Yeast culture *(Saccharomyces cerevisiae)* and its active metabolites affect the cecal microbiome of broilers

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Abstract

Yeast cultures (YCs) are defined as promising feed additives that maintain the health of birds and improve growth performance by modulating gut microbiota. YCs contain effective metabolites such as glycine, fructose, inositol, galactose, and sucrose. This study investigated the effects of YCs and their effective metabolites on carcass traits and cecal microflora in broilers. A total of 280 one-day-old mixed-sex Arbor Acres broilers were randomly allocated to seven groups. The basal diet (control DZ) was supplemented with various proportions of glycine, fructose, inositol, galactose, and sucrose (Groups A, B, and C), 24-hour grown *Saccharomyces cerevisiae* cultures (Group D) (YC), and a commercial yeast culture product (SZ) at concentrations of 0.1% and 1% (Groups E and F). Bodyweight of broilers was correlated positively with proportions of *Proteobacteria in* Group C and *Lactobacillus* and *Roseburia* in Group B (*P* <0.05). Broilers fed diets supplemented with YC or its active metabolites had the highest proportions of bacteria involved in nucleotide metabolism, and amino acid and carbohydrate metabolism. These results suggested that the dietary addition of YC could alter the proliferation of beneficial bacteria in broilers.

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Introduction

Numerous yeast and yeast products have been fed to livestock for more than a century (Stone, 2006; Saied *et al.*, 2011; Bilal *et al.*, 2020). Recently the addition of antibiotics to livestock diets to promote production efficiency has been restricted and has declined. Therefore, research aimed at developing alternatives has increased significantly, with particular emphasis on potential uses of natural feed additives (Chand *et al.*, 2019; Saeed *et al.*, 2019; Tufail *et al.*, 2019). A yeast culture is one such natural additive (Kondo *et al.*, 2014). Yeast culture (YC) is a natural yeast fermentation product, containing a small amount of yeast biomass and a large number of fermentation metabolites. The yeast cells are used as a tool to produce cellular metabolites during production of the YC (Poppy *et al.*, 1995). A number of studies have confirmed that YC can modulate the animal gut microbiota, promote a beneficial balance of the microbial population of the gastrointestinal tract, and inhibit the survival of pathogens, thereby enhancing the immune capacity of animals, and may ultimately play a role in maintaining animal health and improving performance. For instance, dietary YC not only modulated the gut immune response of nursery pigs (Shen *et al.*, 2009), but it also affected intestinal mucosal morphology and ileal villus development in broilers (Zhang *et al.*, 2005; Bai *et al.*, 2013). It has been reported that YC could improve bodyweight gain and feed conversion efficiency (Hooge *et al.*, 2004; Stanley *et al.*, 2004; Shurson *et al.*, 2018; Stanley *et al.*, 2016).

Nutritional and health effects of YC are achieved through the fermentation of extracellular metabolites that have high thermal stability (Shurson *et al.*, 2018; Liu *et al.*, 2018). However, there are many types of metabolites in YC and most have unknown effects (Shurson *et al.*, 2018; Kondo *et al.*, 2014), except for a few with known nutritional value, such as organic acids, oligosaccharides, and amino acids Thus Sun *et al.*

(2020) screened glycine, fructose, inositol, galactose, and sucrose as potential metabolites of YC with gas chromatography-mass spectrometry. Therefore, this study aimed to determine and compare the effects of YC and its active metabolite supplementation on carcass characteristics and the cecal microbiota of broilers to verify further whether dietary supplementation with the effective compounds of YC could alter intestinal health.

Materials and methods

The animal trial was approved by the Experimental Animal Committee of Jilin Agricultural University (JLAU-ACUC2017-007, Changchun, China). This study was conducted at a commercial broiler chicken farm (Changchun, China). A total of 280 one-day-old mixed-sex Arbor Acres broilers were collected and randomly assigned to seven groups, with five replicates for each group, and each replicate consisting of eight broilers (n = 40 broilers/group). The treatments consisted of control group (DZ), in which broilers were fed a basal diet. In Groups A, B, and C broilers were fed a basal diet supplemented with various proportions of glycine, fructose, inositol, galactose, and sucrose. In Group D (YC), broilers were fed a basal diet supplemented with 24-hour- grown *Saccharomyces cerevisiae* cultures at a concentration of 0.192%. Groups E and F were fed a basal diet supplemented with a commercial yeast culture product (SZ) at concentrations of 0.1% and 1% (Table 1). The components of the basal diet and the relative ratios of the five additives are given in Sun *et al.* (2020).

