

Review: The roles and functions of glutamine on intestinal health and performance of weaning pigs

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The gut is composed of a single layer of intestinal epithelial cells and plays important roles in the digestion and absorption of nutrients, immune and barrier functions and amino acid metabolism. Weaning stress impairs piglet intestinal epithelium structural and functional integrities, which results in reduced feed intake, growth rates and increased morbidity and mortality. Several measures are needed to maintain swine gut development and growth performance after weaning stress. A large body of evidence indicates that, in weaning piglets, glutamine, a functional amino acid, may improve growth performance and intestinal morphology, reduce oxidative damage, stimulate enterocyte proliferation, modulate cell survival and death and enhance intestinal paracellular permeability. This review focuses on the effects of glutamine on intestinal health in piglets. The aim is to provide evidentiary support for using glutamine as a feed additive to alleviate weaning stress.

Keywords: swine, weaning stress, functional amino acid, barrier function, epithelial cell renewal

Implications

Piglets are subject to constant nutritional, psychological and environmental stresses during weaning, which can result in significant swine industry economic losses. Developing strategies to reduce the negative consequences of post-weaning stress is of great concern. Glutamine is an important functional amino acid. It alleviates growth suppression and repairs weaning piglet intestinal epithelium. This article summarizes recent researches which highlight the potential use of glutamine in pig nutrition and underlying mechanisms involved. This review helps to provide references for glutamine use in swine production.

Introduction

The gastrointestinal tract plays crucial roles in digestion and absorption of nutrients, providing barrier functions, as well as amino acid metabolism (Wijten *et al.*, 2011). Its health is vitally important to host growth and development. With the widespread application of early weaning, piglets are

immediately exposed to many environmental and psychosocial stressors that impact on gut structure and function (Spreeuwenberg *et al.*, 2001; Yin *et al.*, 2014). These changes cause abnormal digestion and absorption, poor growth performance and increased diarrhea rates (Montagne *et al.*, 2007). Exploring different nutritional factors, management and strategies that focus on promoting weaning pig growth are critical to optimizing performance. Glutamine has always been classified as a nonessential amino acid when assigning swine nutritional requirement. One of the major reasons for this is that glutamine naturally occurs in conventional feed ingredients such as corn grain, sorghum grain, soybean meal and meat and bone meal. Natural glutamine constitutes about 10% of the amino acid content of total dietary protein (Wu, 2014). Also, post-weaning pigs weighing around 5 to 10 kg have high net glutamine synthetic rates (up to 1149 mg/kg BW per day) in their extraintestinal tissues (Wu, 2014). However, all tissues utilize glutamine (Wu *et al.*, 2010), and there is growing evidence that pigs have a dietary requirement for glutamine to achieve maximum growth performance and facilitate normal intestinal physiology, particularly in hypercatabolic states (Wang *et al.*, 2008; Blachier *et al.*, 2013; Wu, 2014). As swine nutrition practices have

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Table 1 *The common effects of weaning on piglet performance and intestinal development and function*

Piglets	Weaning phase	Significant result	References
14-day weaning	Days 1, 3, 5, 7	↓ body weight; ↓ diamine oxidase and D-lactate concentrations ↓ PCNA-positive cells; ↓ AKP activity; ↓ E-cadherin, occludin and zonula occludens (ZO)-1 mRNA or protein abundances	Wang <i>et al.</i> (2016b)
19-day weaning	Days 1, 2, 7	↓ organ weight; ↓ small intestine length ↓ lactase, maltase, dipeptidyl-peptidase 4; amino-peptidase N activities ↑ transepithelial electrical resistance; ↑ [3H]mannitol; ↑ serum CRF	Montagne <i>et al.</i> (2007)
21-day weaning	Days 0.5, 1, 2, 4, 7	↓ average cumulative intake; ↓ goblet cells numbers ↑ T lymphocytes numbers; ↑ metalloproteinase stromelysin expression ↓ jejunal villus height; ↑ crypt depth; ↓ MHC class I RNA expression	Mccracken <i>et al.</i> (1999)
21-day weaning	Days 0, 2, 5, 8, 15	↓ relative weight of the small intestine; ↑ increases in intestinal permeability ↓ pancreatic parameters; ↓ villus height	Moeser <i>et al.</i> (2007)
21-day weaning	Days 0, 2, 5, 8, 15	↓ villous height; ↑ crypt length; ↓ lactase activity ↓ 5-HT, theophylline and HRP fluxes	Boudry <i>et al.</i> (2004)

PCNA = proliferating cell nuclear antigen; AKP = alkaline phosphatase; CRF = corticotropin-releasing factor; MHC = major histocompatibility complex; 5-HT = 5-hydroxytryptamine; HRP = horseradish peroxidase.

rapidly changed, the understanding of the underlying mechanisms needs to keep pace. This article provides an overview on the effects of weaning on gastrointestinal structure and function, the effects of glutamine on piglet performance and gut health and information on the underlying mechanisms, and how they are influenced by glutamine.

