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Specific roles of threonine in intestinal mucosal integrity and barrier function

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1. ABSTRACT

Threonine is the second or third limiting amino acid in swine or poultry diets. This nutrient plays a critical role in the maintenance of intestinal mucosal integrity and barrier function, which can be indicated by intestinal morphology, mucus production (number of goblet cells), transepithelial permeability, brush border enzyme activity, and growth performance. Dietary threonine restriction may decrease the production of digestive enzymes and increase mucosal paracellular permeability. A large proportion of dietary threonine is utilized for intestinal-mucosal protein synthesis, especially for mucin synthesis, and there is no oxidation of threonine by enterocytes. Because mucin proteins cannot be digested and reused, intestinal mucin secretion is a net loss of threonine from the body. Luminal threonine availability can influence synthesis of intestinal mucins and other proteins. Under pathological conditions, such as ileitis and sepsis, threonine requirement may be increased to maintain intestinal morphology and physiology. Collectively, knowledge about the role of threonine in mucin synthesis is critical for improving gut health under physiological and pathological conditions in animals and humans.

2. INTRODUCTION

Threonine (also known as α-amino-β-hydroxybutyric acid) was first isolated from fibrin by McCoy, Meyer, and Rose (1). It is well-known as the second or third limiting amino acid in poultry or swine diet (2, 3). Since 1970's, numerous studies have focused on the requirement, efficacy, and metabolism of threonine (4-7). Adequate threonine is needed to support optimum growth and immune function of animals, while threonine excess or deficiency can reduce feed intake, decrease growth rate, and impair immune function (8, 9). Recently, many researchers have investigated the relationship between intestinal threonine metabolism and intestinal health in animals and humans (9-11). The intestine, a highly secretary and proliferative tissue, plays a multitude of functions, such as nutrient digestion and absorption, and immune defense from pathogens and toxins (12). To a large degree, the gut function depends on the intestinal mucosa integrity. The intestinal mucosa, composed of columnar epithelial cells, lamina propria and muscular mucosa (12), can secret considerable amounts of digestive hydrolases and protect the organisms from harmful substances (13-15). The purpose of this review is to provide an insight into the

critical role of threonine in intestinal mucosal integrity and barrier function.

3. THE INTESTINAL MUCOSAL INTEGRITY AND BARRIER FUNCTION

3.1. The intestinal mucosal integrity

Intestinal mucosal integrity can be assessed by intestinal morphology, mucus production (number of goblet cells), transepithelial permeability, brush border enzyme activity, and growth performance (16). Small intestinal integrity, which is most commonly evaluated by histological measurements of villus height, villus surface area, and crypt depth (16). The intestinal mucus covers the mucosa with a semisolid gel to function as a diffusion barrier for the solutes with low molecular weight and as a physical barrier for microorganisms and their toxins (17). The actual mucus production can hardly be measured directly. However, it can be estimated indirectly by numbers of goblet cells (16). Transepithelial permeability can be determined using passive diffusion of a marker or Ussing chambers (18, 19). The increase in transepithelial permeability can decrease the intestinal mucosal integrity. As a result, pathogens and toxins may cross the mucosal epithelial barrier. The activities of brush border enzymes (including sucrase, lactase, maltase, and isomaltase) are also the indicators of intestinal mucosal integrity and function. In addition, the mass of intestine and mucosae, as well as their daily gain, can be indicative of intestinal mucosal integrity (16). Many factors can affect the intestinal mucosal integrity, such as the route of nutrient administration, sources and levels of energy and protein intake, and specific dietary components (e.g., amino acids, fatty acids, and probiotics). Among these factors, amino acids have the most profound effects.

3.2. The intestinal mucosal barrier function

The intestinal mucosal barrier acts as the first defense line against the luminal hostile environment (20). Under physiological conditions, this barrier only allows minute quantities of intact antigens to penetrate into the mucosa to down-regulate inflammation. Under pathological conditions, this barrier may be impaired. As a result, excessive antigens pass through the epithelial layer and result in chronic gastrointestinal inflammation (21, 22). Therefore, the intestinal mucosal barrier function is crucial for animal and human health.