Table 1 Additions to basal diet to form diets that were fed to broilers

Treatment	Percentages of additives to yeast cultures fermented 24 hours					YC	SZ
Diet	Glycine	Fructose	Inositol	Galactose	Sucrose		
Control (DZ)	_	_	_	_	_	_	_
A	4.13	0.630	0.530	0.840	0.110	_	_
B	3.72	0.560	0.480	0.760	0.090	_	_
C	4.54	0.690	0.580	0.920	0.120	_	_
D	_	_	_	_	_	0.192%	_
E	_	_	_	_	_	_	0.1%
F	_	_	_	_	_	_	1.0%

YC: yeast culture; SZ: commercial yeast culture product

The *Saccharomyces cerevisiae* (JLAU-Borui No. 2012) was obtained from JLAU-Borui Dairy Science and Technology R&D Centre of Jilin Agricultural University (Changchun, China). Its active components (i.e., glycine, fructose, inositol, galactose, sucrose) in 24-hour-grown YC were screened through metabolomic and animal experiments (Sun *et al.*, 2020). The proportions of glycine, fructose, inositol, galactose, and sucrose in the feed were believed to be most beneficial. In Group A the proportions of these components were the same as in 24-hour-fermented YC.

The broilers were raised in wire-floored cages in an environmentally controlled room with continuous light. They had access to feed and water ad libitum. During the first seven days, the temperature was kept at 32 - 35 °C, and then gradually reduced to 22 °C. To evaluate the carcass characteristics, two broilers of similar bodyweight from each replicate were killed at 42 days by cervical dislocation. The weights of the breast, thigh, pancreas and abdominal fat were recorded, and the dressing percentage was calculated relative to the live bodyweight.

Cecal samples were collected from five broilers from each group at 42 days and stored at -80 °C. DNA was isolated from each 220 mg sample with the Fast DNA SPIN Extraction Kit (MP Biomedicals, Santa Ana, CA, USA) and stored at -20 °C. The quantity of the DNA was determined with a Nano Drop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and the quality was evaluated with agarose gel electrophoresis.

Polymerase chain reaction (PCR) amplification of V3–V4 regions of the bacterial 16S rRNA gene was performed with the forward primer 515F (5'–GTGCCAGCMGCCGCGGTAA–3') and the reverse primer 907R

(5'–CCGTCAATTCMTTTRAGTTT–3'). The PCR cycling conditions consisted of an initial denaturation at 98 °C for 2 min, followed by 25 cycles of denaturation at 98 °C for 15 seconds, annealing at 55 °C for 30 seconds, and extension at 72 °C for 30 seconds, with a final extension of 5 min at 72 °C. PCR amplicons were purified with Agencourt AMPure beads (Beckman Coulter, Indianapolis, IN, USA) and quantified with the PicoGreen dsDNA assay kit (Invitrogen, Carlsbad, CA, USA). Equal amounts of the amplicons were pooled, and pair-end 2 ×300 bp sequencing was performed with the Illumina MiSeq platform with the MiSeq reagent kit at Shanghai Personal Biotechnology Co., Ltd. (Shanghai, China). The broiler 16S rRNA gene sequence was deposited in the NCBI SRA database with accession number SRP238826.

Sequence reads were analysed with the quantitative insights into microbial ecology (QIIME, v1.8.0) tool. After chimera detection, the UCLUST algorithm was used to cluster the remaining high-quality sequences into operational taxonomic units (OTUs) at 97% sequence identity. The classification of OTUs was performed by BLAST searching the representative sequences against the Greengenes database (DeSantis *et al.*, 2006) using the best hit. Alpha diversity indices, including Chao1, Shannon, Simpson, and abundance-based coverage estimator (ACE), were used to calculate expected OTUs based on observed OTUs. Principal component analysis (PCA) was performed with genus-level compositional profiles. The taxonomic compositions and proportions were visualized with MEGAN and GraPhlAn software packages. The functions of microbes were assigned by the Kyoto encyclopaedia of genes and genomes (KEGG) tool and predicted by phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt) software (Langille *et al.*, 2013).

Data were analysed with SPSS 20.0 software (SPSS Inc., Chicago, Illinois, USA) for analysis of variance and results expressed as means ± SE. Groups were compared with Duncan's multiple comparison test, and significance was taken at $P < 0.05$. Linear regression was used to examine the association between the bodyweight and microbial taxa. An absolute value of the correlation coefficient closer to 1 indicated a stronger relationship.

Results and Discussion

No differences were detected between the groups and the control in the relative weights of the breast, thigh, pancreas, and abdominal fat, and dressing percentage at 42 days old. Relative weight of the spleen was affected significantly by the treatments, with A, B and C having heavier spleens than DZ ($P < 0.05$) (Table 2).

