Dietary Tryptophan Modulate the Composition of the Ileum and Cecum microbiota in Weaned Piglets after Lipopolysaccharide Challenge

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Abstract

Background: The gut microbiota plays a critical role in metabolism and growth of piglets, and was modifiable by dietary tryptophan (Trp) in previous study. However, no studies focused on the investigation of whether additional dietary Trp supplementation would modulate the composition of ileum and cecum microbiota of piglets challenged by lipopolysaccharide (LPS). We conducted to investigate whether dietary tryptophan could alleviate dysbacteriosis of piglets after challenged with LPS.

Methods: A total of 40 28 days old male weaning piglets were randomly allotted to five groups, include Con group (basal diet), LPS group (basal diet), 0.2% Trp group (0.2% Trp diet), LPS+0.2% Trp group (0.2% Trp diet) and LPS+0.4% group (0.4% Trp diet). On day 10, 20 and 29, Con and 0.2% Trp groups were injected with saline, LPS, LPS+0.2% Trp and LPS+0.4% Trp groups were injected with LPS respectively. The experiment lasted for 30 days.

Results: These results showed that the major three phyla in ileum were Firmicutes, Proteobacteria and Actinobacteria, in cecum were Firmicutes, Bacteroidetes and Proteobacteria. Dietary with 0.2% Trp could attenuate the effect of LPS on the alpha diversity (diversity and richness) in ileum ($P \leq 0.05$). The alpha diversity of cecum microbiota was not affected by LPS or Trp. The relative abundance of *Turicibacte* ($P \leq 0.05$) and *unclassified_f__Peptostreptococcaceae* ($P \leq 0.05$) were decreased but *Lactobacillu* ($P \leq 0.05$) was increased in ileum by LPS. Compared with LPS group, the relative abundance of *Actinobacillus* ($P=0.07$) was decreased in LPS+0.2% Trp group, and the level of *Blautia* ($P=0.08$) was increased in LPS+0.4% Trp group. The complexity of ileum microbiota was decreased by LPS. But the complexity of ileum microbiota was increased in LPS+0.2% and LPS+0.4% groups. The relative abundance of *Lactobacillu* was negative correlation with the majority of genus in ileum and positive correlation with the antioxidant ability of liver ($P \leq 0.05$).

Conclusions: These finding indicated that Trp could alleviate dysbacteriosis induced by LPS, and ileum microbiota might have a self-protective mechanism to resist the injury induced by LPS through enriching *Lactobacillu*.

Background

Early-weaning-induced stress cause diarrhea, thereby increasing mortality and reducing growth performance in piglets [1]. It caused the pig industry great economic loss and also represent a serious threat to farm animal welfare [2]. One of the main causes of diarrhea is gastrointestinal disorder. Several hypotheses have been put forth regarding the role of dysbiosis. One possibility is that dysbiosis disrupts production of microbial metabolites that are utilized by the intestinal epithelial cells for maintaining barrier integrity, which could elevate bacterial endotoxins in circulation, and trigger a pro-inflammatory cytokine cascade in the liver [3]. Recently, it is gradually realized that dietary transition and environmental changes during weaning period are linked to modifications in piglets intestinal microbiota which could be involved in the etiology of post-weaning diarrhea and enteric infections [4, 5].

The mammalian intestinal microbiota is composed of trillions of microbes that facilitate host health, including colonization resistance against gastrointestinal disorders [6]. Growing evidence suggests the potential of utilizing targeted reconstitution of the gut microbiota against gastrointestinal disorders [1, 7]. Recent studies have showed that probiotics, such as *Lactobacillus frumenti* and *Lactobacillus reuteri* could enhance the intestinal barrier and defense diarrhea of piglets [1, 8]. The gut microbiota could also increase the performance of pig through regulating the feed intake[9], feed efficiency[10] and oxidative stress[11] of host. Thus, maintenance and restoration of host intestinal microbiota homeostasis has shown beneficial effect in health and performance of pig.