4. THE MAINTENANCE OF INTESTINAL MUCOSAL INTEGRITY

The maintenance of intestinal mucosal integrity mainly depends on the mucosal barrier defense which is composed of specific immunological responses and non-specific barrier mechanisms (14, 23, 24).

4.1. Specific immunological responses of intestinal mucosa

The specific immune system in the intestinal mucosa largely differs from other immune systems of the body (25). The specific immunological responses of the intestinal-mucosal immune system include (a) expression of immunoglobulin A on the apical luminal surfaces; and (2) the sensitized lymphocytes on Peyer's patches and lymphoid follicles, as well as in the lamina propria and the intramucosal epithelium (23, 26).

4.2. Non-specific barrier mechanisms of intestinal mucosa

In addition to the specific immunological responses, the maintenance of intestinal mucosal integrity depends on the non-specific barrier mechanisms which consist of the mucosal-epithelial regenerating capacity, intercellular junctions between the epithelial cells, and the mucus gel layer (15). The mucosal epithelium has high regeneration capacity, owing to the potentially powerful ability of pluripotent stem cells for migration, proliferation and differentiation (27). During the repair of mucosal injury, the epithelial cell restitution is normally achieved by the pluripotent stem cells (28).

The intercellular junctions, including tight junctions, adherent junctions and desmosomes, are also the key components of the non-specific mucosal barrier mechanisms (29, 30). These junctions, formed from transmembrane proteins and nonmembrane proteins, can seal the paracellular space and regulate the intestinal mucosal permeability to macromolecules, such as endotoxins and other bacterial byproducts (14, 31). The mucus gel layer may protect the intestinal mucosa against digestive secretions, pathogens and physico-chemical damage (32-34). The mucus has the viscoelastic and polymer-like properties that are derived from the major gel-forming glycoprotein components, namely mucins. Mucins are secreted by intestinal goblet cells and can be broadly classified into neutral and acidic subtypes. Acidic mucins are further divided into sulfated (sulfomucins) or non-sulfated (sialamucins) groups (35, 36). Because of the analogs between the mucins and the glycoprotein of the enterocyte membrane, they can act as competitors to the binding of many foreign antigens (37, 38). In 2006, Ven der Sluis *et al.* (39) reported that the deficiency of MUC2, a kind of mucins containing high levels of threonine, could lead to colon inflammation in MUC2 knockout mice. Additionally, the mucus gel layer participates in filtering luminal nutrients and can affect the digestion and absorption of nutrients. Furthermore, the mucosa can produce a broad spectrum of antimicrobial agents, such as antimicrobial peptides, to maintain mucosal integrity (40, 41).

5. METABOLIC FATE OF THREONINE IN THE INTESTINE

5.1. Intestinal threonine uptake

Studies with both humans and pigs have shown that 20-70% of the first-pass metabolism of dietary essential amino acids is consumed by the portal-drained viscera (PDV), including the intestines, pancreas, spleen, and stomach (42, 43). Recent studies showed that large amounts (40-60%) of dietary threonine were extracted by the PDV (dominated by the intestine) in first pass metabolism, while the values for other essential amino acids were 30-60% (42, 44-46). In infant studies involving

dual stable-isotope tracer techniques, the intestinal first-pass threonine metabolism was 82% and 70% for partial enteral feeding and full enteral feeding, respectively (47). These values might have been overestimated possibly due to methodological problems, because the efficiency of utilization of dietary threonine for protein accretion in neonates is approximately 60-70%. Dawson *et al.* demonstrated that threonine uptake by the colonic mucosa of humans with carcinoma was higher than that in the normal mucosa (48). In addition, intestinal inflammation enhanced gastrointestinal threonine uptake in enterally fed mini-pigs (49). Likewise, the study conducted by Bertolo *et al.* indicated that the whole-body threonine requirement was decreased by 60% in piglets receiving total parenteral nutrition (TPN) compared with that in piglets receiving enteral nutrition (50). Furthermore, dietary threonine deficiency caused a decrease in intestinal goblet cell numbers and mucin content, which cannot be reversed by intravenous administration of threonine (10). These data indicate that the intestine takes up a large amount of threonine from the lumen but not from arterial blood.

