Effects of Early Sub-Therapeutic Antibiotic Administration and its Subsequent Withdrawal on Body Composition, Gut Microbiota and Metabolite Profiles in a Pig Model

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Research

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Abstract

Background: Antibiotic exposure in early life has shown to be a significant risk factor for later fat accumulation in human. However, whether early sub-therapeutic antibiotic (STA) exposure affects body composition and its mechanisms remains unclear. The present study used a combination of comparative slaughter method, microbiota, and metabolomics measurement to investigate the effects of early STA administration and its subsequent withdrawal on body composition, colonic microbiota and metabolite profiles in a pig model. The piglets were fed the same basal starter diet added with STA (STA) or without STA (CON) for two weeks during the administration period, and then all piglets were switched to the same nursery diet without STA during the withdrawal period until they reached approximately 25 kg body weight.

Results: Results showed that STA did not significantly improve piglet growth performance during the administration period and the withdrawal period. Piglets treated with the STA had a lower body water deposition (g/d) during the withdrawal period, and tended to have increased body lipid deposition (g/d) during the withdrawal period and the whole period than CON group. It was found that STA was initially effective in decreasing the abundance of pathogenic bacteria during the administration period, such as *Alloprevotella*, *Bacteroides*, *Solobacterium*, and *Sutterella*. However, they could not continue the effect during the withdrawal period, leading to the rebound of pathogenic bacteria such as *Alloprevotella* and the increase of the abundance of other pathogenic bacteria like *Oscillibacter*. Remarkably, STA treatment decreased the abundance of *Blautia* that play a potential protective role against obesity either during the administration period or the withdrawal period. Metabolomic analysis indicated that STA mainly altered amino acid metabolism, lipid metabolism, and carbohydrate metabolism during the two periods. Furthermore, Spearman's correlation analysis showed that the gut microbiota was highly correlated with microbial metabolites changes.

Conclusion: These results suggest that STA administration may alter tissue deposition through reshaping the gut microbiota and their metabolite profiles.

Background

Antibiotics have been widely used in the therapy of bacterial infections in pediatric patients, significantly reducing their morbidity and mortality [1]. Also, it has been proposed as a supplement to the refeeding program for malnourished children [2] due to the enteric infection leads to a substantial part of malnutrition [3]. However, full (therapeutic) dose antibiotic exposure during early life has found to be a significant risk factor for later fat accumulation [4] that contribute to the development of metabolic syndrome, including type 2 diabetes mellitus, fatty liver, and cardiovascular disease [5]. Nowadays, the risk of fat accumulation associated with early administration to antibiotics has become a vital health problem for humans[6]. However, whether early sub-therapeutic antibiotic (STA) administration affects body composition and its mechanism remains unclear.

It has been reported that antibiotics can alter gut microbiota and its metabolism [7], and the causal role of gut microbiota in modulating fat accumulation has been demonstrated by coloning the gut microbiota from obese mice[8] or humans[9] in germ-free mice. Gut microbiota functions as an organ with many metabolism, immunology, and endocrine-like effects that crucial for human health [10, 11]. The pathogenesis of gut microbiota involved in the fat accumulation may be through its influence on energy balance, nutrient absorption, inflammatory pathway, and the gut-brain axis [12]. However, the effects of early STA administration and its subsequent withdrawal on gut microbiota and bacterial metabolites are poorly understood.

We utilized piglets as a model to determine the relationship between gut microbiota of host and STA exposure due to their similarities in anatomy and size to human infants [13, 14]. In the present study, an integrated approach combination of comparative slaughter method, 16S rRNA gene sequencing, and liquid chromatograph-mass spectrometry (LC-MS) technique were utilized to investigate the impact of early STA administration and its subsequent withdrawal on body chemical composition, gut microbial composition and metabolome of a piglet's model.

Materials And Methods

The procedures of the present study were followed the Chinese guidelines for animal welfare, and approved by the Animal Care and Use Committee of the Guangdong Academy of Agricultural Sciences (GAASIAS-2016-017).

Animals, diets and experimental design

Fifty 21-day-old Duroc × Landrace × Yorkshire weaned piglets with average initial body weight (BW) of 6.39 ± 0.02 kg were randomly allocated into a control group (CON) and a STA group with 5 replicates in each, comprising 5 piglets in each replicate. The entire experiment was divided into two periods: the administration period and the withdrawal period. The schematic diagram for the experimental design was shown in Fig. 1. During the administration period, piglets in the CON group were fed a basal starter diet, and those in the STA group were fed a basal starter diet supplemented with 30 mg/kg bacitracin methylene disalicylate, 75 mg/kg chlortetracycline, and 300 mg/kg calcium oxytetracycline for 2 weeks. During the withdrawal period, all piglets were switched to the same nursery diet without STA until they reached an average target BW of approximately 25 kg. Diets were formulated to meet or exceed the National Research Council recommendations [15]. The piglets had *ad libitum* access to feed and water, and the nursery diet is different from the starter diet. The ingredient and nutrient composition of the nursery diet and starter diet are presented in Table S1.

Sampling

Prior to implementing dietary treatments, an additional five weaned piglets with similar initial BW as the experimental pigs were slaughtered to determine initial body composition. Piglets were weighed at the

start and the end of administration period and withdrawal period to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR). After fasting for 12 h, five piglets (every group with 5 replicates in each, 1 piglet form each replicate) were anesthetized with sodium pentobarbital and slaughtered by exsanguination at the end of the administration period and the withdrawal period. Colonic digesta was immediately snap-frozen in liquid nitrogen and stored at -80℃ until further microbiome and metabolome analysis. The empty gastrointestinal tract, visceral organs, blood, and carcass were stored at -20°C for experimental detection. The frozen body components were sheared into small parts in a double-shaft crusher (model L-SP380, LiWill Co. Ltd., Zhengzhou, China) and put in a commercial grinder with an 18-mm die (model SG-130, Yusheng Co., Langfang, China) and then minced in a tiny grinding mill (model GN-130, Yusheng Co., Langfang, China). After homogenizing the body components with a kitchen mixer, subsamples were obtained for chemical analysis.

Growth performance and body composition analysis

During the whole feeding trial, ADG, ADFI, and FCR were calculated. Body composition subsamples were analyzed according to AOAC [16] for water content determined using a convection oven at 105 ℃; crude protein content was calculated as total N content × 6.25 and total N content was determined in a Kjeltec analyzer (model 8400, FOSS Analytical AB, höganäs, Sweden); crude fat content were measured by automatic extractor analyzer (model XT 15i, Ankom Technology Co., Macedon, NY); ash content was determined in a muffle furnace at 550 ℃. The deposition of water, protein, lipid, and ash in the piglets' body was calculated by dividing the difference in body chemical composition between the end and beginning of each trial period by the corresponding trial day [17]. For example: body lipid deposition (g/d)=[(final body lipid composition content (%) × final BW) – (initial body lipid composition content (%)× initial BW)]/(corresponding trial day).

