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Effects of immunomodulatory peptides derived from a soil bacterium on caecal microbiota of broilers challenged with *Clostridium perfringens*

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Abstract

Brevibacillus texasporus peptide (BT peptide) is immunomodulatory in poultry and is proposed as a feed additive substitute for antibiotics. In the present study, we performed 16S rRNA gene sequencing to compare development of the caecal microbial communities in *Clostridium perfringens*-challenged broilers fed a basal diet only or diets containing the BT peptide (48 ppm) or an antibiotic mixture (20 ppm zinc bacitracin and 40 ppm colistin sulphate). A total of 240 chicks were randomly assigned to these three treatments, each group consisting of 80 birds. Birds were challenged once per day between 4 and 10 d posthatch. A total of 65 caecal samples were collected from 6–8 randomly selected birds in each treatment group f The composition of microbial communities was clearly distinguishable over time. Treatments with challenge and the antibiotic mixture were associated with increased diversity and with higher relative abundances of *Alistipes* sp. CHKCI003 and *Faecalibacterium* and lower abundance of *Escherichia coli*. At the end of the trial, the caecal microbiota in broilers supplemented with BT peptide were dominated by members of Bacteroidaceae. Predicted function analysis revealed marked enrichment of genes involved in ion-coupled transporters and sugar and biotin metabolism in the BT peptide treatment. The results suggest that BT peptide and commonly-used antibiotics have different influences on modulating the composition of caecal microbiota in broilers.

Keywords: antibiotics, broiler chicken, BT peptide, *Clostridium perfringens* challenge, 16S rRNA sequencing

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Introduction

Probiotics and their functionally valuable products have been shown to be a promising option to modulate the host's immune system and have been suggested as an alternative to antibiotics for maintaining animal health (Kanmani *et al.*, 2013). Gram-positive soil bacteria, such as *Streptomyces*

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and *Amycolatopsis*, can produce antimicrobial peptides (AMPs), and some peptides encrypted in the microbial metaproteome act as natural effectors of the innate immune response (Lewies *et al.*, 2019; Chen & Lu, 2020). *Brevibacillus* spp., established in 1996 and formerly known as the *Bacillus brevis* cluster (Panda *et al.*, 2014), are rod-shaped, gram-positive bacteria and most of them are strict aerobes. *Brevibacillus* spp. are widely spread in nature and some strains have been applied as probiotics for a long time (Sanders *et al.*, 2003). It is well documented that members of the genus, *Brevibacillus,* produce antibacterial, antifungal, and anti-invertebrate agents and can be a source of diverse enzymes of great biotechnological interest (Yang *et al.*, 2016). One group of these metabolites, *Brevibacillus* AMPs, is synthesized through ribosomal or nonribosomal pathways; the latter involves nonribosomal peptide synthetases (NRPSs). It is interesting to note that the majority of currently-identified *Brevibacillus* AMPs are synthesized using NRPS machinery (Yang & Yousef, 2018).

A group of small cationic AMPs, named *Brevibacillus texasporus* peptides (BT peptides), was isolated in 2005 from a soil bacterium, *B. laterosporus,* with the initial intention of seeking novel antibiotics (Wu *et al.*, 2005). BT peptide has been found to be biosynthesized using NRPS and has been purified to determine its sequence. Subsequent studies have revealed that the 13-residue BT peptide shares high similarity of amino acid composition with the so-called nonribosomal linear lipopeptides secreted from different *B. laterosporus* strains, including Bogorols A-E and Brevibacillin (Barsby *et al.*, 2006; Yang *et al.*, 2016; Yang & Yousef, 2018). Looking into the amino acid sequence alignment, the conserved ornithine in position 3 and lysine residues in positions 7 and 10 make this family of peptides cationic at physiological pH. Several studies suggest that BT peptide has immunomodulatory properties that prime innate immunity and enhance leukocyte bactericidal activity to combat pathogenic infection when provided as a feed additive for broilers (Kogut *et al.*, 2007; Kogut *et al.*, 2010; Kogut *et al.*, 2012). However, there are no studies, to our knowledge, examining the effects of BT peptide on gut microbiota composition of the host so far.