5.2. Intestinal threonine utilization

The intestine is the major site of amino acid utilization and plays an active role in amino acid metabolism (51-53). The amino acids taken up by the intestine can be utilized for protein synthesis, or oxidation into CO_2 for ATP production, or conversion into other amino acids and metabolic substrates (54). Threonine has two metabolic fates in the intestine: (a) incorporation into mucosal proteins [including mucosal cellular proteins and secretary proteins (e.g. mucins)); and (b) catabolism (e.g., oxidation to CO_2) by luminal bacteria (42, 45, 55, 56). Schaart *et al.* (2005) observed that intestinal threonine oxidation in piglets only accounted for 2-9% of the total threonine utilization, while threonine incorporation into mucosal proteins accounted for 71% of the total threonine utilization (46). Thus, threonine extracted by the intestine is primarily used for the mucosal protein synthesis (55). In addition, the peptide backbone of mucins contains large amounts of threonine that represents 28-35% of the total amino acid residues (57-61). Therefore, a large proportion of the threonine extracted by the intestine is used for mucin production. However, mucin proteins cannot be digested and their amino acids cannot be reutilized by the body (44, 59). Thus, the intestinal mucin secretion represents a net loss of threonine from the animal.

6. THREONINE AND INTESTINAL MUCOSAL INTEGRITY AND FUNCTION

It is reported that some specific amino acids, especially threonine, are of critical importance to intestinal mucosal integrity (52, 62). A large amount of dietary threonine taken up and utilized by the intestinal mucosa may aid in maintaining the integrity and function of the intestinal mucosa.

6.1. The role of threonine in maintaining the intestinal mucosal integrity

There is experimental evidence supporting the notion that the availability of dietary threonine can affect intestinal morphology. For example, in 0- to 21-day-old broilers, dietary threonine supplementation significantly increased the weight of duodenum and jejunum, as well as the villous height, epithelial thickness, goblet cell numbers and crypt depth in the duodenum, jejunum, and ileum (63). In neonatal piglets, feeding a threonine-deficient diet (0.1 g threonine/kg body weight per day; fed intra-gastrically) markedly decreased villus heights and villus height-to-crypt depth ratios, compared with the threonine-adequate diet (10). In addition, dietary threonine deficiency (6.5 g threonine/kg diet) in early-weaned piglets induced villus atrophy and reduced villous height, crypt depth, villous height to crypt depth ratio, despite no effects on intestinal weight and length (64). Furthermore, Hamard *et al.* reported that dietary threonine deficiency induces villous hypotrophy in weaned piglets (65). Recently, Wang *et al.* found that either deficiency or excess of dietary threonine dramatically reduced villous area and crypt depth, and induced villous atrophy (66). Additionally, dietary threonine imbalance can increase the apoptosis rate of intestinal epithelial cells (66). These findings indicate that dietary threonine availability is of crucial importance for maintaining the intestinal mucosal structure integrity.

Recently, a large number of studies have focused on the role of dietary threonine availability in the intestinal mucin synthesis in different animal models (Table 1). For example, compared with no threonine perfusion, infusion of threonine (56 mg/g of an amino acid mixture) into isolated porcine gut loops markedly increased the fractional synthesis rates of mucins and total mucosal proteins (66%/day versus 42%/day, and 414%/day versus 323%/day, respectively) (67). This demonstrates that the de novo synthesis of intestinal mucins and mucosal proteins critically depends on the availability of threonine in the intestinal lumen. In addition, piglets fed a deficient or excess dietary threonine (0.37% and 1.11% true ileal digestible (TID) threonine, respectively) remarkably decreased the total amount of mucin in duodenum and mucin-2 mRNA expression in the duodenum and jejunum, and greatly changed the mucin subtypes, compared with piglets fed the optimal level (0.89%) of dietary TID threonine (66). In rats, feeding a diet containing 30% of the threonine requirement for growth severely decreased the mucin fractional synthesis rate in the duodenum, ileum, and colon, but not mucin mRNA expression or intestinal mucosal protein synthesis, compared with the control diet. These data suggest that intestinal mucin synthesis can be substantially impaired by dietary deficiency or excess of threonine (68). Likewise, in 2-day-old piglets, the threonine-deficient diet (0.1 g threonine/kg body weight per day; fed intra-gastrically) severely reduced the total mucin content in the duodenum and colon, as well as acidic mucin subtypes in the small intestine, compared with the threonine adequate diet (0.6 g threonine/kg body weight per day; fed intra-gastrically). In addition, piglets fed a threonine-deficient diet plus intravenous infusion of threonine (0.5 g/kg body weight per day) had smaller goblet cells (10). These data indicate that dietary threonine deficiency can decrease intestinal mucin production. Moreover, threonine supplied by oral route is preferred for the maintenance of the intestinal integrity and barrier function (10).