The intestinal flora can actively utilize dietary amino acids for protein synthesis and modify the amino acid profile in the plasma of the host [12]. Dietary supplementation with amino acids could influence the composition and diversity of the intestinal microbiota, thus improving intestinal function [13]. Trp is considered as the second-limiting amino acid in most corn-based diets of swine [14]. Some previous studies have shown that dietary Trp level could regulate feed intake[15], growth [16], intestinal integrity[17] and oxidative stress[14] of pigs. Interestingly, recent studies reported that dietary Trp altered intestinal microbial composition and diversity, improved intestinal barrier and downregulated expression of inflammatory cytokines in the intestine of weaned piglets [18, 19]. In addition, increasing dietary tryptophan level could improve feed intake and growth performance of weanling pigs orally challenged with *Escherichia coli* K88 [16]. However, few studies focused on the investigation of ileum and cecum microbiota of weaning piglets at the same time and it still remains largely unknown how the intestinal microbiota is modified by Trp supplementation in weaning piglets challenged by LPS. Therefore, the present study was conducted to investigate the effect of dietary Trp on gut microbiota composition through using an LPS-challenged piglet model.

Results

Effect of Trp and LPS on Alpha Diversity of Ileum and Cecum Microbiota

We assessed the effects of Trp on the gut microbiota composition by sequencing the bacterial 16S rRNA. A total of 1,638,794 and 1,675,579 high-quality sequences were obtained in the ileum and cecum contents, respectively (Table 1). Overall, in the ileum and cecum, 369 and 859 operational taxonomic units (OTUs) were detected based on the nucleotide sequence identity of 97% between reads, respectively.

Table 1

The alpha diversity in each group of pig. A: non-challenged control group; B: LPS-challenged control group; C: control + 0.2% Trp treatment group; D: LPS + 0.2% Trp treatment group; E: LPS + 0.4% Trp treatment group. ACE: abundance-based coverage estimator.

Item	A	B	C	D	E	SEM	<i>P</i>
Ileum							
Sequens	46167	44891	40861	45021	44694	656.33	0.076
Shannon	2.02 ^{ab}	1.71 ^a	2.38 ^b	1.98 ^{ab}	1.69 ^{ab}	0.11	0.259
Simpson	0.299 ^{ab}	0.342 ^a	0.207 ^b	0.305 ^{ab}	0.389 ^a	0.03	0.322
Chao1	178.03 ^{ab}	116.76 ^a	242.92 ^b	182.24 ^{ab}	126.92 ^a	17.46	0.081
ACE	191.69 ^{ab}	124.43 ^a	276.52 ^b	192.69 ^{ab}	136.21 ^a	19.04	0.151
Cecum							
Sequens	48619	49257	47698	46192	39484	931.77	0.006
Shannon	4.02	4.09	3.90	4.09	4.04	0.06	0.814
Simpson	0.045	0.058	0.064	0.052	0.055	0.004	0.701
Chao1	494.32	516.73	482.57	521.71	509.53	12.00	0.768
ACE	491.85	513.88	474.5	519.88	499.96	11.85	0.839

The alpha diversity was estimated by diversity (Shannon and Simpson index) and richness (Chao1 and ACE indexes). In ileal samples, the diversity and richness of gut microbiota were decreased in LPS-challenged control group, but increased in the Trp treatment group. Moreover, fed LPS-challenged piglets with a 0.2% Trp diet restored the diversity and richness of gut microbiota to the level of control group. But fed LPS-challenged piglets with a 0.4% Trp diet showed no effect on diversity and richness of gut microbiota. The LPS and Trp showed no effect on the alpha diversity of cecum microbiota.