Intestinal development and health after weaning

A multitude of factors contribute to post-weaning stress, including nutritional (change from sow's milk to solid feed), psychological (mixing of different litters and removal from the sow), environmental (moving from farrowing crates to nursery pens) and others (Moeser *et al.*, 2007; Yin *et al.*, 2014). Weaning stress is generally accompanied by reduced feed intake, decreased nutrient utilization, poor growth performance and increased disease susceptibility (Montagne *et al.*, 2007). Several articles demonstrate that weaning stress also negatively impacts intestinal development and function (Montagne *et al.*, 2007). This review highlights the effects of weaning stress on intestinal epithelial development and its redox status. The common effects of weaning on piglet performance and intestinal development are provided in Table 1.

Intestinal epithelium developmental changes after weaning

The gut includes crypts and villi, which are formed by four principal cell types: absorptive enterocytes, mucus-producing goblet cells, hormone-secreting enteroendocrine cells and lysozyme-containing Paneth cells (Chen *et al.*, 2018a). The porcine intestinal epithelium structure is shown in Figure 1. The homeostasis and renewal of the intestinal epithelium involve

stem cell proliferation at the base of the crypt bottom, cell population expansion in the middle of the villus, terminal differentiation in the upper villus and senescent cell extrusion at the villus tip (Yang *et al.*, 2016b). This intricate process of epithelial cells along the crypt-villus axis is accompanied by functional specialization in the small intestine (Yang *et al.*, 2016a). The proliferation, differentiation and apoptosis of intestinal epithelial cells play important roles in intestinal development, maintenance and recovery from stress-induced tissue damage. In weaning piglets, the altered balance between crypt cell proliferation and epithelial cell apoptosis causes intestinal villus atrophy and crypt hyperplasia resulting in a loss in nutrient absorptive surfaces (Montagne *et al.*, 2007). There is a decrease in enterocyte differentiation post-weaning accompanied by the loss of digestive enzymes, such as lactase and sucrase, and thus a decrease in intestinal digestive capacity (Pluske *et al.*, 1997).

Post-weaning distinctive intestinal functional characteristics

An increase in reactive oxygen species (ROS) can induce oxidative stress during animal growth and development, and weaning is often associated with oxidative stress and decreased antioxidant capacity in the intestine and other tissues of pigs (Wang *et al.*, 2008; Yin *et al.*, 2014). For example, weaning pigs at 14 days of age resulted in oxidative injury to lipid, protein and DNA and increased the plasma concentrations of damage biomarkers such as malondialdehyde, protein carbonyl and 8-hydroxydeoxyguanosine (Yin *et al.*, 2014). Concurrently, the activity of many antioxidant enzymes, such as superoxide dismutase, catalase and glutathione peroxidase, is also reduced due to weaning

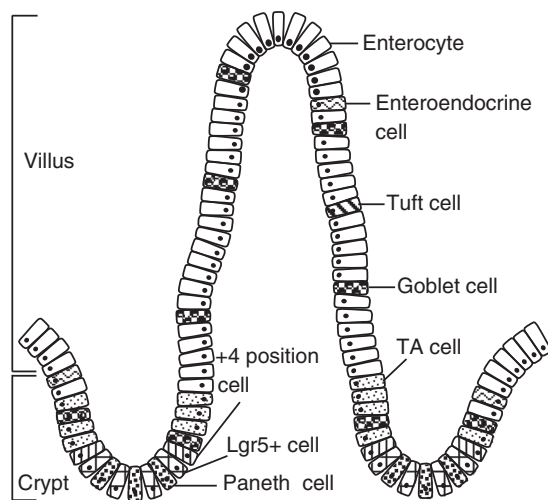


Figure 1 Porcine intestinal epithelium structure. Intestinal stem cells (ISCs) located at the base of the crypt, which can be identified by marker gene *Lgr5* (leucine-rich-repeat-containing G-protein-coupled receptor 5). The +4 position cell resides at the fourth position from the base of the crypt, above the Paneth cell. Paneth cells are located between *Lgr5*-positive (*Lgr5*⁺) cells. The daughter cells of ISCs migrate up the crypt-villus axis and pass through a progenitor stage, forming transient amplifying (TA) cells, before becoming differentiated cells (enterocyte, enteroendocrine cell, tuft cell, goblet cell).