Necrotic enteritis (NE) in broiler chickens is a gastrointestinal disease of economic importance worldwide caused by *Clostridium perfringens* strains (Yitbarek *et al.*, 2012; Lacey *et al.*, 2018). Subclinical NE is characterized by poor performance with no clinical sign of the disease (Wang *et al.*, 2017). Most of the economic losses are related to the subclinical form and the high cost of preventing the disease with antibiotics (Shojadoost *et al.*, 2012). In recent years, countries and regions where in-feed antimicrobials or antibiotic growth promoters have been banned have experienced an increase in disease outbreaks in broiler flocks (Van Immerseel *et al.*, 2004). Thus, it has spurred interest in investigating how it can be prevented by countermeasures other than the use of antibiotics. In this study, we applied an NE challenge model with broiler chickens to investigate the role of BT peptide in gut bacterial community structure shifts and compared it with the antibiotics commonly used for eradication of the pathogenic bacteria. High-throughput sequencing of 16S rRNA genes was performed to follow the response of the caecal microbial communities to *C. perfringens* challenge.

Materials and Methods

All procedures involving animals were carried out according to experimental protocols approved by the Animal Ethics Committee of COFCO Nutrition and Health Research Institute (permit number: AR-15-0701).

A total of 240 one-day-old, male, Arbor Acres broiler chickens were obtained and transferred from a

commercial hatchery (Huadu Broiler Breeding, Beijing, China) and randomly distributed into three treatments. Each group of 80 birds was housed in individual cage compartments within a climate-controlled room. Each compartment had its own feeder, drinker, and brooding lamp for warmth, and plastic mesh was used for the cage floor. The ambient relative humidity inside the room was ~50%, and the environmental temperature was 32°C at placement. Temperature was reduced with age to provide comfort. Treatments were as follows: basal starter diet (coccidiostat- and antibiotic-free, referred to as CP hereafter), 20 ppm zinc bacitracin + 40 ppm colistin sulphate (AB), and 48 ppm BT peptide (BT). The feeding period lasted 18 d. These dose selections were based on previously published studies of BT peptide in broiler diets (Kogut *et al.*, 2007; Kogut *et al.*, 2010). Feed and water were supplied *ad libitum* and the chickens were maintained on a 23-hour lighting program. The corn and soybean meal-based diets were formulated to meet NRC (1994) requirements. The composition of the diet and nutrient levels are presented in Table 1.

Table 1 Ingredients and nutrient composition of the diet (as-fed basis) fed to broilers for 18 days

	Value
Ingredient, %	
Corn	57.2
Soybean meal, 46% CP	34.6
Soybean oil	3.0
Dicalcium phosphate	1.7
Limestone	1.4
2% Premix[a]	2.0
Antioxidant	0.1
Nutrient composition	
Metabolizable energy, MJ/kg	12.33
Crude protein, %	21.00
Lys, %	1.20
Met, %	0.50
Ca, %	1.05
Available P, %	0.45

[a] The premix supplied the following per kg of complete feed: vitamin A, 12,500 IU; vitamin D3, 2,500 IU; vitamin K3, 2.65 mg; vitamin B1, 2 mg; vitamin B2, 6 mg; vitamin B12, 0.025 mg; vitamin E, 30 IU; biotin, 0.0325 mg; folic acid, 1.25 mg; pantothenic acid, 12 mg; niacin, 50 mg; copper, 8 mg; zinc, 75 mg; iron, 80 mg; manganese, 100 mg; selenium, 0.15 mg; iodine, 0.35 mg

Clostridium perfringens challenge was adapted from published methods (Liu *et al.*, 2010) with some modifications. Briefly, a chicken *C. perfringens* type A field strain was obtained from the China Veterinary Culture Collection Center (Beijing, China) and cultured anaerobically in cooked meat medium (Aobox, Beijing, China) overnight at 37 °C. At 4–10-d posthatch, all the birds were orally gavaged once per day at approximately the same time of morning with the actively growing cultures of this pathogenic *C. perfringens* (2.0×10^8 colony forming units/ml, 1.0 ml/bird). Plate count of viable *C. perfringens* was done by performing serial dilution of the culture in sterile saline and plating on sulphite-polymyxin-sulfadiazine agar (Aobox). The plates were incubated anaerobically at 37 °C for 18–24 h and colonies

typical to *C. perfringens* were counted.

