



Effects of Xylanase in Corn- or Wheat-Based Diets on Cecal Microbiota of Broilers

Jian Wang¹, Heng Cao¹, Chengling Bao¹, Yajing Liu¹, Bing Dong¹, Chunlin Wang¹, Zhenda Shang², Yunhe Cao^{1,2*} and Suozhu Liu^{2*}

¹State Key Laboratory of Animal Nutrition, College of Animal Science and Technology, China Agricultural University, Beijing, China, ²College of Animal Science, Tibet Agricultural and Animal Husbandry University, Linzhi, China

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*Correspondence:

Yunhe Cao
caoyh@cau.edu.cn
Suozhu Liu
liusuozhu@xza.edu.cn

Specialty section:

This article was submitted to
Systems Microbiology,
a section of the journal
Frontiers in Microbiology

Received: 11 August 2021

Accepted: 21 September 2021

Published: 13 October 2021

Citation:

Wang J, Cao H, Bao C, Liu Y,
Dong B, Wang C, Shang Z,
Cao Y and Liu S (2021) Effects of
Xylanase in Corn- or Wheat-Based
Diets on Cecal Microbiota of Broilers.
Front. Microbiol. 12:757066.
doi: 10.3389/fmicb.2021.757066

Xylanase has been demonstrated to improve growth performance of broilers fed wheat- or corn-based diets due to its ability to degrade arabinoxylans (AX). However, content and structure of AX in corn and wheat are different, comparing effects of xylanase on cecal microbiota of broilers fed corn- or wheat-based diets could further elaborate the mechanism of the specificity of xylanase for different cereal grains. Thus, a total of 192 one-day-old broilers were randomly allotted into four dietary treatments, including wheat-soybean basal diet, wheat-soybean basal diet with 4,000 U/kg xylanase, corn-soybean basal diet, and corn-soybean basal diet with 4,000 U/kg xylanase to evaluate interactive effects of xylanase in corn- or wheat-based diets on broilers cecal microbiota during a 6-week production period. The results indicated that bacterial community clustering was mainly due to cereal grains rather than xylanase supplementation. Compared with broilers fed wheat-based diets, corn-based diets increased alpha-diversity and separated from wheat-based diets ($p < 0.05$). Xylanase modulated the abundance of specific bacteria without changing overall microbial structure. In broilers fed wheat-based diets, xylanase increased the abundance of *Lactobacillus*, *Bifidobacterium*, and some butyrate-producing bacteria, and decreased the abundance of non-starch polysaccharides-degrading (NSP) bacteria, such as *Ruminococcaceae* and *Bacteroidetes* ($p < 0.05$). In broilers fed corn-based diets, xylanase decreased the abundance of harmful bacteria (such as genus *Faecalitalea* and *Escherichia-Shigella*) and promoted the abundance of beneficial bacteria (such as *Anaerofustis* and *Lachnospiraceae_UCG_010*) in the cecum ($p < 0.05$). Overall, xylanase supplementation to wheat- or corn-based diets improved broilers performance and cecal microbiota composition. Xylanase supplementation to wheat-based diets increased the abundance of butyrate-producing bacteria and decreased the abundance of NSP-degrading bacteria. Moreover, positive effects of xylanase on cecal microbiota of broilers fed corn-based diets were mostly related to the inhibition of potentially pathogenic bacteria, and xylanase supplementation to corn-based diets slightly affected the abundance of butyrate-producing bacteria and NSP-degrading bacterium, the difference might be related to lower content of AX in corn compared to wheat.

Keywords: xylanase, wheat, corn, broiler, cecal microbiota

INTRODUCTION

Xylanase has been widely applied in the broiler industry due to its ability to degrade arabinoxylans (AX) in poultry diets (Bautil et al., 2019). Corn and wheat are dominant feed grains for broilers diets; however, concentration and structure of AX in corn and wheat are different (Cowieson, 2010). At the present, most reports have focused on positive effects of xylanase on performance in broilers fed viscous wheat-based diets (Gonzalez-Ortiz et al., 2017; Liu and Kim, 2017; Wang et al., 2021). However, benefits of xylanase on growth performance of broilers supplemented non-viscous corn-based diets also were recorded (Khadem et al., 2016). Xylanase can reduce chyme viscosity, release nutrients from inside cell wall, and xylo-oligosaccharides (XOS) as a result of AX degradation in the gastrointestinal tract (GIT), which may then act like a prebiotic (Gonzalez-Ortiz et al., 2017; Petry et al., 2021). Previous studies have demonstrated that the dominant mode of action responsible for benefits of xylanase in corn- or wheat-based diets may be distinct and impacts of xylanase on growth performance, nutrient utilization, digesta characteristics, and cecal volatile fatty acid (VFA) concentrations of broilers fed corn- or wheat-based diets were compared (Kiarie et al., 2014; Munyaka et al., 2016; McCafferty et al., 2019a,b).

Cecal microbiota has the most complex microbiota in GIT which was associated with the health of broilers (Yadav and Jha, 2019). The GIT microbiota is shaped by both the environment and the broiler itself (Liu et al., 2021). Environmental changes, especially dietary factors, such as feed components and additives, have been widely demonstrated to regulate GIT microbiota (Shang et al., 2018). Cecal VFA concentrations are considered to be associated with bacterial fermentation in the large intestine (Ríos-Covián et al., 2016). Xylanase changed VFA concentrations in broilers cecum when the diets were corn- or wheat-based (McCafferty et al., 2019a,b). We hypothesized that supplemental xylanase might exert positive effects on cecal microbiota of broilers fed corn- or wheat-based diets by different gut bacterial communities.

16S rRNA gene sequencing analysis is a tool that has been widely applied to evaluate the diversity of GIT microbiota (Shang et al., 2018). To further elaborate the specificity of xylanase for feed ingredients, and the impacts of xylanase on cecal microbiota, we conducted the following experiment by feeding broilers corn- or wheat-based diets for a 6-week period and assessed the bacterial composition by 16S rRNA gene sequencing.

MATERIALS AND METHODS

The present study was approved by the Laboratory Animal Welfare and Animal Experimental Ethical Inspection Committee of China Agricultural University (Beijing, China).

Preparation of Xylanase

The xylanase (2,000,000 U/kg) used in this experiment was preserved by our laboratory. An acidic xylanase gene (*xynA*)

cloned from *Aspergillus sulphureus* was codon-optimized (GenBank accession number: EF114744) and expressed in *Pichia pastoris* (Cao et al., 2007). A disulfide bridge and proline residue substitutions were introduced to create xylanase mutants (AT-xynA) aiming to improve the thermostability of AT-xynA. Moreover, construction of a double-copy expression strain increased AT-xynA produced in *Pichia pastoris* (Wang et al., 2020). AT-xynA exhibited a resistance to an acidic environment (over 70% residual activity remained after 120 min incubation at pH 2.2) and was stable at high temperature (about 50% residual activity remained after 9.5 min incubation at 70°C; Yang et al., 2017a). High-density fermentation of the xylanase was processed in a 15-L fermenter as previously described (Yang et al., 2017a). The actual xylanase product was obtained by mixing 66% of the liquid fermentation broth produced above with 34% soybean meal and then air dried for 24 h. This resulted in a xylanase preparation containing approximately 2,000,000 units (U) of xylanase per kg. One unit of xylanase activity is defined as the amount of enzyme releasing 1 μmol of xylose per min under pH 3.0 and 50°C (Yang et al., 2017a).

Experiment Animals, Management, Design, and Diets

Individually numbered metal tags were attached to the wings of 192 1-day-old Arbor Acres broilers (46.14 ± 0.42 g) for individual identification. Broilers were vaccinated with Newcastle disease vaccine on d 7 and 28, and vaccinated with an inactivated infectious bursal disease vaccine on d 14 and 21. The broilers were raised in an environmentally controlled room, and they were exposed to constant light. The initial room temperature for 1-day-old broilers was controlled at about 33°C, and the temperature reduced 3°C/week until 24°C as the age of broilers increased. Broilers were randomly assigned to one of four diet treatments, and each treatment had six replicates with eight broilers. Broilers were fed an early phase diet (d 1 to 21) and a late phase diet (d 21 to 42). Dietary treatments consisted of 2 × 2 factorial design including two cereals (corn and wheat) and two enzyme treatments (control and xylanase): (1) wheat-soybean-based diet (WCON), (2) wheat-soybean-based diet with 4,000 U/kg xylanase (WXY), (3) corn-soybean-based diet (CCON), and (4) corn-soybean-based diet with 4,000 U/kg xylanase (CXY). The composition of experimental diets was shown (Table 1), and nutrient levels of diets satisfied National Research Council (National Research Council, 1994).