(Yin *et al.*, 2014). Glutathione in reduced form (GSH) is an antioxidant that can directly react with reactive-oxygen species, generating oxidized glutathione (GSSG) through glutathione peroxidase. In turn, GSSG can be catalyzed into GSH by glutathione reductase. Since the reduced and oxidized forms of glutathione are simultaneously present in cells, the ratio of GSSG : GSH is an indicator of oxidative stress, that not only determines cellular redox status but also controls intracellular concentrations of both oxygen-reactive and nitrogen-reactive species (Cruzat *et al.*, 2018). In 28-day-old pigs, weaning reduced feed intake (−36%), BW gain (−47%) and small intestine weight (−26%) over the 7-day post-weaning. Meanwhile, weaning increased the expression of genes (+52% to +346%) related to oxidative stress (i.e. glutathione peroxidase-1 and -4) and the GSSG : GSH ratio (+59%) when compared with piglets maintained on the sow (Wang *et al.*, 2008). Moreover, pigs weaned at 21 days of age grew more slowly (−89%), had shorter villus height (−42%) than piglets maintained on the sow (Wang *et al.*, 2014). In addition, weaning can result in increased intestinal epithelial permeability by decreasing the relative expression of tight junctions including occludin and claudins (Wang *et al.*, 2014). These affect the absorption and transportation of nutrients, disrupt barrier functions and may cause additional intestinal inflammatory responses (Wang *et al.*, 2014).

Glutamine may affect intestinal function of weaning pigs

Glutamine is considered the most abundant free amino acid in the body, because plasma and tissue glutamine concentrations are 10 to 100 fold higher than any other amino acids (Cruzat *et al.*, 2018). Weaning affects intestinal amino acid

metabolism (Sève *et al.*, 1986) with many of these changes related to glutamine metabolism. For example, in enterocytes of weaned pigs, L-glutamine is oxidized approximately eight times less slowly than in enterocytes isolated from suckling pigs (Darcy-Vrillon *et al.*, 1994). Weaning also results in reduced glutamine concentrations in the jejunal lumen fluid (−70%), jejunal tissue (−38%), and plasma (−30%) (Wang *et al.*, 2008). However, the gut preferentially extracts glutamine as a source of energy within intestinal epithelial cells. For example, approximately 67% of dietary glutamine is catabolized by the small intestine (i.e. enterocytes, lymphocytes and bacteria) in swine, with the remaining glutamine entering the portal circulation (Wu *et al.*, 2011). Furthermore, glutamine is the only amino acid in arterial blood that is taken up by the small intestine of swine. Thus, although the small intestine represents only 3% to 4% of BW, it utilizes 30% of total arterial glutamine in pigs (Wu *et al.*, 2011). Therefore, glutamine may affect intestinal development, structure and function and growth performance of weaning piglets. Therefore, the potential of feeding higher dietary glutamine levels to weaning pigs, the underlying mechanisms and how they are influenced by glutamine deserves further research.

Glutamine metabolism in the small intestine

The gut derives glutamine from both the diet and blood. The small intestine is the principal organ for glutamine uptake and metabolism into the body. L-glutamate is the principal ingested form that exerts its physiological function in the gut (Blachier *et al.*, 2013). Dietary glutamine is taken up from the intestinal lumen and transported across the apical membranes of the intestinal enterocyte by a variety of amino acid transport systems (i.e. Na⁺-dependent transport systems) to cytosol and mitochondria. Following that, L-glutamine enters the mitochondria, where it is degraded to L-glutamate and ammonia by the phosphate-dependent glutaminase, which is very abundant in enterocyte mitochondria in both villus and crypt cells (Pinkus and Windmueller, 1977). Dietary glutamate serves primarily as an important energy source for rapidly dividing porcine mucosal cells (Reeds *et al.*, 1996 and 2000; Janeczko *et al.*, 2007). For example, in pigs weaned at 21 days of age and administered three- to fourfold the normal level of dietary glutamate, the majority (70% to 80%) of absorbed glutamate was utilized by the gut, and a large proportion of this served as an oxidative fuel for the mucosa (Janeczko *et al.*, 2007).

Second, as a protein synthesis precursor, glutamine has many metabolites, including glutamate, alanine, aspartate, α-ketoglutarate, arginine and proline (Blachier *et al.*, 1999). Transamination (amino acid synthesis and interconversion) appears to be the principal route by which enteral glutamate is converted to α-ketoglutarate in enterocytes in weaning pigs (Madej *et al.*, 1999). Glutamate is metabolized into alanine and α-ketoglutarate in the presence of pyruvate, and into aspartate and α-ketoglutarate in the presence of oxaloacetate. Then, α-ketoglutarate enters the Krebs cycle

