RESEARCH REPORT
SWINE HEALTH

Title: Fecal microbiome transplantation as a preventive strategy against gut-dysbiosis and porcine post-weaning diarrhea (**#19-216 IPPA)**

Investigator: Shankumar Mooyottu

Institution: Iowa State University

Date Submitted: 09/09/2021

Industry Summary: Post-weaning diarrhea (PWD) caused by enterotoxigenic *Escherichia coli* (ETEC) is an economically important disease in weaned piglets. Currently, antibiotics are used in the swine industry to control enteric infections, including PWD in piglets. Recent studies have confirmed that disruption of the gut microbiome (gut dysbiosis) induced during weaning transition is a critical factor involved in PWD pathogenesis. Gut dysbiosis has been found to be invariably implicated in both initiation and clinical manifestation of PWD. Therefore, stabilizing the gut microbiome during the weaning transition could be an effective strategy for controlling PWD. Recently, fecal microbiome transplantation (FMT) has been shown to prevent gut-dysbiosis and control serious enteric diseases in humans. However, the application of these strategies is minimally explored in the swine industry. This proposal investigated the use of FMT as a preventive strategy against gut- dysbiosis and ETEC infection in weaning piglets. In this project, we tested our central hypothesis that gut microbiome dysbiosis and PWD in piglets could be ameliorated by the transplantation of adult pig gut microbiota to the piglets at weaning. The results from our experiment indicated that FMT could beneficially alter the abundance of specific bacterial communities in piglet gut, increase the diversity of the gut microbiome, and reduce the clinical outcome of ETEC infection in weaning piglets.

Key Findings:

- FMT reduced the severity of diarrhea in ETEC infected piglets
- FMT increased gut microbiome diversity in weaning piglets
- FMT induced a favorable shift in the gut microbial abundance

Keywords: ETEC, postweaning diarrhea, FMT, piglet, weaning

Scientific Abstract:
Post-weaning diarrhea (PWD) caused by enterotoxigenic Escherichia coli (ETEC) is an economically important disease in weaned piglets. Currently, antibiotics are used in the swine industry to control enteric infections, including PWD in piglets. Recent studies have confirmed that disruption of the gut microbiome (gut dysbiosis) induced during weaning transition is a critical factor involved in PWD pathogenesis. Gut dysbiosis has been found to be invariably implicated in both initiation and clinical manifestation of PWD. Therefore, stabilizing the gut microbiome during the weaning transition could be an effective strategy for controlling PWD. Recently, fecal microbiome transplantation (FMT) has been shown to prevent gut-dysbiosis and control serious enteric diseases in humans. However, the application of these strategies is minimally explored in the swine industry.

This proposal investigated the use of FMT as a preventive strategy against gut- dysbiosis and ETEC infection in weaning piglets. In this project, we tested our central hypothesis that gut microbiome dysbiosis and PWD in piglets could be ameliorated by the transplantation of adult pig gut microbiota to the piglets at weaning. For this experiment, a fecal microbiome mixture prepared from six 3-month-old healthy piglets was transplanted to the piglets at 4th and 7th days postweaning (n=6), and the piglets were challenged with a F18 positive field isolate of ETEC (EC) on 7th day post-FMT. The clinical score and diarrhea score were recorded daily for 8 days post-infection and the fecal samples were analyzed for ETEC load and microbial diversity and abundance using qPCR and Illumina MiSeq-based 16s rRNA sequencing technology respectively. FMT group had a significantly lower mean diarrhea score and lesser histologic lesions compared to controls. In addition, FMT significantly increased the abundance of beneficial bacterial communities such as Firmicutes and *Bifidobacteria* in the gut and significantly increased the gut microbial diversity. Together, this study suggests that FMT could be a promising strategy to prevent gut dysbiosis and ETEC infection in weaning piglets, which could be tested at field level.