Sample Collection and Processing

Average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) of broilers from d 1 to 42 were calculated based on body weight and feed intake. One broiler from each replicate was slaughtered on d 14, 28, and 42 for collection of cecal digesta. The cecal digesta was snap-frozen in liquid nitrogen and then stored at -80°C.

Cecal Microbiota Analysis

For microbial diversity analysis, total genomic DNA from cecal digesta samples was extracted by using the EZNA stool

TABLE 1 | Composition and nutrient levels of basal diets (% , as fed basis).

Items	1 to 21 d of age		22 to 42 d of age	
	Corn	Wheat	Corn	Wheat
Ingredients				
Corn	61.15	–	67.99	–
Wheat	–	62.16	–	67.28
Soybean meal, 43% crude protein	28.00	25.80	22.00	19.47
Fish meal, 64.6% crude protein	3.71	3.46	2.60	4.62
Soybean oil	3.00	4.96	3.60	5.56
Dicalcium phosphate	1.28	1.02	1.38	0.83
Limestone	1.26	1.40	1.10	1.16
Salt	0.30	0.30	0.30	0.30
L-lysine HCl, 78%	–	–	0.12	–
DL-Methionine, 98%	0.15	0.14	0.07	0.02
L-Threonine, 98%	0.04	0.01	0.09	0.01
Chromic oxide	0.25	0.25	0.25	0.25
Vitamin-mineral premix ¹	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00
Calculated nutrient content²				
Metabolizable energy, kcal/kg	3,052	3,050	3,157	3,150
Crude protein	20.08	20.95	19.37	20.36
Calcium	1.00	1.00	0.90	0.90
Available phosphorous	0.45	0.45	0.35	0.35
Digestible lysine	0.86	0.86	0.73	0.73
Digestible methionine	0.30	0.30	0.28	0.28
Digestible threonine	0.63	0.63	0.56	0.56

¹Vitamin A, 11,000 IU; vitamin D, 3,025 IU; vitamin E, 22 mg; vitamin K₃, 2.2 mg; thiamine, 1.65 mg; riboflavin, 6.6 mg; pyridoxine, 3.3 mg; cobalamin, 17.6 µg; nicotinic acid, 22 mg; pantothenic acid, 13.2 mg; folic acid, 0.33 mg; biotin, 88 µg; choline chloride, 500 mg; iron, 48 mg; zinc, 96.6 mg; manganese, 101.76 mg; copper, 10 mg; selenium, 0.05 mg; iodine, 0.96 mg; and cobalt, 0.3 mg.

²Values were calculated according to the National Research Council (1994).

DNA kit (Omega Biotek, Norcross, GA). The concentration of DNA was quantified by a NanoDrop 2000 spectrophotometer (Thermo Scientific, MA, United States), and the quality of DNA was analyzed by electrophoresis with 2% agarose gels. Then, the V3-V4 region of the 16S rRNA gene was amplified using genomic DNA of cecal digest samples as the template, and 338F (5'barcodeACTCCTACGGGAGGCAGCAG3') and 806R (5'GGACTACHVGGGTW TCTAAT3') as the primers. The PCR reaction conditions were as: initial denaturation at 95°C for 3 min, then 27 cycles of 95°C for 30 s, 55°C for 30 s and 72°C for 45 s, and finally 72°C for 5 min. The PCR products were purified by the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA). The purified amplicons were pooled in equimolar concentrations and then sequenced on Illumina MiSeq platform. Trimmomatic (version 3.29) was applied to quality-filter raw Illumina fastq files. Operational taxonomic units (OTUs) were clustered with a similarity of 97% using UPARSE (version 7.0).¹ The raw data have been submitted to the NCBI Sequence Read Archive (SRA) database (PRJNA753623).

¹<http://drive5.com/uparse/>

Statistical Measurements

To determine interaction effects of cereal type and xylanase supplementation on growth performance and microbiota alpha-diversity of broilers, a two-way ANOVA was performed using SAS (version 9.2, 2008). Each treatment contained six pens, and the pen was considered as an experiment unit. $p < 0.05$ was considered significant, and $0.05 \leq p \leq 0.10$ was regarded a trend. When a significant effect was observed, the data were further analyzed by one-way ANOVA (version 9.2, 2008). The rarefaction curves of OTUs were calculated by QIIME to determine the sequencing coverage across samples. Alpha-diversity was calculated by Sobs (the observed OTUs), Chao (the Chao 1 estimator), Ace (the ACE estimator), and PD (Phylogenetic diversity). Principal coordinate analysis (PCoA) on Weighted UniFrac distance was conducted. Analysis of similarity (ANOSIM) with 999 permutations was used to assess the similarity between bacterial community structure by measuring R value (between 0 and 1). $R = 0$ indicates that two groups are similar, and $R = 1$ suggests two groups are different. The linear discriminant analysis effect size (LEfSe) method was applied for the analysis of significant differences in abundance of microbiota community.

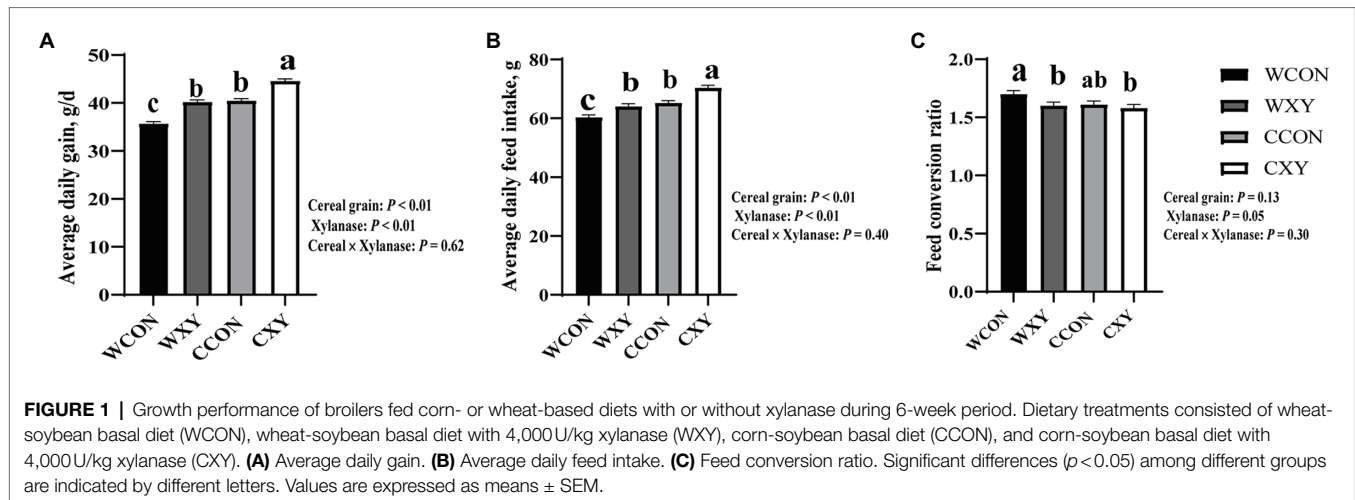
RESULTS

Growth Performance

The ADG, ADFI, and FCR of broilers from different dietary treatments were shown in **Figure 1**. From 1 to 42 d of age, ADG and ADFI of broilers from WXY and CCON groups were higher than broilers from WCON group ($p < 0.05$); however, ADG and ADFI of broilers from CXY group were higher compared with broilers from WXY to CCON groups ($p < 0.05$). Broilers from WCON group exhibited higher FCR compared to broilers from WXY and CXY groups ($p < 0.05$), and FCR of broilers from CCON group was similar to those from WCON, WXY, and CXY groups ($p > 0.05$). Additionally, cereal main effect influenced ADG and ADFI ($p < 0.05$), the broilers receiving corn-based diets showed higher ADG and ADFI than those fed wheat-based diets ($p < 0.05$). Xylanase main effect also influenced ADG and ADFI ($p < 0.05$), and tended to influence FCR ($p = 0.05$), xylanase supplementation to broilers diets could improve ADG, ADFI, and FCR of broilers ($p < 0.05$). However, no interactive effects of cereal type and xylanase were observed on broilers growth performance.