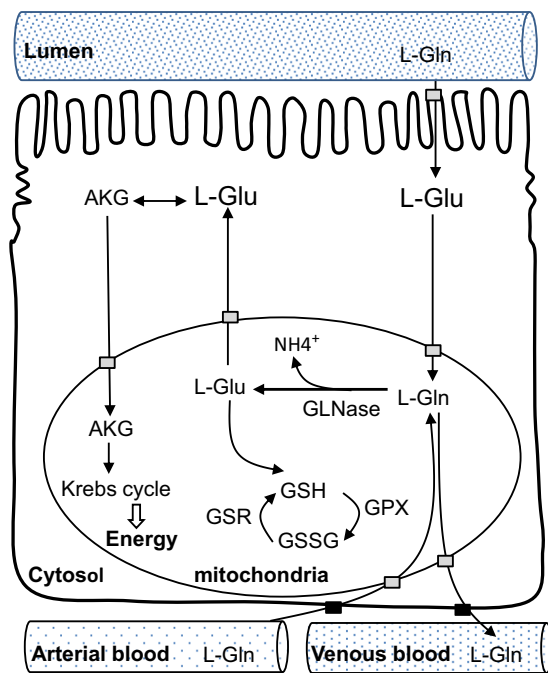


Figure 2 Schematic view of catabolic metabolism of glutamine in the small intestine, adapted from Blachier *et al.*, (2013). AKG = alpha-ketoglutarate; Gln = glutamine; GLNase = phosphate-dependent glutaminase; Glu = glutamate; GPX = glutathione peroxidase; GSH = reduced form of glutathione; GSR = glutathione reductase; GSSG = oxidized form of glutathione.

to be oxidized to produce energy. Third, less than 10% of total glutamine extracted by the pig gut is used for protein synthesis (Stoll *et al.*, 1998). Lastly, one consequence of glutamine metabolism is to produce glutathione via glutamate. Together with L-cysteine and glycine, enteral L-glutamate is the precursor for the synthesis of glutathione, and among these three amino acids, glutamate probably represents the most important step (Cruzat *et al.*, 2018). Most of the metabolites of glutamine are oxidized with the remaining being exported through the hepatic portal vein to the whole body (Blachier *et al.*, 2013). Note that while cellular glutamine can be metabolized into purines, pyrimidines for the biosynthesis of nucleotides, glutamate cannot replace this function. Conversely, glutamate and ammonia can be synthesized into glutamine by glutamine synthetase in the skeletal muscles or liver, but this reaction consumes energy (Cruzat *et al.*, 2018). A schematic view of catabolic metabolism of glutamine in small intestine is shown in Figure 2.

Glutamine regulates intestinal function of weaning pigs

Numerous studies have suggested that glutamine has positive effects on piglet growth performance, intestinal morphology, epithelial cell renewal, antioxidant capacity and barrier functions (Wu *et al.*, 1996; Domeneghini *et al.*, 2006; Wang *et al.*, 2008 and 2014; Molino *et al.*, 2012; Zhu *et al.*, 2015; Jiang *et al.*, 2017).

Glutamine improves piglet health, growth and intestinal morphology

Rhoads *et al.* (1991) reported that glutamine is an effective oral rehydration solution for rotavirus-induced diarrhea. Compared to normal newborn piglets, the rotavirus-induced diarrhea piglets had decreased villus height (−65%), thinner mucosa and increased thymidine kinase activity (+700%) (Rhoads *et al.*, 1991). Meanwhile, the activities of lactase specific (−20%), alkaline phosphatase (−38%) and Na⁺/K⁺-ATPase (−50%) were all reduced in response to rotavirus-induced diarrhea (Rhoads *et al.*, 1991). Moreover, L-glutamine (30 mmol/l) was found to stimulate *in vitro* absorption of sodium and chloride in rotavirus-infected pigs, returning the rates those observed in uninfected tissue (Rhoads *et al.*, 1991). However, that study did not further investigate whether glutamine affects the villus height and mucosal enzyme activities of infected pigs. In another study, piglets that were weaned at 21 days and supplemented with 0.5%, 1%, 2% and 4% monosodium glutamate for 21 days exhibited a dose-dependent reduction in the incidence of diarrhea in the first week after weaning (Rezaei *et al.*, 2013). These data indicate that glutamine or glutamate can alleviate diarrhea. Other studies demonstrate that glutamine or glutamate serves as conditionally essential amino acids, and that supplementation with either can improve weaning piglet growth performance (Wu *et al.*, 1996; Molino *et al.*, 2012) and intestinal epithelium structure and function (Rezaei *et al.*, 2013; Wang *et al.*, 2014). The reported effects of glutamine on the performance, gastrointestinal structure and function of weaned pigs are shown in Table 2. The evidence from these published studies using pure glutamine or a mixture of glutamine and glutamate shows beneficial effects between 0.5% and 2.0% (by weight) throughout the post-weaning period, particularly during the first 2 weeks after weaning (Wu *et al.*, 1996; Domeneghini *et al.*, 2006; Hsu *et al.*, 2010; Molino *et al.*, 2012; Teixeira *et al.*, 2014; Wang *et al.*, 2014). However, some studies also showed that there were no improvements on growth performance of post-weaning piglets supplemented with glutamine or glutamate. For example, a corn soybean meal diet supplemented with 1.0% glutamine did not improve villus height in piglets weaned at 18 days of age, but did improve feed efficiency (Kitt *et al.*, 2002). Di *et al.* (2003) reported that when pigs weaned at 21 days of age were fed with 0.5% glutamine for 28 days, they showed a significant positive effect on ileal morphology and mucosal cell proliferation (lymphatic follicles and epithelial cells, macrophages and intra-epithelial lymphocytes). However, there were no significant differences among the groups in growth performances (Di *et al.*, 2003). In spite of the positive effects of glutamine on small intestinal villus morphology and plasma net xylose absorptive concentration in 28-day-old weaning pigs, 1% or 2% glutamine supplementation in a corn-soybean meal diet did not significantly affect growth performance during the 21-day treatment period (Hsu *et al.*, 2010). Similar results were obtained by (Amorim *et al.*, 2018) who also did not observe any significant effects of 1% glutamine or 1% glutamate on growth performance variables in pigs fed a rice grit and