Introduction:

Combating post-weaning diarrhea in pigs and the need of non-antibiotic strategies: Post-weaning diarrhea (PWD) caused by enterotoxigenic *Escherichia coli* (ETEC) is a major and economically important disease in weaned piglets [1]. It is estimated that *E. coli* are responsible for over 50% of all losses in weaned pigs [2]. The pathogenesis of PWD is complex, which involves managemental, environmental, and host factors [1, 3]. Currently, antibiotics are widely being used for controlling enteric diseases, including ETEC in pigs [4]. The overuse of antibiotics is directly linked to the emergence of antibiotic-resistant bacteria and poses a significant threat to both human and animal health [5]. Thus, the use of antibiotics in the industry is currently being re-examined by regulatory agencies [6, 7]. In this scenario, alternate, environmentally friendly and economically viable approaches are required to control PWD in commercial swine operations. We propose 'fecal microbiome transplantation' as a potential interventional strategy to prevent PWD and promote gut health in weaning piglets. A strategic research collaboration was made for addressing different aspects of this project and to meet our specific objectives. The principal investigator Dr. Mooyottu has extensive experience in interventional microbiology, animal models of infection, gut-microbiome analysis and veterinary pathology. The co-investigator Dr. Ramirez is an established swine disease investigator with immense experience in conducting experimental pig trials.

Gut-dysbiosis – a key factor in pathogenesis of PWD: In commercial swine production, weaning is one of the most critical events in a pig's life cycle, which involves abrupt dietary, environmental and social changes [8]. Recent studies confirm that disruption of gut microbiome (gut-dysbiosis) induced by an abrupt change in dietary and environmental conditions is one of the major predisposing factors of post-weaning enteric diseases, most importantly PWD [9]. After birth, the piglet's gut is quickly colonized with maternal and environmental bacterial flora, and its composition and diversity play a key role in gut health and susceptibility to enteric infections [10]. Early piglet gut microbiome is defined and shaped by a milk rich intestinal environment, and thus a sudden transition to cereal and high protein rich diet at weaning results in dramatic changes in the gut microbiome [11]. Such alterations in the gut microbiome result in the proliferation and intestinal colonization of a dominating pathogenic strain of ETEC, establishing a crucial link between gut-dysbiosis in the pathogenesis of PWD [9]. Furthermore, most recent studies on PWD indicate that ETEC infection can also directly induce and worsen gut-dysbiosis in piglets, which subsequently mediates diarrhea and intestinal inflammation [12]. These studies establish gut-dysbiosis as a key factor in PWD pathogenesis, with involvement in both initiation and manifestation of the disease.

Alterations in gut microbiome during weaning transition: Several studies have been conducted to characterize the major changes of the microbial communities in swine gut that occur during the weaning transition. The most significant gut-microbiome alterations reported were 1) loss of gut microbiome diversity, 2) a shift from Firmicutes to Bacteroidetes with reduced abundance of *Lactobacillaceae* group, and 3) increased abundance of Proteobacteria, especially the members of *Enterobacteriaceae*, which include pathogenic *E. coli* [9, 13]. A dramatic increase in *E. coli* population, *Enterococcus* spp, and members of Clostridia are reported by various investigators [9, 14]. Furthermore, commercially used feed antibiotics can also cause a drastic change in both gut microbiome

composition and diversity, by exerting a broad-spectrum inhibitory effect on both pathogenic and beneficial bacteria [13]. Interestingly, fecal microbiome transplantation FMT has been found to reverse most of these changes in terms of gut microbial abundance and diversity in animals and humans [15, 16].

