in weaned piglets (Novais et al., 2021).

Oxidative stress-induced mitochondrial dysfunction in piglet intestines is also attributed to ER stress (Ringseis et al., 2016). Li et al. (2022b) found that oxidative stress induces dysfunction of porcine jejunal mitochondria, as evidenced by decreased membrane potential, ATP content, respiratory chain complex activity, and expression of mitochondrial biogenesis-related genes, and increased expression of genes related to mitochondrial-related apoptosis (caspase-3 and Cyt-c). Furthermore, oxidative stress triggered ERS, leading to the accumulation of misfolded and unfolded proteins, and enhanced ER-mitochondria interaction. Excessive MAM formation may cause mitochondrial Ca<sup>2+</sup> overload and dysfunction by increasing calcium ion transfer from the ER to mitochondria. Oxidative stress reduces Sirtuin 3 (Sirt3) activity, a crucial deacetylase in mammalian mitochondria regulating mitochondrial function and metabolic enzyme activity (Dabke and Das, 2020; Ma et al., 2022b). Concurrently, oxidative stress reduces the expression levels of Mfn2 and OPA1, while significantly increasing the expression level of MFF, indicating the inhibition of mitochondrial fusion, accelerated mitochondrial fission, and imbalanced mitochondrial fusion and fission, triggering mitophagy (Ma et al., 2022b). PWD is usually caused by pathogen infection. LPS, a type of bacterial endotoxin, significantly disrupts the intestinal energy state, manifested as a reduction in ATP, ADP, and total adenine nucleotide content, adenylate energy charge, and key enzyme activities of the TCA cycle, as well as an increase in the AMP/ATP ratio (Pi et al., 2014). Xia et al. (2020) conducted a study in which they infected weaned piglets and IPEC-J2 cells with *Salmonella*, and observed that this infection caused mitochondrial damage and impaired mitophagy in the ileum and IPEC-J2 cells. This was evidenced by the colocalization of mitochondria with LC3, as well as the high expression of autophagy-related proteins PINK1, sequestosome 1 (SQSTM1/p62), optineurin (OPTN), and LC3, as determined by immunofluorescence.

The aforementioned research suggests that weaning disrupts the oxidative balance in the intestine of piglets, which damages both the intestinal barrier and mitochondrial function. Mitochondrial dysfunction, in turn, exacerbates the occurrence and development of intestinal diseases.

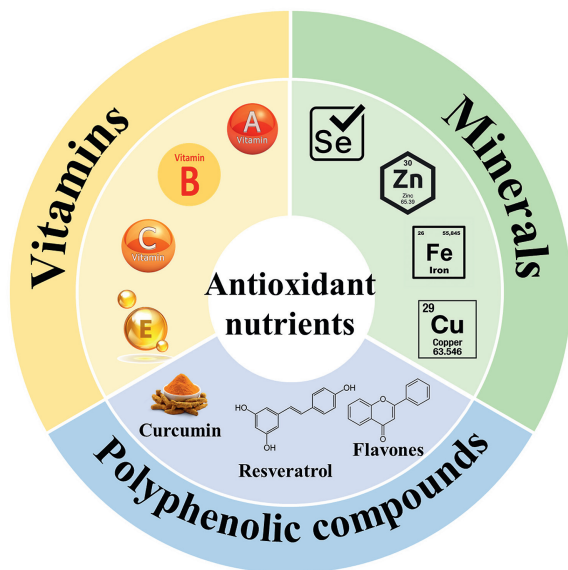
#### 5. Antioxidant nutrient-targeted mitochondrial approaches

As the gut of weaned piglets is not fully developed, their ability to resist stress is weak. When exposed to oxidative stress, the gut is vulnerable to damage, and ingested substances and pathogens can easily pass through the multiple barriers of the gut, further promoting intestinal oxidative stress through activating mitochondrial stress and inflammatory reactions (Wen et al., 2023). Therefore, effective strategies need to be developed to improve the metabolic health of piglets and enhance their health status after weaning. In this section, we review the use of antioxidant nutrients (vitamins, minerals, and polyphenolic compounds) in weaned pig farming and describe their efficacy in protecting mitochondrial function and maintaining gut health (Fig. 3).