Cecal Microbiota Analysis

In this experiment, a total of 3,211,886 high-quality sequence reads of samples, with the average read length of 412 base (bp), were obtained for subsequent analysis. In total, 1,146 distinct OTUs with a 97% identity were generated that fell into 13 phyla, 24 classes, 44 orders, 70 families, and 175 genera. Rarefaction curves based on the observed OTUs approached asymptotic for each dietary treatment on d 14, 28, and 42, indicating the availability of sufficient numbers of OTUs to represent each microbiota community, and age-linked increasing



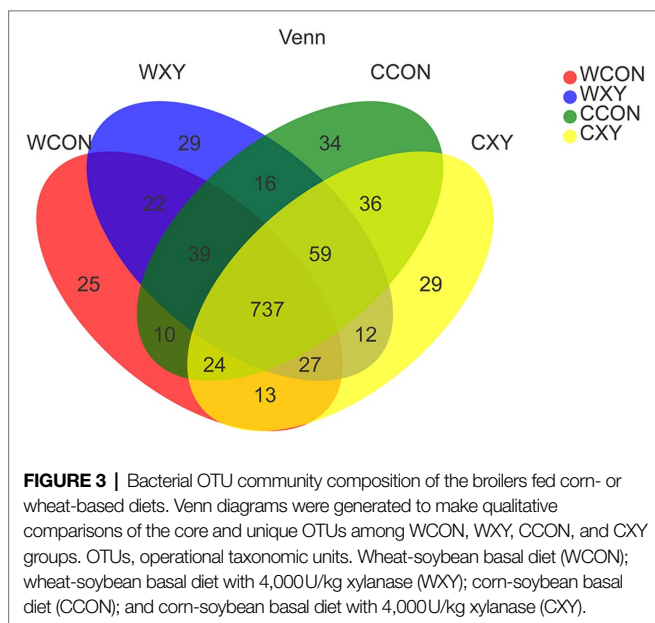
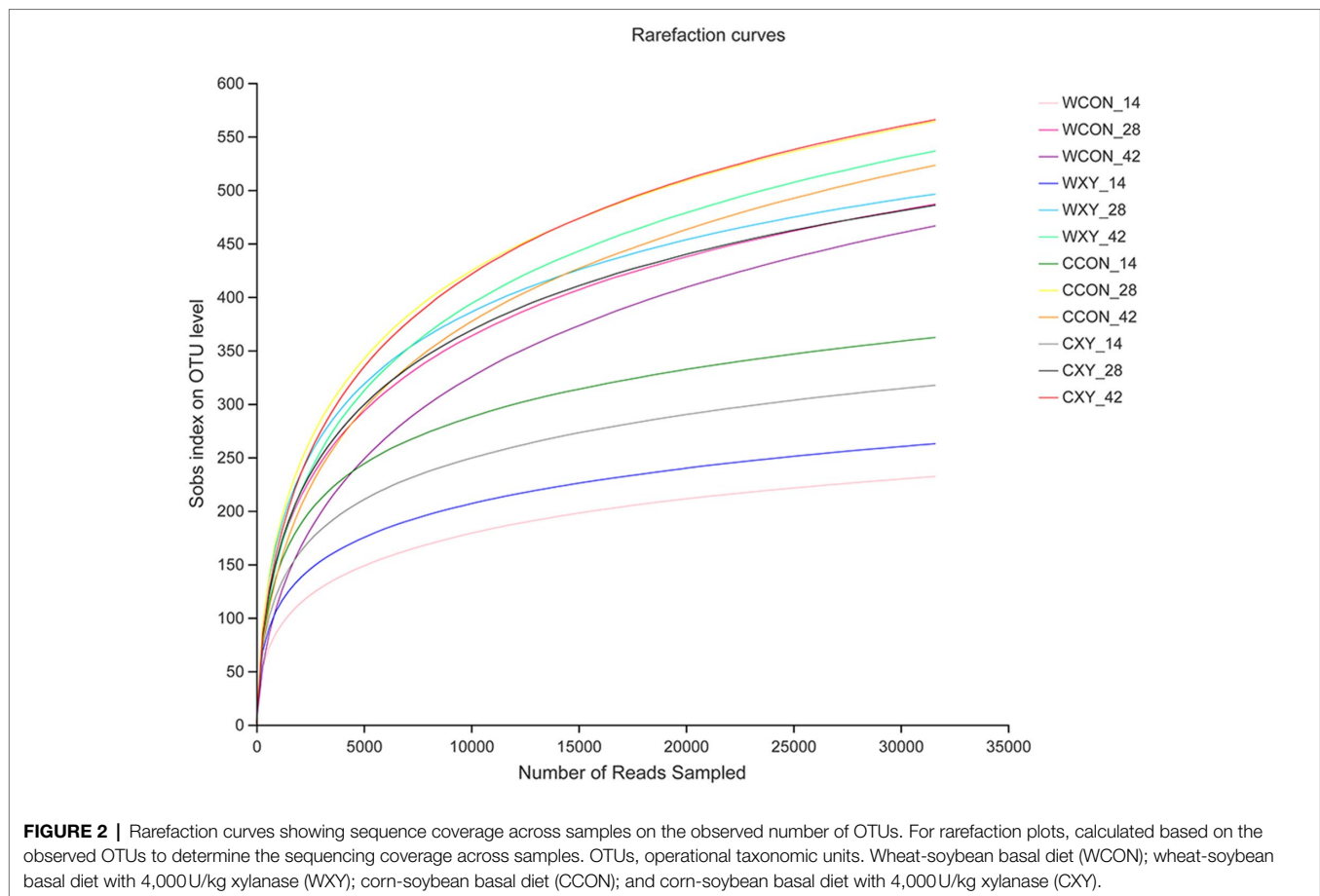
trend in the number of observed OTUs was observed in broilers (Figure 2).

Venn diagrams were generated to make qualitative comparisons of the core and unique genera between WCON, WXY, CCON, and CXY groups (Figure 3). The core OTUs of the four treatments were 737, and the number of unique genera for WCON, WXY, CCON, and CXY groups was 25, 29, 34, and 29, respectively. As shown in Table 2, there was not a significant interaction between cereal type and xylanase supplementation in cecal microbiota alpha-diversity of broilers ($p > 0.05$). On d 14, cereal main effect influenced indices of Sobs, Ace, Chao ($p < 0.05$), and PD ($p = 0.08$), and the broilers fed corn-based diets exhibited higher Sobs, Ace, Chao, and PD values compared to those receiving wheat-based diets ($p < 0.05$). On d 42, PD values were also significantly affected by the cereal type ($p < 0.05$), and broilers from CXY exhibited significantly higher PD values compared with broilers from WCON to WXY groups ($p < 0.05$). The PCoA was performed by using Weighted UniFrac distance to determine β -diversity of microbial communities between different groups (Figure 4). Although the addition of xylanase in wheat- or corn-based diets improved growth performance of broilers, no distinct clustering due to xylanase supplementation was denoted. However, PCoA of the OTU data for chickens fed wheat- or corn-based diets revealed a less pronounced but distinct variation between cereal grains (ANOSIM, $R = 0.1572$, $p = 0.003$).

To further elaborate effects of xylanase supplementation to wheat- or corn-basal diets on broilers cecal microbiota, we then compared the bacterial community at phylum and family levels across four dietary treatments at each sampling time point. At the phylum level (Figure 5A), microbiota of cecal microbiota community was dominated by Firmicutes, Bacteroidetes, Proteobacteria, Tenericutes, and Actinobacteria independent of sampling time point and xylanase supplementation. Two major phyla (Firmicutes and Bacteroidetes) accounted for more than 90% of the cecal bacterial community at each time point in four treatments. The other phyla, such as Proteobacteria, Tenericutes, and Actinobacteria, were represented at relatively low abundance. Bacteroidetes increased in abundance with time

in four dietary treatments; however, the relative abundance of Firmicutes decreased with age. At the family level (Figure 5B), *Ruminococcaceae*, *Lachnospiraceae*, *Clostridiales_vadinBB60_group*, *Lactobacillaceae*, *Peptococcaceae*, and *Erysipelotrichaceae* assigned to Firmicutes phyla, *Porphyromonadaceae*, *Rikenellaceae*, and *Bacteroidaceae* assigned to Bacteroidetes phyla, and *norank_o_Mollicutes_RF9* which belongs to Tenericutes phyla were dominant families in the cecum. At 14 days of age, *Ruminococcaceae* was the most abundant family in WCON, CCON, and CXY groups, and *Lachnospiraceae* was the most abundant family in WXY group. At 28 days of age, *Ruminococcaceae* was the most abundant family, and at 42 days of age, *Porphyromonadaceae* was the dominant family in four dietary treatments.