Microbiome transplantation – a radical approach to prevent gut-dysbiosis and PWD: Fecal microbiome transplantation (FMT) has been widely accepted as 'the future of gastroenterology and gut health' in human and veterinary medicine [16, 17]. A majority of important infectious and non-infectious diseases such as *C. difficile* infection (CDI), inflammatory bowel disease (IBD), ulcerative colitis and Crohn's diseases are recently identified to be directly linked to imbalances in gut microbiome [18]. Thus, FMT is widely being tested and used for treating dysbiosis and important enteric diseases. Management of CDI in humans (and equines) is the most notable example for successful use of FMT as a radical therapeutic strategy against dysbiosis and enteric infections. CDI is invariably associated with gut-dysbiosis; however, antibiotics are widely used for controlling this disease despite constant relapse of the disease and the emergence of antibiotic-resistant *C. difficile* strains. Recently, FMT has been established as the most effective treatment against CDI with superior remission rate and low relapse compared to antibiotic treatment [19]. PWD in piglets has similar pathogenesis as CDI in terms of gut-dysbiosis and subsequent initial pathogen colonization [20]. Thus, stabilizing the gut microbiome by transplanting mature and healthy gut-microflora to weaning piglets could potentially prevent gut-dysbiosis and PWD. However, no such studies have been conducted so far to test this method. Since gut-dysbiosis has already been established to have a critical and indispensable role in PWD incidence and pathogenesis [1], we hypothesized that effective restoration of gut microbiome by FMT during weaning period could potentially prevent PWD in piglets.

Objectives:

1) Determine the effect FMT in reducing ETEC carriage and PWD in piglets
2) Determine the effect of FMT on gut microbiome of weaned piglets using high throughput 16s rRNA sequencing

Materials & Methods:

Preparation of fecal microbiome mix: Fecal microbiome mix for this study will be prepared from selected healthy 3–4-month-old donor pigs as per a recently published protocol [21]. Briefly, fresh fecal material from healthy donor pigs will be homogenized with sterile normal saline in mixer, and the fecal materials will be diluted five times with adequate amount of buffer solution. The suspension will be filtered through sterile sieves and administered orally to the recipients or mixed with 10% sterile glycerol to store at –80°C.

Effect of FMT on ETEC infection and diarrhea in weaned piglets: Twenty-four suckling piglets weaned and weighed at three weeks of age, will be randomly divided into four experimental groups (n=6 per group), which will be housed in separate pens in groups with ad-libitum access to starter diet and drinking water. The treatment groups include: Control group (C), Microbiome transplantation control Group (FMT), ETEC control group (ETEC) and Microbiome transplantation + ETEC group (FMT+ETEC).

FMT and FMT+ETEC group will be provided with a fecal microbiome mix (5 ml solution orally) on the first and third day of the experiment (The 'microbiome mix' has been found to be efficiently delivered by mixing with drinking water, feed or by direct oral gavage twice [21]. Since this is a proof-of-concept study, we administer the microbiome mix as an oral gavage to quantify the effect of FMT in a controlled setting. If found effective, microbiome mix can be administered as a single oral dose at weaning and/or mixing with drinking water or feed, based on practicality in field settings). On approximately day 10th of the study (10-day post weaning), animals in ETEC and FMT+ETEC groups will be challenged using oral intragastric tube with approximately 10^{10} CFU of bacteria (F18 positive ETEC clinical isolate with Stx2). Control groups will be administered with phosphate buffer saline (PBS) orally.

Animals will be weighed and observed for diarrhea for 7 days, and fecal samples will be collected at different time points (0, 2, 4, and 7-day post-infection). Three piglets each will be sacrificed on 4th and 7th-day post-infection, and the intestinal samples will be collected for histopathology. The number of

pigs showing diarrhea will be recorded daily throughout the duration of the study. The severity of diarrhea will be evaluated using standard fecal consistency score system [22]. Both individual and pen fecal scores will be recorded everyday post ETEC challenge. Diarrhea index will be calculated as the sum of feces score/total number of pigs.

Quantification of ETEC and histopathology: Total DNA will be isolated from 200 mg of feces using QIAamp Fast DNA Stool Mini Kit (QIAGEN, Carlsbad, CA) as per manufacturer's instruction. Real-Time PCR will be performed using CybrGreen Q-PCR Kit (New England BioLabs, Ipswich, MA) in a QuantStudio 3 PCR machine (Thermo Fisher Scientific, Waltham, MA) as per previously published protocol and oligonucleotide primers [23]. Total DNA isolated from 200 microliters of *E. coli* suspension of known titers will be used as a standard, and the absolute quantification of F18 positive ETEC will be performed by standard curve method [23]. The mid-jejunal segments (5 cm in length) will be collected, opened longitudinally and rinsed thoroughly with physiological saline at 4°C before fixing in 4% paraformaldehyde for subsequent histological analysis. Intestinal villus height and villus crypt depth will be measured using Image-Pro Plus 6.0 image processing and analysis system (Media Cybernetics, Bethesda, MD).