Table 2 Broiler carcass characteristics at 42 days old

Group	Dressing percentage	Relative weights, %							
		Eviscerated carcass	Breast	Thigh	Spleen	Bursa	Gizzard	Pancreas	Abdominal fat
DZ	93.52	70.20	76.14	6.92	0.15[a]	0.20	1.36	0.21	1.12
A	93.47	74.36	81.31	7.61	0.19[b]	0.21	1.45	0.22	1.14
B	94.35	70.70	83.61	6.81	0.19[b]	0.18	1.39	0.21	1.17
C	93.47	74.00	88.38	7.10	0.20[b]	0.20	1.41	0.20	1.02
D	93.51	75.86	88.08	7.35	0.18[ab]	0.19	1.40	0.20	0.93
E	92.53	74.60	87.84	7.02	0.17[ab]	0.21	1.39	0.19	0.85
F	93.77	75.41	86.79	7.44	0.19[ab]	0.17	1.43	0.22	0.97
SE	0.002	0.001	2.058	0.001	0.053	0.054	0.258	0.041	0.000
P-value	0.680	0.340	0.110	0.660	0.030	0.570	0.060	0.610	0.410

[a,b] Means in the same column with a common superscript were not different at probability $P = 0.05$
DZ: control group, A, B, and C: basal diet supplemented with various proportions of glycine, fructose, inositol, galactose, and sucrose; D: basal diet supplemented with 192% S. cerevisiae cultured for 24 hours; E and F: basal diet supplemented with a commercial yeast culture product at concentrations of 0.1% and 1%, respectively

Previous work had shown that improved growth performance was attributable to YC active metabolites, which affected the proportions of amino acids, sugar, and organic acids (Sun *et al.*, 2020). At cellular level, nutrition depends on the feed ingredients provided, the absorption rate of the nutrients, and the

digestion of nutritionally active substances that are released and dissolved, and which are absorbed by cells to affect gene expression and cell function. It was reported that carbohydrates and amino acids in feeds could regulate gene expression, thereby facilitating animal health and growth (Adams, 2006). Chickens require dietary sugars, such as sucrose, fructose, and galactose, which could replace corn as the primary energy source (Hussein *et al.*, 2018). Dietary sugars are highly metabolizable, thereby providing instant energy. They also have a pleasing aroma and make feed more palatable, which can increase feed intake in broilers. In animal systems, sucrose, and not starch, is considered the optimal energy source, and glucose can replace the fatty acids in the feed. Wang *et al.* (2014) reported that in ovo use of glucose and fructose may improve the immunity of chicks. On the other hand, as a nonessential amino acid in animals, glycine works with other amino acids in the synthesis of creatine, glutathione, bile acids, nucleic acids, and uric acids (Corzo *et al.*, 2004). Pirgozliev *et al.* (2017) also demonstrated that dietary myo-inositol, which is critical to cell survival, can improve the intestinal health and growth of broilers. However, in another study, inositol supplementation did not affect the mineral availability and growth of broiler chickens (Pirgozliev *et al.*, 2018). in addition, the spleen is an important immune organ in birds. Ahiwe *et al.* (2019) reported an increase in spleen weight after supplementation with yeast and its derivatives in broilers, consistent with the current results, which showed that supplementation with component C increased the spleen weight. These findings may have been caused by increased immune activity in chicken fed YC active metabolites.

A total of 1,599,212 high quality sequences were generated from V3–V4 regions of the 16S rRNA gene with an average of 45,691 reads per subject (range 27,853 - 157,129). The assembled sequences had an average length of 425 bp. The number of operational taxonomic units (OTUs) and the sample richness and diversity are shown in Table 3. Chao1 and ACE diversity indices were lower in A, C, D, and E than in the control or F (P <0.05). However, there were no significant differences in Shannon and Simpson indices among the groups (P >0.05). Figure 1 shows the relationships by PCA among microbial communities after various treatments. The contribution rates of principal component factor (PC) 1 and PC2 were 32.28% and 24.39%, respectively. The two principal components explained 56.7% of the composition of the microbiota at the genus level. After supplementation with YC or active metabolites, the overall composition of the cecal microbial environment was different from that of the control. However, there was no apparent separation in microbial genera among Groups A, B, C, and E, indicating that active metabolites induced changes that were similar to those induced by the YC product.