Table 2 The reported effects of glutamine on the performance, gastrointestinal structure and function of weaned pigs

Weaning age	Experiment treatment	Response to supplementation	References
Suckling and piglets weaned at 28 days old	Basal diet supplemented with 0.5% monosodium glutamate to creep feed for suckling pigs on day after farrowing until weaning, and to weaning pigs during first 13 days after weaning, respectively	Increased suckling pigs feed intake by 36%, but no increase in weaning weight; increased weaning pigs daily food intake by 10% and daily BW gain by 7% during first 13 days after weaning	Gatel and Guion (1990)
21 days old	Corn and soybean meal basal diet supplemented with either 0, 0.2%, 0.6% or 1.0% free glutamine, for 14 days	1% glutamine improved jejunal villus height by 33% during the first week, increased gain: feed ratio by 25%, increased plasma concentrations of glutamine by 24%, glutamate by 74% and alanine by 35% during the second week	Wu <i>et al.</i> (1996)
12.5 days old	Six treatments including corn, whey, oat basal diet (control), basal diet supplemented either arginine, glutamate, citrulline, ornithine or polyamines, at levels of 0.93%, 6.51%, 0.94%, 0.90% and 0.39%, respectively, for 12 days	Pigs receiving 6.51% glutamate significantly increased duodenum villus height, when compared with control group or polyamine group. That indicating that glutamate prevented weaning-induced intestinal villus atrophy	Ewtushik <i>et al.</i> (2000)
21 days old	Corn soybean meal, barley meal basal diet supplemented with 0, 0.5% glutamine for 28 days	Increased ileal villus height by 23.5% and crypt depth by 51.4% and decreased the ratio of villus height to crypt depth by 18.3%, increased the number of mitotic mucosal cells by 4.4% and decreased apoptosis of enterocytes by 5.6% and lymphatic follicles by 25.4%	Domenechini <i>et al.</i> (2006)
28 days old	Corn soybean meal basal diet supplemented with either 0, 1% or 2% glutamine for 21 days	Glutamine groups tended higher villus height than the control group in duodenum and jejunum. 2% glutamine supplementation increased plasma net xylose absorptive concentration by 10% and 21%, respectively, in days 7 to 14 after weaning	Hsu <i>et al.</i> (2010)
21 days old	Phase 1: 21 to 35 days, adding 0.8% mixer of glutamine and glutamate in diets with no lactose or with 4.0% or 8.0% lactose inclusion; phase 2: 36 to 49 days, all animals received the same diet but with no lactose inclusion in corn and soybean meal basal diet	0.8% mixer of glutamine and glutamate improved 7% BW and 10% weight gain of pigs from 21 to 49 days old and increased the villus height in duodenum by 7.7%, jejunum by 10.4% and ileum by 6.6% at 49 days of age	Molino <i>et al.</i> (2012)
21 days old	Corn soybean meal basal diet supplemented with 0, 0.5%, 1%, 2% and 4% monosodium glutamate for 21 days	4% monosodium glutamate increased BW 7.8%, daily gain 31.6%, jejunal villus height 31.9%, crypt depth 8.1%, reduced diarrhea incidence 78.6% and enhanced jejunal concentrations of adenosine triphosphate 54.8%, deoxyribonucleic acid 35.0%, ribonucleic acid 30.7%, during the first week after weaning	Rezaei <i>et al.</i> (2013)
21 days old	Corn soybean meal basal diet supplemented with 0, 1% L-glutamine compared with piglets reared by sows, for 7 days	Weaned pigs in control group grew (over 7 days) 89% slower than sow-reared piglets. Those offered a diet with 1% glutamine grew 68% slower than sow-reared piglets. Similar effects of glutamine on villus height were also observed in the jejunum tissues. Glutamine had the adverse effect of weaning on growth, villus height but not totally overcoming the stress of weaning on intestinal structure and function	Wang <i>et al.</i> (2014)
21 days old	Mixtures of glutamine and glutamate (the ratio between glutamine and glutamate was unknown); five treatments: basal diet supplementation with 0, 0.5%, 1.0%, 1.5% mixtures and positive control (basal diet +4% porcine plasma) for 21 days in corn and soybean meal-based diet	1.0% mixtures improved weight gain by 68.3%, feed intake by 29.8%, decreased feed conversion ratio by 29.7% and diarrhea score by 12.1% compared with porcine plasma control group during the first week after weaning. 1.0% mixtures also reduced duodenal crypt depth 17.3% and ileum digestive content pH 2.2%, increased villus : crypt ratio 36.8% at age 28 days, when compared to the porcine plasma diet	Teixeira <i>et al.</i> (2014)