Samples were collected in the afternoon for each sampling time. At 4-d (start of challenge, referred to as T1 hereafter), 11-d (1-d post-challenge, T2), and 17-d (7-d post-challenge, T3) posthatch, 6–8 birds from each treatment were randomly selected and humanely euthanized by cervical dislocation. Thus nine sampling groups were generated (CP1, CP2, CP3, AB1, AB2, AB3, and BT1, BT2, BT3) according to treatment and sampling sequences. Mucoid gut contents and sparse occurrence of focal necrosis in the small intestine with no obvious mortality implied a successful challenge. From each chicken, the caecum was removed and the caecal luminal contents were collected in a sterilized tube. The samples were flash frozen and stored at −80 °C until further analysis.

Bacterial genomic DNA of each sample was extracted using the QIAamp Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. DNA concentration and purity were monitored by agarose electrophoresis and determined by ultraviolet absorption analysis using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, USA). The V4 hypervariable region of the 16S rRNA bacterial gene (515-806 bp) was amplified using specific primers using the barcodes of the Phusion High-Fidelity polymerase chain reaction (PCR) Master Mix (New England Biolabs, Ipswich, USA). PCR amplicons from each sample were pooled in equimolar amounts and purified using QIAquick Gel Extraction Kit (Qiagen). Sequencing libraries were generated using TruSeq DNA PCR-Free Sample Prep Kit (Illumina, San Diego, USA) and assessed by the Qubit 2.0 Fluorometer (Thermo Fisher Scientific) and Agilent 2100 Bioanalyzer system according to the standard protocols. The final library was paired-end sequenced at 2 × 250 bp on the Illumina HiSeq 2500 platform. The raw sequence data are available at the NCBI Sequence Read Archive (SRA) database (Accession Number: SRP144400).

Raw sequences were demultiplexed and quality-filtered using QIIME (version 1.7.0, http://qiime.org) (Caporaso *et al.*, 2010) to eliminate all low quality sequence reads under specific filtering conditions (Bokulich *et al.*, 2013). The resulting trimmed sequences were filtered to remove singleton reads and then grouped into operational taxonomic units (OTUs) with 97% identity threshold using the USEARCH software based on the UPARSE algorithm (version 7.1, http://drive5.com/uparse) (Edgar, 2013); chimeric sequences were identified and removed using UCHIME (Edgar *et al.*, 2011). The taxonomy of OTU-representative sequences was analysed using the RDP Classifier (version 2.2, http://rdp.cme.msu.edu) (Wang *et al.*, 2007) against the Silva (SSU128) 16S rRNA database, using a confidence threshold of 70%. The resulting OTU table was used to determine taxonomic relative abundances of each sample.

Data analyses were performed using SPSS Statistics software (version 24.0, IBM Corporation, Armonk, USA) and R (version 3.3.1, http://www.r-project.org). Non-parametric statistical methods were used to analyse the data sets when they did not follow a normal distribution. Differences in α-diversity for the caecal microbiota of broilers were tested using the Kruskal–Wallis H test with Dunn's post hoc test and Bonferroni correction for multiple comparisons. Differences in microbiota composition (as assessed by β-diversity metrics) were tested using analysis of similarities (ANOSIM) or permutational multivariate analysis of variance (PERMANOVA, 999 permutations) at the OTU level in the R vegan package (version 2.4-4, http://CRAN.R-project.org/package=vegan). Differences in the abundance of

taxa were tested using Kruskal–Wallis H test with *p*-values adjusted (p_{adj}) for multiple testing using the false discovery rate (FDR) procedure. Linear discriminant analysis effect size (LEfSe) (Segata *et al.*, 2011) was performed to identify the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways differentially represented among groups.

Results and Discussion

BT peptide has been proposed as a feed additive for poultry to achieve effective control of pathogens affecting food safety. Feeding a BT peptide-supplemented diet in day-old broiler chickens induced the up-regulation of the innate immune response, reduced pathogenic bacterial colonization of the intestine, and primed the caecal tissue for increased immune gene expression in response to *Salmonella enterica* serovar Enteritidis infection (Kogut *et al.*, 2007; Kogut *et al.*, 2010). Researchers have realized that the large numbers of microorganisms residing in the gastrointestinal tract have a highly co-evolved relationship with the host's immune system (Hooper *et al.*, 2012), supported by the fact that disturbances in the bacterial microbiota result in dysregulation of immune cells (Round & Mazmanian, 2009). In particular, it is well understood that the interaction between the gut immune system and commensal microbes in chickens starts immediately after hatching (Crhanova *et al.*, 2011). Since BT peptide is not absorbed in the intestine (Kogut *et al.*, 2012), we considered the effect of its supplementation on diversity and function of the avian gut microbiota.