To further probe how AT-xynA modulated GIT microbial community, alterations in cecal microbiota were also analyzed by the LefSe method on d 14, 28, and 42. On d 14 (Figure 6A), in WCON and WXY groups, the abundance of genus *norank_f_Lachnospiraceae*, *Shuttleworthia*, *Ruminococcus_torques_group*, and *Sellimonas* (family *Lachnospiraceae*) was higher in WXY group ($p < 0.05$). The abundance of genus *Ruminococcaceae_UCG_004*, *unclassified_f_Ruminococcaceae*, *Anaerotruncus*, and *Butyrivibrio* (family *Ruminococcaceae*) was also higher in WXY group, while the abundance of family *Ruminococcaceae* was higher in WCON group ($p < 0.05$). The relative abundance of genus *Defluviitaleaceae_UCG_011* (family *Defluviitaleaceae*), *Clostridium_sensu_stricto_1*, *Anaerofustis* (family *Eubacteriaceae*), and *norank_f_Erysipelotrichaceae* was higher in WXY group ($p < 0.05$). The phylum Actinobacteria, order *Coriobacteriales*, and genus *Bifidobacterium*, *Gordonibacter*, and *Senegalimassilia* were also higher in WXY group ($p < 0.05$). On d 14 (Figure 7A), in CCON and CXY groups, higher relative abundance of *Ruminococcus_gauvreauii_group*, *Eubacterium_brachy_group*, and *Family_XIII_UCG_001* was observed in CXY group ($p < 0.05$). The abundance of genus *Lachnospiraceae_FCS020_group*, *Faecalitalea*, *Eubacterium_hallii_group*, *Flavonifractor*, *Intestinimonas*, *Candidatus_Soleaferrea*, and *Ruminococcaceae_NK4A214_group* was higher in CCON group ($p < 0.05$). The abundance of genus *Christensenellaceae_R_7_group* (family



Christensenellaceae), *Defluviitaleaceae_UCG_011* (family *Defluviitaleaceae*), *unclassified_f_Bacillaceae* (order *Bacillales*), and *norank_o_Mollicutes_RF9* (order *Mollicutes_RF9*) was

increased in CCON group ($p < 0.05$). The abundance of phylum Proteobacteria including family *Enterobacteriaceae* and family *Desulfovibrionaceae* was also higher in CCON group ($p < 0.05$).

On d 28 (**Figure 6B**), in WCON and WXY groups, the phylum Firmicutes were higher in WXY group, while the phylum Bacteroidetes were higher in WCON group ($p < 0.05$). The genus *Anaeroplasma* belonged to order *Anaeroplasmatales* and the genus *Odoribacter*, *Hydrogenoanaerobacterium*, and *Ruminococcaceae_V9D2013_group* were higher in WXY group ($p < 0.05$). On d 28 (**Figure 7B**), in CCON and CXY groups, the abundance of class *Clostridia* including genus *Anaerotruncus*, *Tyzzerella*, and *Caproiciproducens*, as well as the phylum Lentisphaerae, was higher in CXY group ($p < 0.05$). The abundance of genus *Alistipes*, *Eubacterium_ventriosum_group*, *norank_f_Peptococcaceae*, *Senegalimassilia*, and *norank_f_Lachnospiraceae* was higher in CCON group ($p < 0.05$).

On d 42 (**Figure 6C**), in WCON and WXY groups, the abundance of genus *Eubacterium_hallii_group*, *Eubacterium_ventriosum_group*, *Gordonibacter*, and *Parabacteroides* was higher in WXY group ($p < 0.05$). The abundance of genus *Anaerotruncus*, *unclassified_f_Ruminococcaceae*, *Subdoligranulum*, and *Caproiciproducens* (family *Ruminococcaceae*) was also higher in WXY group ($p < 0.05$). Higher abundance of the genus *Clostridium_sensu_stricto_1* belonging to family *Clostridiaceae_1*, genus *Defluviitaleaceae_UCG_011* belonging to family *Defluviitaleaceae*,

TABLE 2 | Effects of xylanase on alpha-diversity of cecal bacterial community in broilers fed wheat- or corn-based diets¹.

	WCON	WXY	CCON	CXY	SEM	Analysis of Variance		
						Cereal grain × Xylanase	Cereal grain	Xylanase
d 14								
The observed OTUs	232.00 ^c	262.67 ^{bc}	362.00 ^a	317.33 ^{ab}	24.46	0.16	< 0.01	0.78
The ACE estimator	271.49 ^b	311.52 ^{ab}	420.13 ^a	368.80 ^{ab}	32.44	0.20	0.01	0.87
The Chao ¹ estimator	276.91 ^b	315.58 ^b	422.40 ^a	374.52 ^{ab}	34.35	0.16	0.01	0.68
Phylogenetic diversity	17.63	22.61	28.00	24.90	3.15	0.24	0.08	0.77
d 28								
The observed OTUs	486.67	496.00	564.00	485.67	35.22	0.25	0.37	0.36
The ACE estimator	579.62	567.51	667.31	569.31	29.94	0.19	0.17	0.10
The Chao ¹ estimator	581.69	564.43	686.76	570.28	34.81	0.19	0.15	0.10
Phylogenetic diversity	32.74	32.99	35.40	32.96	1.92	0.50	0.51	0.58
d 42								
The observed OTUs	466.33	536.33	523.00	565.67	32.98	0.69	0.23	0.13
The ACE estimator	588.28	648.24	645.53	655.60	41.29	0.56	0.46	0.42
The Chao ¹ estimator	598.90	662.40	667.68	656.46	43.40	0.41	0.49	0.57
Phylogenetic diversity	32.33 ^b	34.91 ^a	35.74 ^{ab}	42.11 ^a	2.05	0.38	0.03	0.10

¹Alpha diversity analysis for bacterial community determined by 16s RNA sequencing. OTUs, operational taxonomic units. Wheat-soybean basal diet (WCON); wheat-soybean basal diet with 4,000 U/kg xylanase (WXY); corn-soybean basal diet (CCON); and corn-soybean basal diet with 4,000 U/kg xylanase (CXY).

^{a-c}Means with a row with no common superscript differ significantly.