Effect of Trp and LPS on the Composition of Ileum and Cecum Microbiota

The relative abundance of gut microbiota and the principal components analysis (PCA) are showed in Fig. 1. The Trp treatment group had significantly deviated from three LPS-challenged groups in ileum microbiota. The composition of control group was similar with the Trp treatment group both in ileum and cecum (Fig. 1C, F). After filtering the relative abundance of phyla lower than 0.1% in all groups, 5 and 7 phyla were identified in the ileum and cecum microbiota, respectively (Fig. 1A, D). Firmicutes, Proteobacteria and Actinobacteria were three major phyla in ileum microbiota, and Firmicutes,

Bacteroidetes and Proteobacteria were three major phyla in cecum microbiota. LPS increased the level of Firmicutes and decreased the level of Proteobacteria and Actinobacteria. However, 0.2% Trp diet decreased the level of Firmicutes and increased the level of Proteobacteria and Actinobacteria in ileum. But in the cecum, the LPS and Trp had little effect on the relative abundance of microbiota at phyla level.

At the genus level, a total of 13 and 27 taxa were defined as the most abundant in ileum and cecum, respectively (≥1% of the total sequences). *Lactobacillus* and *Clostridium_sensu_stricte_1* were two major genera in ileum, *Lactobacillus* and *Prevotella_9* were two major genera in cecum (Fig. 1B, E). We found that the relative abundance of microbiota was significantly influenced by LPS and Trp in ileum and cecum. In the ileum content, compared with control group, 2 genera were significantly affected by LPS ($P \leq 0.05$), and 4 genera were little affected by LPS ($P \geq 0.1$), *Olsenella* was little affected by 0.2% dietary Trp ($P = 0.09$). Compared with LPS-challenged group, *Actinobacillus* was little decreased in LPS + 0.2%Trp group ($P = 0.07$), *Blautia* was little increased in LPS + 0.4% Trp group ($P = 0.08$) (**Figure S1 A-D**). In the cecum content, compared with control group, 3 genera were significantly affected by LPS ($P \leq 0.05$), *Terrisporobacter* was little decreased by LPS ($P = 0.07$); *Blautia* was significantly increased by 0.2% dietary Trp ($P \leq 0.05$), *Prevotellaceae_NK3B31_group* was little decreased by 0.2% dietary Trp ($P = 0.05$). Compared with LPS-challenged group, *norank_f__Veillonellaceae* was significantly decreased in LPS + 0.2% Trp or LPS + 0.4%Trp groups ($P \leq 0.05$) (**Figure S2 A-D**).

Bacterial Interaction Network Analysis

Co-occurrence networks in ileum and cecum were generated for all piglets and each group individually, based on their 50 most abundant genera (Fig. 3A and **Figure S3**). The overall topological properties of these network were calculated in order to distinguish the genus correlations at different groups. For the number of nodes, edges and correlations, the LPS + 0.4%Trp group showed the highest values, the LPS and 0.2%Trp groups showed lower values. This result indicating that the network topology existed more complex in ileum microbiota of piglets after the treated with LPS and Trp.

Bacterial core members were easily found in network interactions among five groups, including *Lactobacillus*, *Clostridium_sensu_stricto*, *Terrisporobacter*, *Streptococcus*, *Actinobacillus* (Fig. 3A). Interestingly, in the control group, the *Clostridium_sensu_stricto* is the key genera negatively correlated with the dominant genera (Fig. 3B). However, in the LPS, LPS + 0.2%Trp and LPS + 0.4%Trp groups, *Lactobacillus* is the key genus negatively correlated with the relative abundance of the dominant genera (Fig. 3C, 3E and 3F).

Effect of LPS and Trp on the abundance of *Lactobacillus* in Ileum

As the results of Fig. 2 and Fig. 3 shown, the relative abundance of *Lactobacillus* of piglets was significantly increased by LPS, and the *Lactobacillus* is a key genus negatively correlated with the

dominant genus. Then, we analyzed the relative abundance of *Lactobacillus* of ileum at the species level (Fig. 4). As the results shown that, the relative abundance of *Lactobacillus_amylovorus*, *s_unclassified_g_Lactobacillus*, *s_uncultured_bacterium_g_Lactobacillus* and *s_Lactobacillus_pontis* were increased by the challenge of LPS. Compared with the 0.2%Trp group, the relative abundance of *Lactobacillus_amylovorus* and *s_uncultured_bacterium_g_Lactobacillus* were significantly increased in LPS, LPS + 0.2%Trp and LPS + 0.4%Trp groups ($P \leq 0.05$).