Microbiome analysis

Total genomic DNA was extracted from colonic digesta by QIAAMP Powerfecal DNA Kit (Qiagen, Hilden, Germany) follow the manufacturer's instructions and its concentration was determined by the Nanodrop2000 (Thermo Fisher Scientific, Waltham, MA, USA). The 16SrRNA gene of V3-V4 region was amplified by the universal forward primer 338F (5'-ACTCCTRCGGAGGCAGGCAG-3') and reverse primer 806R (5'-GGACTACCVGGATCTAAT-3'). The PCR amplicon was extracted by the QIAGEN Gel Extraction Kit (Qiagen, Hilden, Germany) following the manufacturer protocol. The TruSeq Generated Sequencing Library ® by using a free sample preparation kit for DNA PCR that following the manufacturer's instructions and index code (Illumina, San Diego, CA, USA). The library quality was evaluated by Qubit@2.0 fluorimeter and AgilentBioanalyzer2100 system. Finally, the library was sequenced on the Illumina-Novaseq platform to produce 250bp paired end readings. Bioinformatics analysis performed based on the description of previous studies[18].

Microbial Metabolite Measurement

The procedure of microbial metabolite measurement as our previously describe [19] using Vanquish UHPLC system (Thermo Fisher) coupled with an Orbitrap Q Exactive series mass spectrometer (Thermo

Fisher). Briefly, the homogenate of grounded colonic digesta was centrifuged. The obtained supernatant was diluted with LC-MS grade water and centrifuged for 10 min, and finally injected into the LC-MS/MS system analysis. Raw UHPLC-MS/MS data was analyzed by Compound Finder 3.1 (CD3.1, Thermo Fisher Scientific Waltham, MA, USA) to peak alignment, peak pick-up, and quantification of each metabolite.

Statistical analysis

The data were analyzed by Student's t-test if the date fitted a Gaussian distribution, using SAS 9.4 (SAS Inst., Inc., Cary, NC). or by Wilcoxon test if the data were not normally distributed. Data are expressed as means ± standard deviation (SD). Correlations between gut microbiota and metabolite profiles were analyzed by Spearman's correlation test. Significant differences were declared at $P < 0.05$ and tendencies declared at $0.05 < P < 0.10$.

Results

Effects of STA administration and its subsequent withdrawal on growth performance

Effects of STA administration and its subsequent withdrawal on growth performance of piglets were given in Table 1. We found that STA treatment had no influence ($P > 0.05$) on ADG and ADFI during the administration period, withdrawal period and the whole period than CON group, except that the FCR within the withdrawal period tended to be higher ($0.05 < P < 0.10$) in the STA group than in the CON group. Moreover, the experimental days to reach target BW between the two groups is insignificant ($P > 0.05$).

Effects of STA administration and its subsequent withdrawal on the body chemical composition (% of empty BW)

The average body chemical composition of piglets slaughtered at the start of the experiment on a percentage basis was (% of empty BW) 69.7 ± 1.82, 15.6 ± 0.35, 10.8 ± 1.85 and 3.04 ± 0.13 for water, protein, lipid and ash, respectively. Effects of STA administration and its subsequent withdrawal on body chemical composition of piglets are presented in Table 2. At the end of the administration period and the withdrawal period, no difference ($P > 0.05$) between the two groups was observed on the body chemical composition of piglets.

Effects of STA administration and its subsequent withdrawal on tissue deposition (g/d)

Effects of STA administration and its subsequent withdrawal on tissue deposition are presented in Table 3. During the administration period, no differences ($P > 0.05$) for tissue deposition were observed between

the two groups. Only when calculated as tissue deposition per day, there is a significant effect on water deposition ($P < 0.05$) in the withdrawal period and tendencies for increased lipid deposition ($0.05 < P < 0.10$) drawn by the effect seen in the withdrawal period and the whole period.

Effects of STA administration and its subsequent withdrawal on gut microbiota structure

To evaluate the influence of STA administration and its subsequent withdrawal on gut microbiota structure, the sequences of the 16S rRNA gene were amplified. After administration period, no significant difference ($P > 0.05$) in α-diversity indices, including observed species, Ace, Shannon and Simpson index, was found between the two groups (Fig. 2A). PCoA and NMDS plots based on Bray Curtis distance were performed to assess the differences in beta-diversity, and the results showed that the two groups were well-separated (Fig. 2C). At the end of the withdrawal period, the STA group had lower species richness and diversity indices compared with the CON group, as reflected by the decreased ($P < 0.05$) observed species and Ace index (Fig. 2B). However, the Shannon and Simpson index did not differ ($P > 0.05$) between the CON and STA groups (Fig. 2B). For beta-diversity, the results indicated significant differences between the two groups (Fig. 2D).

Venn analysis identified 262 and 205 unique operational taxonomic units (OTUs) of CON and STA group, respectively, and 463 shared OTUs in the two groups at the end of the administration period (Fig. 3A); and 395 and 222 unique OTUs in the CON and STA group, respectively, and 783 shared OTUs in the two groups at the end of the withdrawal period (Fig. 3B). At the end of the administration period, compared with the CON group, the relative abundance of *Alloprevotella*, *Sphingomonas*, *Bacteroides*, *Solobacterium*, *Blautia*, *Massilia* and *Sutterella* were dramatically declined ($P < 0.05$) in the STA group (Figs. 3C). After the withdrawal period, STA treatment enhanced ($P < 0.05$) the relative abundance of *Alloprevotella* and *Oscillibacter*, and decreaced ($P < 0.05$) the relative abundance of *Blautia*, *Succinivibrio*, *Corynebacterium*, *Methanosphaera*, *Desulfovibrio* and *Holdemanella* than CON treatment (Fig. 3D).

Effects of STA administration and its subsequent withdrawal on metabolite profiles

To further explore the impact of STA administration and its subsequent withdrawal on the gut microbiota, LC-MS was used to analyze the metabolite profiles in the CON and STA groups. The PLSDA model showed that the STA group separated from the CON group either at the end of the administration period (Fig. 4A) or at the end of the withdrawal period (Fig. 4B). Based on the criteria of VIP > 1 and $P < 0.05$ and fold change ≥ 1.20 or ≥ 0.83, 25 and 36 metabolites were identified at the end of the administration period (Fig. 4C) and the end of the withdrawal period (Fig. 4D), respectively.

At the end of the administration period, 10 metabolites (nicotinic acid, isobutyryl-L-carnitine, prostaglandin B2, phosphatidylglycerol (3:0/18:1), lysophosphatidylethanolamine 22:5, fatty acid esters of hydroxy fatty acids (FAHFA) (16:0/18:0), FAHFA (18:0/20:2), gibberellin A4, xanthine and 4-

chlorophenol) were upregulated, whereas 15 metabolites (inositol, L-cysteinesulfinic acid, phenylacetylglycine, phenylacetylglutamine, 5-hydroxylysine, nicotinuric acid, indoleacrylic acid, biocytin, imidazoleacetic acid, tomatidine, 7α-hydroxytestosterone, kinetin, picolinamide, mevalonolactone and feruloylcholine) were downregulated in the STA group compared with the CON group (Fig. 5A). At the end of the withdrawal period, 18 metabolites (D-arabinose, D-mannitol, coniferin, proline, L-lysine, L-cystathionine, S-adenosylhomocysteine, stearic acid, erucic acid, 11β-hydroxyandrosterone, nervonic acid, adenosine diphosphate ribose, nicotinamide adenine dinucleotide, uric acid, xanthine, 3,5-dihydroxybenzoic acid, salicylic acid and hydroxypyruvic acid) were enriched, while 18 metabolites (D-raffinose, 5-hydroxy-L-tryptophan, FAHFA (2:0/24:2), FAHFA (4:0/22:0), FAHFA (14:0/22:3), FAHFA (17:1/22:3), FAHFA (18:2/17:2), FAHFA (20:2/22:3), elaidic acid, ginsenoside C, 13,14-dihydroretinol, (R)-3-hydroxy myristic acid, 7α-hydroxypregnenolone, calcitriol, estriol, cyclic adenylic acid, bilirubin and N,N-dimethylaniline) were reduced in the STA group compared with the CON group (Fig. 5B). These differentiated metabolites mainly included amino acid relatives, lipid relatives, carbohydrates, nucleotides, and others.