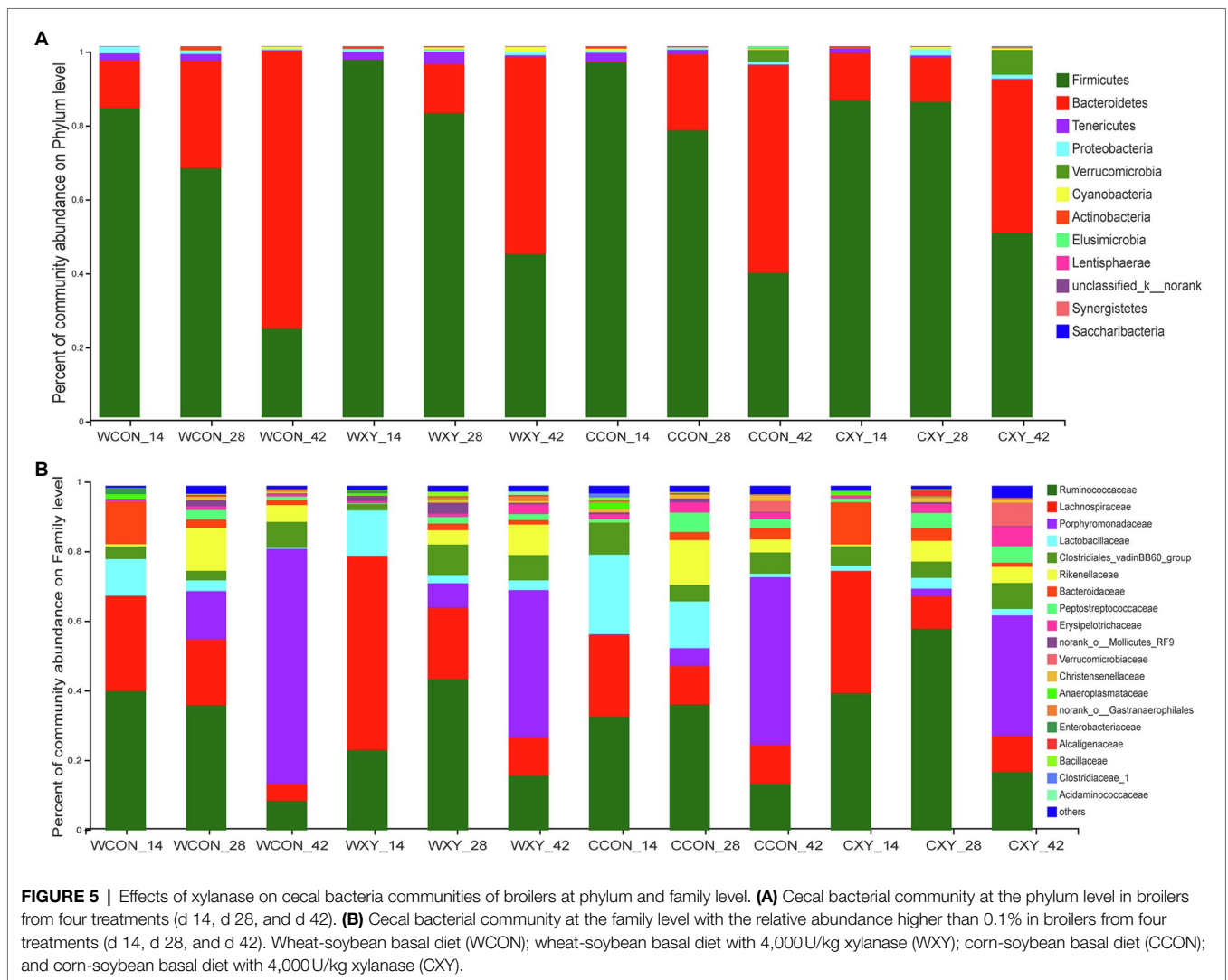
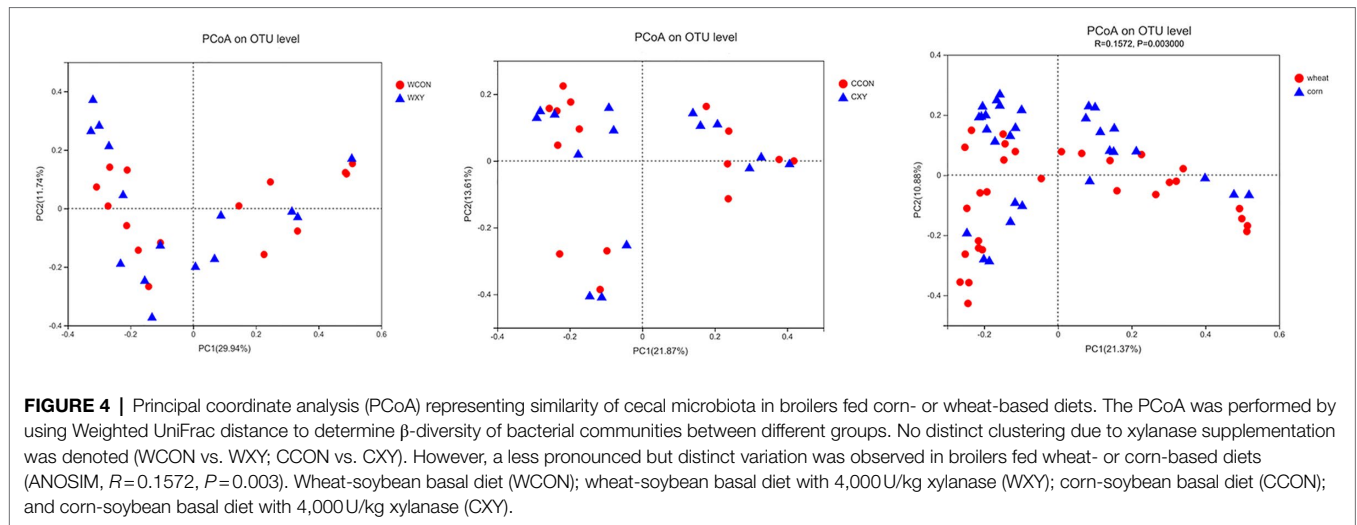
and genus *Lactobacillus* from class *Bacilli* was observed in WXY group ($p < 0.05$). On d 42 (Figure 7C), in CCON and CXY groups, the abundance of class *Erysipelotrichia*, genus *Lachnospiraceae_UCG_010*, *Subdoligranulum*, *Anaerofustis*, and *norank_f_Runmicoccaceae* was higher in CXY group, while the abundance of genus *Ruminiclostridium_1*, *Senegalimassilia*, *Flavonifractor*, and *norank_f_Erysipelotrichaceae* was higher in CCON group ($p < 0.05$).

The cecal microbiota of broilers fed corn- or wheat-based diets was also compared by the LefSe method (Figure 8). The abundance of genus *Lachnospiraceae_UCG_010*, *Tyzzera*, *Shuttleworthia*, *Eubacterium_ventriosum_group*, *Ruminococcaceae_UCG_013*, *Ruminococcaceae_UCG_004*, *Oscillospira*, *Anaerofilum*, *Butyricoccus*, *Ruminococcaceae_UCG_008*, *Ruminococcaceae_UCG_005*, *Intestinimonas*, *Ruminococcus_2*, *norank_f_Ruminococcaceae*, *Oscillibacter*, and *Ruminiclostridium_9* was higher in broilers fed corn-based diets ($p < 0.05$). The abundance of genus *Christensenellaceae_R_7_group* (*Christensenellaceae* family), *Romboutsia* (*Peptostreptococcaceae* family), genus *unclassified_f_Propionibacteriaceae* (*Peptococcaceae* family), *Erysipelatoclostridium*, *Turicibacter*, *Dielma*, *Erysipelotrichaceae_UCG_003*, *Faecalitalea* (family *Erysipelotrichaceae*), *Elusimicrobium* (phylum *Elusimicrobia*), *unclassified_f_Peptococcaceae* (family *Propionibacteriaceae*), *Eggerthella*, *Gordonibacter* (family *Coriobacteriaceae*), *Methylococcus* (family *Methylococcaceae*), and *Methylophilus* (family *Methylophilaceae*) was higher in broilers receiving corn-based diets ($p < 0.05$). Moreover, higher abundance of the phylum *Proteobacteria*, order *Bifidobacteriales*, family *Bifidobacteriaceae*, genus *unclassified_f_Lachnospiraceae*, *Marvinbryantia*, *Bifidobacterium*, and *Lactococcus* was observed in broilers receiving wheat-based diets ($p < 0.05$).