Table 3 Number of operational taxonomic unit and indexes of cecal microbial diversity based on the sampling of one bird from each of five replicates of each treatment group

Group	Operational taxonomic units	ACE	Chao1	Shannon	Simpson
DZ	290.4 [b]	2177 [c]	1964 [c]	7.48	0.954
A	225.4 [ab]	1521 [a]	1350 [a]	7.33	0.950
B	269.0 [ab]	1913 [bc]	1908 [bc]	7.79	0.974
C	241.0 [ab]	1300 [a]	1283 [a]	7.90	0.975
D	242.6 [ab]	1464 [a]	1494 [a]	7.06	0.931
E	220.8 [ab]	1398 [a]	1368 [a]	7.51	0.966
F	186.4 [a]	1653 [b]	1734 [b]	7.65	0.971
SE	11.1	67	53	0.11	0.005
P-value	0.03	<0.01	<0.01	0.56	0.42

[a,,b,c] Means in the same column with a common superscript were not different at probability P =0.05
DZ: control group, A, B, and C: basal diet supplemented with different proportions of glycine, fructose, inositol, galactose, and sucrose; D: basal diet supplemented with 192% S. cerevisiae cultured for 24 hours; E and F: basal diet supplemented with a commercial yeast culture product at concentrations of 0.1% and 1%, respectively
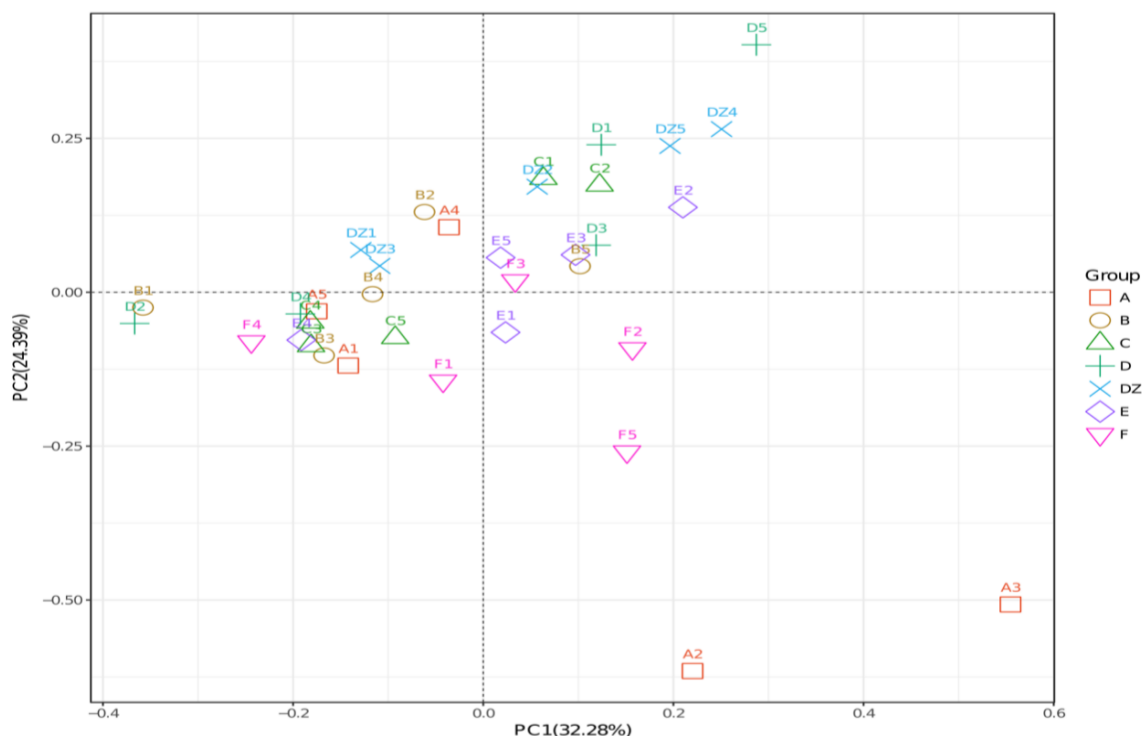
Figure 1 Principal component analysis of the operational taxonomic units of microbiota found in cecal samples from broilers

PC1: first principal coordinate, PC2: second principal coordinate, DZ: control group, A, B, and C: basal diet supplemented with different proportions of glycine, fructose, inositol, galactose, and sucrose; D: basal diet supplemented with 192% S. cerevisiae cultured for 24 hours; E and F: basal diet supplemented with a commercial yeast culture product at concentrations of 0.1% and 1%, respectively

Microbial diversity in the gut is important for digestion and nutrient uptake (Xu *et al.*, 2016). The results indicated that active metabolites reduced the complexity of the cecal microbial environment, as indicated by decreased Chao1 and ACE indices. A previous study revealed a negative correlation between bodyweight gain and intestinal microbial diversity (Han, 2018). The results of PCA indicated a separate microbial cluster between metabolite-supplemented and unsupplemented diets of broilers. This indicated that the microbial environment was affected by the metabolites in the YC. In contrast, Ran *et al.* (2015) illustrated that YC supplementation of the diets of Nile tilapia affected the alpha-diversity of microbiota, but PCA revealed no significant difference.

