Dietary *Saccharomyces cerevisiae* improves intestinal flora structure and barrier function of Pekin ducks

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ABSTRACT This study aimed at investigating the effects of dietary *Saccharomyces cerevisiae* (**SC**) on the intestinal flora structure and barrier function of Pekin duck. A total of 180 1-day-old Pekin ducks were randomly divided into 3 groups with 6 replicates in each group and 10 birds per replicate. The birds in the control group (**CON**) were fed the basal diet, and those in the experimental group were fed the basal diets supplemented with 600 mg/kg SC (**LSC**) and 1,200 mg/kg (**HSC**), respectively. The trial lasted for 42 d. Results showed that LSC and HSC treatments tended to improve the feed conversion efficiency during the trial. The ileum length of birds in the LSC and HSC groups was elevated. Additionally, with 600mg/kg SC supplemented, the mRNA levels of villin, claudin3, and *MUC 2* in d21 were up-regulated, as well as the mRNA levels of villin, claudin3, occludin, *i-FABP, ZO-1*, and *MUC 2* in d42. In addition, dietary SC supplementation improved the α-diversity of the bacteria in cecal chyme and tended to increase the abundance (**RA**) of *Bacteroidetes* ($P = 0.071$). Besides, the RA of *Ruminococcaceae_UCG-014* was raised in the LSC group. Beyond that, the RA of *Proteobacteria* was descended with two levels of SC added. In conclusion, dietary *Saccharomyces cerevisiae*, particularly at 600 mg/kg level, improved the intestinal flora structure and barrier function of Pekin duck.

Key words: *Saccharomyces cerevisiae*, intestinal flora, intestinal barrier, Pekin duck

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INTRODUCTION

The intestine not only is regarded as the largest immune organ, but also serves as a selective barrier to maintain homeostasis of the body (Burrello et al., 2018; Di Tommaso et al., 2021). In the process of poultry growth, various stresses can easily impair the intestinal barrier leading to enterogenic infection, and even systemic inflammatory response to inhibit growth performance. Therefore, the growth potential of livestock and poultry depends largely on a healthy gastrointestinal tract, and the intestinal physical barrier is fundamental to it.

Yeast has long been used as direct-fed microbial in animal diets to improve growth performance, and *Saccharomyces cerevisiae* (**SC**) is the most commonly used one. Previous studies have shown that SC was able to target colonization in the intestine, and its metabolites such as functional amino acids and B vitamins contributed to the development of the intestine (Elghandour et al., 2020; Gil-Rodríguez and Garcia-Gutierrez, 2021). At present, SC has been widely used in animal production. A recent finding described that the fermentate of SC could raise the expression of tight junction proteins (**TJP**) (claudin, occludin, zona occludens-1 (**ZO-1**), and junctional adhesion molecule A (**JAM-A**) of rats intestine (Ducray et al., 2019). Another study in broilers also has shown that oral administration of *Saccharomyces boulardii* elevated the mRNA levels of claudin2, claudin3, and occludin, and this result was confirmed in intestinal ultrastructure under Transmission Electron Microscope (Rajput et al., 2013). Several disease models were also used to demonstrate the beneficial effect of SC on gut barrier function. SC was reported to be able to improve barrier function by preventing the LF82-induced expression of pore-forming claudin2 (Sivignon et al., 2015), and it could relieve the decreased protein expression of ileum claudin induced by *Escherichia coli* in piglets (Che et al., 2017). Besides, some findings also suggested that SC helped to improve the growth performance (Bidura et al., 2019), immunity function antioxidant properties (Nelson et al., 2020), and gut microbial community structure (Wang et al., 2016) of broilers.

Although numerous studies have demonstrated that SC was helpful to the host intestine, this has not been

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confirmed in Pekin ducks. We hold it would be interesting to study the effect of dietary SC on the intestinal flora and barrier function of Pekin ducks.

MATERIALS AND METHODS

The present study was approved by the ethics committee and conducted according to the Guidelines for Experimental Animals of China Agricultural University (Beijing, China). The animal welfare number was AW10211202-2-2.