DISCUSSION

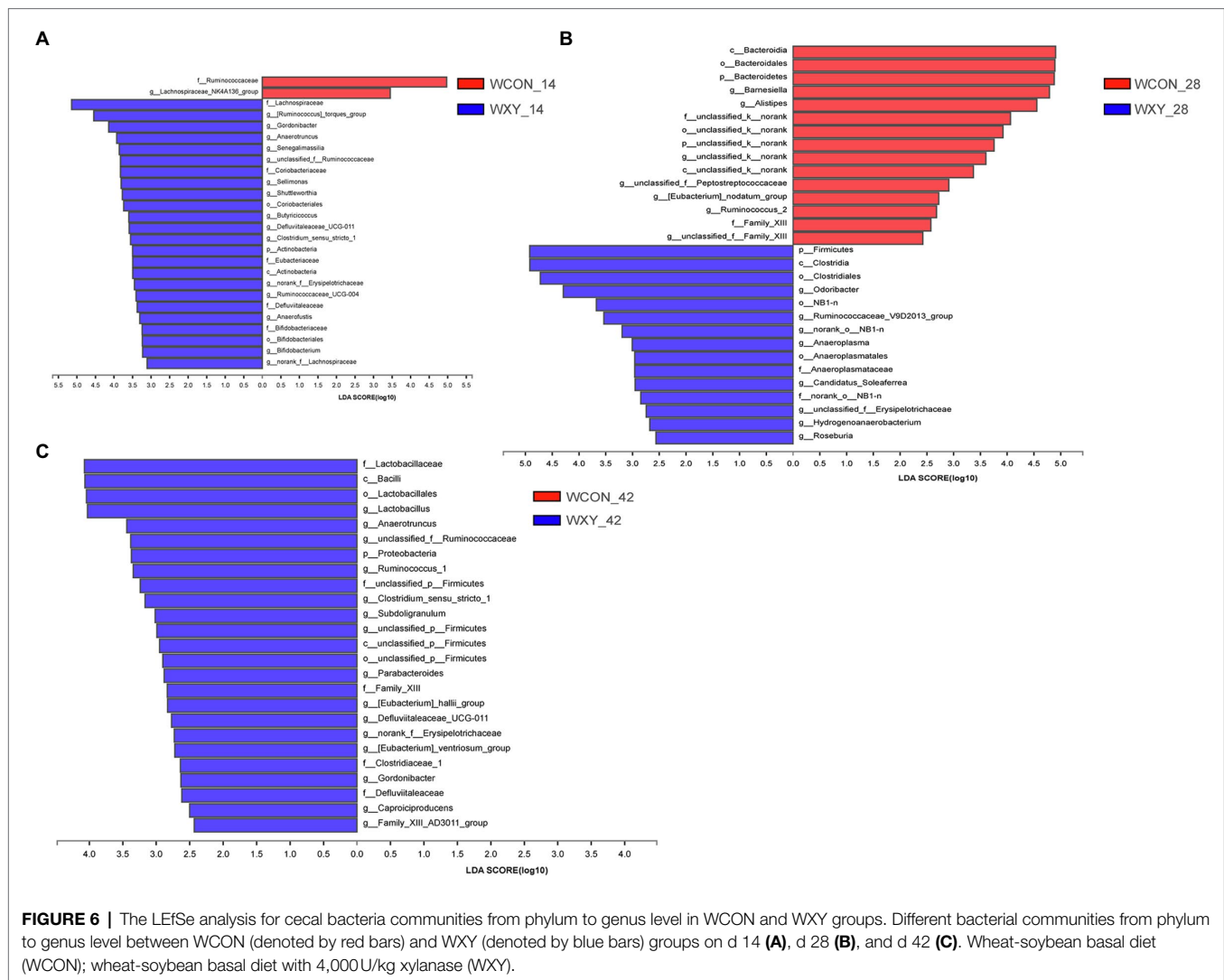
In the current study, broilers receiving corn-based diets showed higher ADG and ADFI than broilers supplemented wheat-based diets. Masey-O'Neill et al. (2014) also reported that feeding broilers with corn-based diets increased body weight gain and feed intake compared to broilers fed wheat-based diets from d 1 to 28. This observation might be related to the higher concentration of soluble NSP in viscous wheat. The soluble NSP is known to exhibit antinutritive effects by increasing intestinal digesta viscosity, affecting gut transit time, thus impair nutrient absorption and utilization of broilers (Kiarie et al., 2014). However, similar to our results, it has been reported that feeding broilers with wheat-based diets supplemented with xylanase could improve growth performance, the improvement might be associated with the alleviation of detrimental effects of soluble NSP on digesta viscosity (Gonzalez-Ortiz et al., 2017; Liu and Kim, 2017). Moreover, in this study, xylanase improved growth performance of broilers receiving corn-based diets. Benefits of xylanase on performance of broilers fed non-viscous corn-based diets also have been reported, indicating that there are other mechanisms besides this mode of viscosity reduction (Khadem et al., 2016). The primary mode of action may depend on the type of cereal grain, thus 16S rRNA gene high-throughput sequencing in this study was used to elaborate effects of xylanase on cecal microbiota of broilers supplemented corn- or wheat-based diets on d 14, 28, and 42.

The generated rarefaction curves have reached plateau with the Sobs index, suggesting that 16S rRNA sequencing libraries could represent most of OTUs present in all the samples sequenced in this study. As shown in Venn diagram, the number of OTUs in cecal microbiota among four dietary treatments was similar, and most of them shared. When comparing effects of xylanase on cecal microbiota alpha-diversity of broilers fed



wheat- or corn-based diets, there was not a significant interaction between cereal type and xylanase supplementation in alpha-diversity, and xylanase main effect also did not affect

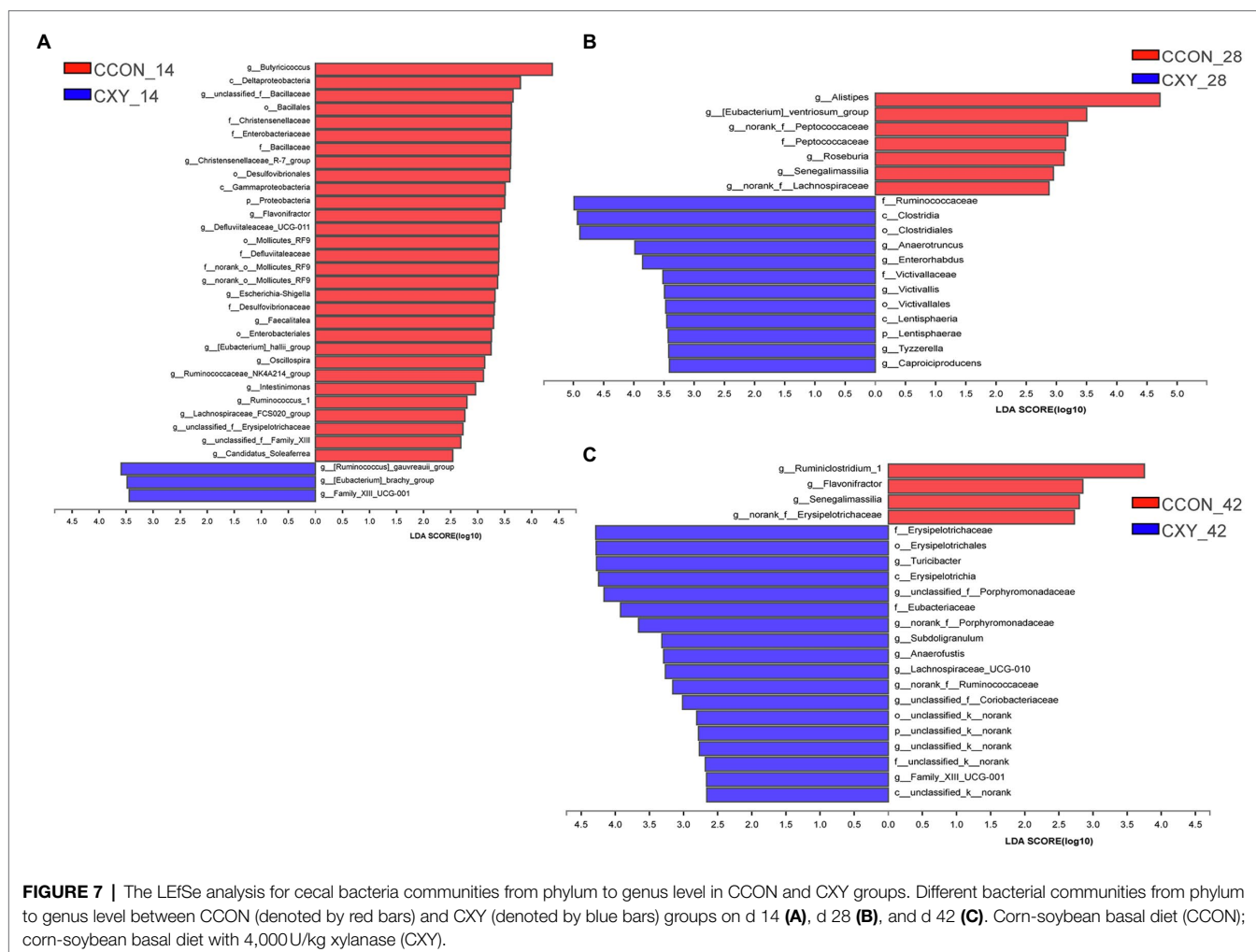
alpha-diversity of broilers. However, we found that broilers receiving corn-based diets had richer and more diverse microbiota than broilers fed wheat-based diets on d 14 and 42, indicating



that microbiota of broilers receiving corn-based diets exhibited higher richness. The increase in alpha-diversity has been reported to be beneficial to the health of broilers, the increased diversity might be able to promote the stability of microbiota (Díaz Carrasco et al., 2018; Dhillon et al., 2019). The analysis of PCoA based on dietary treatments did not exhibit distinct clustering patterns, the composition of GIT microbiota between WCON and WXY groups was similar, and beta diversity was also not different between CCON and CXY groups. However, the phylogenetic distances clearly revealed that type of cereal grain affected beta diversity, suggesting a distinction in microbial community structure of broilers receiving corn-based diets relative to broilers receiving wheat-based diets.