In the present study, MetaboAnalyst (http://www.metaboanalyst.ca/) was used to perform metabolic pathway enrichment analysis. Results showed that STA administration had significant effects on the glycerophospholipid metabolism, ascorbate and aldarate metabolism, taurine and hypotaurine metabolism, vitamin metabolism, amino acid metabolism and galactose metabolism (Fig. 6A); antibiotic withdrawal mainly affected the purine metabolism, amino acid metabolism, vitamin metabolism, galactose metabolism and biosynthesis of unsaturated fatty acids (Fig. 6B).

Realtionship between microbiotal and metabolites

To detect the relationship between the colon microbiome and its metabolites, we utilized the Spearman's correlation analysis for metabolites with VIP > 1 and bacterial genera with significant differences between the STA and CON groups. At the end of the administration period, the relative abundance of *Alloprevotella* and *Sutterella* was positively correlated with inositol. The relative abundance of *Bacteroides* and *Solobacterium* had a positive correlation with biocytin, tomatidine, 7α-hydroxytestosterone, inositol, picolinamideand and feruloylcholine, while they had a negative correlation with phosphatidylglycerol (3:0/18:1), lysophosphatidylethanolamine 22:5, FAHFA (16:0/18:0) and FAHFA (18:0/20:2). The relative abundance of *Blautia* showed a positive correlation with inositol and feruloylcholine, and a negative correlation with prostaglandin B2 and FAHFA (18:0/20:2) (Fig. 7A). At the end of the withdrawal period, the relative abundance of *Alloprevotella* and *Oscillibacter* was positively correlated with proline and negatively correlated with FAHFA (4:0/22:0) and elaidic acid. The relative abundance of *Blautia*, *Succinivibrio* and *Holdemanella* showed a negative correlation with proline, L-cystathionine, uric acid, salicylic acid and hydroxypyruvic acid, and a positive correlation with 5-hydroxy-L-tryptophan, FAHFA (4:0/22:0), FAHFA (20:2/22:3), elaidic acid, ginsenoside C, 13,14-dihydroretinol, (R)-3-hydroxy myristic acid and bilirubin (Fig. 7B).

Discussion

It has been reported that early antibiotic exposure might program later body composition and therefore might be a determinant of obesity risk, which was associated with alteration in gut microbiota composition and metabolites. Since antibiotics have been used to promote growth in animal[20] and have been proposed as therapeutic regimes for malnutrition in humans [21], we firstly studied the effect of STA on pig growth. It was found that under well-controlled environmental conditions, STA did not significantly improve piglet growth performance during the administration period, which was consistent with other studies [22, 23]. It is generally assumed that growth-promoting mechanism of antibiotics is related to its ability to reduce clinical and subclinical infections under sanitary challenges [20]. A meta-analysis involving more than 900 infants also showed that the positive effects of antibiotics were most prominent in the youngest and most malnourished children, but they were often less dramatic and not statistically significant in children without the disease [24]. A previous report [25] found that STA affect subsequent performance negatively, and this may be that early STA administration increased their susceptibility to pathogens during the withdrawal period as discussed later. While, in the present study, STA administration didn't affect subsequent performance during the withdrawal period.

In the present study, STA did not significantly alter body composition and tissue deposition of piglets at the end of the 2-week administration period, which contrasts with the previous report [26] that STA increased fat mass in young mice after 7-week exposure. This may be attributed to differences in the STA type and dose or the shorter STA administration time, which have not yet been shown to be different in body composition between the groups. During the withdrawal period and the whole period, piglets in the STA group tended to have a higher body lipid deposition than those in the CON group.

It was found that during the administration period, STA treatment increased abundances of several harmful bacteria/pathogenic bacteria (*Alloprevotella*, *Bacteroides*, *Solobacterium* and *Sutterella*) compared with CON group. *Alloprevotella* was considered an opportunistic pathogen microorganism that causes infections in the host [27, 28]. It was reported that individuals with the *Bacteroides* enterotype increased susceptibility to disease [29]. *Solobacterium* was positively correlated with colorectal cancer [30]. Previous studies showed that rather than directly induce substantial inflammation, *Sutterella* can degrade IgA to impair the functionality of the intestinal antibacterial immune response [31, 32]. However, during the withdrawal period, STA treatment increased the abundances of several harmful bacteria, for example, *Alloprevotella* and *Oscillibacter* and decreased abundances of several beneficial bacteria such as *Succinivibrio* and *Desulfovibrio*. For example, *Oscillibacter* has been reported that promotes metabolic diseases and gut dysbiosis [33]. *Succinivibrio* was reported to be lower in humans with environmental enteric dysfunction (a causative factor of childhood stunting) [34]. *Desulfovibrio* is significant in sugar metabolism and is negatively associated with inflammation markers [35, 36]. These results suggested that STA were initially effective in decreasing the abundance of pathogenic bacteria during the administration period, but they were not able to continue the effect during the withdrawal period, leading to the rebound of pathogenic bacteria such as *Alloprevotella* and the increase of the abundance of other pathogenic bacteria. Evidence has shown that antibiotic administration, especially in early childhood, increases susceptibility to intestinal infections after antibiotic cessation [37].

Remarkably, we found that STA treated decreased the abundance of *Blautia* that play a potential protective role against obesity either during STA administration or its subsequent withdrawal period. *Blautia*, a common acetic acid-producing bacterium, may suppress insulin-mediated fatty deposits in adipocytes and promote the metabolism of unbound lipids and glucose in other tissues by activating the G protein-coupled receptors GPR41 and GPR43, thereby alleviating obesity-related diseases [38]. A previous study found that *Blautia* is the only intestinal microorganism negatively correlated with visceral fat accumulation, and adiposity biomarker for metabolic disease risk [39]. In a study of differential microbiota between lean and fat line chickens, Blautia was significantly reduced in the latter [40]. Similarly, significant depletion of *Blautia* was observed in obese children [41, 42].