Microbiome analysis: DNA will be extracted from 0.25 g of fecal samples from all treatment groups using the MoBio PowerMag Soil 96 well kit (QIAGEN, Carlsbad, CA). The microbiome analysis will be set up as a completely randomized design with treatments done in replicates of six. Partial bacterial 16S rRNA genes (V4) will be amplified using 30 ng of extracted DNA as template. The cleaned pool will be sequenced on MiSeq (Illumina, San Diego, CA). Forward and reverse reads from the paired-end sequencing will be first merged using the fastq.join script. Qiime 1.8 will then be used for additional data analysis. Comparisons of specific OTUs between treatment groups will be made at the phylum, order, and genus level using OTUs detected in at least 25% of samples in a given group included in the analysis. Biological effect sizes will be estimated using the linear discriminant analysis effect size (LEfSe) method. A CoVennTree analysis will be performed to assess differences in the microbial population structure between groups.

Statistical analysis: The results of the animal study will be expressed as means ± standard errors of the means (SEM). The differences between the experimental groups will be compared using the analysis of variance (ANOVA). Two-way ANOVA will be used to compare experimental groups across the days. The differences between two groups will be analyzed using unpaired Student's t-test. "N1" Chi-squared test will be used to compare incidence rate between two different treatments. The statistical significance level will be set at a $P < 0.05$. Alpha diversity (chao1) will be compared using a non-parametric two sample *t*-test with 999 Monte Carlo permutations. Beta diversity (Bray-Curtis dissimilarity) will be compared using a two-sided student's two-sample *t*-test with Bonferroni correction. The frequency of detection (group significance) of specific OTU calls within groups will be compared using a Kruskal–Wallis non-parametric analysis of variance, followed by correction for multiple comparison using the Benjamini and Hochberg False Discovery Rate (FDR) method. A FDR of 5% will be utilized to determine significance.

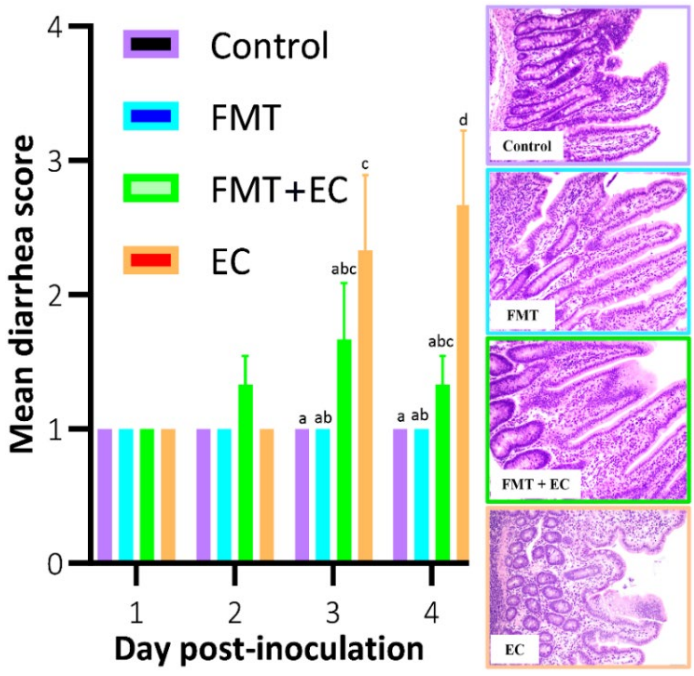

Fig. 1. Effect of FMT on diarrheal score (1-4 dpi) and ileal mucosa (4th dpi) in piglets challenged with F-18+ ETEC. Means that have no superscript in common are significantly different from each other ($P<0.05$)

Results:

FMT reduced the severity of diarrhea in ETEC infected piglets: Results from our pilot study revealed that FMT during weaning transition significantly reduce diarrhea score in ETEC challenged pigs **(Figure 1)**. A fecal microbiome mixture prepared [21] from six 3-month-old healthy piglets was transplanted to the piglets at 4th and 7th days postweaning (n=6), and the piglets were challenged with a F18 positive field isolate of

ETEC (EC) on 7th day post-FMT. The diarrhea score was recorded using a previously published scoring system [24]. Diarrhea peaked at 4th-day post-challenge in the EC group, and FMT treatment significantly reduced the mean diarrhea score in FMT treated piglets (FMT+EC) at 4th day post-ETEC inoculation. Histopathology of the ileal samples revealed no significant lesions in Control and FMT group. In ETEC challenged group (EC), numerous adherent bacteria are observed along enterocytes, typical of ETEC infection in piglets. In addition, there was moderate mucosal edema, congestion, lymphoplasmacytic, and occasional neutrophilic infiltration in the lamina propria of the ileum. The endothelium of the mucosal vessels was plump and reactive. Lymphatics in the mucosa and submucosa were moderate to markedly ectatic. Rare crypts contained necrotic epithelial cells and neutrophils. In a few animals, mild villous blunting was noticed. In contrast, in FMT+EC group, the changes were minimal, which include a minimal to patchy adherent bacteria on the villous surface and mild excess number of lymphocytes and plasma cells in the lamina propria.

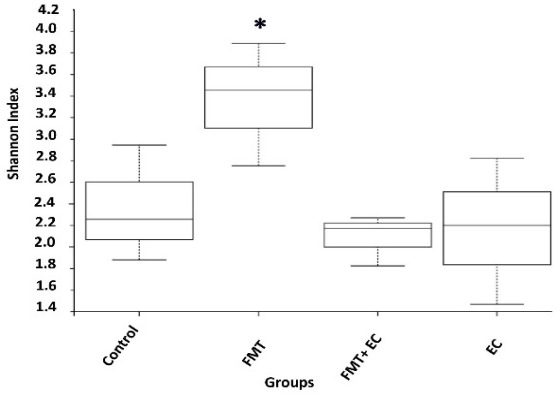

Fig. 2. Effect of FMT on microbial alpha-diversity (jejunum) in piglets infected with ETEC. (* treatment is significantly different from control ($p<0.05$)).

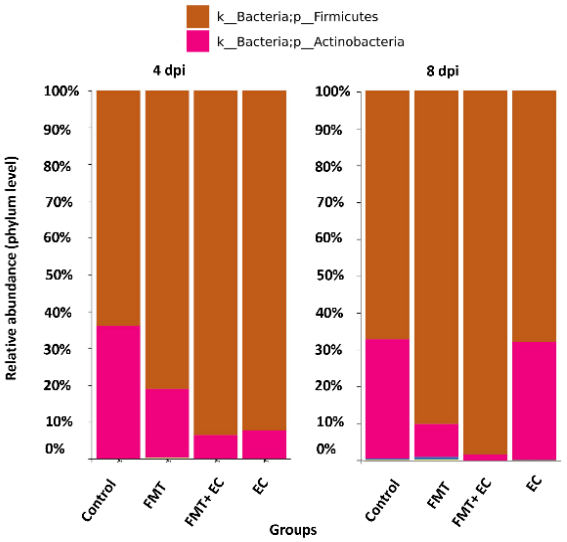

Fig. 3. Effect of FMT on relative abundance of jejunal bacteria communities at phylum level (all treatments are significantly different from control ($p<0.5$) except for EC at 8 dpi).

