Effects of Glutamine on Intestinal Mucosa Renewal in Weaned Piglets and Its Mechanisms: Postprint

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Abstract

Glutamine (Gln) holds significant importance in piglet production. As an essential nutrient and energy substrate for intestinal epithelial cell proliferation, Gln maintains intestinal structural and functional integrity, promotes intestinal mucosal cell renewal, alleviates weaning stress in piglets, and enhances production performance. This article reviews the effects of Gln on intestinal mucosal renewal in weaned piglets and its underlying mechanisms.

Full Text

Effects of Glutamine on Intestinal Mucosal Renewal in Weaned Piglets and Its Mechanisms

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Abstract: Glutamine (Gln) plays a crucial role in piglet production. As an essential nutrient and energy source for intestinal epithelial cell proliferation, Gln maintains intestinal structural and functional integrity, promotes intestinal mucosal cell renewal, alleviates weaning stress, and improves piglet performance. This paper reviews the effects of Gln on intestinal mucosal renewal in weaned piglets and explores the underlying mechanisms.

Keywords: weaned piglets; glutamine; intestinal nutrition; mucosal renewal; effects; mechanisms

To increase annual litter numbers, prevent disease transmission between sows and piglets, and improve economic efficiency, early weaning (3–4 weeks of age) is commonly practiced in commercial production. However, weaning stress adversely affects intestinal tissue morphology, disrupts microecological balance in the digestive tract, impairs immune and enzyme system development [1], and compromises intestinal mucosal cell renewal. Consequently, weaned piglets frequently suffer from "early weaning syndrome," characterized by reduced feed intake, digestive disorders, diarrhea, poor feed utilization, and growth retardation.

Glutamine (Gln) is a common free amino acid in mammalian plasma and maternal milk that serves as a precursor for numerous important substances (including proteins, purines, pyrimidines, and nucleotides) and represents a critical energy source for intestinal epithelial cell proliferation. Therefore, Gln plays a significant role in promoting intestinal mucosal renewal and maintaining intestinal structure.

1.1 Metabolic Pathways of Gln in the Intestine

Gln is a vital substance in material and energy metabolism. Its amino nitrogen can be used to synthesize purines, pyrimidines, and amino sugars, while its carbon chain and amino groups can form other amino acids such as proline, ornithine, and arginine (Figure 1 [Figure 1: see original paper]) [1]. Gln not only enters the tricarboxylic acid cycle for oxidative energy supply [2] but also serves as a precursor for hepatic glycogen synthesis [3], making it a primary energy substrate utilized by the intestine [4].

The metabolic pathway of Gln in the intestine is illustrated in Figure 1. $NADP^+$: nicotinamide adenine dinucleotide phosphate; H_2O_2: hydrogen peroxide; HO_2: hydroperoxyl radical; CO_2: carbon dioxide; NO: nitric oxide; succinyl-CoA: succinyl coenzyme A; acetyl-CoA: acetyl coenzyme A.

Figure 1 The metabolic pathway of Gln in the intestinal tract [5]

1.2.1 Conversion of Gln into Various Amino Acids in the Intestine

In the intestine, Gln can synthesize important amino acids such as citrulline and proline. Citrulline can subsequently be converted to arginine in the kidneys of adult animals [5], a process with significant physiological implications. First, feeding cats, ferrets, and other animals arginine-free diets leads to severe hyperammonemia and even death [6]. Second, the efficiency of citrulline synthesis from Gln is extremely low in some young animals, resulting in growth retardation when arginine is deficient [7]. Arginine derived from Gln or peptide degradation can generate proline [8], and this pathway may represent the primary source of proline in the body [9].

1.2.2 Conversion of Gln into Other Substances in the Intestine

The intestinal tract of weaned piglets can utilize ammonia, Gln, and arginine to produce urea [10]. Research indicates that the intestine contains all enzymes necessary for ammonia conversion to urea [11], but their activity is low, so only about 5% of ammonia derived from Gln is converted to urea [12]. Additionally, Gln can generate ornithine via transaminase action, and through a series of enzymatic reactions, ornithine can produce polyamines [13]. Polyamines exhibit various biological activities, promoting epithelial cell maturation and differentiation while playing crucial roles in maintaining cell membrane structure and function [14].