Table 1. Effects of dietary threonine imbalance and feed administration route on mucosal integrity in animals

Ref. [a]	Treatments [b]	Design [b]	Observations [b]	Remarks
I	Dietary Thr levels: 0.23%, 0.46%, 0.77%, and 1.16%	Animals: male Sprague-Dawley rats, 158 ± 1 g Duration of experiment: 14 days n = 8/treatment	Comparing 0.23% vs. 0.77% Thr, the mucin FSR was lower in the duodenum, ileum and colon; the mucosal protein FSR and mucin mRNA levels did not differ.	Young rats were used in the study.
II	EN 0.1 g/kg/d Thr, EN 0.6 g/kg/d Thr, EN 0.1 g/kg/d Thr + TPN 0.5 g/kg/d Thr	Animals: neonatal piglets, 1~3 d (1.8 kg) Duration of experiment: 8 days n = 7/treatment	Comparing EN 0.1 g/kg/d Thr vs. EN 0.6 g/kg/d Thr, the gut mucosal weigh, mucin content and villus height were reduced; diarrhea occurred in piglets fed the low-Thr diet.	Diarrhea was not due to any apparent disease.
III	EN 0.1 g/kg/d Thr, EN 0.6 g/kg/d Thr, EN 0.1 g/kg/d Thr + TPN 0.5 g/kg/d Thr	Animals: male Yorkshire piglets, 2 d (1.8 ± 0.3 kg) Duration of experiment: 8 days n = 7/treatment	Comparing EN 0.1 g/kg/d Thr vs. EN 0.6 g/kg/d Thr or EN 0.1 g/kg/d Thr + TPN 0.5 g/kg/d Thr, there were higher rates of nitrogen excretion, higher plasma urea and lower plasma threonine; mucosal mass and total crude mucin content were lower in the colons; there were lower numbers of acidic mucin-producing goblet cells in the duodenum and ileum; acidic mucin subtypes were lower in the small intestine, but higher in the colon. Comparing EN 0.1 g/kg/d Thr + TPN 0.5 g/kg/d Thr vs. EN 0.6 g/kg/d Thr, there were smaller colonic goblet cells with more acidic mucins.	Parenteral threonine supply could ameliorate most of the symptoms of dietary threonine deficiency.
IV	Dietary Thr levels: 0.37%, 0.74%, and 1.11%	Animals: weaned crossbred barrows, 21 d Adaptation period: 4 days Duration of experiment: 14 days n = 6/treatment	Comparing 0.37 % or 1.11% Thr vs. 0.74% Thr, the FSR of protein in jejunal mucosal and mucins was reduced; the ASR of protein in the jejunal mucosa and mucins was reduced.	The imbalance of dietary threonine reduced protein synthesis of skeletal muscle.
V	Dietary Thr levels: 0.65%, 0.93%	Animals: weaned crossbred piglets, 7 d Duration of experiment: 14 days n = 11/treatment (experiment 1) or 15/treatment (experiment 2)	Comparing 0.65% Thr vs. 0.93% Thr, in the small intestine, the protein deposition, FSR and amino acid composition of protein did not differ; ubiquitin mRNA level was decreased in the jejunum.	The data on FSR and ubiquitin mRNA levels were derived from Experiment 1. The data on amino acid composition in proteins were derived from Experiment 2.
VI	Dietary Thr levels: 0.65% and 0.93%	Animals: weaned crossbred piglets, 7 d Duration of experiment: 14 days n = 7/treatment	Comparing 0.65% Thr vs. 0.93% Thr, the paracellular permeability was increased in the ileum; the expression of genes encoding MUC1, SGLT1 and ZO-1 was increased.	Synthesis of mucosal and mucin proteins was not measured.
VII	Dietary Thr levels: 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1.0%, and 1.1%	Animals: male broiler chicken, 1 d Duration of experiment: 21 days n = 20/treatment	Thr supplementation affected goblet cell number, epithelial thickness and morphology in the duodenum, jejunum and ileum.	Interactions between crude protein levels and dietary Thr levels were studied. The crude protein levels were 16% and 19%.
VIII	Dietary Thr levels: 0.33%, 0.58%, and 0.82%	Animals: Ross broiler cockerels and White Pekin drakes, 1 d Duration of experiment: 28 days n = 4-8/treatment (experiment 1-4)	With increasing levels of dietary Thr, intestinal crude mucin excretion was increased in broilers and ducklings; intestinal MUC2 mRNA abundance increased as dietary Thr increased in ducklings, but not in broilers.	The data on crude mucins were derived from Experiments 1-4. The data on mucin gene expression were derived from Experiments 3-4.
IX	Intestinal infusion: 0, 21, and 56 mg Thr/g of total amino acids	Animals: Yorkshire Piglets, ~10 kg Duration of experiment: 120 min n = 6/treatment	Increasing the infusion of Thr, the FSR of mucosal and mucin proteins was increased.	A complete mixture of amino acids containing different levels of Thr was continuously infused.

