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Review: A systematic review of the effects of functional amino acids on small intestine barrier function and immunity in piglets

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ABSTRACT

The need to reduce the use of antibiotics and zinc oxide at the pharmacological level, while preserving the performance of postweaning piglets, involves finding adequate nutritional strategies which, coupled with other preventive strategies, act to improve the sustainability of the piglet-rearing system. Amino acids (**AAs**) are the building blocks of proteins; however, they also have many other functions within the body. AA supplementation, above the suggested nutritional requirement for piglets, has been investigated in the diets of postweaning piglets to limit the detrimental consequences occurring during this stressful period. A systematic review was carried out to summarise the effects of AAs on gut barrier function and immunity, two of the parameters contributing to gut health. An initial manual literature search was completed using an organised search strategy on PubMed, utilising the search term "<amino acid> AND <parameter related to intestinal health>". These searches yielded 302 articles (published before October 2021); 59 were selected. Based on the method for extracting data (synthesis of evidence), this review showed that L-Arginine, L-Glutamine and L-Glutamate are important functional AAs playing major roles in gut morphology and immune functions. Additional benefits of AA supplementation, refereed to a supplementation above the suggested nutritional requirement for piglets, could also be observed; however, data are needed to provide consistent evidence. Taken together, this review showed that supplementation with AAs during the weaning phase supported a plethora of the physiological functions of piglets. In addition, the data reported confirmed that each amino acid targets different parameters related to gut health, suggesting the existence of potential synergies among them.

Implications

This article evaluated and analysed using a systematic approach and a synthesis of evidence method the available knowledge regarding the effect of functional amino acids on gut barrier function and immunity of piglets. Among the investigated amino acids, the results highlighted that L-Arginine, L-Glutamine and L-Glutamate play a major role in gut morphology and immune functions of piglets. Overall, the supplementation of these in feed-grade crystalline amino acids, provided alone or in combination, could represent a valuable strategy to contribute to sustaining the piglet's gut health and therefore reduce the use of antimicrobials in the feed.

Introduction

In intensive pig production, weaning is a critical period during which piglets face many changes in their rearing condition. They are removed from the sow, are transitioned from a highly digestible and liquid diet to a less digestible diet, and are mixed with pigs originating from other litters in a new environment (Pluske et al., 1997). These changes generate stress, decreasing water and feed intake (Pluske et al., 1997; Trevisi et al., 2021). For instance, Brooks et al. (2001) have reported that 50% of piglets do not consume even one meal in the first 24 hours postweaning. The accumulation of evidence has suggested that the intestine has an elevated requirement for energy in order to maintain its morphology, integrity, and function (Blachier et al., 2009; Burrin and Stoll, 2009). This demand increases in transition phases along with the need for functional gut development. This explains why it is common to observe notable changes in these three parameters (gut morphology, integrity and function) at weaning, together with microbiota modifications (Pluske et al., 1997; Lallès et al., 2007;

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Trevisi et al., 2021). In fact, it has been reported that just-weaned piglets exhibit villous atrophy, crypt hyperplasia and fewer goblet cells involved in mucin synthesis in the small intestine (Lallès et al., 2004). Modification of the intestine morphology alters transepithelial resistance and depresses enzymatic activities (disaccharidase, protease, lipase) increasing cellular infiltration and decreasing nutrient absorption (Lallès et al., 2004; Lallès, 2008). Signs of intestinal dysbiosis can also be noted and are characterised by a reduction in beneficial bacteria (*Lactobacillus sobrius*, *L. acidophilus* and *L. reuteri*) at the expense of opportunistic pathogens, including enterotoxigenic *Escherichia coli* (***E. coli***) (Gresse et al., 2017).

These numerous changes lead to animal discomfort, diarrhoea, impairment of growth and, sometimes, increased morbidity and mortality. Considering the growing pressure to reduce the use of antibiotics and zinc oxide at the pharmacological level while preserving health and performance, it is necessary to find nutritional strategies to limit the severity of these changes (Vondruskova et al., 2010). Amino acids (**AAs**) are the building blocks of proteins; however, they also have many other functions within the body: cell proliferation, immunity modulation, food intake control and energy provision (Wu, 2009; Le Floc'h et al., 2018; Liao, 2021) and can participate in and regulate the interplay between the host and microbiota (Chalvon-Demersay et al., 2021; Beaumont et al., 2022). Indeed, according to the recent review of Chalvon-Demersay et al. (2021), AAs can support or restore the gut health of piglets defined in four main pillars: (i) epithelial barrier and digestion, (ii) immune fitness, (iii) microbiota balance and (iv) oxidative stress homeostasis. Therefore, amino acid supplementation, especially of functional AAs, defined as those that can take part and regulate key metabolic pathways to improve health, survival and growth, could be a good way of coping with the deleterious consequences occurring at weaning. In this context, the aim of this review was to evaluate and analyse using a systematic approach and a synthesis of evidence method the effects of functional amino acid supplementation on the gut health of piglets at the time of weaning, with a special focus on the barrier function of the small intestine and immunity.