The dominant phyla across all groups were *Firmicutes, Bacteroidetes*, and *Proteobacteria,* which accounted for more than 90% of the total phyla in the cecum (Figure 2). The proportions of *Cyanobacteria* and *Proteobacteria* in Group F and *Actinobacteria* in Group A were higher than those in the other groups. The most abundant genera were *Alistipes, Faecalibacterium, Rikenella, Lactobacillus, Bacteroides, Oscillospira, Ruminococcus*, and unclassified *Ruminococcus* (Figure 3). The proportions of *Bacteroides* in Group A and *Megamonas* and *Coprobacillus* in Group F were significantly higher than in the other groups. In the present study, the most predominant phyla in the cecum of broilers were *Bacteroidetes, Firmicutes, Actinobacteria,* and *Proteobacteria,* which was consistent with (Oakley *et al.*, 2014; Wei *et al.*, 2013) and inconsistent with previous studies (Liu *et al.*, 2018). It was found that the proportions of *Actinobacteria* and *Bacteroides* increased significantly after supplementation with component A. *Actinobacteria* includes probiotic strains *Collinsella* and *Bifidobacterium* (Tremaroli *et al.*, 2012; Liu *et al.*, 2018), whereas *Bacteroidetes* breaks down polysaccharides to improve nutrient utilization, promote immunity, and maintain the microecological balance in the intestine (Bäckhed *et al.*, 2004).
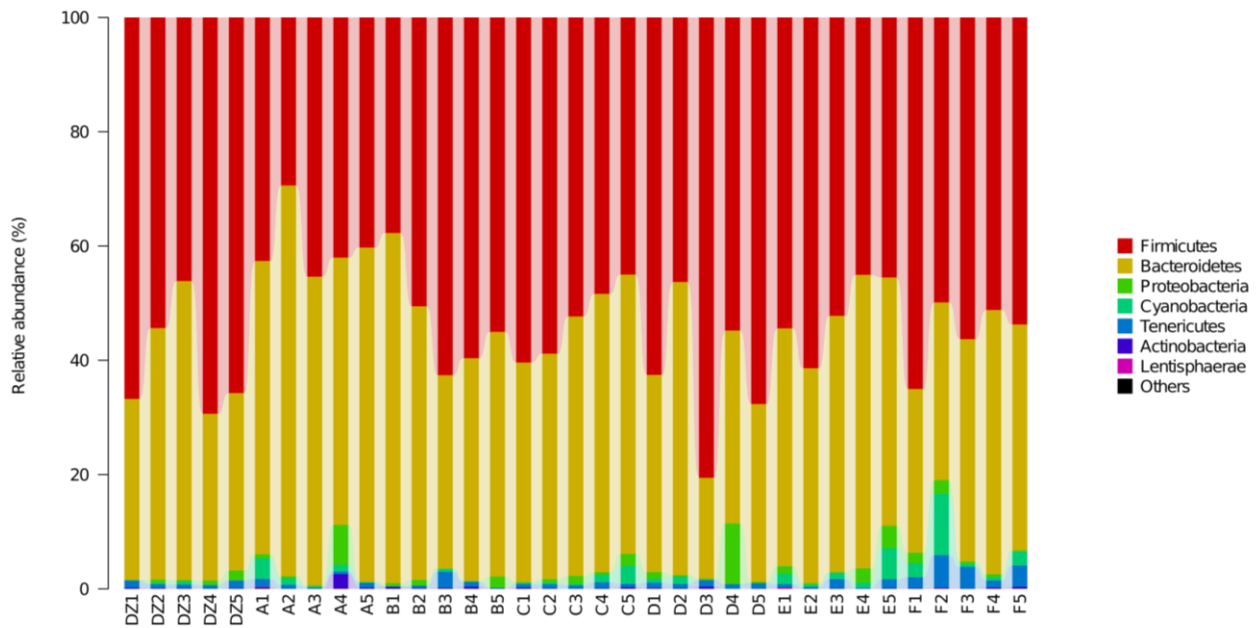
Figure 2 Compositional changes in the cecal microbial environment at the phylum level in 42-day old broilers
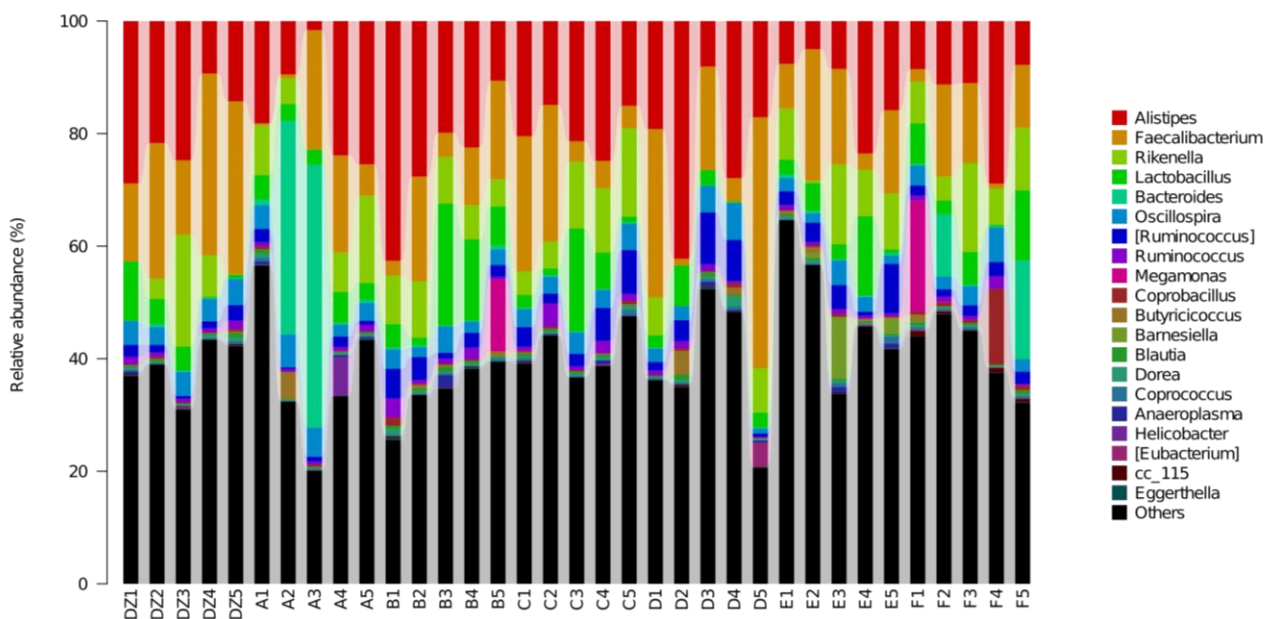