##### 5.1. Vitamins

###### 5.1.1. Vitamin A

Vitamin A plays an important physiological role in regulating animal health, but animals cannot synthesize vitamin A themselves, so it needs to be supplemented through diet to meet



**Fig. 3.** Antioxidant nutrients targeting mitochondria to prevent or treat postweaning diarrhea (PWD) in piglets. The addition of antioxidant nutrients such as vitamins A, B, C, and E, minerals (Se, Zn, Fe, Cu) and polyphenols to piglet diets can effectively reduce intestinal diseases in weaned piglets. Se = selenium; Zn = zinc; Fe = iron; Cu = copper.

daily requirements (Bondi and Sklan, 1984). Compared with the control group (basal diet without vitamin A), supplementing vitamin A (13,500 IU/kg) in the basal diet of weaning piglets significantly increased serum IgA level and GPx activity (Hu et al., 2020). Studies have shown that when vitamin A is depleted, T cells undergo programmed cell death, which is also accompanied by an increase in ROS production, and a sharp decrease in ATP and NAD<sup>+</sup> levels. This evidence highlights a novel regulatory role for vitamin A in mitochondrial energy homeostasis (Chiu et al., 2008). Vitamin A found in fruits and vegetables called provitamin A is represented by carotenoids. Oral administration of 40 or 80 mg/kg  $\beta$ -carotene decreased the level of MDA and increased the activities of SOD and GPx, and significantly inhibited jejunal caspase-3 mRNA levels and decreased the ratio of Bax/Bcl-2 in a dose-dependent manner in piglets (Li et al., 2020).

### 5.1.2. Vitamin B

Several mitochondrial matrix enzymes require cofactors to function. Mitochondrial carrier proteins have been found to transport coenzymes, including thiamine pyrophosphate, coenzyme A, FAD, and NAD<sup>+</sup>, which are structurally similar to nucleotides and are derived from various vitamin B sources (Palmieri et al., 2022). Therefore, vitamin B supplementation is important to maintain mitochondrial homeostasis. Niacin (vitamin B<sub>3</sub>) is an essential vitamin in the diet of weaned piglets. A study by Wang et al. (2022b) showed that supplementing 30 mg/kg niacin in the diet of weaned piglets can rapidly reshape antioxidant status, increase the levels of T-SOD and CuZn-SOD, and mitigate intestinal damage caused by weaning stress. Moreover, higher doses (45 or 75 mg/kg) of niacin can effectively improve immunity and reduce inflammation levels of piglets. Li et al. (2019) studied the effects of adding vitamin B<sub>6</sub> in high crude protein diets on the growth, diarrhea rate, and intestinal morphology and function of weaned piglets. The results showed that the 7 mg/kg vitamin B<sub>6</sub> group had a tendency to reduce diarrhea rate, and vitamin B<sub>6</sub> significantly increased ileal villi height and villi width, and increased expression levels of amino acid transporters which affect protein absorption and metabolism in piglets.

### 5.1.3. Vitamin C

Vitamin C (an electron donor), also known as ascorbic acid (AA), is an important micronutrient with excellent antioxidant capacity and a broad impact on the immune system, which is important for the growth and development of piglets (Skrzypczak et al., 2020). Vitamin C has been shown to have various advantageous effects on mitochondria, including direct ROS elimination and regeneration of other mitochondrial antioxidants in the matrix and membranes. High concentrations of vitamin C were found in mitochondria freshly isolated from different animal cells, and dietary intake of vitamin C increases the mitochondrial content of the vitamin. Although its hydrophilic nature limits its antioxidant response to the matrix, vitamin C can still regenerate  $\alpha$ -tocopherol from the  $\alpha$ -tocopheroxyl radical and impact the membrane compartment (Fiorani et al., 2021). Rey et al. (2017) found that an increase in vitamin C in the diet increased the serum antioxidant capacity (FRAP) and IgM and IgA levels in weaned piglets.