Material and methods

This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (Liberati et al., 2009).

Literature search

A structured search strategy was carried out on PubMed and Google Scholar using the combination of an amino acid and a parameter related to intestinal morphology, integrity, or function as shown in Table 1. Furthermore, articles related to this subject, based on their titles, were obtained from the METEX NOOVISTAGO database and from an additional manual search. The articles were then included or excluded based on the criteria outlined below.

The inclusion criteria were the following: (1) articles published in a peer-reviewed journal before October 2021; (2) articles based on interventional studies conducted on piglets challenged or not, and (3) articles focusing on the effects of proteinogenic amino acid supplemented solely. The exclusion criteria were the following: (1) articles not in the English language (2) letters, commentaries, conference papers, reviews (3) articles in which AAs were not provided orally, and (4) articles in which AAs were supplemented as a combination.

The systematic search in PubMed and Google Scholar retrieved a total of 548 titles of which 208 articles were selected based on full title reading; 94 articles from the manual search were assessed for eligibility and were added to the 208 previous articles. Fifty-nine articles fulfilled the inclusion criteria. The study selection is detailed in Supplementary Fig. S1.

The effects of AAs on the parameters related to the following pillars of gut health: epithelial barrier and digestion, and immune fitness as described previously (Chalvon-Demersay et al., 2021) were extrapolated and analysed. The interplay between dietary AAs, microbiota and host, an additional pillar for gut health, was not considered in the present review, however, for information on this, see the reviews of Beaumont et al. (2022) and Blachier and Wu (2022).

In detail, the following parameters were considered because they were those most commonly studied in the articles:

Epithelial barrier and digestion:

- Villous height, crypt depth and villous height-to-crypt depth (**V:C**) ratio
- Number of goblet cells
- Abundance of mucins
- Severity of diarrhoea
- Transepithelial resistance
- Concentration or expression of tight junctions (Zonulin, Occludin, Claudin-1)
- Activity or concentration or expression of digestive enzymes (Sucrase, maltase, lactase)

Immune fitness:

- Concentration or expression of immunoglobulins (**Ig**) A, G and M and secretory IgA in the gut
- Concentration or expression of pro-inflammatory (Interleukin (**IL**) IL-1β, IL-6, IL-8, tumour Necrosis Factor alpha (**TNF-α**) and anti-inflammatory IL-10 cytokines
- Concentration of intraepithelial lymphocytes

Data extraction and analysis

In addition to the parameters related to epithelial barrier and digestion, and immune fitness, the data related to growth performance, including feed intake and average daily gain, were extracted. When the expression, activity and abundance of a

Table 1
Keywords used for the systematic search of the effects of amino acids on small intestine barrier function and immunity in piglets.

Amino acid		Parameters				
Threonine	Glycine	Development	Gut	Physiology	Gut	Diarrhoea
Arginine	Glutamic acid		Intestine		Intestine	Microbiota
Tryptophan	Methionine	Integrity	Gut	Immunity	Gut	Microflora
Lysine	Glutamate		Intestine		Intestine	Mucin
Leucine	Cysteine	Permeability	Gut	Inflammation	Gut	Mucosa
Proline	Aspartate		Intestine		Intestine	
Glutamine	Amino acid	Barrier	Gut	Health	Gut	
			Intestine		Intestine	

specific protein were available in the same study, activity was favoured over abundance and abundance over expression for data extraction. The percentage of the difference between the supplemented group and the control group within each study was extracted when the comparison was statistically significant ($P < 0.05$). Additional data regarding duration of the test, number of piglets per group, population characteristics (including age, BW), level and type of AA supplementation, characteristics of the control diet, average daily gain, food intake, and type and duration of the challenge were also retrieved. The complete list of articles obtained from the literature search is reported in Supplementary Table S1.

As the data were not consistent for all the parameters extracted, the analysis of the literature search was split into two different methods. The first method, based on the synthesis of evidence methodology, was carried out when at least four different studies reported the data for the parameter. The second method, based on a classical synthesis table, was carried out for the parameters included in less than four studies.

The synthesis of evidence methodology was carried out as previously described in published systematic reviews (Voortman et al., 2015; Chalvon-Demersay et al., 2017). The evidence was deemed to be strong or moderate if at least three-quarters or one-half, respectively, of the studies reported consistent results regarding a specific outcome, under the condition that the compound was tested at least four times in four different trials under similar conditions. The findings were considered to be inconclusive below these thresholds. A scheme of the methods applied is reported in Fig. 1.

Results

Characteristics of the studies included

The different compounds tested were AAs (L-Arginine, L-Asparagine, L-Aspartate, L-Cysteine, L-Glutamate, L-Glutamine, L-Isoleucine, L-Leucine, L-Methionine, L-Serine, L-Threonine, L-Tryptophan); the level of supplementation ranged from 0.04 to 6.51% as the fed-basis. The articles were published between 1996 and 2021. The subjects were piglets with 25.75 ± 1.01 days of age (12.5–46), weighing 7.23 ± 0.21 (3.94–12.23) kg. On average, the duration of the supplementation was 18.66 ± 0.86 days and ranged from 5 to 42 days. The piglets were challenged or not. The different challenges used were the following: *E. coli*, Porcine rotavirus, transport, transport combined with heat stress, low sanitary conditions and diquat (an herbicide) (Supplementary Table S1).