Figure 3 Compositional changes in the cecal microbial environment genus level in 42-day old broilers

In this experiment, birds in Groups B, C and D were heavier at 42 days than those in DZ (Sun *et al.*, 2020). The relationships of microbial abundance with bodyweight (r and *P*-values) are shown in Table 4. In Group B, *Tenericutes* was correlated positively with bodyweight. In Groups C and E, *Proteobacteria* was correlated positively with bodyweight. In B Group, *Lactobacillus* and *Roseburia* were correlated positively with bodyweight. In Group A, *Coprobacillus*, *Blautia* and *Coprococcus* were correlated negatively with bodyweight. In Group C, *Oscillospira* and in Group E, *Megamonas* were correlated positively with bodyweight. In the control group, *Holdemania* and *Anaerofustis* were correlated negatively with bodyweight.

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Table 4 Estimates of the correlation of microbial abundance with bodyweight of broilers fed various diets

	DZ			A			B			C			D			E			F		
Phylum	%	r	*P*	%	r	*P*	%	r	*P*	%	r	*P*	%	r	*P*	%	r	*P*	%	r	*P*
Firmicutes	56.9	0.29	0.64	57.0	0.38	0.53	59.1	0.24	0.69	59.3	0.18	0.77	58.4	0.29	0.64	57.4	-0.55	0.34	54.6	-0.21	0.73
Bacteroidetes	41.0	0.07	0.91	38.7	-0.35	0.56	38.9	-0.82	0.09	37.3	0.15	0.81	37.8	-0.19	0.76	38.0	0.25	0.68	37.5	-0.19	0.77
Proteobacteria	0.67	0.22	0.72	1.65	0.28	0.64	0.72	-0.28	0.65	0.94	0.99	0	2.10	-0.17	0.79	1.61	0.95	0.02	1.06	0.05	0.94
Tenericutes	0.91	0.27	0.66	0.71	-0.69	0.2	0.85	0.88	0.05	0.72	-0.6	0.28	0.92	0.44	0.46	0.84	0.24	0.7	3.28	0.35	0.56
Genus																					
Lactobacillus	3.98	0.07	0.92	3.65	-0.14	0.83	9.70	0.92	0.03	5.83	0.24	0.7	2.99	0.33	0.59	4.96	0.10	0.88	5.60	0.1	0.87
Oscillospira	4.06	0.12	0.85	4.12	-0.19	0.76	2.90	0.55	0.34	3.59	0.93	0.02	3.41	0.46	0.44	2.49	-0.47	0.42	3.51	-0.33	0.58
Megamonas	0	0	0	0.04	0.35	0.56	2.50	0.06	0.93	0.02	-0.5	0.39	0	0	0	0.03	0.89	0.04	3.98	0.06	0.93
Coprbacillus	0.27	0.74	0.16	0.21	-0.92	0.03	0.40	-0.58	0.3	0.27	0.76	0.14	0.32	0.15	0.81	0.22	-0.56	0.32	3.20	-0.6	0.29
Blautia	0.48	0.24	0.70	0.34	-0.93	0.03	0.50	0.45	0.45	0.41	-0.79	0.11	0.46	0.86	0.06	0.29	-0.44	0.45	0.39	0.03	0.96
Coprococcus	0.29	-0.15	0.81	0.28	-0.93	0.02	0.30	0.27	0.66	0.37	0.72	0.17	0.35	0.62	0.26	0.52	0.64	0.25	0.31	0.72	0.17
Roseburia	0.01	0.28	0.65	0.01	0.35	0.57	0.10	0.91	0.03	0.02	-0.5	0.39	0.02	0.22	0.72	0.19	-0.4	0.5	0.17	0.12	0.85
Holdemania	0.01	-0.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Anaerofustis	0.01	-0.99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.01	-0.43	0.47

r: Pearson's correlation coefficient. *P*: probability r ≠ 0, %: microbial abundance

DZ: control group, A, B, and C: basal diet supplemented with various proportions of glycine, fructose, inositol, galactose, and sucrose; D: basal diet supplemented with 192% S. cerevisiae cultured for 24 hours; E and F: basal diet supplemented with a commercial yeast culture product at concentrations of 0.1% and 1%, respectively