Correlation of gut microbiota with growth performance, serum and liver parameters of piglets

In our previous study, we analyzed the performance, serum and liver parameters of piglets. Then, a spearman correlation analysis was performed among the top 50 genera according the relative abundance in all piglets. As shown in Fig. 5, the relative abundance of *Bacillus*, *Turicibacter*, *Clostridium_sensu_strecto-1*, *Terrisporobacter*, *Syntrophococcus* and *Blautia* were significant positive correlated with the average daily gain (ADG) of piglets. However, the relative abundance of *Lactobacillus* was significant negative correlation with the ADG and average daily feed intake (ADFI) of piglets. It is interesting to note that, the relative abundance of *Lactobacillus* was significant positive correlated with the level of SOD, GSH-Px and T-AOC in liver.

Discussion

Host homeostasis with respect to issues of physiology and metabolism is crucially underpinned by the gut microbiota and its metabolites[20]. Researches indicated that the gut microbiota can facilitate metabolite production in two ways, first, the resident species of the gut microbiota use the amino acids produced from food or the host as elements for protein synthesis, and second, conversion or fermentation are used to drive nutrient metabolism[21]. Previous study reported that, the tryptophan-derived bacterial metabolite indole attenuates indicators of inflammation in epithelial cells and liver of host[3]. LPS has been widely used to mimic features of endotoxin-induced acute intestinal injury. LPS irritates the intestine, causing mucosal injury, metabolic disorder, and bacterial translocation. Recent study reported that LPS could induce the disorder of gut[22] and ruminal bacterial[23].

To evaluate the effect of Trp on gut microbiota disorder induced by LPS, ileum and cecum microbiota was extracted for 16S rRNA analysis. Gut microbial community is a mini-ecosystem whose diversity is regarded as a key health indicator of healthy individuals and affected by the health status of the hosts [24]. It has been reported that reduced biodiversity of the gut microbiota is associated with disease status [24, 25]. In the study, we found that the alpha diversity of gut microbiota in cecum is not affected by Trp and LPS. However, in the ileum, it was increased with the supplementation of 0.2% Trp, and decreased with the challenge of LPS in piglets. This may be due to the small-intestine is the main target organ of LPS[26], and amino acids are absorbing in small-intestine[27]. Interesting, we found dietary 0.2% Trp alleviated the decrease of alpha diversity in ileum microbiota challenged by LPS, but dietary 0.4% has no

effect on the reduction of diversity in ileum microbiota caused by LPS. This result consistent with a previous study, which reported the richness and diversity of gut microbiota in 0.2% Trp group higher than that in control and 0.4% Trp groups[19]. These results indicated that the addition of 0.2% Trp to the diet has improved regulation of ileum microbiota diversity than 0.4% Trp treatments regardless of whether the piglets are challenged by LPS.

As previous studies reported, LPS altered bacterial composition at genus level in ileum and cecum[28]. We found that *Turicibacter* in ileum were lower in LPS-challenged pig. In our previous study, we found the abundance of *Turicibacter* was higher in sows with higher performance and low oxidative stress[11]. In agreement, Suchodolski study reported significantly decrease of *Turicibacter* in dogs with Acute Hemorrhagic Diarrhea (AHD) [29]. But the molecular mechanism about *Turicibacter* regulated the health of intestinal remains to be resolve. It is curious that the relative abundance of *Lactobacillus* was significantly increased in LPS, LPS + 0.2%Trp and LPS + 0.4%Trp groups. *Lactobacillus* has been identified as one of the core probiotics in the gastrointestinal tract of pigs[30]. Bu it has been reported that when the absence of epithelial reactive oxygen species (ROS), compensatory adaptation of the *Lactobacillus* confers protection against enteric pathogens by fostering H₂O₂ and downregulating virulence factors[31]. Moreover, in the present study, we found *Lactobacillus* is the key genus negatively correlated with the abundance of the dominant genera in LPS, LPS + 0.2%Trp and LPS + 0.4%Trp groups (Fig. 3). Yang also found that *Lactobacillus* could modulate the intestinal injury induced by LPS in piglets[8]. It suggested that *Lactobacillus* may play a key role in inhibiting the growth of pathogens and maintaining the homeostasis of the intestinal flora when the intestinal injury was induced by LPS of piglets.