According to the data extracted, the synthesis of evidence was carried out for the growth parameters and the intestinal morphological parameters while, for the other parameters, classical synthesis tables were utilised.

Feed intake and average daily gain

The decrease in feed intake is primarily responsible for the intestinal changes that occur at weaning (Lallès et al., 2007). Therefore, the effect of the amino acid supplementations (L-Arginine, L-Aspartate, L-Glutamate, L-Glutamine, L-Threonine, L-Tryptophan) on feed intake and BW gain was reviewed. Based on the synthesis of evidence methodology, it was observed that the supplementation of the majority of the AAs (L-Arginine, L-Aspartate, L-Glutamate, L-Glutamine) did not improve feed intake or average daily gain (Fig. 2). Instead, supplementation with L-Tryptophan was associated with an improvement in both feed intake and average daily gain while supplementation with L-Threonine was associated only with an improvement in feed intake (Fig. 2). For the other AAs, fewer than four studies reported data for feed intake and BW gain.

Epithelial barrier and digestion

The different outcomes related to epithelial barrier and digestion were defined by villous height, crypt depth, V:C ratio, number of goblet cells, the abundance of mucins, the severity of diarrhoea, transepithelial resistance, concentration or expression of tight junctions (Zonulin, Occludin, Claudin-1), and activity or concentration or expression of digestive enzymes (sucrase, maltase, lactase).

Based on the articles collected, the synthesis of evidence was carried out on the morphology parameters, and L-Arginine, L-Glutamate, L-Glutamine, L-Tryptophan as AAs. The results of the synthesis of evidence of the effect of L-Arginine, L-Glutamate, L-Glutamine, and L-Tryptophan on villous height, crypt depth and the V:C ratio in the duodenum, jejunum and ileum are shown in Fig. 3. Based on the synthesis of evidence, it can be concluded that L-Arginine supplementation could consistently improve villous height in the duodenum as >70% of the tests reported that L-Arginine supplementation was associated with an increase in villous height. On the contrary, L-Arginine supplementation had no effect on other segments of the intestine. L-Glutamate supplementation was associated with an improvement in villous height in the duodenum and jejunum, and L-Glutamine supplementation was associated with an improvement in the V:C ratio in the duodenum and villous height in the jejunum. L-Tryptophan did not affect the villous height; however, it consistently reduced crypt depth in the jejunum (Fig. 3).

For the other AAs, fewer than four studies reported data for the morphological parameters; however, some information can be obtained from the literature. Table 2 summarises the effect of AAs not included in the synthesis of evidence on the jejunal

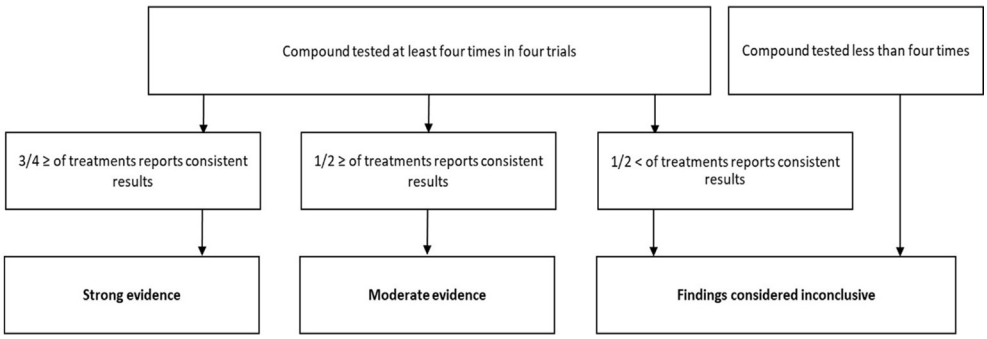

Fig. 1. Methodology followed for the synthesis of evidence to evaluate the effects of amino acids on small intestine barrier function and immunity in piglets.