Kyoto encyclopaedia of genes and genomes (KEGG) analysis revealed that microbes in Group D were involved mainly in xenobiotic biodegradation, metabolism, and nucleotide metabolism (Figure 3). Microbes in Group B were involved mainly in nucleotide metabolism, cofactor and vitamin metabolism, lipid metabolism, and amino acid metabolism. Microbes in Groups A and C were involved mainly in energy metabolism and carbohydrate metabolism. In addition, the fermented YC with many active metabolites can affect the metabolic processes of intestinal microbes. Compared with the control, most metabolic processes, such as xenobiotic degradation, were higher in YC and active metabolite supplementation groups, consistent with the results of a previous study (Stanley *et al.*, 2016). The authors observed differences in amino sugar and nucleotide sugar metabolism pathways in Groups A and C. Amino sugars break down proteins in feed into amino acids for energy (Miska *et al.*, 2014), whereas nucleotide sugars are important for the synthesis of purines and pyrimidines for nucleotides (Rengaraj *et al.*, 2013). As reported, the cecal microbial environment of birds with improved feed efficiency was enriched with microbes functioning in amino acid metabolism (Yan *et al.*, 2017). Therefore, as active ingredients, amino acids and carbohydrates play important roles in the metabolism of nutrients in broilers.
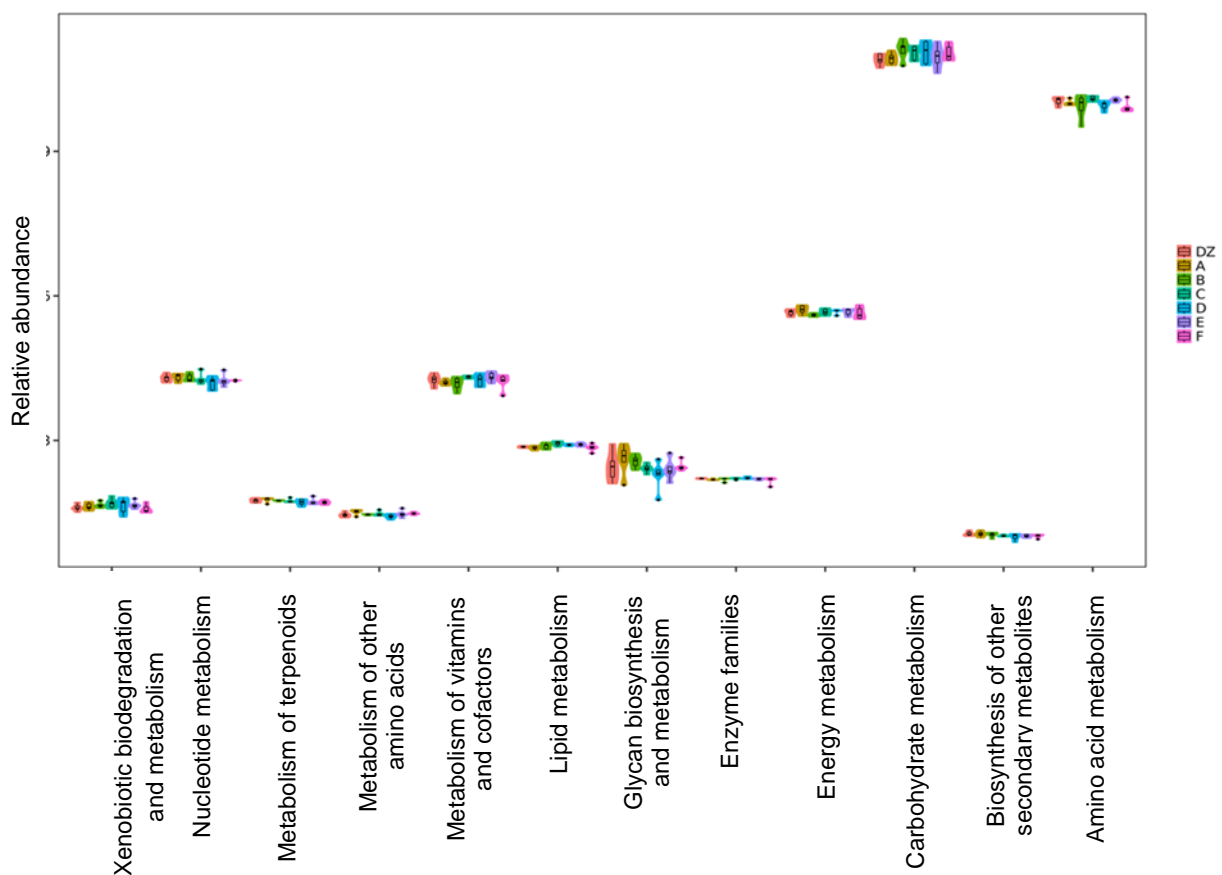

Figure 3 Treatment effects on distribution of pathways identified with the *Kyoto encyclopaedia of genes and genomes*

Megamonas is a commensal organism present in the intestinal tract of mammals and birds (Hooper, 2004). A long-term study reported that the proportion of *Lactobacillus* was higher in healthy individuals than in unhealthy individuals (Isolauri *et al.