[a] References: I: Faure *et al.* 2005 (68); II: Law *et al.* 2000 (72); III: Law *et al.* 2007 (10); IV: Wang *et al.* 2007; V: Hamard *et al.* 2009; VI: Hamard *et al.* 2009 (70); VII: Zaefarian *et al.* 2008 (63); VIII: Horn *et al.* 2009 (69); IX: Nichols and Bertolo 2008 (67). [b] Abbreviations: Thr: threonine; EN: enteral nutrition; TPN: total parenteral nutrition; d: day; min: minute; FSR: fractional protein synthesis rate; ASR: absolute protein synthesis rate; MUC: mucin; SGLT1: sodium/glucose cotransporter; ZO-1: cingulin and myosin light chain kinase.

There are different reports in the literature regarding effects of dietary threonine on intestinal-mucosal protein synthesis, possibly due to different levels of dietary threonine, animal sepsis, and animal ages. For example, in broiler chicken and White Pekin ducklings, Horn *et al.* (69) observed that dietary threonine restriction impaired intestinal mucin synthesis. Moreover, Wang *et al.* (70) reported that the excess of dietary threonine reduced the synthesis of mucosal proteins and mucins in piglets. However, Hamard *et al.* demonstrated that in early-weaned piglets, a low threonine diet (6.5 g threonine/kg diet) didn't affect the fractional synthesis rate of intestinal mucosal proteins, in comparison with the control diet (9.3 g threonine/kg diet) (64). Under pathological conditions, such as ileitis and sepsis, threonine requirement is enhanced because of the increase in mucin synthesis to maintain intestinal mucosal integrity. For instance, mucin fractional synthesis rate was higher in adult mini-pigs with ileitis induced by direct administration of trinitrobenzene sulfonic acid into the ileum, in comparison with the control group (114%/day versus 61%/day) (49). This indicates that intestinal inflammation would increase mucin synthesis to protect the gut, which may

Table 2.. Effects of threonine levels on intestinal mucosal integrity in animals and humans under unhealthy conditions