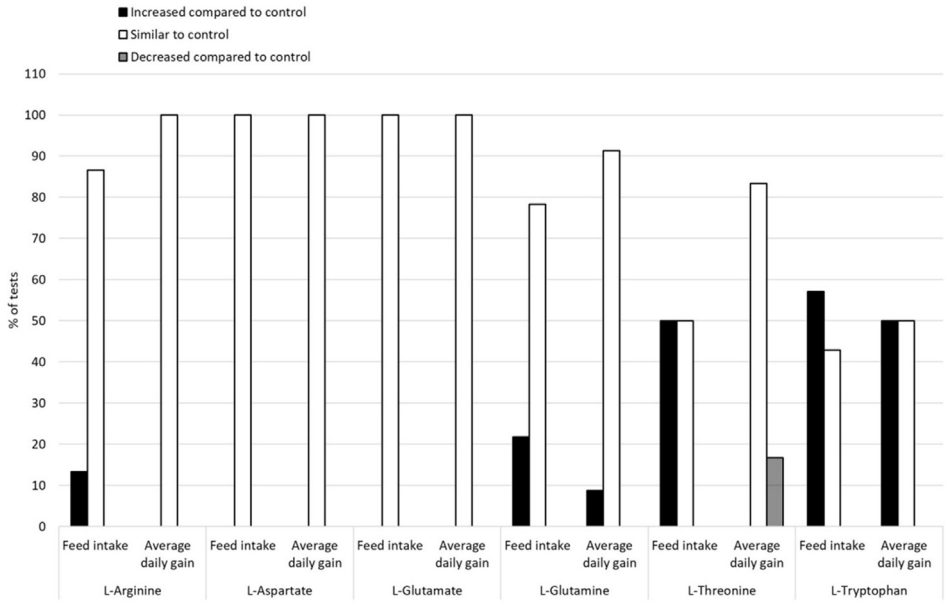

Fig. 2. Synthesis of evidence of the effects of L-Arginine, L-Aspartate, L-Glutamate, L-Glutamine, Threonine, and L-Tryptophan on feed intake and average daily gain of postweaning pigs.

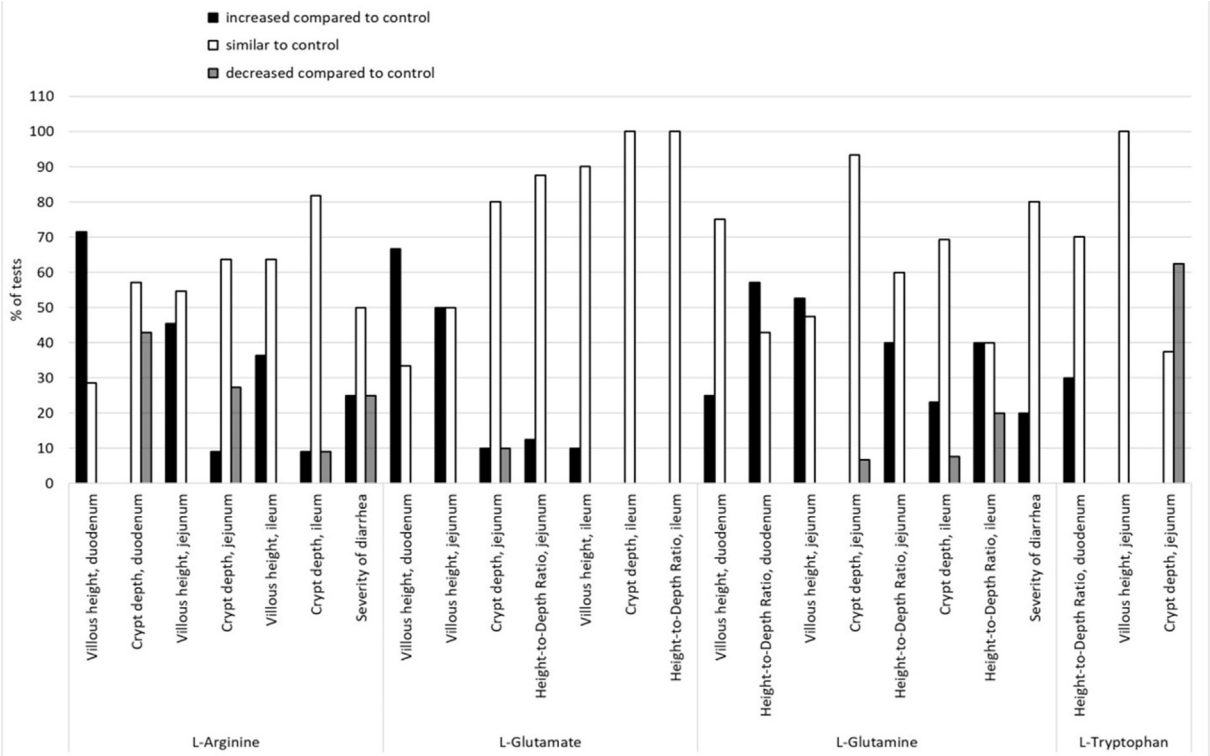

Fig. 3. Synthesis of evidence of the effects of L-Arginine, L-Glutamate, L-Glutamine, and L-Tryptophan on the parameters of the gut morphology in postweaning pigs.

mucosa. L-Aspartate and L-Cysteine have also been suggested as modulators of the V:C ratio, promoting its increase in the jejunum (Pi et al., 2014; Song et al., 2016; Wang et al., 2017). Results for L-leucine and L-threonine supplementation are inconsistent as a positive effect of L-leucine on villous height and height-to-depth ration has been reported by Mao et al. (2015) under challenge situation while no effect has been reported by Sun et al. (2015); similarly, Ren et al. (2014) have reported a positive effect of L-threonine on villous height and height-to-depth ration, while according to Trevisi et al. (2015), no effect has been reported on the jejunal mucosa.