soybean-based diet. Different diets, weaning age, experimental duration or dosage of glutamine may contribute to differing growth performance responses to glutamine or glutamate supplementation (Kitt *et al.*, 2002; Di *et al.*, 2003; Amorim *et al.*, 2018).

Glutamine and cellular redox state

Glutamine metabolism can provide glutamate required for cellular glutathione synthesis. Glutathione can directly react with ROS and thereby protect cellular macromolecules such as DNA, proteins and lipids against oxidative stress (Cruzat *et al.*, 2018). Glutamine has been shown to preserve total glutathione levels after injury/ischemia to gut (Harward *et al.*, 1994). In response to cobalt chloride (375 µmol/kg BW)-generated oxidative stress, rats showed increased liver thiobarbituric acid reactive substances content (+50%) and decreased GSH (−40%) activity compared to control values (Gonzales *et al.*, 2005). The level of antioxidant enzymes catalase, glutathione peroxidase and superoxide dismutase in liver also was decreased by about 30% after cobalt injection, while that of hepatic heme oxygenase-1 induction was increased over 14-fold after cobalt treatment. However, these dramatic effects of cobalt on enzyme activities, including thiobarbituric acid reactive substances and GSH, were not observed in rats that had been pretreated with dietary glutamine (300 mg/kg BW). Also, the marked increase in the liver heme oxygenase-1 (protein expression and activity) due to cobalt challenge was partially prevented by glutamine administration (Gonzales *et al.*, 2005). These data indicate that glutamine supplementation prevents glutathione depletion in the liver of rats, thus exerting protective effects during oxidant stress. Similarly, in pigs weaned at 21 days age and supplemented with 4% monosodium glutamate, there was an increase in jejunal GSH (+56%) and a decrease in GSSG (−24%) and the ratio of GSH : GSSG (−54%) during the first week after weaning (Rezaei *et al.*, 2013). Dietary glutamate also alleviated intestinal oxidative stress in weaning pigs, when animals were challenged by the potent oxidants diquat and hydrogen peroxide (Yin *et al.*, 2015b; Duan *et al.*, 2016). Diquat is an herbicide that generates superoxide anions through redox cycling and has been used to induce oxidative stress *in vivo* (Lv *et al.*, 2012). A diquat injection to piglets significantly decreased final BW (−22%), average daily weight gain (−125%) and feed intake (−57%) (Yin *et al.*, 2015b). Meanwhile, supplementation with 2% glutamate after diquat treatment partially restored final BW to 91.2%, average daily feed intake to about 50%, and average daily weight gain to 19% of control levels (Yin *et al.*, 2015b). Diquat disrupted antioxidant enzymes balances, such as decreased serum nitric oxide about onefold, elevated serum malondialdehyde by about 80%. Diquat also decreased serum concentrations of threonine (−42%), serine (29%), glycine (26%) and glutamate (−5.5%) (Yin *et al.*, 2015b). Pigs treated with 2% glutamate increased nitric oxide and reduced malondialdehyde. Meanwhile, 2% supplemental glutamate significantly enhanced serum glutamate level by 21% and associated amino acid transporters expression.

Glutamate supplementation increased serum threonine, serine and glycine concentrations, although they did not return completely to control values (Yin *et al.*, 2015b).