Ref. [a]	Treatment [b]	Design	Observations	Remarks
I	Dietary Thr levels: 0.57%, 1.07%, and 2.07%	Animals: male Sprague-Dawley rats, 10 months of age Duration of experiment: 20 days n = 8/treatment Unhealthy condition: intestinal inflammation	Comparing 1.07 or 2.07% Thr vs. 0.57% Thr, the number of MUC2-containing goblet cells was increased in the surfaced epithelium of the ulcerated area; mucin synthesis and production in the colon was enhanced; the mucosal mass was increased; the gut microbiota was reequilibrated.	Intestinal inflammation was induced by the treatment of dextran sulfate sodium
II	Dietary Thr levels 140 mg/kg/d Thr, i.g. 4 μmol/kg/d L-[^{15}N]Thr, i.v. 4 μmol/kg/d L-[U-^{13}C]Thr	Animals: Pitmann-Moore minipigs, 10 mo Duration of experiment: 7 days n = 4/treatment Unhealthy condition: ileitis	Comparing ileitis mini-pigs vs. normal mini-pigs, intestinal mucin synthesis and PDV utilization of Thr were increased.	Ileitis was induced by the direct administration of Trinitrobenzene sulfonic acid into ileum
III	i.g. 2.1 μmol/g weight L-[U-^{13}C]Thr	Animals: Female MUC2 knockout or normal mice, 8 weeks of age Duration of experiment: 120 min n = 17/treatment Unhealthy condition: intestinal inflammation	Comparing normal mice vs. MUC2 knockout mice, there were no differences in the concentration of free or protein-bound Thr in both serum and colon; however, there was higher rate of Thr oxidation.	MUC2 knockout could induced the intestinal inflammation in mice
IV	i.v. 500μmol/100g weight L-[U-^{13}C]Thr	Animals: male Sprague-Dawley rats, 300 g body weight Duration of experiment: 2 days n = 14/treatment Unhealthy condition: sepsis	Comparing sepsis rats vs. normal rats, Thr utilization was increased by the mucosa for mucin synthesis.	The sepsis of rats was induced by injecting live E. coli via a tail vein in day 2 and 6
V	i.v. 500 μmol/100 g weight L-[U-^{13}C]Thr	Animals: male Sprague-Dawley rats, 300 g body weight Duration of experiment: 6 days n = 12/treatment (d 2) or 14/treatment (d 6) Unhealthy condition: sepsis	Comparing sepsis rats vs. normal rats, the mucin content and mucosal protein synthesis were increased; plasma protein ASR was increased.	The sepsis of rats was induced by injecting live *E. coli* via a tail vein in day 2 and 6
VI	i.v. 4.6 mg/kg weight Thr	Men: 30~43 years of age Duration of experiment: 150 min n = 7 or 8/treatment Unhealthy condition: HIV seropositive	Comparing HIV patient vs. normal men, there was a selective deficiency in threonine.	A complete amino acid mixture containing different levels of Thr was continuously perfused
VII	Thr levels D,L-[G-^{3}H]Thr 100 μCi/mL	Tissue: intestinal biopsies of patients Duration of experiment: 0~60 min n = 6/treatment Unhealthy condition: carcinoma	Comparing the intestine of carcinoma patients vs. the intestine of patients with no known intestinal disease, Thr uptake was increased; most of the Thr was incorporated into the immature cells at the bottom of the crypt.	Intestinal biopsies of patients were cultured as the model of *in vitro* experiment.
VIII	Dietary Thr levels: 0.51%, 0.58%, 0.65%, 0.72%, 0.79%, and 0.86%	Animals: male Ross × Ross chickens, 21 days of age Duration of experiment: 21 days n = 8/treatment Unhealthy condition: unclean environment	Comparing the unclean environment vs. clean environment, the basal need for Thr by broilers was increased; the relative thymus weight was higher; monocyte NO production was decreased; the higher needs for Thr of broilers could reflect the resulting changes in mucin production.	A good gradient of Thr in the diets; Thr may influence immunity in chickens.
IX	Dietary Thr level: 0.63%	Animals: male piglets, 10 kg body-weight Duration of experiment: 16 days n = 6/treatment Unhealthy condition: anti-nutritive factors in diets	Comparing diets containing anti-nutritive factors vs. the control diet, Thr digestibility was lower; the reduction in apparent Thr digestibility was correlated to an increase in intestinal mucin production.	A short-term study; luminal Thr is crucial for mucin production by the gut.