Results regarding the effect of AAs on the other parameters related to the epithelial barrier and digestion are limited (Supplementary Table S2; Table 3). For L-Arginine, no information regarding the expression of mucins, zonulin, occludin and claudin were available. L-Arginine supplementation was not related to any

Table 2
The effect of the supplementation of L-Aspargine, L-Aspartate, L-Cysteine, L-Leucine, L-Methionine and L-Threonine on villous height, crypt depth, height-to-depth ratio of jejunum in postweaning pigs.

Item	Villous height	Crypt depth	Height-to-Depth Ratio
L-Aspargine			
	→(0.5%, Chen et al., 2016)	→(0.5%, Chen et al., 2016)	→(0.5%, Chen et al., 2016)
	↑(1%, Chen et al., 2016)	→(1%, Chen et al., 2016)	↑(1%, Chen et al., 2016)
	→(0.5–1%, Wang et al., 2015)	↓(0.5–1%, Wang et al., 2015)	↑(0.5–1%, Wang et al., 2015)
L-Aspartate			
	↑15% (0.5%, Pi et al., 2014)	→(0.5%, Pi et al., 2014)	↑16% (0.5%, Pi et al., 2014)
	↑14% (1%, Pi et al., 2014)	↓14% (1%, Pi et al., 2014)	↑32% (1%, Pi et al., 2014)
	→(0.5% Wang et al., 2017)	→(0.5% Wang et al., 2017)	↑6% (0.5% Wang et al., 2017)
	→(1% Wang et al., 2017)	→(1% Wang et al., 2017)	↑8% (1% Wang et al., 2017)
	→(2%, Yin et al., 2015)	→(2%, Yin et al., 2015)	→(2%, Yin et al., 2015)
L-Cysteine			
	↑23% (0.25% Song et al., 2016)	→(0.25% Song et al., 2016)	↑35% (0.25% Song et al., 2016)
	↑26% (0.5% Song et al., 2016)	↓12% (0.5% Song et al., 2016)	↑44% (0.5% Song et al., 2016)
L-Leucine			
	→(1% Mao et al., 2015)	→(1% Mao et al., 2015)	→(1% Mao et al., 2015)
	↑27% (1% Mao et al., 2015)	↓20% (1% Mao et al., 2015)	↑58% (1% Mao et al., 2015)
	→(7% Sun et al., 2015)	→(7% Sun et al., 2015)	→(7% Sun et al., 2015)
L-Methionine			
	↑7% (0.12%Chen et al., 2014)	→(0.12%Chen et al., 2014)	↑10% (0.12% Chen et al., 2014)
	→(0.04% Zeitz et al., 2017)	→(0.04% Zeitz et al., 2017)	→(0.04% Zeitz et al., 2017)
	→(0.08% Zeitz et al., 2017)	→(0.08% Zeitz et al., 2017)	→(0.08% Zeitz et al., 2017)
L-Threonine			
	↑23% (0.75–1.11% Ren et al., 2014)	↓21% (0.75–1.11% Ren et al., 2014)	↑58% (0.75–1.11% Ren et al., 2014)
	→(0.05% Trevisi et al., 2015)	→(0.05% Trevisi et al., 2015)	
		→(0.37% Wang et al., 2010)	
		→(0.52%, Wang et al., 2010)	
		→(0.74%, Wang et al., 2010)	

The percentage following the arrow represents the percentage difference with the control diet.
The percentage shown inside the square bracket represents the dose of supplemental amino acid included in the diet.
→ = similar to the control; ↑ = increased significantly relative to the control; ↓ = decreased significantly relative to the control.

improvement in the level of intestinal maltase, sucrase and lactase, except in the ileum where 0.5% of L-arginine supplementation was associated with an increased activity of sucrase by 43% in relation to the control (Liu et al., 2009) (Table 3). L-Glutamate supplementation could be effective mainly in the tight junctions (zonulin, occludin and claudin) in which expression was increased predominantly in the jejunum (Liu et al., 2002; Qin et al., 2018; Kyoung et al., 2021), but not in the ileum (Qin et al., 2018; Kyoung et al., 2021) in response to the supplementation. No consistent effect of L-Glutamate could be observed for the expression of maltase, sucrase and lactase (Table 3), and for the number of goblet cells as the response varied according to the part of the intestinal tract, the supplemented dose and the studies (Ewtushik et al., 2000; Qin et al. 2018). Scarce or no information regarding the effect of L-Glutamate on the expression of mucins, and transepithelial resistance was available. The effect of the supplementation of L-Glutamine on the gut parameters was mainly investigated in relation to the epithelial barrier function; however, no consistent results could be observed. According to Wang et al. (2015) and McConn et al. (2020), no improvement in the zonulin level could be obtained with L-Glutamine supplementation in the jejunum while Xing et al. (2017) observed a significant increase in its expression. In addition, according to Wang et al. (2015) and Xing et al. (2017), occludin expression in the jejunum was improved by L-Glutamine supplementation, but not according to Ewaschuk et al. (2011). Regarding L-Tryptophan, its effect on the gut epithelial barrier and digestion parameters was not significant either for the goblet cells or for the expression of the tight junction while its effect on mucins, maltase, sucrase and lactase has not yet been investigated. Regarding L-Asparagine, L-Cysteine, Glycine, L-Isoleucine, L-Leucine, L-Methionine, L and L-Threonine, data are still scarce and limited to few studies which, however, report promising results (Supplementary Table S2 and Table 3).