### 5.1.4. Vitamin E

In mitochondrial membranes, the lipid-soluble antioxidant vitamin E plays an important role by reacting more rapidly with peroxy radicals than polyunsaturated fatty acid molecules, thus protecting the membrane from excessive oxidative damage (Napolitano et al., 2019). It is generally recommended to supplement a high level of vitamin E in the post-weaning diet to avoid disease in piglets. Researchers evaluated the plasma oxidative status indices of piglets before and after weaning and found that piglets with diarrhea had significantly higher hydrogen peroxide and oxidative stress indices compared to those without diarrhea, and a lower concentration of vitamin E. These results indicated that plasma vitamin E concentration before weaning was related to post-weaning growth (Buchet et al., 2017). Chen et al. (2019) reported the effect of different doses of  $\alpha$ -tocopherol (the main form of dietary vitamin E in humans and pigs) on intestinal morphology in weaned piglets and found that vitamin E did tend to reduce jejunal crypt depth and villi width. In addition, the inclusion of a high level of vitamin E (250 mg/kg) in piglet diets was found to elevate the levels of  $\alpha$ -tocopherol in both serum and liver, enhance antioxidant capacity, and reduce muscle oxidation in comparison to low-dose vitamin E (40 mg/kg) supplemented groups. Thus, it is recommended to provide piglets with high-dose vitamin E-enriched diets for an extended period to enhance their oxidative status after weaning (Rey et al., 2017). Additionally, supplementing maternal diets with high levels of vitamin E can increase piglet body weight at weaning, while also enhancing the humoral immune function and antioxidant activity (including total antioxidant capacity and catalase) of both sows and piglets (Wang et al., 2017).

## 5.2. Minerals

### 5.2.1. Selenium (Se)

Se plays a vital role in pig nutrition by promoting the synthesis of selenoproteins, and the level of Se is closely related to the expression levels of selenoproteins (key components of the body's oxidation system) and the maintenance of redox homeostasis (Xing et al., 2019). Moreover, Se is involved in mitochondrial biogenesis and boosts the electron transfer system function (Wesselink et al., 2019). Li et al. (2022a) investigated the impact of Se deficiency on the apoptosis of gastric tissue cells in pigs and its underlying mechanism. The results showed that Se deficiency decreased the antioxidant capacity of weaned piglets, increased cell apoptosis in porcine gastric tissue, and upregulated the expression of caspase-3, caspase-9, AIF, and endonuclease G (EndoG), indicating that Se deficiency can induce cell apoptosis in porcine gastric tissue

through the mitochondrial pathway triggered by oxidative stress. However, sodium selenite ( $\text{Na}_2\text{SeO}_3$ ), which has been used as a feed additive in the past, is not the best form of Se for animals, mainly due to its low bioavailability and possible environmental pollution when excreted into the environment (Surai, 2021). Lv et al. (2020) found that Se-enriched yeast can improve the digestibility of nutrients and inhibit inflammation and oxidative stress more effectively than  $\text{Na}_2\text{SeO}_3$  by inducing the activity of lymphocytes and the expression levels of antioxidant enzymes. In addition, Se nanoparticles (SeNPs) have been proposed as a safer, more stable, and more effective Se delivery system for meeting the micronutrient requirements of animals (Yang and Yang, 2023). SeNPs are superior to traditional Se in synthesizing selenoproteins such as GPx and can also reshape the digestive system to enhance nutrient digestion and absorption (Chen et al., 2022). Our research group conducted a study on the effects of adding either biogenic SeNPs or  $\text{Na}_2\text{SeO}_3$  (0.3 mg/kg) to the diet of early-weaned piglets on their growth performance and intestinal health. SeNPs significantly improved the growth performance and reduced the incidence of diarrhea when compared to  $\text{Na}_2\text{SeO}_3$  in piglets. This protective mechanism may be attributed to SeNPs enhancing the utilization of Se in piglets, increasing the mRNA levels of selenoproteins, thereby improving the antioxidant capacity (especially the activity of Se-containing antioxidant enzymes) and immunity, and further maintaining mitochondrial structure and function. Analysis of intestinal flora and serum metabolites found that SeNPs significantly increased the abundance of *Holdemanella* bacteria producing SCFAs (Qiao et al., 2023).