[a] References: I: Faure *et al.* 2006 (11); II: Rémond *et al.* 2009 (49); III: Van Der Sluis *et al.* 2009 (73); IV: Faure *et al.* 2004 (74); V: Faure *et al.* 2007 (75); VI: Laurichesse *et al.* 1998 (76); VII: Dawson and Filipe 1982 (48); VIII: Corzo *et al.* 2007 (77); IX: Myrie *et al.* 2003 (78). [b] Abbreviations: Thr: threonine; i.v.: intravenous administration; i.g.: intragastric administration; d: day; min: minute; wk: week; mo: month; ASR: absolute protein synthesis rate; MUC: mucin; PDV: portal-drained viscera; NO: nitric oxide; HIV: human immunodeficiency viurs.

necessitate a greater amount of dietary threonine. Besides, Faure *et al.* demonstrated that sepsis increased mucin fractional synthesis rate and absolute synthesis rate in rats. Collectively, dietary threonine availability is a major determinant of intestinal mucin production.

As mentioned above, intestinal paracellular permeability can be used to assess the intestinal mucosal integrity. With the increase in paracellular permeability, the intestinal integrity and epithelial barrier may decrease. A moderate threonine deficiency (6.5 g threonine/kg diet) increased the intestinal mucosal paracellular permeability in the ileum of piglets, and changed the expression of genes related with the regulation of intestinal mucosal paracellular permeability, such as tight junction protein ZO-1, cingulin, and myosin light chain kinase (65). Furthermore, the digestive enzymes contain abundance of threonine which accounts for 5-11% of the total amino acid residues. Research findings have shown that dietary threonine restriction decreased the production of digestive enzymes (71). Thus, we can speculate that dietary threonine availability may the digestion and absorption of dietary nutrients. Collectively, both intestinal paracellular permeability and brush border enzyme activities are important indicators of the intestinal mucosal integrity.

6.2. Threonine and the intestinal mucosal barrier function

Dietary threonine imbalance influences the intestinal-mucosal integrity and barrier function. In 2000, Law *et al.* (72) reported that dietary threonine deficiency (0.1 g threonine/kg body weight per day; fed intra-gastrically) resulted in diarrhea in piglets. Recently, studies with animals and humans with intestinal inflammation (11, 49, 73), sepsis (74, 75), colonic carcinoma (48), HIV

infection (76) or other types of immunological challenge (77, 78) revealed an increase in threonine requirement by the intestinal mucosa due to enhanced synthesis of intestinal proteins (Table 2). Under these pathological conditions, supply of threonine in regular diets designed for healthy animals may be inadequate for the maintenance of intestinal mucosal integrity, leading to the impairment of intestinal barrier function. Interestingly, some of these studies also showed increasing dietary threonine provision with or without other amino acids enhanced mucin synthesis and re-equilibrated the gut microbiota to benefit gut function (11, 49, 75).

Threonine is a major component of plasma immunoglobulins in animals and humans (79-81). Some studies (8, 12, 82-84) with different animal species demonstrated that dietary threonine levels influenced plasma antibody concentrations and whole-body immune function. Furthermore, results of our research indicated that dietary threonine supplementation improved the intestinal morphology and specific immunological responses in the piglets challenged with *E. coli* $K88^+$ (data no published). These findings suggest that dietary threonine availability is of great importance for supporting both intestinal-mucosal and whole-body immunity.

7. CONCLUSION AND PERSPECTIVES

Intestinal mucosal integrity, which is essential for nutrient digestion and absorption, as well as mucosal barrier function (e.g., protecting the host from gut-related diseases), critically depends on adequate provision of dietary threonine. Deficiency or excess of dietary threonine is deleterious to the intestinal mucosal integrity and barrier function. While considerable advances have been made in threonine nutrition research, much remains to be learned about the signaling pathways through which dietary threonine regulates villous height, crypt depth, goblet cell numbers, and mucin synthesis. Moreover, because many factors can affect the intestinal mucosal integrity, including the route of nutrient administration, the source and level of energy and protein intake, and specific dietary component, attention should be paid to interactions between threonine and these factors. Solving such problems requires combined applications of modern high-throughput and high-efficient technologies, such as genomics, proteomics, and metabolomics (85-88). This is expected to be a challenging but fruitful area of investigation in protein nutrition.

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Abbreviations: Thr: threonine; i.v.: intravenous administration; i.g.: intra-gastric administration; d: day; min: minute; wk: week; mo: month; ASR: absolute protein synthesis rate; MUC: mucin; PDV: portal-drained viscera; NO: nitric oxide; HIV: human immunodeficiency viurs

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