Immune fitness

Results regarding the effect of AAs (L-Arginine, L-Cysteine, L-Glutamate, L-Glutamine, L-Glycine, L-Isoleucine, L-Serine, L-Threonine and L-Tryptophan) on the parameters related to immune fitness are reported in Supplementary Table S3. It should be noted that these parameters have been less investigated than the parameters related to epithelial barrier and digestion in the literature; however, some indications could be observed.

L-Arginine did not contribute to any activation of pro- or anti-inflammatory cytokines while no information regarding the Ig production was available. L-Cysteine supplementation from 0.25 to 0.50% was able to reduce the expression of IL-6, IL-8 and TNF-α in both the jejunum and the ileum; however, the results should be considered cautiously as they come from a single study (Song et al., 2016). L-Glutamate supplementation was associated with an increase in the level of IL-1β and IL-6 (Kyoung et al., 2021), and in the number of intraepithelial lymphocytes in piglets challenged with deoxynivalenol but not in piglets under normal healthy conditions (Wu et al., 2014). Regarding L-Glutamine, the studies of Ewaschuk et al. (2011), Xing et al. (2017) and McConn et al. (2020) suggested that its supplementation was not effective in modulating the levels of IgG, IgM, IL-1β, IL-6, IL-8, IL-10 and TNF-α, while it increased the level of secretory IgA in the jejunum (Xing et al., 2017). Similarly, the level of secretory IgA in the jejunum was also suggested to increase with the supplementation of Glycine (Ji et al., 2021); furthermore, Glycine was suggested to reduce the levels of IL-1β, IL-6 and TNF-α but only in the jejunum (Ji et al., 2021). Two studies investigated the effect of L-Threonine on immune fitness parameters (Ren et al., 2014; Trevisi et al., 2015) and according to them, its supplementation did not improve Ig production in the intestine. Finally, some studies suggested that L-Serine, but not L-Isoleucine, supplementation was associated

Table 3
The effect of the supplementation of L-Arginine, L-Asparagine, L-Aspartate, L-Glutamate and L-Glutamine on the gut parameters related to digestible function of postweaning pigs.

Amino acids	Maltase	Sucrase	Lactase
L-Arginine			
	duo: →(0.5%, Liu et al., 2009) →(1%, Liu et al., 2009) jej: →(0.93%, Ewtushik et al., 2000) →(0.5%, Liu et al., 2009) →(1%, Liu et al., 2009) il: →(0.5%, Liu et al., 2009) →(1%, Liu et al., 2009)	duo: →(0.5%, Liu et al., 2009) →(1%, Liu et al., 2009) jej: →(0.5%, Liu et al., 2009) →(1%, Liu et al., 2009); il:↑43% (0.5%, Liu et al., 2009) →(1%, Liu et al., 2009)	duo: →(0.5%, Liu et al., 2009) →(1%, Liu et al., 2009); jej: →(0.93%, Ewtushik et al., 2000) →(0.5%, Liu et al., 2009) →(1%, Liu et al., 2009) il: →(0.5%, Liu et al., 2009) →(1%, Liu et al., 2009)
L-Aspargine			
	jej: →(0.5–1%, Wang et al., 2015)	jej and il: →(0.5–1%, Wang et al., 2015)	jej and il:↑ (0.5–1%, Wang et al., 2015)
L-Aspartate			
	duo: →(0.5%, Pi et al., 2014) jej: →(0.5%, Pi et al., 2014) →(1%, Pi et al., 2014) il: →(0.5%, Pi et al., 2014)↑23% (1%, Pi et al., 2014)	jej:↑54% (0.5%, Pi et al., 2014) →(1%, Pi et al., 2014) il: →→(0.5%, Pi et al., 2014)↑37% (1%, Pi et al., 2014)	jej:↑74% (0.5%, Pi et al., 2014) →(1%, Pi et al., 2014); il:↑26% (0.5%, Pi et al., 2014)↑ 20% (1%, Pi et al., 2014)
L-Glutamate			
	jej: →(6.5% Ewtushik et al., 2000)↑23% (1%, Qin et al., 2018)↑29% (2%, Qin et al., 2018); il:↑32% (1%, Qin et al., 2018) →(2%, Qin et al., 2018)	jej: →(6.5% Ewtushik et al., 2000) →(1%, Qin et al., 2018) →(2%, Qin et al., 2018); il:↑34% (1%, Qin et al., 2018) →(2%, Qin et al., 2018)	jej: →(1%, Qin et al., 2018) →(2%, Qin et al., 2018); il: →(1%, Qin et al., 2018)↑239% (2%, Qin et al., 2018)
L-Glutamine			
	duo: →(2%Pluske et al., 1996)	duo: →(2%Pluske et al., 1996)	jej: →(1%, Cabrera et al., 2013)