#### 5.2.2. Zinc

PWD has long been treated with diets supplemented with high pharmaceutical doses of zinc oxide (ZnO) because of its excellent antimicrobial activity (Wei et al., 2020). In addition, sufficient intake of Zn is crucial for minimizing susceptibility to bacterial infections and preserving the integrity of intestinal TJ proteins. Moreover, Zn exhibits diverse antioxidant effects, including serving as a cofactor for Cu/Zn superoxide dismutase to facilitate the conversion of superoxide radicals into less harmful  $\text{H}_2\text{O}_2$ , and suppressing NADPH oxidase to attenuate the production of ROS (Baholet et al., 2022). Moreover, Zn serves as a cofactor for the Zim17 protein within the mitochondria, which in turn interacts with Ssc1 to facilitate both protein importation and protein folding processes within the matrix (Pierrel et al., 2007).

However, the bioavailability rate of ZnO is relatively low, leading to a considerable proportion of the administered Zn being excreted through feces, which can result in significant soil pollution. It also increases bacterial resistance. For these reasons, the addition of pharmacological doses of ZnO to weaned piglets has been phased out in Europe as of June 2022 (Papadomichelakis et al., 2023). Therefore, there is an urgent need to find another form of Zn to eliminate the negative effects of ZnO. Zhang et al. (2022a) investigated the effects of tetrabasic zinc chloride (TBZC) as a substitute for ZnO on the growth capability and gut microbiota of weaned piglets. It was found that feeding ZnO and TBZC diets both reduced the incidence of diarrhea in piglets, but piglets fed TBZC diets had higher crude protein digestibility and total energy, and increased fecal acetate and propionate levels. Diao et al. (2021) conducted a study to assess the impact of dietary supplementation with 100 mg/kg zinc sulfate, zinc glycinate (Gly-Zn), and zinc lactate on growth performance of weaned piglets. The results indicated that the incidence of diarrhea was lower in the zinc lactate group compared to the control group, while both the Gly-Zn and zinc lactate groups demonstrated higher jejunal villi height relative to the control group.

#### 5.2.3. Iron

Iron deficiency leads to impaired immune response, increased infection risk, and stunted growth in piglets (Dong et al., 2020). Iron is a plentiful metal within mitochondria and is stored as mitochondrial ferritin (FtMt) in the mitochondrial matrix. The formation of polynuclear sulfur bridge iron-sulfur (Fe/S) clusters, which are crucial for OXPHOS, in the cristae membrane of mitochondria is dependent on iron. Fe/S clusters are present in respiratory complexes I, II, and III, and are essential for their proper function (Tang et al., 2021). In addition, complexes II-IV of the respiratory chain require the heme cofactor, which is also iron-containing (Pierrel et al., 2007). Ma et al. (2022a) observed that dietary supplementation with 2,000 mg/kg ferrous glycinate chelate (FGC) led to improvements in the growth performance and feed intake of weaned piglets, as well as a decrease in the incidence of early diarrhea. Additionally, FGC supplementation increased the activities of SOD and CAT, while reducing levels of superoxide and MDA. Furthermore, FGC supplementation was found to regulate the microbiome composition of piglets in the early stage, promoting the growth of *Tezzerella*, a bacterium that produces SCFAs, while reducing the abundance of potentially pathogenic bacteria. However, excessive iron intake (tolerable upper intake levels, 3000 mg/kg) increases the content of ROS and MDA in the duodenal mucosa of weaned piglets, inducing the generation of cytosolic and mtROS and reducing the MMP, which in turn leads to cellular vacuole formation and fibrosis (Ding et al., 2020).