Trp also altered bacterial composition at genus level in ileum and cecum. *Olsenella* is a kind of lactic acid bacterium[32], which is up-regulated by Trp in ileum in our study. Lactic acid is not only an intermediate metabolite of short-chain fatty acid (SCFA) produced by intestinal flora metabolism[33], but also playing an important role in maintaining homeostasis within the gut community, by preventing colonization and infection of incoming bacterial pathogens[34, 35]. Comparing with the control group, the relative abundance of *Prevotellaceae_NK3B31_group* decrease in the cecum of Trp group. A recent study reported the *Prevotellaceae_NK3B31_group* was dominant in the low feed conversion ratios pigs cecum[36]. In addition, Trp supplementation is associated with reduced relative abundances of *Actinobacillus* in ileum and increased relative abundance of *norank_f_Veillonellaceae* in cecum of piglets challenged with LPS. Lower proportions of *Actinobacillus* was reported with the higher weight gain of piglets[37]. Interestingly, the average daily gain of piglets was increase with the addition of tryptophan in our study (unpublished data). *Veillonellaceae*, a family with known lactate utilizing species[38], which is associated with conversion of lactate to propionate[34]. In our study, lactic acid bacterium, such as *Lactobacillus* and *Olsenella* were significantly enriched in piglets challenged with LPS. These results suggested that the increase of *Veillonellaceae* may be induced the elevating of source of nutrients.

In general, the greater the stability of the microbiome in a given environment, the greater complexity of these relationships, since these organisms and environments are less susceptible to microbial

invasion[39]. Our results revealed that the complexity of ileum microbiota in LPS group were reduced, but increased by the treated with 0.2% and 0.4% Trp. It suggested that Trp might increase the complexity and stability of ileum microbiota in pig challenged with LPS, and inhibit the invasion of pathogenic bacterium. Moreover, we observed that *Lactobacillus* is the key genus negatively correlated with the abundance of the dominant genus in piglets challenged with LPS, and the relative level of *Lactobacillus* in ileum was increased after the challenge of LPS. It is worth noting that the *Lactobacillus_amylovorus* is the most abundant strain of *Lactobacillus* in the ileum of piglets. Previous study had reported that *Lactobacillus_amylovorus* inhibited the inflammatory signaling triggered by enterotoxigenic *Escherichia coli* in intestinal Caco-2 and pig explants [40]. It also demonstrated that *Lactobacillus_amylovorus* could improve host metabolism and relieve disorder of gut microbiota [41]. These indicate that *Lactobacillus_amylovorus* might proliferate to inhibited the inflammation and alleviates intestinal flora disorders of ileum induced by LPS.

Spearman correlation analysis was used to reveal the relationship between gut microbiota and the growth performance or antioxidant ability of piglets. These results showed that the relative abundance of *Lactobacillu* in ileum was negatively with growth performance but positively with antioxidant parameters of piglets. *Lactobacillu* is probiotics which is beneficial to growth of piglets[42]. In addition, the growth performance of piglets was reduced due to the challenge of LPS (unpublished data), but the relative abundance of *Lactobacillu* was increased to alleviate dysbacteriosis of ileum induced by LPS. Therefore, the negative correlation between *Lactobacillu* and growth performance do not mean that *Lactobacillu* are detrimental to the health of piglets. Liver injury and oxidative stress were induced by LPS in previous report [43]. Furthermore, previous study had shown *Lactobacillu* could protective from oxidative and metabolic hepatic injury [44]. A possible positive correlation between *Lactobacillu* and antioxidant ability is that *Lactobacillu* was proliferate to alleviate oxidative damage of liver when piglets challenge with LPS.