Glutamine and intestinal epithelial cells renewal

Glutamine and intestinal epithelial cell proliferation and differentiation. Glutamine has long been recognized as having an important role in cell survival and proliferation (Ehrensvarid *et al.*, 1949). Glutamine promotes intestinal cell proliferation in rats (Swaid *et al.*, 2013), piglets (Wang *et al.*, 2008) and various cell lines (Chen *et al.*, 2018b). A recent study with weaning mice by Chen *et al.* (2018a) found that 10 mg/ml glutamine promotes Lgr5 (leucine-rich-repeat-containing G-protein-coupled receptor 5, also known as Gpr49), a stem cell marker, expression in intestinal cells. Another study found that Lgr5 stem cell growth is closely associated with epidermal growth factor (EGF), Wnt3 and the Notch-ligand. These are all essential signals required for stem cell proliferation and differentiation (Sato *et al.*, 2011). Further work is necessary to determine if and how glutamine changes these signal pathways to promote Lgr5 stem cell development. Some studies indicate that supplemental glutamine had no effect on lysozyme and angiogenin expressions that are Paneth cell markers (Liu *et al.*, 2017; Chen *et al.*, 2018a). For example, glutamine at a dosage of 2% did not promote mRNA expression in other Paneth cell-associated factors such as α-defensins (cryptdin-1, -4 and -5), lysozyme C and angiogenin 4 in the ileum of mouse models (Liu *et al.*, 2017). Glutamine at a dosage of 10 mg/ml fed to weaning mice did not affect the number of absorptive enterocytes, Paneth cells, goblet cells, enteroendocrine cells or the gene expression of these cell markers (Chen *et al.*, 2018a). However, the *in vitro* administration of 10 mmol glutamine increased chromogranin A and mucin 2 expression. These are differentiation markers for enteroendocrine and goblet cells, respectively (Chen *et al.*, 2018b). This apparent discrepancy may result from differences between *in vivo* and *in vitro* studies. Whether glutamine affects weaning piglet intestinal cell differentiation warrants further investigation.

Glutamine and intestinal cell apoptosis. Various factors, such as glutamine deprivation (Papaconstantinou *et al.*, 1998), toxins (Wu *et al.*, 2014) and inflammation (Crespo *et al.*, 2012), have been reported to induce intestinal cell apoptosis. Glutamine administered rectally to rat with colitis reduced activating transcription factor 6 (ATF6), activating transcription factor 4 (ATF4) and spliced X-box-binding protein-1 (XBP-1) mRNA levels. These are transmembrane proteins in the endoplasmic reticulum that have pro-endoplasmic reticulum stress effects. Glutamine (25 mg/dl) administration inhibited the pro-apoptotic protein expression of phospho-p53 and cytochrome c, and decreased activities of apoptotic markers including caspase-9, caspase-8 and caspase-3 (Crespo *et al.*, 2012). Double immunofluorescence staining showed co-localization of C/enhancer binding protein homologous protein (CHOP) and cleaved caspase-3 in colon sections (Crespo *et al.*, 2012). When 2% glutamate was given to piglets weaned at

28 days of age, plasma superoxide dismutase and glutathione peroxidase activities were increased after being challenged with deoxynivalenol for 30 days (Wu *et al.*, 2014). Significantly increasing proliferating indexes and markedly decreasing caspase-3 activities in the jejunum and the ileum were observed in toxin-challenged piglets treated with 2% glutamate (Wu *et al.*, 2014). These results indicate that glutamine is beneficial to alleviate intestinal cell apoptosis.

Glutamine and intestinal cell endoplasmic reticulum stress. Glutamine (0.45 g/l) lessened endoplasmic reticulum stress in intestinal porcine epithelial cell line J2 (IPEC-J2) treated with tunicamycin, an endoplasmic reticulum stress inducer (Jiang *et al.*, 2017). First, IPEC-J2 was exposed to tunicamycin and spontaneous endoplasmic reticulum stress was observed. It was exhibited by upregulation of the glucose-regulated protein 78 (GRP78). Second, prolonged tunicamycin treatment induced apoptosis mediated by CHOP, and this was accompanied by GRP78 downregulation (Jiang *et al.*, 2017). Third, stressed cells that were treated with 0.45 g/l L-glutamine maintained a high level of GRP78 while CHOP-mediated apoptosis was alleviated. The inositol requiring enzyme1 α (IRE1 α)-XBP1 axis was also activated and cell proliferation promoted. Finally, a specific inhibitor of the IRE1 α -XBP1 axis reversed the protective effect of L-glutamine by blocking the expression of IRE1 α /XBP1s (Jiang *et al.*, 2017). These findings indicate that L-glutamine facilitates intestinal health partly through modulating endoplasmic reticulum stress and CHOP-mediated apoptosis. These data give some insight into the mechanism by which glutamine repairs intestinal damage in weaned piglets.

Glutamine and intestinal cell autophagy. Glutamine affects autophagy and coordinates cell growth and proliferation in various cells, including cancer cells (Nicklin *et al.*, 2009) and porcine intestinal cells (Zhu *et al.*, 2015). Glutamine deprivation in porcine intestinal epithelial cells (IPEC-1) increased the autophagy marker light chain 3B-II in a time-dependent manner and decreased cell growth number in a time-dependent manner (Zhu *et al.*, 2015). Glutamine supplementation at 5 mmol/l increased cell growth and cellular protein content and repressed IPEC-1 cell light chain 3B-II protein abundance (Zhu *et al.*, 2015). Glutamine deprivation reduced mammalian target of rapamycin (mTOR) activation and its downstream protein p70S6 kinase. It reduced mitogen-activated protein kinase (MAPK) phosphorylation and its downstream extracellular signal-related kinase (ERK) 1/2 protein (Zhu *et al.*, 2015) indicating that glutamine may suppresses cell autophagy by activating mTOR and MAPK signaling pathways. Nuclear factor kappa B (NF- κ B) and nuclear factor erythroid 2-related factor 2 (Nrf2) are both known to act as transcription factors in stress adaptation and tissue protection (Ringseis *et al.*, 2016). Weaning piglets exposed to hydrogen peroxide were found to form cell autophagosome in the jejunum (Yin *et al.*, 2015a). Nuclear factor kappa B was triggered along with Nrf2, and Keap1, a Kelch-like ECH-associated 23 protein 1, signaling pathway (Yin *et al.*, 2015a). When 6-week-old pigs were