In order to define the microbiome of broilers challenged with *C. perfringens* and to evaluate the impact of BT peptide on the broiler microbiome in comparison with an antibiotic mixture, we reproduced subclinical necrotic enteritis disease in hatched broiler chicks using a consecutive 7-d *C. perfringens* challenge; the development in the composition of caecal microbiota was followed longitudinally (Fig. 1a). Caecal digesta samples were processed and sequenced to generate 3,535,329 clean reads using the 16S rRNA gene's V4 hypervariable region. After OTU clustering and rarefying to 37,970 reads per sample, 1,635 OTUs were retained for downstream analyses.

The caecal microbiota profiles of challenged broilers at phylum level are shown in Fig. 1b. It can be observed that the phylum, Proteobacteria, was highly abundant in the T1 samples of all the three treatments but declined to a small portion in the T2 and T3 samples, where it was replaced by another phylum, Bacteroidetes, in these samples.

Next, alpha diversity was calculated to describe the within-sample bacterial richness and diversity using observed OTUs and the Shannon index. When examining within different time points, the AB1 group was found to have a higher number of observed OTUs than CP1 at the T1 sampling time (p = 0.037; Fig. 2a). Alpha diversity indices in different treatments were compared to evaluate the impact of the challenge. Both bacterial community richness (p = 0.018; Fig. 2b) and diversity (p = 0.002; Fig. 2c) notably increased over time in the CP treatment. Moreover, the Shannon index in the AB treatment also exhibited a gradual increase over the course of treatment (p = 0.029; Fig. 2d).

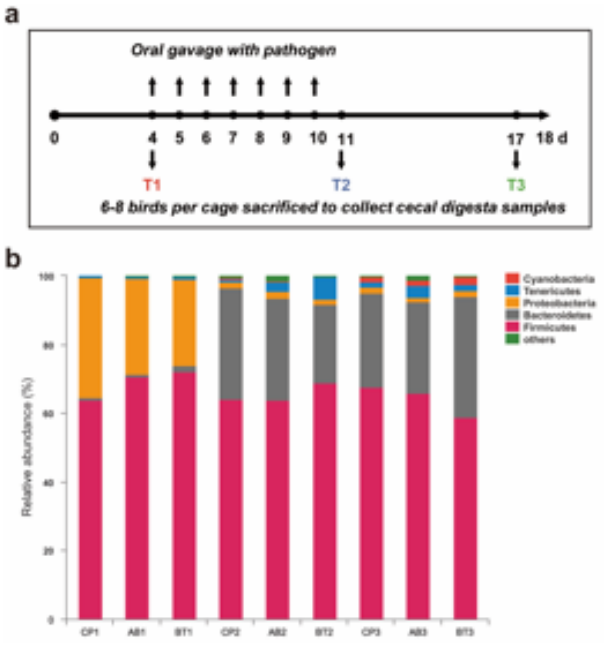

Figure 1 Microbial community composition of the caecum of broiler chickens challenged with *C. perfringens*. (a) Scheme of sampling strategy. T1–T3 represent the three sampling time points in this study, generating a total of nine groups for the following analyses. (b) Bar plots show the mean values of relative abundance for the most abundant phyla in the nine sampling groups. Only phyla present in at least 1% of the samples are shown separately.