1.3 Current Status and Prospects of Gln Research in Intestinal Nutrition

Weaning stress severely reduces feed intake in piglets, causing diarrhea and insufficient nutrient supply. Due to high metabolic intensity and energy demands of intestinal mucosal cells, weaning stress often places these cells in a "starvation state," impairing normal physiological functions and inhibiting proliferation and differentiation, ultimately leading to mucosal atrophy [15]. Dietary Gln supplementation effectively promotes intestinal cell proliferation, prevents mucosal atrophy, ensures intestinal structural and functional integrity, and alleviates weaning stress [16].

Studies show that dietary L-glutamine (L-Gln) supplementation in weaned piglets increases villus height in the duodenum and jejunum, enhances jejunal mucosal glutamate oxaloacetate transaminase activity, elevates glutamine synthetase mRNA expression levels, and upregulates mRNA expression of tight junction proteins occludin and zonula occludens-1 (ZO-1) [17]. Furthermore, L-Gln supplementation increases plasma concentrations of multiple amino acids (including glutamate, arginine, histidine, isoleucine, leucine, methionine, phenylalanine, and threonine) and enhances mRNA expression of amino acid receptors and transporters in jejunal mucosa, such as the ion-sensitive receptor, metabotropic glutamate receptor 1, and metabotropic glutamate receptor 4 [18]. These findings demonstrate that dietary L-Gln supplementation improves jejunal integrity, promotes expression of amino acid receptors and transporters, and facilitates nutrient digestion and absorption in the jejunum of weaned piglets.

However, Gln has limitations including thermal instability and low solubility [19], restricting its practical application. Gln dipeptides, formed by combining Gln with other amino acids, offer advantages over free Gln including higher water solubility, better stability, and improved absorption rates, showing excellent potential in animal production. Common Gln dipeptides include alanyl-glutamine (Ala-Gln) and glycyl-glutamine (Gly-Gln) [20], though industrial processing complexity and high costs remain challenges [21].

2 Effects of Gln on Intestinal Mucosal Renewal in Weaned Piglets and Its Mechanisms

Intestinal mucosa maintains a dynamic balance between cell proliferation and apoptosis, typically renewing every 3–8 days [22]. Gln deficiency accelerates intestinal epithelial cell apoptosis and impedes cell proliferation by selectively activating caspases, the key enzymes in apoptosis. Once activated, caspases degrade intracellular proteins, leading to cell death [23]. Based on Gln metabolic characteristics, its mechanisms for promoting intestinal mucosal cell proliferation likely involve two aspects: first, Gln oxidation provides energy required for mucosal cell proliferation; second, Gln serves as a precursor for substances needed during cell proliferation [24].

2.1 Gln as the Primary Energy Source for Intestinal Mucosa and Nitrogen Donor for Purine and Protein Synthesis

Research indicates that glucose and Gln provide energy in a 62:38 ratio in the proximal intestine [25], highlighting Gln' s important role in intestinal energy supply. Insufficient Gln availability affects intestinal mucosal cell growth and development. In intestinal mucosal cells, oxidation of 1 mol Gln generates 2 molecules of carbon dioxide (CO_2) and 9 mol adenosine triphosphate (ATP), while the remaining three carbons that do not form CO_2 can participate in other energy metabolic pathways [26]. Additionally, Gln serves as a nitrogen source for synthesizing other amino acids in the intestine, primarily because Gln can be converted to glutamate via glutaminase, which subsequently generates alanine, proline, arginine, and ornithine through transaminase action [27]. During purine and pyrimidine synthesis, Gln not only provides energy but also acts as a precursor, specifically controlling nucleotide synthesis efficiency in proliferating cells [28]. Studies suggest Gln promotes DNA and RNA synthesis in intestinal cells, possibly because Gln can be converted to arginine, which stimulates growth hormone (GH) secretion [29].

2.2 Gln Inhibits Cell Apoptosis

Weaning stress induces excessive apoptosis of intestinal mucosal cells in piglets, disrupting mucosal homeostasis, impairing structure and function, and causing intestinal barrier dysfunction with increased permeability. Gln can inhibit excessive apoptosis of intestinal mucosal cells, thereby mitigating effects of weaning stress and intestinal diseases on barrier function [30]. Apoptosis is directly controlled by related genes, while external stimuli can regulate apoptosis through signal transduction pathways that affect gene expression [31]. Key apoptosis-related genes in mammals include tumor necrosis factor (TNF) receptor superfamily member 6 (Fas) and its ligand (FasL), and the B-cell lymphoma-2 (Bcl-2) gene family. These can be classified as pro-apoptotic genes [such as Bcl-2-associated X protein (Bax)] or anti-apoptotic genes (such as Bcl-2) [32]. During apoptosis, mitochondria release cytochrome C, which activates apoptotic protease activating factor-1 (Apaf-1) to encode cysteine proteinase-3 (caspase-3),