The percentage following the arrow represents the percentage difference with the control diet.
The percentage shown inside the square bracket represents the dose of supplemental amino acid included in the diet.
Abbreviations: duo = duodenum; jej = jejunum; il = ileum.
→ = similar to the control; ↑ = increased significantly relative to the control; ↓ = decreased significantly relative to the control.

with a downregulation of inflammation markers (TNF-α) in the jejunum and ileum (Mao et al., 2018; Zhou et al., 2018).

Discussion

The aim of this systematic review was to clarify the effects of AA supplementation refereed to a supplementation above the suggested nutritional requirement for piglets, on epithelial barrier and digestion, and immune fitness in piglets with the final aim of highlighting the useful use of specific AAs as feeding intervention to sustain piglets to sustain piglets in the current scenario of requested reduction of the use of zinc oxide and antibiotic use. In order to evaluate this, a systematic approach and a synthesis of evidence method have been used in the present review. The synthesis of evidence reported that L-Arginine, L-Glutamate, and L-Glutamine were particularly efficient for promoting intestinal morphology (villous height, V:C ratio). The contribution of L-Arginine to gut health and barrier can be connected to its activity as signalling molecules able to activate the mammalian target of rapamycin (**mTOR**), mitogen-activated protein (**MAP**) kinase and ribosomal protein S6 kinase (p70s6) in the enterocytes (Rhoads et al., 2004; 2008) and to its metabolism and conversion into L-Ornithine via arginase. Ornithine is in turn, metabolised via ornithine decarboxylase to produce polyamines, including putrescine, spermidine and spermine, which are known to protect the intestinal mucosa and to have a trophic action favouring cell proliferation and migration (Timmons et al., 2012). L-Ornithine can also be converted into L-Proline which is involved in cell migration and represent an additional pathway for promoting the intestinal morphology improvement connected to L-Arginine. In addition, the effect of L-Arginine on cell proliferation is related to its catabolism into nitric oxide, which can regulate the tight junction proteins in endothelial cells and the epithelial cell of the intestine with both beneficial and deleterious effects (Alican and Kubes, 1996). The present review also suggested that supplementations >1.2% for L-Arginine could have deleterious effects on intestinal health. Excess L-Arginine is thought to induce endothelin-1 and prostaglandin synthesis in the intestine, leading to a greater incidence of diarrhoea (Maurer et al., 2004; Gookin et al., 2008; Zhan et al., 2008).

L-Glutamine plays many roles related to immunity, development and energy homeostasis in the intestine (Cruzat et al., 2018; Luise et al., 2022). In fact, glutamine can be used as a substrate for the production of nucleic acid, nucleotide, adenosine triphosphate, nicotinamide adenine dinucleotide phosphate and CO_2 (Cruzat et al., 2018), and it can therefore favour an increase in villous height, and the V:C ratio, as previously reported in *in vitro* and *in vivo* studies on rats (Wirén et al., 1998; Boza et al., 2000; Yamauchi et al., 2002). In line with the present results, one study reported that the supplementation of L-Glutamine decreased intestinal permeability and restored the expression of occludin in rats with methotrexate-induced mucositis (Beutheu et al., 2014). The beneficial effect of L-Glutamine is also related to its signalling molecule activity. Its beneficial effect on gut morphological parameters is related to its capacity to increase the functional integrity of the mitochondria, via the activation of heat shock proteins (**HSPs**),

particularly HSP72, which is expressed by enterocytes; furthermore, it can act to prevent the cellular apoptosis under heat stress via the regulation of the mTOR and p38 MAP kinase pathways (Wischmeyer 2002).

The effect of L-Glutamine in the intestine can be mimicked by L-Asparagine as the latter can serve as a carbon donor for L-Glutamine synthesis. This would explain the positive effect of L-Asparagine on the intestinal tight junction in piglets as highlighted in the present review; however, this effect was tested in only one study (Chen et al., 2016). In line with this hypothesis, it has been shown that L-Asparagine is sufficient to suppress the apoptosis induced by L-Glutamine depletion in rapidly proliferating cells (Papaconstantinou et al., 1998; Zhang et al., 2014). L-Glutamine can also contribute to the modulation of the immune cell function and cytokine production as reviewed by Newsholme (2001). The regulation of cytokine expression by L-Glutamine could be connected to its function as an enhancer of the HSP72 expression (Wischmeyer, 2002). However, according to the studies of Ewaschuk et al. (2011), Xing et al., (2017) and McConn et al. (2020) analysed in the present review, no consistent effect of L-Glutamine was observed on these parameters, mainly due to the lack of studies investigating this aspect.