Metabolomics, an effective method to detect the variant metabolites and biochemical pathways [43, 44], was utilized to further explore gut microbiota metabolism in response to STA administration and its subsequent withdrawal. The PLS-DA model was a clear separation of colonic metabolites between the STA and CON groups either within the administration period or within the withdrawal period, suggesting significant differences in the metabolic profiles due to different treatments. During the administration period, STA significantly altered glycerophospholipid metabolisms, as reflected by the increased concentrations of phosphatidylglycerol (3:0/18:1) and lysophosphatidylethanolamine 22:5. Previous studies showed that glycerophospholipid is vital in the strengthening intestinal barrier [45, 46]. The compounds involved in amino acid metabolisms, such as L-cysteinesulfinic acid, phenylacetylglycine, phenylacetylglutamine and 5-hydroxylysine, were dramatically decreased in the STA group compared to the CON group, which suggests lower nitrogen sources left for the microbial fermentation of the large intestine. A previous report found that antibiotics could upregulate the gene expression of amino acid transporters and receptors in the small intestine and thereby improve the absorption of amino acids [47]. However, during the withdrawal period, piglets in the STA group showed higher amino acid relatives like proline, L-lysine, L-cystathionine and S-adenosylhomocysteine in comparison with the CON group, indicating an increased amount of protein derived substrate for microbial fermentation in the colon, which was consistent with a previous study [48]. This could be harmful to host health due to the possible formation of a range of toxic and harmful products from protein fermentation, such as ammonia, indoxyl sulfate and trimethylamine oxide [49]. Meanwhile, in the present study, STA significantly altered the compounds involved in FAHFAs metabolism, indicating by an enhanced concentrations of FAHFA (16:0/18:0) and FAHFA (18:0/20:2) during the administration period, and a decrease in the concentrations of FAHFA (14:0/22:3), FAHFA (17:1/22:3), FAHFA (18:2/17:2) and FAHFA (20:2/22:3) than CON during the withdrawal period. FAHFAs are a novel class of bioactive lipid, forming by esterification hydroxyl group of a hydroxy fatty acid and the carboxyl group of a fatty acid [50]. Previous studies have shown that palmitic acid esters of hydroxy stearic acids and the family of polyunsaturated FAHFAs have anti-inflammatory and immunomodulatory effects [51, 52]. Besides, several new short-chain FAHFAs (SFAHFAs) of acetic acid or propanoic acid esterified long-chain hydroxy fatty acids tended to be lower in mice fed with a high-fat diet than those fed with a regular diet [53].Similarly, STA treatment decreased the concentrations of several SFAHFAs like FAHFA (2:0/24:2) and FAHFA (4:0/22:0) during the withdrawal period, which coincided with higher content of body lipid and lower abundance of *Blautia*, a bacterium

with a protective role against obesity. These results suggest that STA plays beneficial roles in gut health during the administration period, but it may exert harmful effects on gut health during the withdrawal period. Simultaneously, most compounds involved in carbohydrate metabolisms like D-arabinose, D-mannitol and coniferin were increased by STA treatment than the CON treatment during withdrawal period, indicating that most carbohydrates can be fermented by gut microbiota in the colon.

The gut microbiota and microbial metabolites are vital for promoting intestinal immunity balance. In this study, we found correlations between the gut microbiota and microbial metabolites. However, whether the effects of STA induced on gut microbiota and microbial metabolites would influence the host immunity and its mechanism was still unknow. In the future, we will further study after STA administration the relationship between gut microbiota, microbial metabolites, corresponding metabolite receptors and host immunity.

Conclusions

This study utilized the comparative slaughter method, microbial and metabolite measurement and found that STA administration may alter tissue deposition through reshaping the gut microbiota and their metabolite profiles. These results may be helpful for the future application of the STA on human nutrition.

Declarations

Acknowledgements

Not applicable

Authors' contributions

Conceptualization, Z.J. and L.W.; methodology, Z.J. and L.H.; software, L.H. and Y.X.; validation, Z.J. and L.W.; formal analysis, L.H. and Y.Q.; data curation, H.X. and X.W.; writing—original draft preparation, L.H.; writing—review and editing, S.C., Y.Q. and L.W.; visualization, S.C.; supervision, X.Y. and K.G.; project administration, L.H.; funding acquisition, Z.J. All authors have reviewed and approved the final manuscript.

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Availability of data and materials

The datasets supporting the conclusions of this article are included within the article.

Ethics approval and consent to participate

The experimental protocols and procedures performed in this study were approved by the Animal Care and Use Committee at Guangdong Academy of Agricultural Sciences (GAASIAS-2016-017).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1. Effects of STA administration and its subsequent withdrawal on initial BW, ADG, ADFI and FCR of piglets [1]

Item	CON	STA	*P*-value
Administration period (day 0 to 14)			
Initial BW, kg	6.39 ± 0.07	6.39 ± 0.04	1.000
ADG, kg/d	0.32 ± 0.03	0.35 ± 0.04	0.331
ADFI, kg/d	0.42 ± 0.03	0.43 ± 0.05	0.588
FCR	1.29 ± 0.05	1.26 ± 0.03	0.256
BW at end of period, kg	10.9 ± 0.40	11.2 ± 0.57	0.373
Withdrawal period (day 15 to target BW)			
ADG, g/d	0.53 ± 0.01	0.51 ± 0.02	0.253
ADFI, g/d	0.86 ± 0.02	0.87 ± 0.05	0.614
FCR	1.64 ± 0.03	1.71 ± 0.06	0.079
Final BW, kg	24.9 ± 0.37	25.2 ± 1.16	0.638
Whole period (day 0 to target BW)			
ADG, g/d	0.46 ± 0.02	0.46 ± 0.02	0.865
ADFI, g/d	0.71 ± 0.01	0.73 ± 0.05	0.525
FCR	1.56 ± 0.04	1.60 ± 0.03	0.150
Days on experiment	40.8 ± 1.50	41.5 ± 1.73	0.537

[1] ADG, average daily gain. ADFI, average daily feed intake. FCR, feed:gain. Data are expressed as means ± SD. CON, control group. STA, sub-therapeutic antibiotic group.

Table 2. Effects of STA administration and its subsequent withdrawal on body chemical composition (% of empty BW) of piglets [1]

Item	CON	STA	*P*-value
End of the administration period			
Water, %	70.9 ± 0.78	71.1 ± 0.56	0.645
Protein, %	16.3 ± 0.23	16.0 ± 0.34	0.105
Lipid, %	9.01 ± 0.98	9.01 ± 0.27	1.000
Ash, %	3.10 ± 0.42	3.13 ± 0.06	0.907
End of the withdrawal period			
Water, %	69.4 ± 0.62	68.5 ± 1.32	0.217
Protein, %	16.8 ± 0.30	16.6 ± 0.30	0.401
Lipid, %	10.7 ± 1.00	11.2 ± 1.32	0.532
Ash, %	2.82 ± 0.37	2.88 ± 0.22	0.791

[1] Data are expressed as means ± SD. CON, control group. STA, sub-therapeutic antibiotic group.

Table 3. Effects of STA administration and its subsequent withdrawal on tissue deposition (g/d) of piglets [1]

Item	CON	STA	*P*-value
Administration period (day 0 to 14)			
Water, g/d	235 ± 18.1	252 ± 29.1	0.305
Protein, g/d	55.8 ± 4.18	56.7 ± 6.53	0.820
Lipid, g/d	20.9 ± 2.24	22.9 ± 3.68	0.346
Ash, g/d	10.3 ± 0.79	11.2 ± 1.28	0.214
Withdrawal period (day 15 to target BW)			
Water, g/d	358 ± 7.6	339 ± 9.3	0.018
Protein, g/d	89.9 ± 2.08	87.2 ± 2.65	0.156
Lipid, g/d	62.9 ± 1.75	66.4 ± 2.40	0.053
Ash, g/d	13.6 ± 0.26	13.7 ± 0.35	0.861
Whole period (day 0 to target BW)			
Water, g/d	316 ± 9.5	309 ± 16.6	0.494
Protein, g/d	78.2 ± 2.38	76.7 ± 4.03	0.557
Lipid, g/d	48.4 ± 1.46	51.6 ± 2.72	0.085
Ash, g/d	12.5 ± 0.37	12.8 ± 0.69	0.444

[1] Data are expressed as means ± SD. CON, control group. STA, sub-therapeutic antibiotic group.