Conclusions

These studies suggested that Trp could relieve the toxic effect of LPS through improving the diversity and complexity of ileum microbiota in piglets. And the *Lactobacillu* might proliferate to inhibit the growth of pathogenic bacteria and alleviate oxidative damage of liver when piglets were challenged with LPS. These finding indicated that ileum microbiota might have a self-protective mechanism to resist the injury induced by LPS through enriching *Lactobacillu*.

Methods

Animals

Piglets were obtained from the Hunan New Wellful Coompany Yongan farm and the study was carried out in the Union Experimental Center of Institute of Subtropical Agriculture, Chinese Academy of Sciences and Hunan New Wellful Company (Liuyang, China). A total of 40 28 days old male piglets (Yorkshire ×

Landrace, initial body weight 7.79 ± 0.75 kg) were randomly divided into five groups with eight replicate pens for each treat group and one pig for each pen. Piglets were individually caged in 1.80×1.10 m pens and allowed ad libitum access to feed and water in an environmentally controlled house.

The experiment included five treatments: (1) non-challenged control (A: piglets fed a control diet and injected with sterile saline); (2) LPS-challenged control (B: piglets fed the same control diet and injected with LPS); (3) control + 0.2% Trp treatment (C: piglets fed with a 0.2% Trp diet and injected with sterile saline); (4) LPS + 0.2% Trp treatment (D: piglets fed with a 0.2% Trp diet and injected with LPS); (5) LPS + 0.4% Trp treatment (E: piglets fed with a 0.4% Trp diet and injected with LPS). On the morning of day 10, 20, 29, the challenged groups were injected intraperitoneally with LPS (80 ug/kg BW), A and C groups were injected intraperitoneally with sterile saline (80 ug/kg BW). All animals were euthanized by intravenous injection of an overdose of sodium pentobarbital at the end of the 30-day trial. The ileum and cecum contents were collected and placed in liquid nitrogen and then were stored at -80°C until laboratory analyses.

Dna Extraction And 16s Ribosomal Rna (rrna) Amplification

Ileum and cecum digesta samples were used to extract Genomic DNA with the E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) according to the standard protocol. The V3-V4 region of the bacterial 16S rRNA gene was amplified using 16S rRNA universal primers targeting. PCR reaction mixture were performed in triplicate, consisted of 10 ng template DNA, 4 μL 5 $\times$ FastPfu Buffer, 2 μL dNTPs (2.5 mmol/L), 0.8 μL reverse primer (5 $\mu\text{mol/L}$), 0.8 μL forward primer (5 $\mu\text{mol/L}$), 0.4 μL FastPfu Polymerase, and ddH₂O in a final volume of 20 μL . PCR amplification program was as follows: an initial activation step with 95°C for 3 min, followed by 27 cycles at 95°C for 30 s, 55°C for 30 s, and 72°C for 45 s and a final extension at 72°C for 10 min. Samples that contained no template and those that contained known 16S rRNA gene sequences were used as positive and negative controls in the PCR reactions.