The beneficial effect of L-Aspartate and L-Glutamate supplementation on the intestine is mainly based on their capacity to fuel enterocytes with energy. Moreover, it has been shown that almost all dietary aspartate and glutamate are oxidised by enterocytes (Wu, 1998). The beneficial effect of L-Glutamate could also depend on the fact that it could avoid L-Glutamine utilisation by inhibiting its oxidation in enterocytes (Blachier et al., 1999; He et al., 2016). Furthermore, L-Glutamate has been shown to play a crucial role as an excitatory neurotransmitter in the small intestine; its supplementation was able to modulate the intestine expression of amino acid transporters and some taste receptors, facilitating the repair of intestinal architecture in weaning pigs (Lin et al., 2014). In addition, recently, it has been suggested that supplementation of a mixture of L-Glutamate and L-Glutamine (6 kg/tonne in a ratio 25 + 75% and 50 + 50%) can improve the barrier function of post-weaning piglets, notable by an increase in the number of goblet cells and a reduction in the probability of having intraepithelial lymphocytes in the jejunal mucosa, more than the single AA alone (Luise et al., 2022).

L-Leucine may exert beneficial effects on outcomes related to the intestine morphology and immune function, although its effect has been investigated in only one study in piglets (Mao et al., 2015). In addition to its role as a precursor of L-Glutamine (especially in the muscle) (Wu, 2009), L-Leucine and the other branched-chain AAs (**BCAAs**) can increase protein synthesis and decrease proteolysis via activation of the mTOR signalling pathway in the intestine of piglets and humans (Torrazza et al., 2010; Coëffier et al., 2011). The regulation of mTOR is also the main mechanism through which BCAAs and L-Leucine can influence intestinal immune function (Powell et al., 2012).

L-Threonine has been considered to be an important amino acid for gut health due to its relevant role in the structure of mucins. These constitute the mucus layer which is crucial for non-immune gut barrier functionality (Burrin and Stoll, 2002). It has been suggested that L-Threonine can increase the mucins level, promoting the goblet cell differentiation via modulation of the genes associated with the Notch-Hes1-Math1 pathway as observed in intrauterine growth retarded weanling piglets (Zhang et al., 2019). However, according to Trevisi et al. (2015), it appeared that L-Threonine supplementation could not modify the production of mucins under *E. coli* challenge conditions. It should be noted that the effect of L-Threonine deficiency on intestinal health is well known and, in the studies included in our review, a requirement of threonine was always maintained (Hamard et al., 2009; 2010). In addition, it has been suggested that L-Threonine, like L-Glutamine, could act on the HSPs, affecting apoptosis in the intestinal cells (Baird et al., 2013); therefore, an imbalance in L-Threonine (deficiency or excess) could increase apoptosis and villous atrophy (Wang et al., 2010). Finally, L-Threonine can regulate pro-inflammatory and anti-inflammatory interleukins via mTOR pathway, however, studies in piglets are still limited (Ren et al., 2014).

Overall, the present systematic review provides a critical evaluation of the effects of amino acid supplementation on the intestinal health of piglets, with a special focus on the barrier function of the small intestine and immunity. Despite a strong effort to be rigorous, this study had some limitations. First, some AAs were not tested sufficiently on different outcomes and could not be included in the synthesis of evidence. Second, an arbitrary threshold for the synthesis of evidence was applied; this threshold was based on the Authors' experience taken from the published studies, the number of trials, and the parameters investigated. Some parameters related to intestinal health could have yielded some interesting information; however, they were not considered due to the scarce number of times they were studied in the articles (expression of amino acid transporters, mTOR phosphorylation, heat shock protein activity). In addition, the number of articles was not sufficient to investigate the potential effect of other relevant factors which could modulate the intestinal digestion barrier and immunity function including age, BW and dose of supplementation. Finally, the effect of the combination of AAs was not evaluated in the present review; however, recent articles suggested a beneficial effect of the mixture of different AAs on the gut barrier function and immunity of piglets. In particular, beneficial effects on the gut barrier and immunity were observed by the combination of L-Glutamine and L-Glutamate (Cabrera et al. 2013; Luise et al., 2022) and by the combination of L-Arginine, BCAA and L-Cystine, in a weight ratio of 42:33:25 (Prates et al., 2021) in weaned pigs.

Conclusion

In conclusion, the present review showed that L-Arginine, L-Glutamine and L-Glutamate were important functional AAs playing a major role in the gut morphology and in the gut immune functions in weaning pigs. The availability of the feed-grade crystalline form of these AAs provided alone or in combination will be of special interest in young animals, especially regarding the new perspective of reducing the use of antimicrobials in feed. Finally, to fully optimise the AA utilisation as a dietary strategy to sustain the gut health of piglets, further insights regarding the interplay between functional AAs, gut microbiome metabolism, host and microbial bidirectional exchange of AAs, and activation of signalling pathways on the intestinal mucosa are crucial.

Supplementary material

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Ethics approval

Not applicable.

Data and model availability statement

None of the data were deposited in an official repository. No new datasets were created.

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