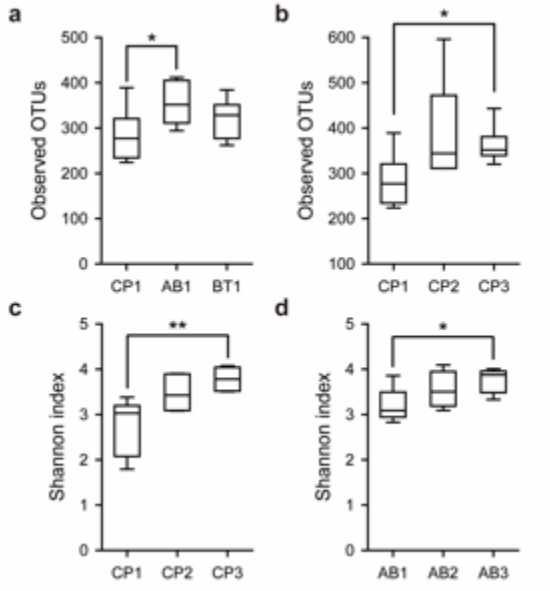

Figure 2 Differences in bacterial community richness and diversity between treatments and sampling times. The bacterial abundance was represented by the numbers of operational taxonomic units (OTUs) and compared showing the impact of treatment on (a) T1 sampling time, or (b) impact of challenge in CP treatment. The Shannon index was used to estimate the diversity of the caecal microbiota of the challenged birds in (c) CP or (d) AB treatment. *, P <0.05; **, P < 0.01; Diets: basal diet (CP), BT peptide (48 ppm), antibiotic (AB) mixture (20 ppm zinc bacitracin and 40 ppm colistin sulphate); Times: 4-d and 11-d posthatch, 7-d post-challenge (17-d posthatch)

Bacterial community composition was different between sampling groups formed by treatments and times when measured using ANOSIM distances (weighted UniFrac, r = 0.514, p = 0.001). These differences were recapitulated using two dimensional, non-metric, multidimensional scaling analysis (NMDS) of the data based on weighted UniFrac similarity distances, which revealed a clear grouping structure of taxonomic composition over sampling time (Fig. 3a). The caecal microbiota were investigated at the genus level to identify the 30 most abundant taxa (Fig. 3b). The heat map graphically indicates that the caecal bacterial community in the nine sampling groups varied, especially over the course of the experiment, which supports the ANOSIM and NDMS analyses.

The reciprocal relationship of Proteobacteria and Bacteroidetes observed between T1 and T2/T3 can be partially explained by the abundance of the genus, *Escherichia-Shigella,* in the first week of age and *Bacteroides* thereafter, which shares strong similarity with findings of previous research (Waite & Taylor, 2014; Johnson *et al.*, 2018). The core bacterial microbiota of the broiler caecum are proposed to comprise *Escherichia-Shigella* at 0 and 7 days of age and *Bacteroides*, Erysipelotrichaceae, *Faecalibacterium*, Lachnospiraceae, *Oscillospira*, *Rikenella*, *Ruminococcus*, *Streptococcus* and *Lactobacillus* spp. after 14 days of age (Stanley *et al.*, 2014; Johnson *et al.*, 2018). Temporal changes in the chicken caecal microbiota suggest that taxonomic richness and diversity typically increase from day of hatch to maturity (Oakley & Kogut, 2016), as revealed by the alpha diversity analysis.

To single out the contribution of individual OTUs to the dissimilarity among samples, taxonomic changes that differed markedly over time in each treatment were assessed (Fig. 4). Surprisingly, the abundance of OTU284 (*Escherichia coli*) decreased >20-fold from T1 to T2 and T3 sampling times in both CP (p_{adj} = 0.036) and AB (p_{adj} = 0.049) treatments. This trend coincided with the increases in the abundance of OTU1111 (*Alistipes* sp. CHKCI003) and OTU1175 (*Faecalibacterium*) over the time course of the study. In the CP treatment, OTU1092 (Ruminococcaceae) and OTU1052 (*Lactobacillus*) were observed to increase from sampling times, T1 to T2, and then decline from sampling times, T2 to T3. In contrast, the abundance of OTU1052 decreased over time in the AB treatment groups (p_{adj} = 0.045); OTU304 (*Lachnoclostridium*) showed a similar pattern of reduced abundance (p_{adj} = 0.043).

To assess the impact of different treatments on the caecal microbial community of challenged broiler chickens, the Bray–Curtis similarity-based principal coordinate analysis (PCoA) was performed on sequencing data of T3 samples and the first three coordinates are shown in Fig. 5a (representing 63% of the total variance). This analysis indicates a clear division between the CP3 and BT3 groups. The results of the PERMANOVA analysis at the OTU level also reveal a modest global variation in bacterial composition among different treatment groups (Bray–Curtis, r^2 = 0.222, p = 0.013). The data were analysed using ternary plots to show the relative distribution of OTUs among T3 samples (Fig. 5b). Each OTU was categorized as a group representative OTU based on whether there was a defined (10% or above) increase in its relative abundance in this treatment group compared with the other two groups. Our results suggest that CP3 and AB3 had a higher relative abundance of Ruminococcaceae, whereas BT3 harboured a high relative abundance of Bacteroidaceae, which was solely contributed by OTU810 (*Bacteroides dorei*), and a less relative abundance of Ruminococcaceae. OTUs from the families Rikenellaceae and Porphyromonadaceae were mostly associated with the CP3 bacterial community, whereas Clostridiales_vadinBB60_group was associated with the AB3 community.