16s Rrna Sequencing And Microbiota Analysis

Amplicons were extracted from 2% agarose gels, then purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified with QuantiFluor™-ST (Promega BioSciences LLC, Sunnyvale, CA, USA). All procedure was carried out with the standard protocols. Finally, Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA) was used to pool and pair-end sequenced (2×300). All raw reads were screened according to barcode and primer sequences with Quantitative Insights Into Microbial Ecology (QIIME, version 1.17), the following criteria: 1) The 300 bp reads were truncated at any site receiving an average quality score < 20 over a 10 bp sliding window; 2) the truncated reads that were < 50 bp were abandoned; 3) Sequences that overlap shorter than 10 bp, or reads containing ambiguous characters, or > 2 nucleotide mismatch in primer matching were removed. Operational taxonomic units (OTUs) were clustered according to the standard of cut-off of 97% similarity using UPARSE (version 7.1,

<http://drive5.com/uparse/>). The chimeric sequences was identified and removed with UCHIME. RDP Classifier (<http://rdp.cme.msu.edu/>) was used to analyze the phylogenetic affiliation of each 16S rRNA gene sequence, against the silva (SSU115) 16S rRNA data base with confidence threshold of 70%. A principal components analysis (PCA) was performed to find clustering patterns of piglets in five groups.

Statistical Analyses

The normal distribution of the data was calculated with the Kolmogorov-Smirnov test. Principal components analysis (PCA) was performed to find the clustering patterns among the five groups of OTU-level microbiota abundance data. The differences of gut microbiota relative abundance among five groups was evaluated using the Wilcoxon rank-sum test. Network analysis was plotted using Networkx. The Spearman correlation analysis was used to determine the relationship between relative abundance of genus and growth performance, serum and liver parameters of piglets. These results were considered statistically significant at $P < 0.05$.

List Of Abbreviations

Trp: Tryptophan; LPS: Lipopolysaccharide.; OTUs: Operational taxonomic unit; ADG: Average daily gain; ADFI: Average daily feed intake; SOD: Superoxide dismutase; GSH-Px: Glutathione peroxidase; T-AOC: Total antioxidant capacity; AHD: Acute Hemorrhagic Diarrhea; SCFA: Short-chain fatty acid; ROS: Reactive oxygen species; PCA: Principal components analysis

Declarations

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Author's Contributions

WW and YL contributed to the study design. YY, XD, LF and YYJ conducted the animal experiments. LY, LF, ZY, JX and YH executed the lab analysis. HW analyzed experiment results and wrote the manuscript. WW revised the paper. All authors had read and approved the manuscript.

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Ethics approval and consent to participate

The procedures of this present study was approved by the Animal Ethics Committee of South China Agricultural University (SCAU-10564).

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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treatment group; D: LPS+0.2% Trp treatment group; E: LPS+0.4% Trp treatment group. ACE: abundance-based coverage estimator.

Figure 2

Differences in relative abundance of 10 major bacterial genera between five groups in ileum (A) and cecum (B). A: non-challenged control group; B: LPS-challenged control group; C: control+0.2% Trp treatment group; D: LPS+0.2% Trp treatment group; E: LPS+0.4% Trp treatment group. ACE: abundance-based coverage estimator, *, $P < 0.05$; **, $P < 0.01$.

Figure 3

The network analysis on genus level in ileum. A: all ileum digesta samples. B: non-challenged control group; C: LPS-challenged control group; D: control+0.2% Trp treatment group; E: LPS+0.2% Trp treatment group; F: LPS+0.4% Trp treatment group. Size and color of the nodes represent relative abundance of the ileum microbiota and species, respectively. Lines in red and green denote positive and negative correlations, respectively. The width reflects the strength of the correlation.

Figure 4

The relative abundance of 6 major bacterial species belongs to *Lactobacillus* in ileum. A: non-challenged control group; B: LPS-challenged control group; C: control+0.2% Trp treatment group; D: LPS+0.2% Trp treatment group; E: LPS+0.4% Trp treatment group. ACE: abundance-based coverage estimator, *, P<0.05.

Figure 5

Correlation analysis between the relative abundance of ileum with performance and serum parameters of pig. A: non-challenged control group; B: LPS-challenged control group; C: control+0.2% Trp treatment group; D: LPS+0.2% Trp treatment group; E: LPS+0.4% Trp treatment group. ACE: abundance-based coverage estimator, *, $P < 0.05$; **, $P < 0.01$.

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