FMT increased gut microbiome diversity in weaning piglets: To assess the basic microbiome changes associated with FMT in weaning piglets, we collected jejunal and ileal contents from all treatment groups and performed 16s rRNA sequencing. A significant increase in the alpha diversity (as indicated by a higher Shannon index) of the jejunal microbiome was observed in piglets transplanted with fecal material (FMT) at 4th day post-ETEC infection (11th-day post-FMT) compared to Control (**Figure 2**). A reduced gut microbiome diversity in weaning piglets has been implicated in ETEC colonization of the small intestine [25]. Similar results were also obtained from ileal and colon contents (data not shown). Our preliminary results suggest that FMT could improve weaning-associated reduction in gut microbiome diversity in piglets. However, a significant difference in diversity was not observed in FMT+EC and EC group, which could be attributed to the direct effect of ETEC on dysbiosis, as reported previously [12].

FMT induced a favorable shift in the gut microbial abundance: Alterations in the abundance of different gut bacterial populations have been reported during weaning transition. These alterations include decrease in the proportion of beneficial bacteria and increase in the abundance of unfavorable bacteria [14, 26]. To assess the effect of FMT on the abundance of major bacterial communities in the piglet gut, we analyzed different operational taxonomic units (OTUs) obtained from 16s rRNA sequencing results. Our results revealed an increase in the abundance of specific bacterial communities at different taxonomic levels. For example, FMT significantly increased the jejunal Firmicute population in weaning piglets compared to control at both 4th and 8th day post-infection (dpi) ($p< 0.05$), which is considered as favorable in terms of gut health [9, 14]. Moreover, at 8th dpi, the relative

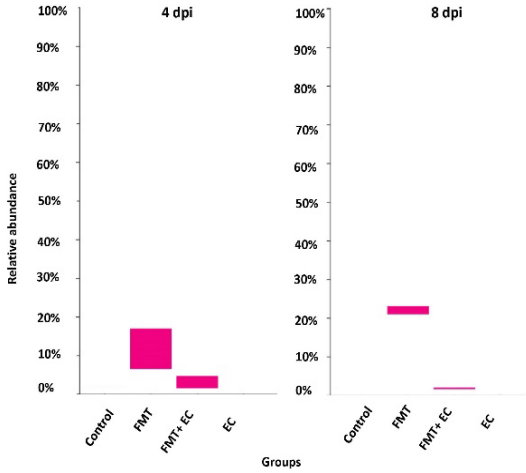

Fig. 4. Relative abundance of jejunal *Bifidobacteria* in different treatment groups (all treatments are significantly different from control ($p<0.5$).

abundance of Firmicutes was significantly increased in FMT + EC group compared to the challenge control group (EC) (**Figure 3**). Additionally, a significant increase in the abundance of the specific bacterial species in genus *Bifidobacteria* was observed in piglets transplanted with fecal microbiome compared to controls ($p<0.05$) (**Figure 4**). Members of the genus *Bifidobacteria* have been demonstrated to prevent weaning associated intestinal infections and improve gut-health in piglets [27-32]. Thus, our preliminary results suggest that FMT favorably alters the gut microbial communities during the weaning transition, which could potentially prevent PWD in piglets.

Discussion: Currently, limited strategies are available for controlling ETEC infection and PWD in piglets, and the use of feed antibiotics for this purpose has several disadvantages [13]. Recent advances in the field of the gut microbiome have expanded our understanding of the gut-pathogen interactions during enteric diseases. This knowledge revolution facilitated the implementation of novel therapeutic and preventive strategies such as FMT in human medicine. However, the application of these strategies is minimally explored in the swine industry. We proposed to fill this knowledge gap by investigating the efficacy of FMT against PWD and understanding the gut-microbial dynamics during this procedure. Our results suggests that FMT could beneficially alter the abundance of specific bacterial communities in piglet gut, increase the diversity of the gut microbiome, and reduce the clinical outcome of ETEC infection in weaning piglets. Since gut-dysbiosis has already been established to have a critical and indispensable role in PWD predisposition and pathogenesis [1]. Moreover, FMT has been experimentally shown to promote gut immunity, adiposity, and growth performance in agricultural animals, including pigs [33-35]. Nonetheless, FMT is a promising method in terms of cost and practicality, as the inexpensive fecal microbiome mix can be easily administered orally by mixing with feed or drinking water [21]. Together, our study suggests that FMT could be a promising strategy to prevent gut dysbiosis and ETEC infection in weaning piglets, which could be tested at field level.