Figures

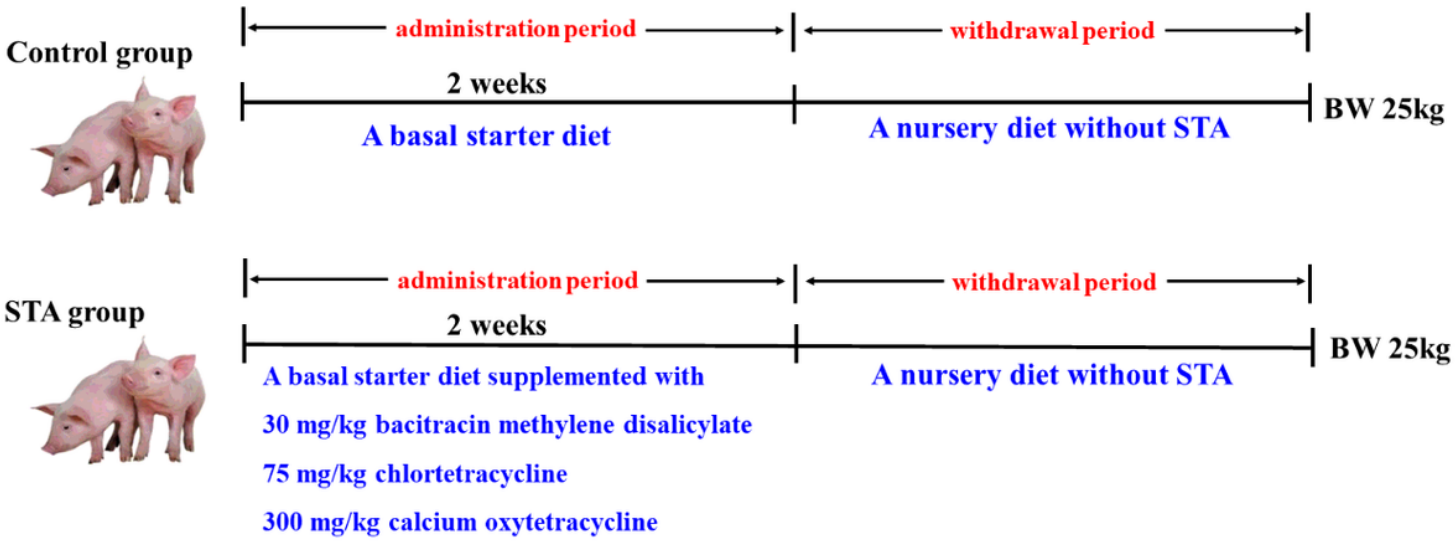

Figure 1

A schematic diagram for the experimental design. Fifty 21-day-old Duroc × Landrace × Yorkshire weaned piglets with average initial body weight (BW) of 6.39 ± 0.02 kg were randomly allocated into a control group (CON) and a STA group with 5 replicates in each, comprising 5 piglets in each replicate. The entire experiment was divided into two periods: the administration period and the withdrawal period. During the administration period, piglets in the CON group were fed a basal starter diet, and those in the STA group were fed a basal starter diet supplemented with 30 mg/kg bacitracin methylene disalicylate, 75 mg/kg chlortetracycline, and 300 mg/kg calcium oxytetra-cycline for 2 weeks. During the withdrawal period, all piglets were switched to the same nursery diet without STA until they reached an average target BW of approximately 25 kg.

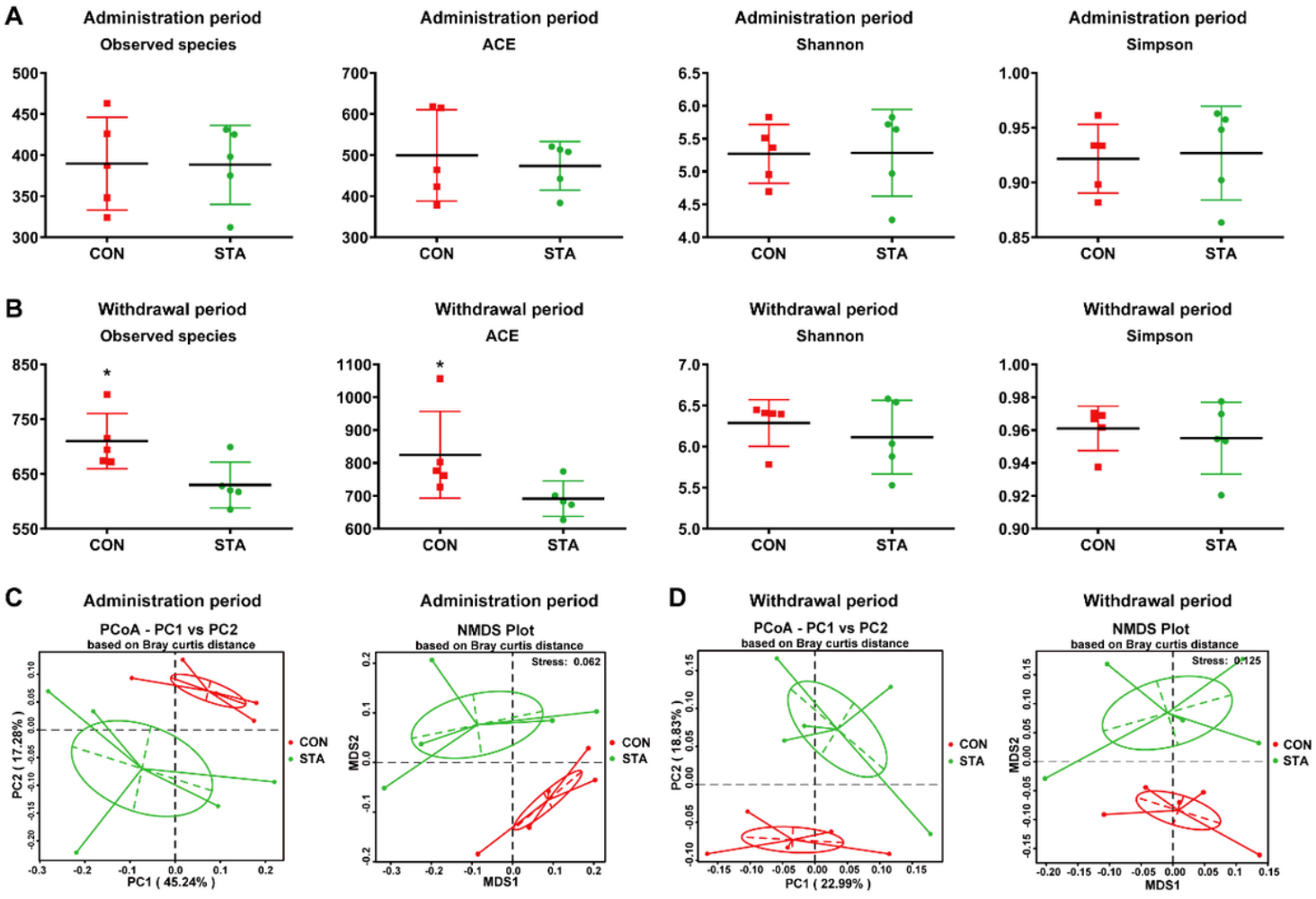

Figure 2

Effects of STA administration and its subsequent withdrawal on gut microbiota diversity in piglets. The α-diversity indices, including observed species, Ace, Shannon and Simpson index at the end of the administration period (A) and the end of the withdrawal period (B). Data are expressed as means ± SD. Each point represents one sample. The β-diversity visualized in PCoA plot and NMDS plot at the end of the administration period (C) and the end of the withdrawal period (D). CON, control group. STA, sub-therapeutic antibiotic group.