Experiment Design and Birds Management

A total of 180 1-day-old male (**d1**) Pekin ducks (mix) with similar body weights (59.59 ± 0.55 g) were reared on a plastic net at the Poultry Experiment Base of China Agricultural University (Zhuozhou, Hebei). Ducklings were randomly reassigned to 3 groups. Each group had 6 replicates with 10 ducks per replicate. The basal diet met the nutrient standards of ducks recommended by the National Research Council (NRC, 1994) (Table 1). Owing to the previous study on chicken (added into the basal diet at 10^{10} CFU/kg) (Tengfei et al., 2021) and the recommended dose by the manufacturer (600 mg/kg, basic diet added with 1.2×10^{10} CFU/kg SC), the dose treatment (basic diet added with 600 mg/kg SC) and the double-dose treatment (basic diet added with 1,200 mg/kg SC) were set up to test whether it is able to exert beneficial effects equally. Therefore, there were 3 dietary treatments in the experiment as follows: **CON** (the corn-soybean basic diet), **LSC** (basic diet added with 600 mg/kg SC), **HSC** (basic diet added with 1,200 mg/kg SC). The bacterial strain SC (2.0×10^{10} CFU/g, batch No. WYYLRSJ100) was purchased from Guangzhou Weiyuan Biotechnology Co., Ltd. The initial temperature was maintained at 35°C for the first week, and then gradually reduced to 22°C. Ducks had free access to diet and water. Continuous light was provided throughout the whole trial.

At the end of day 21 and 42, body weight (**BW**) and feed intake (**FI**) were recorded as an average per pen. Average body weight (**ABW**), average daily gain (**ADG**), average daily feed intake (**ADFI),** and the feed conversion ratio (**FCR**) were calculated for the periods of d1-d21, and d1-d42. After that, 12 ducks (2 birds per pen) with a BW close to the ABW of each treatment were selected for blood sample collection and then slaughtered after anesthesia to obtain duodenum, jejunum, ileum, and the chyme of cecum. A ruler with an accuracy of 0.01 cm was used to measure the length of the duodenum, jejunum, and ileum.

Serum Diamine Oxidase and D-lactic acid Analysis

After blood was obtained from the jugular veins, the serum was harvested by centrifugation at 3000 × g and 4°C for 15 min. The kits from Nanjing Jian cheng Bioengineering Institute Co., Ltd. were used to measure the levels of serum diamine oxidase (**DAO**) and D-lactic acid (**D-LA**).

Gene Expression Analysis

Referring to the method described by Li et al. (2022). Ilea were placed into plastic collection tubes and stored at −80°C. Total RNA of ileum tissue was extracted using Trizol (Takara Biotechnology, Dalian, China). The purity and concentration of total RNA were measured by NanoDrop One (Thermo Fisher Scientific, Massachusetts, USA). Subsequently, RNA was reverse-transcribed to complementary DNA using PrimeScript RT reagent Kit (Takara Biotechnology, Dalian, China).

Table 1. Composition and nutrient levels of the basal diets (air-dry basis).

	contents (%)			Levels	
Items	1−21d	22−42d	Nutritional parameters	1−21d	22−42d
Corn	56.00	60.24	ME MJ/kg	12.31	12.53
Soybean meal	32.69	24.67	Crude protein %	19.52	16.83
Wheat middling	5.00	9.00	Lysine%	1.12	0.87
Soybean oil	2.10	1.80	Methionine%	0.46	0.39
Phytases	0.02	0.02	Calcium %	0.88	0.89
Dicalcium phosphate	1.00	1.60	Available phosphorus %	0.29	0.39
Limestone powder	1.50	1.20	Total phosphorus %	0.54	0.62
DL-Methionine	0.15	0.12	Methonine+Cysteine	0.79	0.69
L-Lysine	0.20	0.10			
Vitamin premix[1]	0.02	0.02			
Trace element premix[2]	0.20	0.20			
NaCl	0.35	0.30			
Choline chloride (50%)	0.24	0.20			
Ethoxyquin (33%)	0.03	0.03			
Maifanite	0.50	0.50			
Total	100	100			

[1]Vitamin premix (provided per kilogram of feed) the following substances: vitamin A, 12,500 IU; vitamin D_3, 3,500 IU; vitamin E, 20 IU; vitamin K_3, 2.65 mg; vitamin B_1, 2 mg; vitamin B_2, 6 mg; vitamin B_{12}, 0.025 mg; vitamin E, 30 IU; biotin, 0.0325 mg; folic acid, 12 mg; pantothenic acid, 50 mg; niacin, 50 mg.

[2]Trace element premix (provided per kilogram of feed) the following substances: copper, 6 mg; zinc, 40 mg; iron, 80 mg; manganese, 100 mg; selenium, 0.15 mg; iodine, 0.35 mg.

Table 2. Primer sequences used for gene expression analysis.