To further probe how xylanase modulates GIT microbial community of broilers receiving wheat- or corn-based diets, the variations in relative abundance of microbiota were analyzed. The development of GIT microbiota in broilers might be a continuous process of microbial community succession. In the current study, the development of microbiota community from an immature phase to a mature phase in four dietary treatments

was clearly shown. As previously reported, cecal communities for all the broilers were dominated by members of Firmicutes and Bacteroidetes phylum (Ilaria et al., 2020). Firmicutes accounted for most of the cecal microbiota in young broilers, while Bacteroidetes were only enriched at the end of productive life. Butyrate-producing bacteria mostly belong to Firmicutes phylum (Chakraborti, 2015; Dai et al., 2021). Representatives of Bacteroidetes phylum mainly degrade polysaccharides producing propionate (Yang et al., 2013; Polansky et al., 2016). In quickly growing, young birds, butyrate is highly required to provide energy for the growth of intestinal cells, while adult birds have fully developed intestines and need less amount of butyrate (Petra et al., 2014; Onrust et al., 2015). Higher abundance of Bacteroidetes in adult broilers represented an effective balance between energy obtained from available nutrients and sustained growth (Ocejo et al., 2019). The alteration of the proportion of Firmicutes and Bacteroidetes indicates the change of produced butyrate in broilers cecum, thus can explain the results in this study that Bacteroidetes gradually increased throughout the production period.



When comparing cecal microbiota of broilers fed wheat-based diets, compared with WXY, higher abundance of family *Ruminococcaceae* on d 14 and phylum Bacteroidetes on d 28 was observed in WCON group. *Ruminococcaceae* has been reported to be related to cellulose-degrading capacity, and *Ruminococcaceae* is enriched in genes encoding for endo-1,4-beta-xylanase and cellulase (Biddle et al., 2013; Dai et al., 2020). Phylum Bacteroidetes also contain a high number of genes encoding for glycosyl hydrolases, such as β -xylosidase, endo-1,4- β -xylanase, and α -N-arabinofuranosidase, which makes Bacteroidetes become the primary phylum for the degradation of complex polysaccharides in GIT (Zhang et al., 2018a). Furthermore, it has been demonstrated that *Bacteroides* of the phylum Bacteroidetes was related to AX degradation (Zhang et al., 2018a,b). Dietary supplementation of xylanase to broilers could effectively hydrolyze AX in small intestines, thereby reducing the availability of polysaccharides to *Ruminococcaceae* and Bacteroidetes in the cecum. However, the abundance of microbiota associated with arabinoxylan-degrading capacity was not significantly different between broilers from CCON to CXY groups, we speculated that the difference might be related to the lower contents of arabinoxylans in corn.

Additionally, compared with WXY, the increased abundance of *Ruminococcaceae* in broilers from WCON group on d 14, and compared with CXY, higher abundance of order *Mollicutes_RF9* in CCON group on d 14 was observed. *Ruminococcaceae* has been demonstrated to be sensitive to starch, increased abundance of *Ruminococcaceae* in broilers from WCON group might also be related to lower ileal starch digestibility, because the addition of xylanase has been reported to increase starch digestibility (Choct et al., 1999; McCafferty et al., 2019a). The metagenome-assembled genomes of *Mollicutes_RF9* group also have the CAZyme related with starch degradation, thus lower concentration of undigested starch flowing into the cecum might limit the growth of *Mollicutes_RF9* (Ma et al., 2019).

Compared to WCON, higher abundance of family *Lachnospiraceae* (containing *norank_f_Lachnospiraceae*, *Shuttleworthia*, *Ruminococcus_torques_group*, and *Sellimonas*) on d 14, phylum Firmicutes, genus *Hydrogenoanaerobacterium*, and *Ruminococcaceae_V9D2013_group* on d 28, genus *unclassified_f_Ruminococcaceae*, *Anaerotruncus*, *Caproiciproducens*, and *Subdoligranulum* on d 42 was observed in WXY group. *Lachnospiraceae* has been demonstrated to

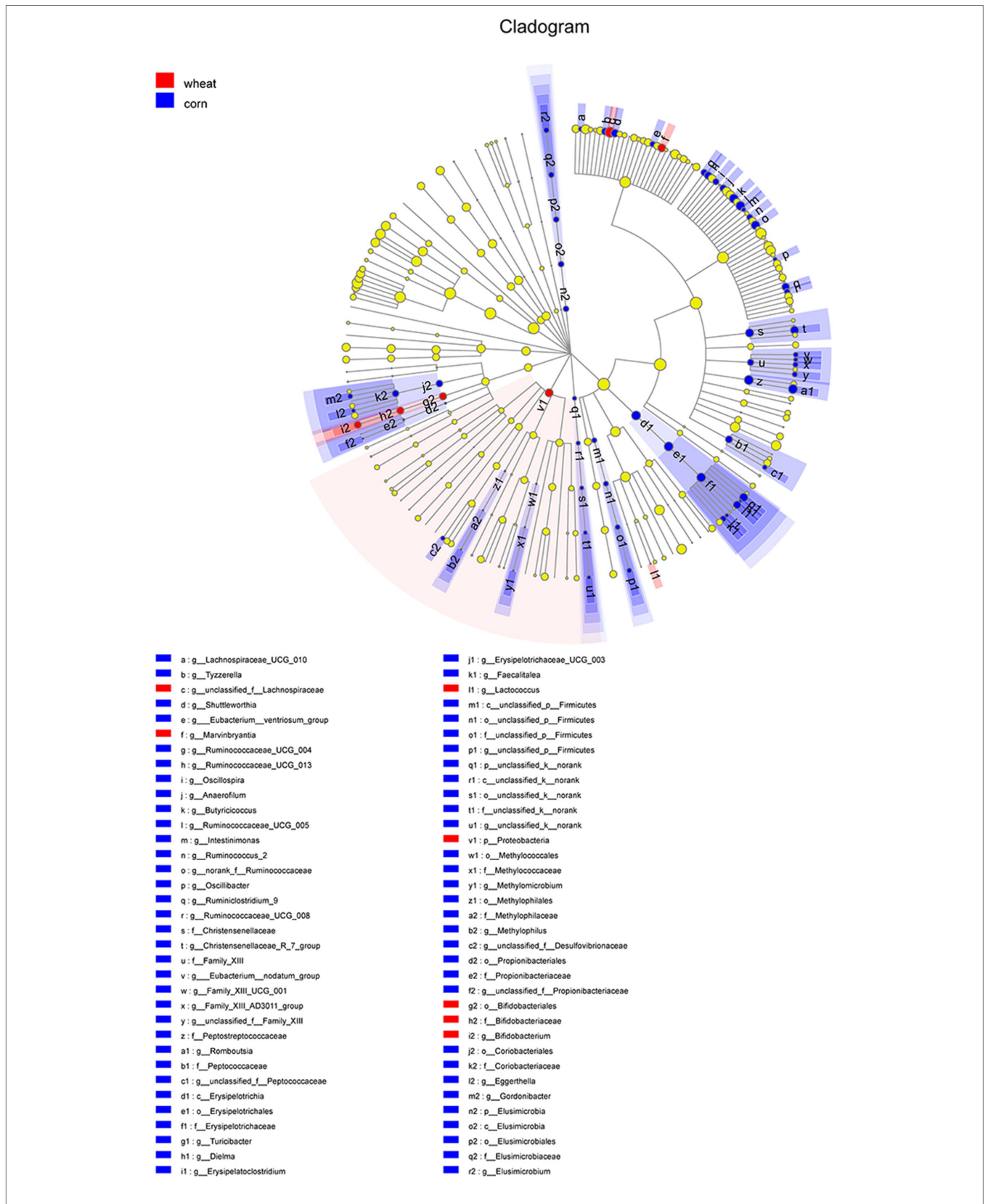


FIGURE 8 | The LEfSe analysis for cecal bacteria communities from phylum to genus level in broilers fed wheat- or corn-based diets. The circles from inner to outer represent distinct bacteria from phylum to genus level, respectively. The yellow dots inserted in the circle demonstrated no significant differences in bacteria communities between two dietary treatments.