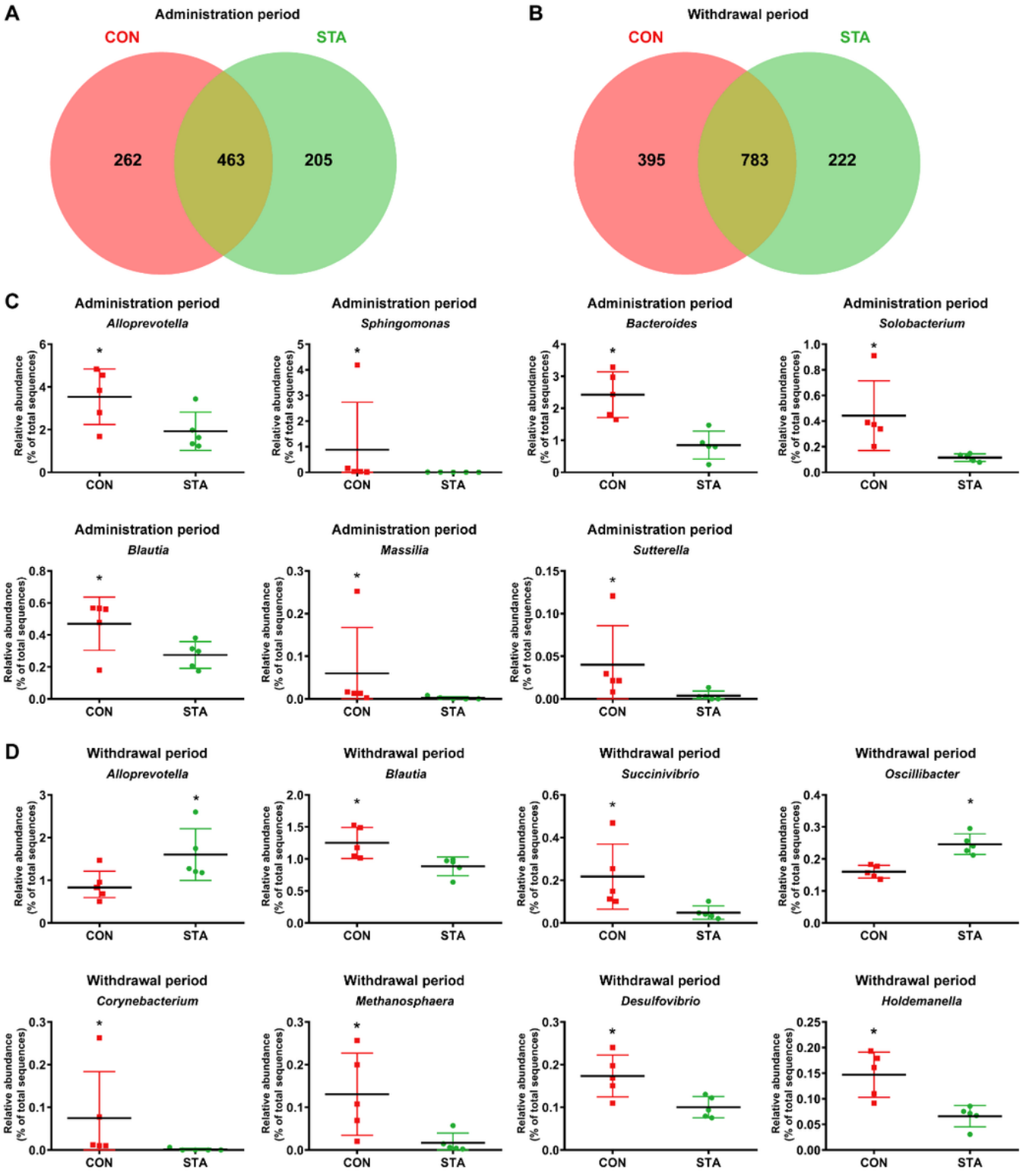

Figure 3

Effects of STA administration and its subsequent withdrawal on gut microbiota community in piglets. Venn diagram depicting the unique and shared OTUs between the two groups at the end of the administration period (A) and the end of the withdrawal period (B). Scatter dot plot visualizing the significantly changed bacteria genera between groups at the end of the administration period (C) and the

end of the withdrawal period (D). Data are expressed as means ± SD. Each point represents one sample. *, P-value < 0.05. CON, control group. STA, sub-therapeutic antibiotic group.