fed a diet with oxidized fat (rapeseed oil that had been heated to 175°C for 72 h) for 29 days, mRNA concentrations in Nrf2 target genes, including NAD(P)H dehydrogenase, quinone 1, peroxiredoxin 6, glutathione peroxidase 2 and thioredoxin reductase 1 in the duodenal mucosa, were upregulated (Ringseis *et al.*, 2016). Protein concentrations of active or cleaved caspase-3 in the duodenal mucosa increased approximately 30% (Ringseis *et al.*, 2016). The association between glutamine regulation of piglet small intestinal autophagy and Nrf2/Keap1 signal pathway remains unknown.

Glutamine and intestinal paracellular permeability

The intestinal epithelium provides several extrinsic and intrinsic defense mechanisms. One is the permeability barrier that is regulated predominantly by tight junctions (Turner, 2009). The beneficial effect of glutamine on intestinal paracellular permeability has been well described in humans, animals (Wang *et al.*, 2014) and various cell lines (Li and Neu, 2009; Wang *et al.*, 2016a). For example, glutamine depletion results in porcine villus atrophy (Wu *et al.*, 1996). Intestinal permeability was increased by 40% by weaning (Wang *et al.*, 2014), and this was accompanied by villus atrophy and reduced jejunal protein expression, including occludin, claudin-1, zonula occludens-2 (ZO-2) and ZO-3. Dietary 1% L-glutamine supplementation attenuated the weaning stress-induced mucosal barrier dysfunctions although it did not return growth performance, villus morphology, tight junction protein expression (occludin, claudin-1, zonula occludens-2) to levels exhibited by sow-reared piglets (Wang *et al.*, 2014). The underlying mechanism for glutamine's regulatory effect on paracellular permeability may involve multiple signal pathways. Glutamine at 4.0 mmol/l may regulate claudin-expression via the PI3K/AKT pathway in Caco-2 cells (Li and Neu, 2009). Glutamine at 2.0 mmol/l was found to regulate claudin-1, claudin-4 and ZO-1 distributions through activated phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) but via CaMKK2-AMPK, calmodulin-dependent kinase 2 to AMP-activated protein kinase, in IPEC-1 cells (Wang *et al.*, 2016a). One recent study observed that 2.0 mmol/l glutamine prevented EGF receptor degradation and activated EGF receptor tyrosine kinase in intestinal epithelial-6 cells. Glutamine-mediated EGF receptor signaling activated ERK1/2 and inactivated p38MAPK in the heat-stressed cells (Niederlechner *et al.*, 2013). These results suggest that glutamine is an important metabolic checkpoint and may have distinct regulating pathways in different cells. The type of glutamine being added may influence outcomes. When Caco-2 cells are treated with acetaldehyde (600 μ mol), there is a time-dependent decrease in transepithelial electrical resistance and an increase in inulin permeability. However, supplementation with L-glutamine (2 mmol) effectively prevented the acetaldehyde-induced changes in transepithelial electrical resistance and inulin permeability. Interestingly, D-Glutamine (2 mmol) or a glutaminase inhibitor by themselves did not influence transepithelial electrical resistance or inulin flux in control or acetaldehyde-treated Caco-2 cell


monolayers (Seth *et al.*, 2004). Glutaminase is responsible for catalysing glutamine into glutamate and ammonia suggesting that L-glutamine rather than D-glutamine or glutamate protects the barrier function in Caco-2 cell monolayers from acetaldehyde-induced injury.

Conclusions

Despite glutamine not being considered essential in the traditional sense, there is growing evidence that glutamine supplementation alleviates the negative effects induced by weaning. A healthy gut is extremely important for animal growth and development. Glutamine is a functional amino acid involved in many metabolic processes that are especially important for small intestinal health and development. Our review addressed the mechanisms involved and the physiological significance for dietary glutamine in small intestine of weaning pigs. This nutrient is necessary to support maximum growth by reducing weaning piglet intestinal oxidative stress, preventing intestinal epithelium damage and improving normal barrier function.

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Declaration of interest

None.

Ethics statement

All ethical standards have been met.

Software and data repository resources

None.

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