References

1. Luppi A. Swine enteric colibacillosis: diagnosis, therapy and antimicrobial resistance. Porcine health management 2017; 3:16.
2. Gyles CL. Escherichia coli verotoxin and other cytotoxins. Wallingford, United Kingdom: CAB International, 1994.
3. Zhang W. Progress and challenges in vaccine development against enterotoxigenic Escherichia coli (ETEC)-associated porcine postweaning diarrhoea (PWD). 2014; 1:1006-19.
4. Barton MD. Impact of antibiotic use in the swine industry. Curr Opin Microbiol 2014; 19:9-15.
5. Van Boeckel TP, Brower C, Gilbert M, Grenfell BT, Levin SA, Robinson TP, et al. Global trends in antimicrobial use in food animals. Proc Natl Acad Sci U S A 2015; 112:5649-54.
6. Maron DF, Smith TJ, Nachman KE. Restrictions on antimicrobial use in food animal production: an international regulatory and economic survey. Global Health, 2013:48.
7. Zawack K, Li M, Booth JG, Love W, Lanzas C, Gröhn YT. Monitoring Antimicrobial Resistance in the Food Supply Chain and Its Implications for FDA Policy Initiatives. Antimicrob Agents Chemother, 2016:5302-11.
8. Campbell JM, Crenshaw JD, Polo J. The biological stress of early weaned piglets. J Anim Sci Biotechnol, 2013:19.
9. Gresse R, Chaucheyras-Durand F, Fleury MA, Van de Wiele T, Forano E, Blanquet-Diot S. Gut Microbiota Dysbiosis in Postweaning Piglets: Understanding the Keys to Health. Trends Microbiol 2017; 25:851-73.
10. Frese SA, Parker K, Calvert CC, Mills DA. Diet shapes the gut microbiome of pigs during nursing and weaning. Microbiome 2015; 3:28.
11. Kim J, Nguyen SG, Guevarra RB, Lee I, Unno T. Analysis of swine fecal microbiota at various growth stages. Arch Microbiol 2015; 197:753-9.
12. Bin P, Tang Z, Liu S, Chen S, Xia Y, Liu J, et al. Intestinal microbiota mediates Enterotoxigenic Escherichia coli-induced diarrhea in piglets. BMC Vet Res 2018; 14:385.
13. Looft T, Johnson TA, Allen HK, Bayles DO, Alt DP, Stedtfeld RD, et al. In-feed antibiotic effects on the swine intestinal microbiome. Proc Natl Acad Sci U S A 2012; 109:1691-6.
14. Alain BPE, Chae JP, Balolong MP, Bum Kim H, Kang DK. Assessment of fecal bacterial diversity among healthy piglets during the weaning transition. The Journal of general and applied microbiology 2014; 60:140-6.
15. Schenck LP, Beck PL, MacDonald JA. Gastrointestinal dysbiosis and the use of fecal microbial transplantation in Clostridium difficile infection. World JGastrointestPathophysiol 2015; 6:169.