*, 1991). The proportion of *Lactobacillus* increased after YC supplementation, suggesting that it might promote overall health, which may have been because several *Lactobacillus* species can induce butyrate production. *Roseburia* and *Oscillospira* are also butyrate-producing bacteria, with *Roseburia* fermenting an array of carbohydrates. The proportions of both bacteria were shown to correlate positively with antioxidant activity and to correlate negatively with inflammation (Gophna *et al.*, 2017; Wang *et al.*, 2018). In this study, the proportions of *Roseburia* in Group B and *Oscillospira* in Group C were correlated positively with bodyweight. However, bacteria in the gut can produce

harmful substances that affect nutrient absorption. For example, *Holdemania* can break down intestinal mucus and induce intestinal pain (Willems *et al.*, 2015), whereas *Anaerotruncus*, an anaerobic bacterium, lacks metabolic enzymes (Lawson *et al.*, 2015). Both bacteria were found to affect the production performance of broilers in the control group, but not in the YC group, indicating that YC can reduce the production of harmful bacteria and improve the overall health of the intestinal environment. Furthermore, *Coprobacillus,* which is widely distributed in mammalian intestines, was correlated negatively with bodyweight after supplementation with component A. Most *Coprobacillus* strains are non-pathogenic (Takayuki, 2015).

Conclusions

Dietary supplementation of YC could alter the proliferation of beneficial bacteria of broilers through its content of active metabolites. Further studies are needed to understand how these metabolites promote the growth performance of broilers.

Authors' Contributions

ZS, XF and GX were in charge of designing and conducting the experiment and analysing the data. All other authors participated in interpreting the results, and writing and editing the manuscript.

Conflict of Interest Declaration

The authors declare that there is no conflict of interest.

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