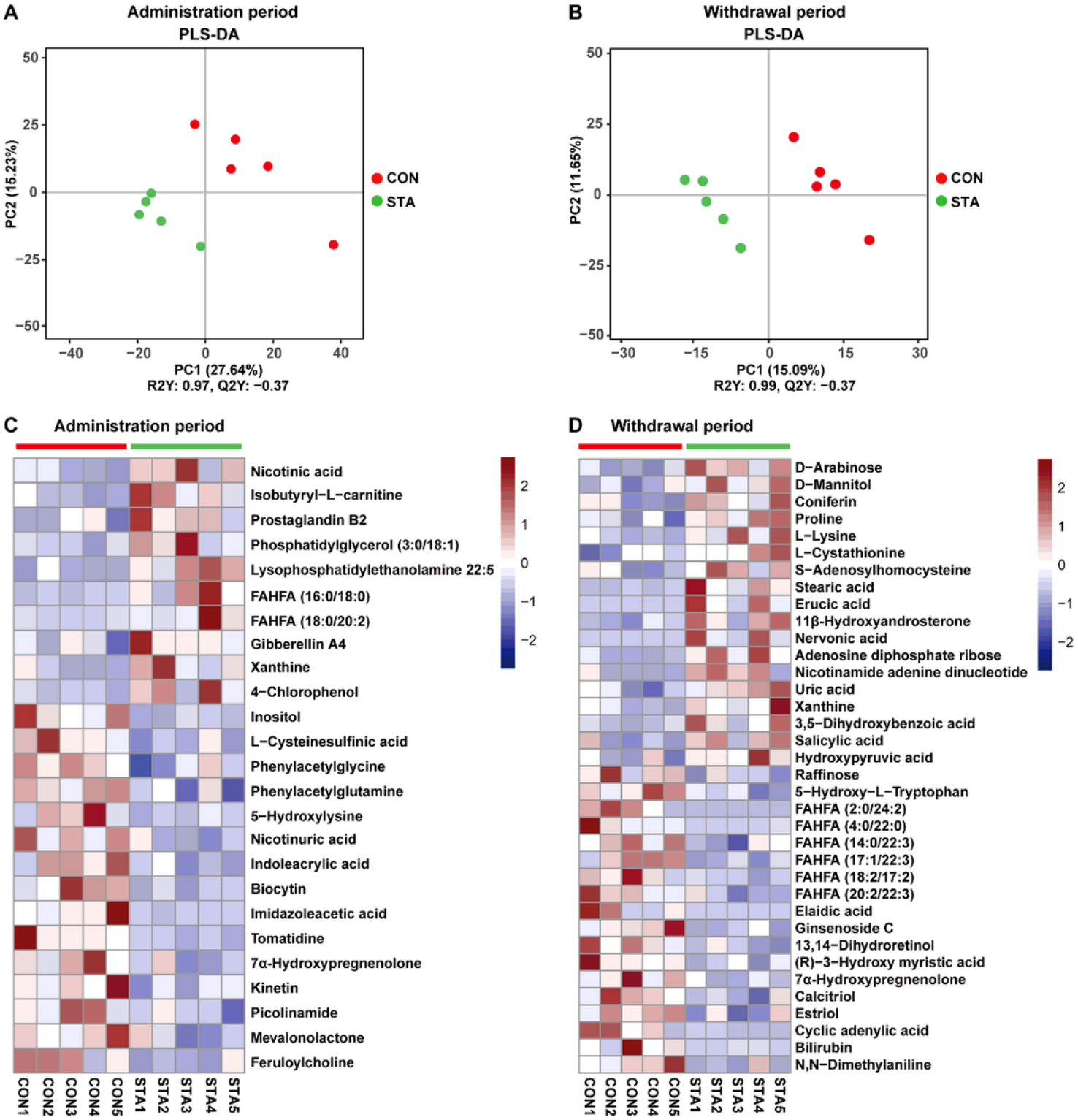

Figure 4

Effects of STA administration and its subsequent withdrawal on colonic metabolism profiles of piglets. Partial least squares discriminant analysis (PLS-DA) between groups at the end of the administration period (A) and the end of the withdrawal period (B). Each point represents one sample. Heatmap

visualizing the identified differential metabolites between groups at the end of the administration period (C) and the end of the withdrawal period (D). CON, control group. STA, sub-therapeutic antibiotic group.

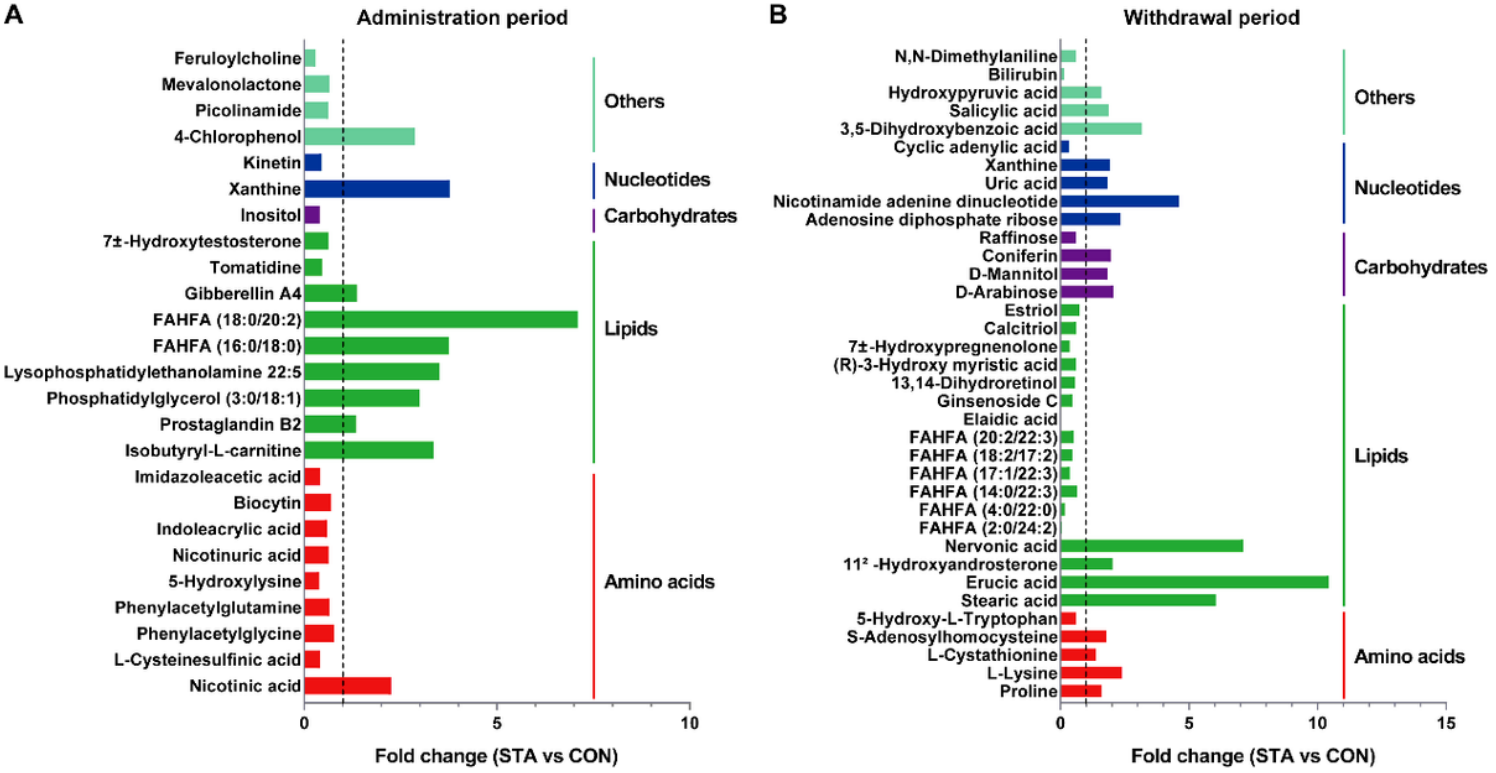

Figure 5

Classification of metabolites with significant difference between the STA and CON groups at the end of administration period (A) and the end of the withdrawal period (B). CON, control group. STA, sub-therapeutic antibiotic group.