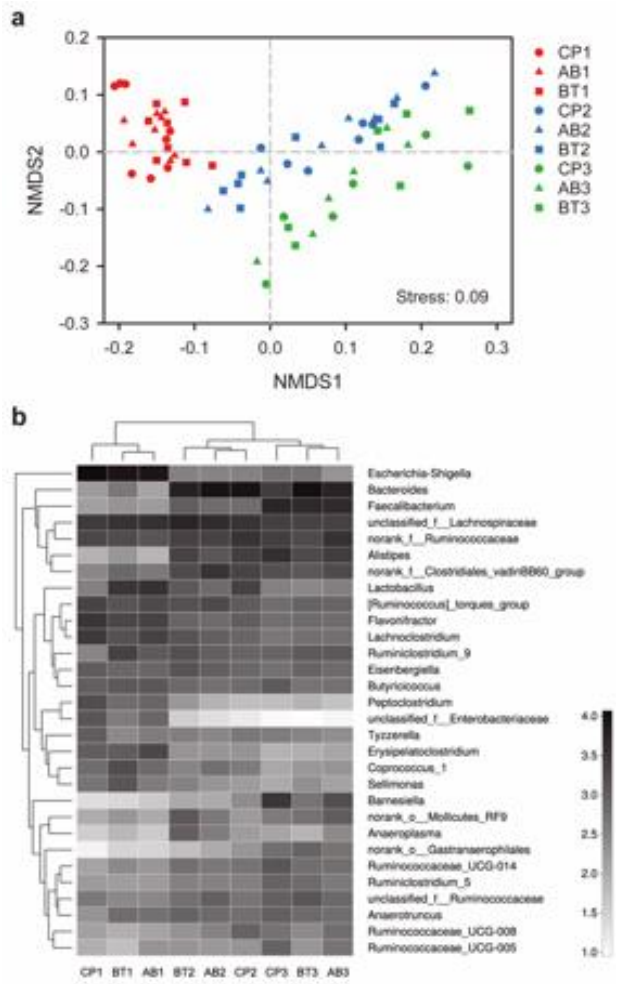

Figure 3 Changes in caecal bacterial community composition over time in the broilers challenged with *Clostridium perfringens*. (a) non-metric, multidimensional scaling analysis (NMDS) ordination based on weighted UniFrac similarity at sampling times, T1 (red), T2 (blue), and T3 (green). (b) Heatmap based on the relative abundance of the thirty most abundant genera in the nine sampling groups. Clustering of groups (top) and taxa (left) were defined using the average pairwise Euclidean distance.
Diets: basal diet (CP), BT peptide (48 ppm), antibiotic (AB) mixture (20 ppm zinc bacitracin and 40 ppm colistin sulphate); Times: 4-d and 11-d posthatch, 7-d post-challenge (17-d posthatch)

To study the functional alterations of the caecal microbiota, we used Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) (Langille *et al.*, 2013) to predict the functional composition profiles from the 16S rRNA sequencing data in all treatment groups at sampling time, T3. It was found that multiple KEGG level three categories were enriched in different treatments (Fig. 5c). The pathways overrepresented in the CP3 group highlighted protein translation (ribosome, aminoacyl-tRNA biosynthesis, translation proteins, and amino acid related enzymes) and four metabolism pathways (peptidoglycan biosynthesis, energy metabolism, terpenoid backbone biosynthesis, and C5-branched dibasic acid metabolism). In contrast, the gut microbiota of the BT3 group were characterized by enrichment of other ion-coupled transporters and metabolism of sugars (galactose, fructose and mannose, and carbohydrate) as well as biotin, whereas the gut microbiota of the AB3 group was characterized by overrepresentation of benzoate degradation and isoflavonoid biosynthesis.