16. Niederwerder MC. Fecal microbiota transplantation as a tool to treat and reduce susceptibility to disease in animals. Vet Immunol Immunopathol 2018; 206:65-72.
17. Filip M, Tzaneva V, Dumitrascu DL. Fecal transplantation: digestive and extradigestive clinical applications. Clujul Med 2018; 91:259-65.
18. Wang JW, Kuo CH, Kuo FC, Wang YK, Hsu WH, Yu FJ, et al. Fecal microbiota transplantation: Review and update. Journal of the Formosan Medical Association = Taiwan yi zhi 2018.
19. Hvas CL, Jorgensen SMD, Jorgensen SP, Storgaard M, Lemming L, Hansen MM, et al. Fecal Microbiota Transplantation is Superior to Fidaxomicin for Treatment of Recurrent Clostridium difficile Infection. Gastroenterology 2019.
20. Kim S, Covington A, Pamer EG. The intestinal microbiota: Antibiotics, colonization resistance, and enteric pathogens. Immunological reviews 2017; 279:90-105.
21. Hu J, Chen L, Tang Y, Xie C, Xu B, Shi M, et al. Standardized Preparation for Fecal Microbiota Transplantation in Pigs. Front Microbiol 2018; 9:1328.
22. R MR, L.Z J, Jung-Woo K, Lin F, A FA, K BS. Passive protective effect of egg-yolk antibodies against enterotoxigenic Escherichia coli K88+ infection in neonatal and early-weaned piglets. FEMS Immunology & Medical Microbiology 1999; 23:283-8.
23. Wang W, Zijlstra RT, Gänzle MG. Identification and quantification of virulence factors of enterotoxigenic Escherichia coli by high-resolution melting curve quantitative PCR. BMC Microbiol 2017; 17:114.
24. Li Q, Burrough ER, Gabler NK, Loving CL, Sahin O, Gould SA, et al. A soluble and highly fermentable dietary fiber with carbohydrases improved gut barrier integrity markers and growth performance in F18 ETEC challenged pigs1. J Anim Sci 2019; 97:2139-53.
25. Pollock J, Hutchings MR, Hutchings KEK, Gally DL, Houdijk JGM. Changes in the Ileal, but Not Fecal, Microbiome in Response to Increased Dietary Protein Level and Enterotoxigenic Escherichia coli Exposure in Pigs. Appl Environ Microbiol 2019; 85.
26. Characterisation of Early-Life Fecal Microbiota in Susceptible and Healthy Pigs to Post-Weaning Diarrhoea. 2017.
27. Abe F, Ishibashi N, Shimamura S. Effect of administration of bifidobacteria and lactic acid bacteria to newborn calves and piglets. JDairy Sci 1995; 78:2838.
28. Trevisi P, De Filippi S, Minieri L, Mazzoni M, Modesto M, Biavati B, et al. Effect of fructo-oligosaccharides and different doses of Bifidobacterium animalis in a weaning diet on bacterial translocation and Toll-like receptor gene expression in pigs. Nutrition 2008; 24:1023-9.
29. Barba-Vidal E, Castillejos L, Lopez-Colom P, Rivero Urgell M, Moreno Munoz JA, Martin-Orue SM. Evaluation of the Probiotic Strain Bifidobacterium longum subsp. Infantis CECT 7210 Capacities to Improve Health Status and Fight Digestive Pathogens in a Piglet Model. Front Microbiol 2017; 8:533.
30. Splichalova A, Trebichavsky I, Rada V, Vlkova E, Sonnenborn U, Splichal I. Interference of Bifidobacterium choerinum or Escherichia coli Nissle 1917 with Salmonella Typhimurium in gnotobiotic piglets correlates with cytokine patterns in blood and intestine. Clin Exp Immunol 2011; 163:242-9.
31. Shu Q, Qu F, Gill HS. Probiotic treatment using Bifidobacterium lactis HN019 reduces weanling diarrhea associated with rotavirus and Escherichia coli infection in a piglet model. JPediatrGastroenterolNutr 2001; 33:171-7.
32. Merrifield CA, Lewis MC, Claus SP, Pearce JT, Cloarec O, Duncker S, et al. Weaning diet induces sustained metabolic phenotype shift in the pig and influences host response to Bifidobacterium lactis NCC2818. Gut 2013; 62:842-51.
33. Brunse A, Martin L, Rasmussen TS, Christensen L, Skovsted Cilieborg M, Wiese M, et al. Effect of fecal microbiota transplantation route of administration on gut colonization and host response in preterm pigs. Isme j 2018.
34. Yang H, Xiang Y, Robinson K, Wang J, Zhang G, Zhao J, et al. Gut Microbiota Is a Major Contributor to Adiposity in Pigs. Front Microbiol 2018; 9:3045.
35. Tsai TC, Maxwell CV, Zhao JC. Fecal microbiota transplant at weaning improves growth performance and alters fecal microbiome in pigs. JAnimSci 2019; 97:175-.