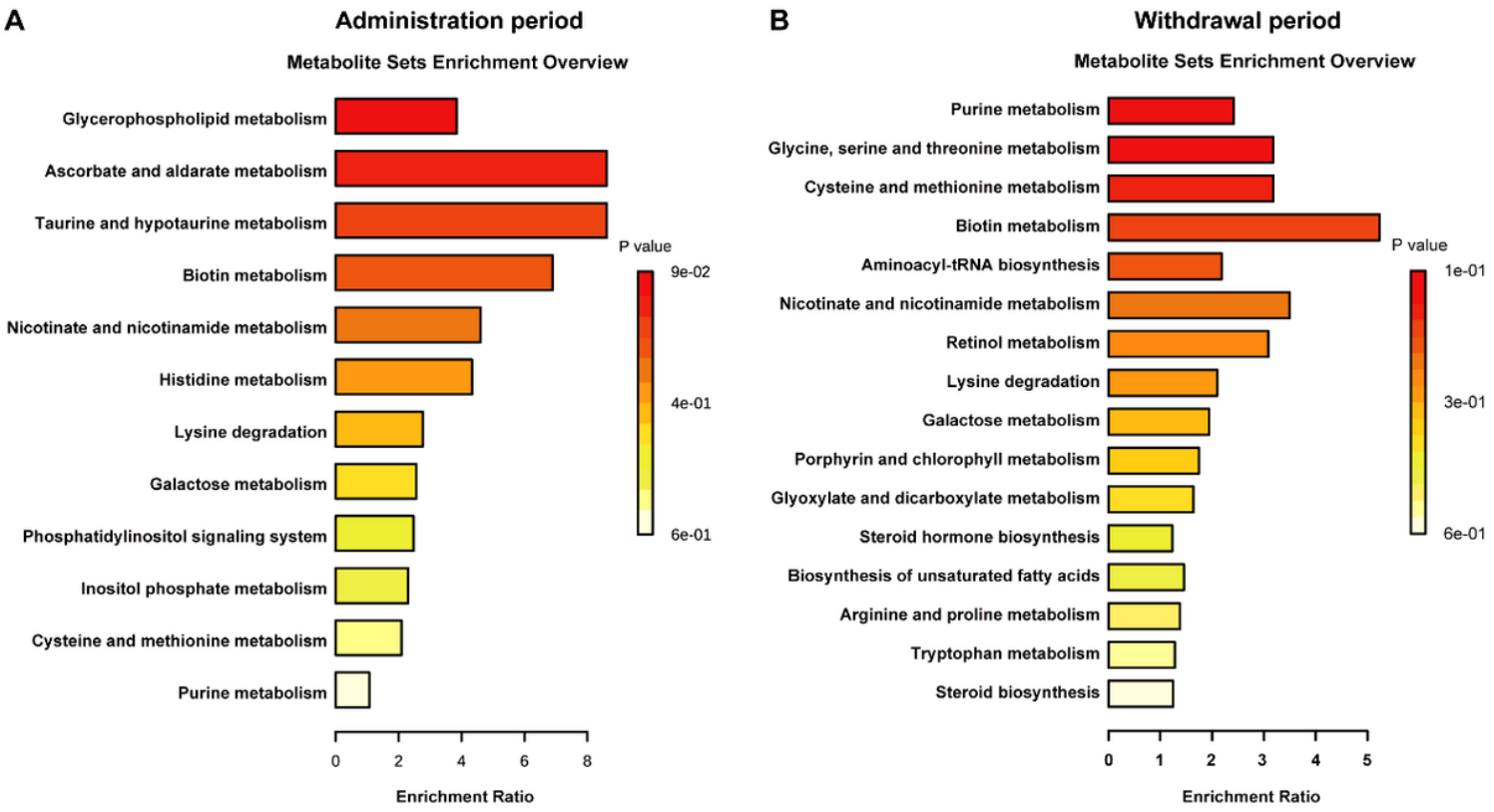

Figure 6

Metabolic pathway enrichment analysis. Overview of metabolites that were enriched in the colon of piglets fed the STA diet compared to piglets fed the CON diet at the end of the administration period (A) after withdrawal period (B). CON, control group. STA, sub-therapeutic antibiotic group.

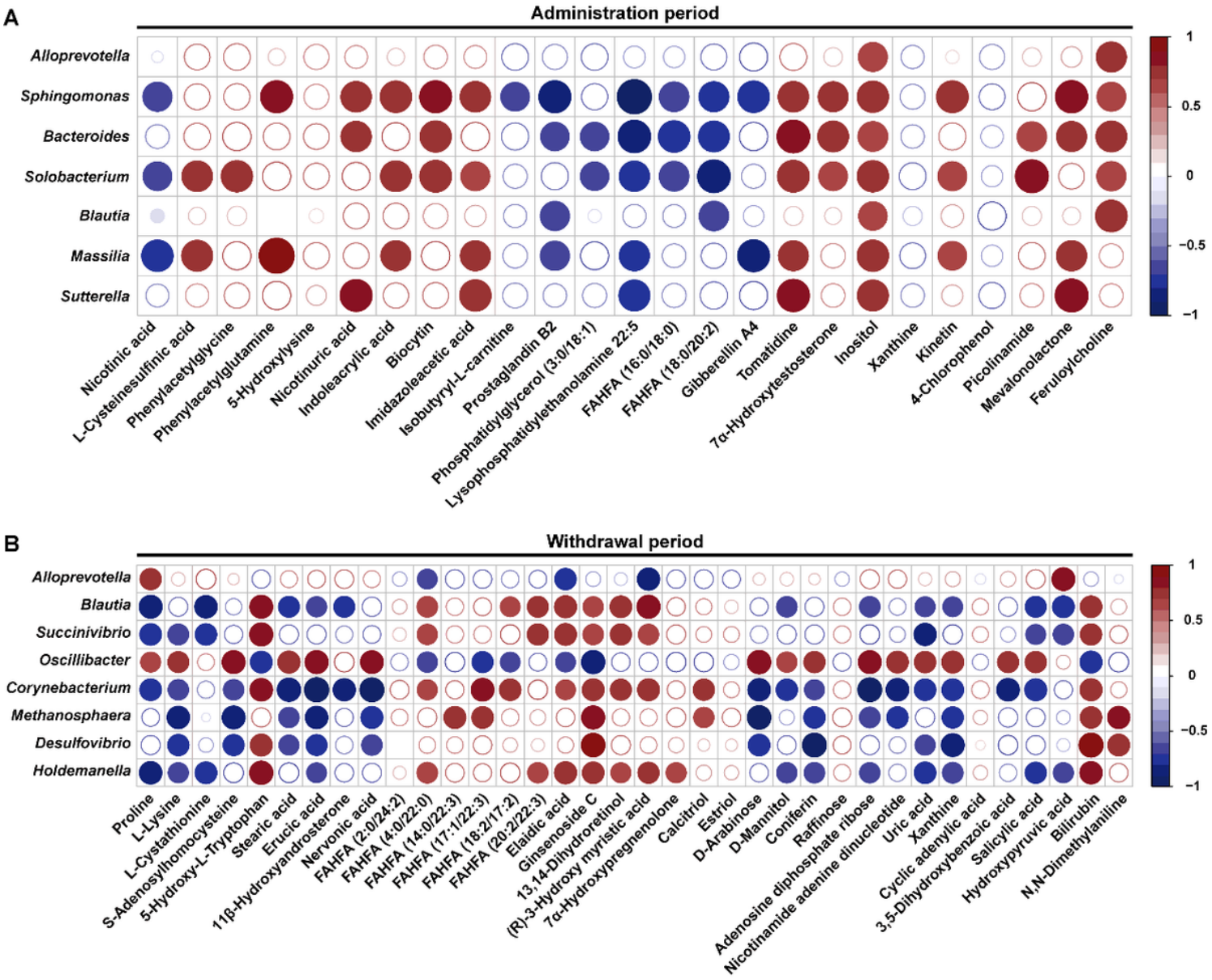

Figure 7

Relationship between colonic microbiota (at the genera level) and metabolites of piglets fed the STA diet or the CON diet at the end of the administration period (A) and the end of the withdrawal period (B). The circle border and circle filling are colored according to Spearman's correlation coefficient distribution and sized based on the correlation coefficient value. Red-filled circle rep-resents significantly positive correlation ($P < 0.05$), blue-filled circle represents significantly negative correlation ($P < 0.05$) and white-filled circle represents no significant correlation ($P > 0.05$). CON, control group. STA, sub-therapeutic antibiotic group.

Supplementary Files

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