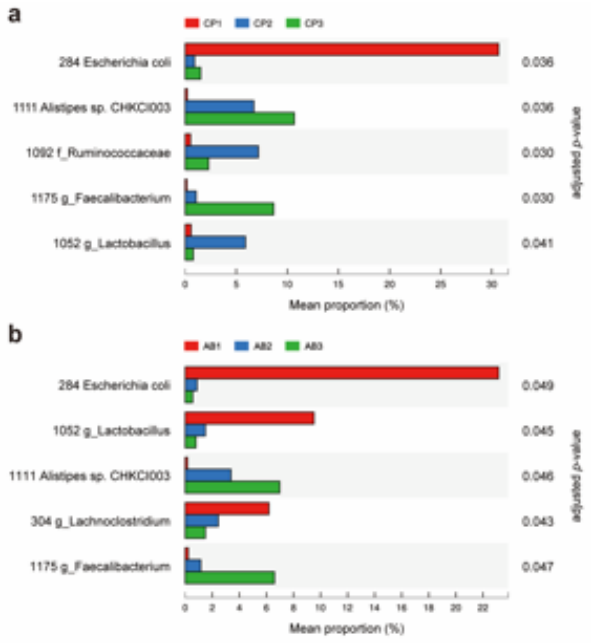

Figure 4 Bar plots identifying significant differences between mean proportions of bacterial taxa in the (a) control diet (CP), and (b) antibiotic (AB) treatments over time (P_{adj} <0.05). Only operational taxonomic units (OTUs) with at least 5% abundance in at least one group were included. Diets: basal diet (CP), BT peptide (48 ppm), antibiotic (AB) mixture (20 ppm zinc bacitracin and 40 ppm colistin sulphate); Times: 4-d and 11-d posthatch, 7-d post-challenge (17-d posthatch)

Analysis of the microbiome at 7-d post-challenge showed that the most abundant organisms were different in the various treatments. For challenged broilers fed with BT peptides, the dominant taxa were *Bacteroides dorei* and Ruminococcaceae. *Bacteroides* colonization of the gastrointestinal tract at an early stage of life is important, given their role in the digestion of complex carbohydrates to fermentation products, which are beneficial to hosts (Martens *et al.*, 2008; Davis-Richardson *et al.*, 2014). The PICRUSt results also revealed that predicted genes from the metagenome related to multiple carbohydrate metabolic pathways were substantially increased in the BT treatment. *Bacteroides dorei*, in particular, was found to exhibit a strong association with the development of autoimmunity in type 1 diabetes in a Finnish cohort of children (Davis-Richardson *et al.*, 2014). The structurally and functionally distinct lipopolysaccharide of *Bacteroides dorei* is assumed to play an important role in immune regulation in human individuals (Vatanen *et al.*, 2016). The current study lacks sufficient data to define the effect of high *Bacteroides* abundance on the broiler immune response to the *C. perfringens* pathogen, and further studies are needed to investigate this potential link.