#### 5.2.4. Copper

Copper is also an essential trace element for animal growth. In commercial feed, it is typically supplemented at a concentration of 10 to 20 mg/kg inorganic copper. In addition, supplementing copper at pharmacological doses (150 to 250 mg/kg) can effectively enhance the growth performance of weaned piglets by increasing the digestibility of energy, fat, and amino acids, as well as reducing the incidence of diarrhea (Yin et al., 2021). This may be related to the regulation of mitochondrial function by copper. In mitochondria, copper is required for the assembly of the Cox4 subunit of cytochrome c oxidase (CcO) and SOD1, where the two mitochondrial-encoded subunits of CcO have a copper center (Pierrel et al., 2007). Intestinal stem cells (ISCs) drive the development and continuous renewal of the intestinal epithelium, which requires a large energy supply. Dietary copper improves intestinal morphology (reduced villi width and higher villi height) in weaned piglets by promoting cell proliferation and modulating ISC activity (Yin et al., 2021).

### 5.3. Polyphenolic compounds

#### 5.3.1. Curcumin

Curcumin is an all-natural compound derived from the root of turmeric and is the most active constituent of polyphenolic curcuminoids (Zheng et al., 2018). Recently, it has been discovered that curcumin possesses antioxidant, anti-inflammatory, and anticancer properties. Due to these effects, curcumin plays an important role in preventing and treating various diseases. Curcumin's antioxidant effects are mainly manifested in its ability to scavenge oxygen-free radicals and increase the activity of SOD, CAT, and GPx (Kocaadam and Şanlıer, 2017). Curcumin has a high ability to scavenge intracellular oxidative molecules, and can easily transfer electrons or easily donate H atoms from two phenolic sites to scavenge free radicals (Barzegar and Moosavi-Movahedi, 2011). Supplementation of 300 mg/kg curcumin in the diet significantly increases the villus height and TJ protein expression in ileum of weaned piglets, and inhibits intestinal oxidative stress through the upregulation of SOD and GPx (Shi et al., 2020). Curcumin also

elevated secretory immunoglobulin A (sIgA) protein expression in the jejunum, augmented the goblet cell count, and attenuated the expression of pro-inflammatory cytokines (Xun et al., 2015). Li et al. (2022b) investigated curcumin's effects on small intestine mitochondria, ER, and MAMs under oxidative stress in weaned piglets. Curcumin improved small intestine morphology and barrier function, mitochondrial structure and function, alleviated ER stress and cell apoptosis and prevented excessive MAM formation and dysregulation. This research suggests that curcumin can alleviate MAM dysregulation and prevent excessive accumulation of mitochondrial  $\text{Ca}^{2+}$ , thereby improving mitochondrial function, reducing ROS generation, alleviating ERS, and mitigating oxidative stress.

### 5.3.2. Resveratrol

Resveratrol is a naturally occurring polyphenol and plant defense toxin that can be produced in response to biotic or abiotic stress. It is present in various dietary sources and has antioxidant, anti-aging, metabolic, and energy-regulating functions (Zeng et al., 2020). Resveratrol is known to enhance mitochondrial function and provide protection against metabolic disorders by activating two key regulatory proteins, SIRT1 and PGC-1 $\alpha$  (Lagouge et al., 2006). Cao et al. (2019) evaluated the mitigation effect of resveratrol on intestinal damage in weaned piglets during oxidative stress and found that resveratrol increased total antioxidant capacity (T-AOC), decreased  $\text{H}_2\text{O}_2$  and MDA levels, and improved intestinal barrier dysfunction by increasing TJ protein expression levels. In addition, resveratrol ameliorated diquat-induced mitochondrial swelling, vacuolization, and cristae dehiscence, decreased ROS production, and increased intestinal MMP, jejunal mitochondria, and mitochondrial complex I-IV activity. Finally, resveratrol enhanced PINK1 and Parkin levels in intestinal mitochondria. These data suggest that resveratrol effectively protects the gut barrier, improves redox status, attenuates mitochondrial damage, and induces mitophagy in piglets challenged by oxidative stress. Similarly, Hong et al. (2022) found that the addition of resveratrol to the diet increased the average daily feed intake of piglets, increased the height of jejunal villi, reduced the level of ROS in mitochondria, and improved the MMP in the jejunum. Another study indicated that co-administration of resveratrol and curcumin enhances the average daily feed intake, intestinal mucosal integrity and function, antioxidant capacity, and mRNA expression of TJ proteins in weaned piglets (Gan et al., 2019).