produce butyrate; in addition, genus *norank_f_Lachnospiraceae*, *Shuttleworthia*, and *Ruminococcus_torques_group* have been observed to be related to better performance (Torok et al., 2011; Lee et al., 2017; McCafferty et al., 2019a; Li et al., 2020). Genera (*Hydrogenoanaerobacterium* and *Ruminococcaceae_V9D2013_group*) from the phylum Firmicutes are also suggested to effectively produce butyrate (Fang et al., 2020; Ilaria et al., 2020; Sun et al., 2020). Genus *unclassified_f_Ruminococcaceae*, *Anaerotruncus*, *Caproiciproducens*, and *Subdoligranulum* (family *Ruminococcaceae*) are also known as short-chain fatty acid producers. Supplemental xylanase could modulate GIT microbiota by increasing XOS concentrations in the cecum, and XOS has been reported to increase the abundance of butyrate-producing bacteria (De Maesschalck et al., 2015; McCafferty et al., 2019a). Moreover, the present result showed that xylanase supplementation to wheat-based diets increased the abundance of *Bifidobacterium* (phylum Actinobacteria) on d 14, and the abundance of *Lactobacillus* on d 42. XOS has been demonstrated to result in better performance of broilers by the stimulation of *Bifidobacterium* and *Lactobacillus* (Oyeagu et al., 2019; González-Ortiz et al., 2020). Although some species of Actinobacteria can lead to diseases, some strains like *Streptomyces* spp. exhibited antibacterial activities (Jing et al., 2018). *Bifidobacterium* as lactate-producing bacteria benefits to the health of GIT (Jing et al., 2018; González-Ortiz et al., 2020). Members of *Lactobacillus* could stimulate butyrate-producing bacteria and inhibit the colonization of pathogenic bacteria (Munyaka et al., 2016; Proszkowiec-Weglarz et al., 2020). When comparing cecal microbiota of broilers fed corn-based diets, compared with CON, higher abundance of order *Clostridiales* on d 28, genus *Anaerofustis*, *Lachnospiraceae_UCG_010*, and *norank_f_Ruminococcaceae* on d 42 was observed in broilers from CXY group. Clostridium-related bacteria within GIT have been demonstrated to be able to produce short-chain fatty acids and act as a barrier against invasion by other potentially pathogenic microbiota (Munyaka et al., 2016). The increase of these butyrate-producing bacteria might also be related to increased XOS concentrations in the cecum.

Furthermore, compared with CON group, lower abundance of family *Christensenellaceae*, *Desulfovibrionaceae*, and genus *Faecalitalea*, *Escherichia_Shigella* on d 14, and higher abundance of family *Erysipelotrichaceae* on d 42 were observed in CXY group. Xylanase supplementation to wheat-based diets could also increase the abundance of genus *Gordonibacter* on d 14 and d 42. The family *Christensenellaceae* was negatively associated with overall GIT health (Richards et al., 2019). *Faecalitalea* which affiliates to *Erysipelotrichaceae* family was related to inflammatory disorders (Dai et al., 2020). In addition, the previous work has reported that the abundance of *Desulfovibrio* (family *Desulfovibrionaceae*) was increased with ulcerative colitis, indicating that *Desulfovibrio* was enriched in an inflammatory environment (Yan et al., 2019). *Enterobacteriaceae* family includes potential pathogens, such as *Escherichia_Shigella*, the abundances of *Escherichia_Shigella* were found to be negatively correlated with growth

performance in broilers (Díaz Carrasco et al., 2018). Moreover, *Erysipelotrichaceae* showed a positive correlation with growth performance (Stanley et al., 2016). *Gordonibacter* was associated with a decrease risk or severity of different diseases, such as cancer and inflammatory bowel disease (Pérez-Burillo et al., 2018). Overall, the results indicated that xylanase supplementation could stimulate the colonization of beneficial bacteria or inhibit the colonization of harmful bacteria in broilers receiving wheat- or corn-based diets.

Additionally, we compared the cecal microbiota of broilers supplemented corn- or wheat-based diets. The abundance of *Lactobacillus* and *Bifidobacterium* increased in the cecal microbiota of broilers receiving wheat-based diets, and the increase may be associated with high contents of AX in wheat. The cecal fermentative ability of broilers depends on type and content of substrates flowing into cecum. Members of *Lactobacillus* contained a range of genes coding for AX-degrading enzymes; therefore, they can be beneficial to the degradation of XOS and AX (Zhang et al., 2018b). It also has been reported that AX and XOS selectively increased the abundance of *Bifidobacterium* spp., a so-called bifidogenic effect exerted by these prebiotics. Also, *Bifidobacterium* spp. have been reported to contain large number of genes coding for AX-degrading enzymes (Rivière et al., 2014). Overall, AX and XOS are preferred substrates of *Lactobacillus* and *Bifidobacterium*, and the growth of *Lactobacillus* and *Bifidobacterium* was stimulated by wheat relative to corn.

In corn-based diets fed broilers, the abundance of butyrate-producing bacterium increased, such as *Lachnospiraceae_UCG_010*, *Shuttleworthia*, *Anaerofilum*, *Ruminococcaceae_UCG_008*, *Ruminococcaceae_UCG_005*, *Ruminococcus_2*, *norank_f_Ruminococcaceae*, *Oscillibacter*, and *Ruminiclostridium_9* comparing to the broilers fed wheat-based diets. This difference may be attributed to the resistant starch content in corn and wheat. Corn contains the content of resistant starch 1.9-fold higher than that in wheat. Resistant starch is fermented by microbiota in large intestine and relates to increased abundance of butyrate-producing bacteria (Bednar et al., 2001; Yang et al., 2017b). Moreover, resistant starch-rich diet was associated with increased circulating propionate concentration in mice (Bai et al., 2021). In the current study, higher abundance of propionic acid-producing bacteria *Propionibacteriaceae* was likely to relate to the higher content of resistant starch in corn.

In wheat-based diets fed broilers, the abundance of Proteobacteria was higher than those fed corn-based diets, which may relate to proteinaceous substrates since wheat-based diets contain about 1% more protein than corn-based diets in this study. Proteobacteria are mainly responsible for utilization of amino acids and possess extensive gene coverage of amino acid responses (Dai et al., 2015; Diether and Willing, 2019). Moreover, the reason that higher abundance of *Peptostreptococcaceae* in the broilers fed corn-based diets was likely due to the different amino acid compositions of corn protein from wheat protein. Zein as the major fraction of corn protein is rich in leucine

(Cowieson, 2005). *Peptostreptococcus* spp. was associated with leucine metabolism in hindgut (Ma and Ma, 2018).

CONCLUSION

Overall, AT-xynA supplementation to wheat- or corn-based diets modulated the relative abundance of specific bacteria without changing the overall microbial structure. We showed that bacterial community clustering was mainly due to cereal grain source rather than xylanase supplementation. The addition of xylanase to wheat-based diets increased the abundance of *Lactobacillus*, *Bifidobacterium*, and some butyrate-producing bacteria, the increase might be related to the release of XOS as a result of AX degradation in GIT. Moreover, the undigested polysaccharides flowing into the cecum resulted in higher abundance of (non-starch polysaccharides) NSP-degrading bacteria in broilers fed wheat-based diets without xylanase supplementation, such as *Ruminococcaceae* and Bacteroidetes. Xylanase supplementation to corn-based diets slightly affected the abundance of butyrate-producing bacteria and NSP-degrading bacterium, the difference might be related to AX concentration and composition in corn and wheat. However, xylanase still increased the abundance of beneficial bacteria and decreased the abundance of harmful bacteria in the cecum of broilers fed corn-based diets. Moreover, beneficial effects of xylanase on cecal microbiome improved growth performance.

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DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: <https://www.ncbi.nlm.nih.gov/>, PRJNA753623.

ETHICS STATEMENT

The animal study was reviewed and approved by the Laboratory Animal Welfare and Animal Experimental Ethical Inspection Committee of China Agricultural University.

AUTHOR CONTRIBUTIONS

YC and SL supervised and provided continuous guidance for the experiments. YC, BD, and CW conceived and designed the experiments. JW, HC, CB, and YL performed the animal experiments. JW, ZS, and BD analyzed the data and wrote the manuscript. All authors have discussed the results and reviewed the manuscript.

FUNDING

Our study was supported by the funds of National Natural Science Foundation of People's Republic of China (no. 31760673) and the Project of introducing talents to Tibet (RXYJ-2019-06).

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