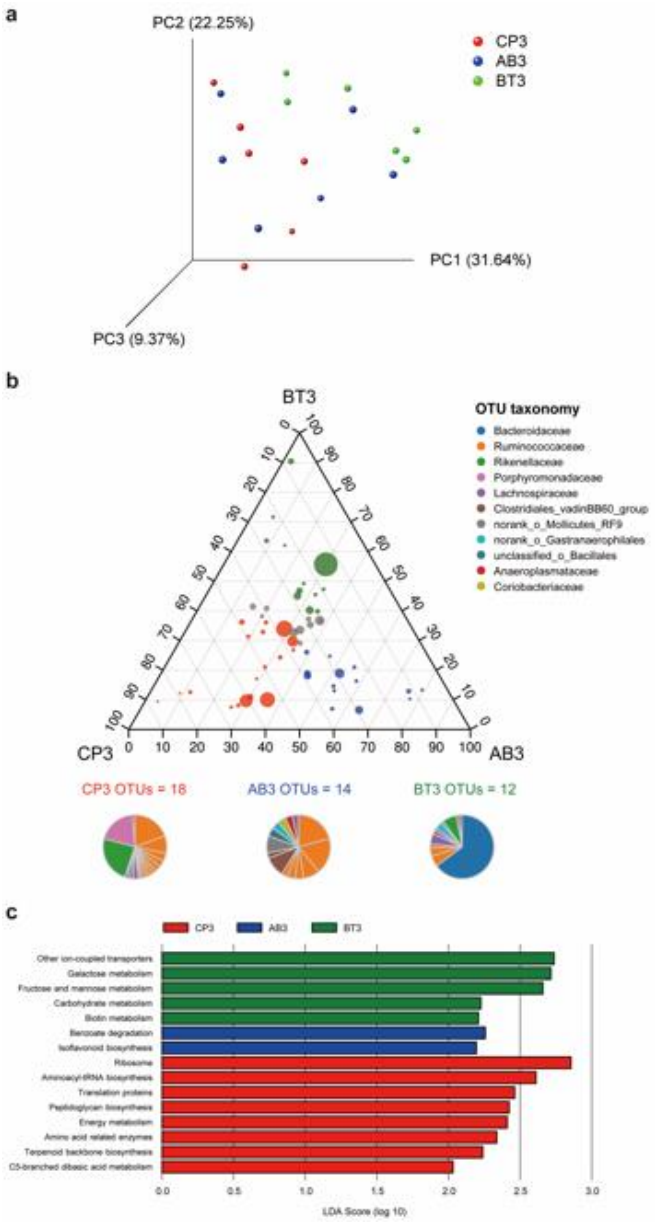

Figure 5 Taxonomic differences and predicted function of the caecal microbiota in the differently-treated broilers challenged with *C. perfringens* at the sampling time, T3. (a) Principal coordinate analysis (PCoA) plot based on Bray–Cutis distance at OTU level illustrating the distribution of microbiota by treatment group. The percentage of variation explained by the principal coordinates is indicated on the axes. (b) Ternary plot representing the relative occurrence of individual OTUs (circles) in T3 samples. The size of the circles is proportional to the mean abundance of each OTU, and the position of the circles is determined by the contribution of the indicated treatments to the total relative abundance. Red, blue, and green circles mark OTUs that are at least 10% more enriched in CP3, AB3, and BT3 than in the other two groups, respectively, and their relative abundance is shown as pie charts and coloured using family-level taxonomic classification. Only OTUs >0.5% abundant in at least one treatment are shown. (c) KEGG level three pathways were differentiated among treatments at sampling time T3, identified by LEfSe with cutoff of linear discriminant analysis (LDA) > 2. Diets: basal diet (CP), BT peptide (48 ppm), antibiotic (AB) mixture (20 ppm zinc bacitracin and 40 ppm colistin sulphate); Times: 4-d and 11-d posthatch, 7-d post-challenge (17-d posthatch)

Various bacteria belonging to Ruminococcaceae were most abundant in both CP and AB treatments at 7 d post-challenge. Members of the family Ruminococcaceae, including *Faecalibacterium*, are known for their ability to decompose plant material and convert it into butyrate and other short-chain fatty acids that can be absorbed and used for energy by the host. Marked enrichment of pathways regulating energy metabolism in the CP treatment also demonstrates a heightened demand for energy by the cells of the intestine in this condition. Furthermore, we identified two *Bacteroidetes* OTUs, OTU1111 (*Alistipes* sp. CHKCI003, a species from the family of Rikenellaceae) and OTU377 (*Barnesiella*, a genus from the family of Porphyromonadacea*e*), which were more enriched in the CP treatment. These two bacteria have been reported to contribute to host immune development. The appropriate proportion of *Alistipes* is suspected to augment intestinal immune maturation (Chung *et al.*, 2012), and administration of the commensal *Barnesiella* could eradicate the infection of vancomycin-resistant *Enterococcus* (Ubeda *et al.*, 2013), suggesting that the microbiota are a required component of the effector response of the host (Belkaid & Hand, 2014). Nevertheless, we need more research to unravel the underlying mechanism of this bacterial community development.

Conclusions

Collectively, our results show that feeding *C. perfringens*-challenged broilers with BT peptide or antibiotics causes a different outcome in the composition of the microbial community in the caecum. Additionally, analysis of the predicted metagenomes indicates functions involved with improved sugar metabolism when supplemented with BT peptide. Future studies should include a more explicit investigation of the host–microbe interaction to depict a detailed picture of the immunomodulatory activity of BT peptide from the perspective of gut microbiota, which may benefit BT peptide as a novel antibiotic alternative for a range of livestock species.

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Authors' contributions

XL, GL, and JR conceived and designed the study. XL, GL, QP, YS, YL, and JR performed the experiments. GL, HL, and JR analysed and discussed the results. JR wrote the paper. All authors have read and approved the final manuscript.

Conflict of interest declaration

The authors declare that they have no competing interests.

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