### 5.3.3. Other polyphenolic compounds

Many polyphenolic compounds have antioxidant functions, including but not limited to chlorogenic acid, flavones, anthocyanins, and quercetin. Chlorogenic acid (CGA) is a prevalent phenolic acid found in fruits, vegetables, grains, and tuber crops. Supplementing CGA in the diet of weaned piglets increased duodenal, jejunal, and ileal villus height and improved serum GPx activity, as well as duodenal GPx and CAT activities. Additionally, feeding piglets with CGA-supplemented diets was observed to increase the abundance of lactic acid bacteria and decrease the abundance of *E. coli* in the colon contents, as well as increase the levels of propionic and butyric acids (Zhang et al., 2018). Eucommia flavonoids (EUF) have been shown to reduce inflammation and oxidative stress in piglets. Yuan et al. (2020) reported that EUF supplementation improved growth performance, reduced diarrhea incidence, increased villus height in the jejunum and ileum, and enhanced the abundance of lactic acid bacteria in the ileum of weaned piglets. Furthermore, Hu et al. (2022) investigated the effects of the metabolites of cyanidin 3-glucoside, ferulic acid (FA), and vanillic acid (VA), on inflammation and intestinal barrier function of weaned piglets challenged by LPS. The findings demonstrated that both VA

and FA were effective in mitigating inflammation and oxidative stress. The addition of quercetin could reduce the fecal score of piglets and improve intestinal injury by increasing TJ protein expression, villus height, and antioxidant capacity indicators. In particular, after dietary quercetin supplementation, the anaerobic characteristics and carbohydrate metabolism functions of gut microbiota were enhanced, which may be due to the increased intestinal antioxidant capacity (Xu et al., 2021).

## 6. Conclusions and perspectives

Given the central role of mitochondria in the regulation of gut homeostasis, we propose a nutritional intervention strategy targeting mitochondria to alleviate gut disorders in PWD piglets. Currently, the addition of certain antioxidant nutrients, such as vitamins A, B, C, and E, minerals (Se, Zn, Fe, Cu, etc.), and polyphenolic compounds to piglet diets has been proven to be effective. However, current research on mitigating oxidative stress in weaned piglets through nutrition has primarily focused on improving antioxidant capacity, but there is limited research on inhibiting ROS over-production and intestinal oxidative stress from an organelle perspective. Further in-depth research on this topic could lead to more effective nutritional interventions for the weaning stress in piglets. Additionally, future studies should explore the effects of products with different active ingredients and consider how the type and dosage of additives can impact antioxidant function. Therefore, the use of different combinations of products needs to be rationally considered and repeatedly tested in practice to determine the most suitable products for the growth and development of weaned piglets. In addition, amino acids may also be candidate additives for combating PWD in piglets. The metabolic function of amino acids is mainly used for the synthesis of tissue proteins, where amino acids such as leucine and glutamate can serve as energy sources for intestinal tissue after weaning. Furthermore, amino acids such as cysteine, glycine, and glutamate, as precursors for GSH synthesis, can regulate mitochondrial redox signaling to maintain the antioxidant status of intestinal mucosal tissue.

Overall, targeting mitochondrial function using antioxidant nutrients may represent a promising approach for the prevention and treatment of PWD in piglets. Further research is needed to optimize dosing strategies and to investigate the long-term effects of this approach on piglet health and productivity. Furthermore, this review provides a new perspective for future research on the important physiological role of organelles in maintaining gut homeostasis, the reconstruction of gut homeostasis in piglets, the repair of intestinal barrier damage, and the prevention and treatment of PWD.

### Author contributions

**Lei Qiao:** Conceptualization, Funding acquisition, Writing-original draft. **Xina Dou:** Supervision. **Xiaofan Song:** Supervision. **Jiajing Chang:** Supervision. **Hongbo Yi:** Funding acquisition, Writing-review & editing. **Chunlan Xu:** Conceptualization, Funding acquisition, Supervision, Writing-review & editing.

### 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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