a 32 kDa protein [33]. Caspase-3 plays a crucial role in the apoptosis mechanism, and increased caspase-3 expression leads to excessive cell apoptosis [34]. Studies have found that Gln can inhibit caspase-3 expression in intestinal mucosal cells, interrupting apoptosis signal transduction pathways and thereby reducing intestinal mucosal cell death [35].

2.3 Gln Participates in Glutathione (GSH) Synthesis

GSH is a small peptide composed of glutamate, cysteine, and glycine that can neutralize free radicals by accepting electrons from hydrogen peroxide (H_2O_2) through the action of glutathione peroxidase (GSH-Px), thereby exerting antioxidant effects and reducing oxidative damage [36]. Glutamate is essential for GSH synthesis, but as a strongly charged molecule, it cannot easily cross cell membranes. In contrast, Gln readily enters cells and produces glutamate through deamination, thus participating in GSH synthesis. During stress and metabolic disorders, insufficient Gln uptake by intestinal mucosal cells leads to inadequate GSH synthesis and accumulation of free radicals and peroxides, compromising antioxidant capacity. Therefore, Gln supplementation increases GSH content and enhances resistance to oxidative damage [37].

2.4 Gln Reduces Pro-inflammatory Cytokine and Intestinal Cytokine-Mediated Immune Cell Factor mRNA Expression

Gln can reduce mRNA expression of neutrophilic chemotactic factor (CINC) mediated by intestinal cytokines. Although CINC has defensive functions against infection, it can also trigger inflammatory responses at infection sites, impairing immune function [38]. Additionally, Gln reduces intestinal TNF levels. TNF exists in two forms (TNF-α and TNF-β); TNF-α affects occludin distribution and phosphorylation levels and serves as an important initiating factor for intestinal mucosal barrier damage caused by stress and disease. Gln promotes tight junctions between intestinal epithelial cells and prevents TNF-α-induced occludin expression abnormalities, thereby protecting intestinal health. Research shows that when premature infants develop intestinal bacterial infections, pro-inflammatory substances such as interleukin-8 (IL-8) are over-secreted or expressed. IL-8 is a chemotactic cytokine that promotes inflammatory cell chemotaxis and acts as an important mediator in inflammatory diseases, accelerating bacterial translocation from blood to intestine and disrupting normal intestinal cell function [39]. Gln supplementation in premature infants suppresses IL-8 expression, demonstrating that Gln can inhibit intestinal inflammatory factor expression and protect intestinal health.

2.5 Gln Upregulates Proto-oncogenes c-fos and c-jun mRNA Expression to Promote Intestinal Mucosal Cell Division and Proliferation

The c-fos gene is a DNA segment in the cell nucleus that can recognize specific DNA sequences to initiate cell proliferation and differentiation. The c-jun gene is a proto-oncogene in the cell nucleus that expresses within minutes after

stimulation by growth factors, gonadotropins, and neurotransmitters [40], earning it the designation of an "immediate early gene." Products of these genes act on DNA sequences to alter DNA synthesis and are therefore considered markers of cell proliferation. In vitro experiments have demonstrated that Gln promotes mRNA expression of proto-oncogenes c-fos and c-jun in small intestinal mucosal cells, thereby stimulating proliferation [41]. The likely mechanism involves Gln stimulating expression of insulin-like growth factor-1 (IGF-1) in the liver and intestinal mucosa, which activates the mitogen-activated protein kinases (MAPKs) signaling pathway to promote c-fos and c-jun expression.

Gln exerts multiple functions in the animal intestine, including maintaining intestinal structural and functional integrity and enhancing intestinal immune function. As the primary energy substrate and important metabolic precursor for intestinal mucosal cells, these findings underscore the significance of investigating Gln' s role and mechanisms in piglet intestinal nutrition and mucosal renewal for alleviating weaning stress. However, current research on Gln' s effects and mechanisms on intestinal mucosal renewal in weaned piglets remains limited, and the specific mechanisms by which Gln inhibits apoptosis are not fully elucidated, leaving considerable scope for future research at the cellular and molecular levels.

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