Gene	Prime sequences (5' - 3')	Product size, bp
Occludin	F GCAGGATGTGGCAG AGGAATA R CTTGTCGTAGTCGC TCACCAT	136
Claudin3	F GGCGTCATCTTCCTGCTCTC R GCTCCCTCTTCTGCGATTCAA	115
ZO-1	F ACCACCACCTCTTCACA ACTAC R ACCATCTGCCTTGCCTTCTG	128
i-FABP	F AAGAATCAAGCAACTTCCGTA R ATACACGTAGGTCTGAACGA	209
Villin	F CCCCTGACTCAAGACATGC TCCA R AGTTCTTGCCCTTCCACACGA	88
MUC 2	F AGTTCTTGCCTAATTCCTCAG TCT R TTGCCGTTCATATCCAGGT TCA	146
GAPDH	F GTAGTGAAGGCTGCTGCTG AT R AGGTGGAGGAATGGCTGTCA	103

ZO-1: zona occludens protein-1; i-FABP: intestinal fatty acid-binding protein; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

The mRNA expression levels of occludin, claudin, *ZO-1, MUC 2*, intestinal fatty acid-binding protein (***i-FABP***), and villin were determined using Applied Biosystems StepOnePlus Real-Time PCR System (Thermo Fisher Scientific, Massachusetts, USA) with TB Green Premix Ex Taq (Takara Biotechnology, Dalian, China). The PCR amplifications were carried out using the following conditions: 2 min at 50°C, 95 °C for 10 min for DNA denaturation, followed by 40 cycles of denaturation at 95°C for 15 s, 60°C for 1 min, and a final extension at 72°C for 10 min. The glyceraldehyde-3-phosphate dehydrogenase (***GAPDH***) genes were used as endogenous controls for normalization of gene expression using the method described by Fu (Fu et al., 2010). The primer sequences were shown in Table 2.

Cecal Microbiota Analysis

Total DNA of cecal chyme was extracted using QIAamp DNA Stool Mini Kit (Qiagen Company, Germany). The concentration of the extracted DNA was assessed using NanoDrop ND-2000C (Thermo Fisher Scientific, Massachusetts), and purity was verified by electrophoresis on the agarose gel. The V3-V4 hypervariable regions of the 16S rRNA gene were amplified with bar-coded primers 338 F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806 R (5′-GGACTACHVGGGTWTCTAAT-3′). High-throughput sequencing analysis of the purified PCR products was then performed by using Illumina Hiseq 2500 platform (Baimaike Company, Beijing, China). Alpha diversity analysis was assessed by Qiime software (Qiime2-2019.7, Nature Biotechnology). The relative abundance (**RA**) of gut bacteria at the phylum and genus level was clarified. R software (Version 2.15.3) was used to draw Venn and PCoA diagrams.

Western-Blot Analysis

The ileum issue was added to the RIPA lysis buffer containing PMSF inhibitors (Beyotime, Shanghai, China) for protein extraction. The protein concentration was determined using a BCA assay (Beyotime, Shanghai, China). Proteins (20 μg per lane) were separated by 10% SDS-PAGE and then transferred onto PVDF membranes (0.22 μm, Millipore Corp, Billerica, MA). The membranes were blocked in 5% milk for 2 h and incubated with the primary antibody (claudin1 and Glyceraldehyde-3-phosphate dehydrogenase (**GAPDH**)) at 4° C overnight. After being washed with TBST, the membranes were incubated with secondary antibody for 1 h and then visualized using ECL substrate. The western blot protein bands were assessed using ImageJ. GAPDH was used to normalize the expression of claudin1.

Statistical Analysis

The data were subjected to one-way analysis of variance (**ANOVA**) using the SPSS 26.0 software (SPSS Inc., Chicago, IL, USA). The mean differences were compared using Duncan's multiple range tests. The correlation between gut bacteria and the gene expression level was determined by Pearson correlation analysis. Differences among groups were considered significant when a value of $P < 0.05$. Results were presented as means with their SEM. Graphpad prism 8.0 software (GraphPad Software Inc., San Diego, CA, USA) was used to graph the data.

RESULTS

Growth Performance and Small Intestine Length

Dietary two doses of SC tended to improve the FCR during the whole trial ($P = 0.091$) (Table 3), and the FCR in the HSC group was significantly reduced during the d1 to d21 ($P < 0.05$). We also found the ileum length of ducks in the LSC and HSC group was raised ($P < 0.05$) (Table 4). These results illuminated to us that SC might be helpful to the intestine to improve the production performance of Pekin ducks. To gain more insight, the next studies were carried out.

Serum D-LA and DAO Level

The levels of DAO and D-LA in serum were not affected by the supplementation of SC (Table 5).

Ileal Physical Barrier Function

With 600 mg/kg SC supplemented, the mRNA levels of villin, claudin3, and *MUC 2* in d21 were up-regulated, as well as the mRNA levels of villin, claudin3, occludin, *i-FABP, ZO-1*, and *MUC 2* in d42. Besides, dietary 1 200 mg/kg SC increased the mRNA levels of claudin3 in d21 and occludin, ZO-1, and MUC 2 in d42 ($P < 0.05$) (Figure 1A, B). Additionally, the protein level of claudin1 was increased by the addition of two levels of SC

Table 3. The results of production performance (n = 6).

Items	CON	LSC	HSC	*P*-value
d0 BW, g	59.75 ± 0.82	59.48 ± 0.34	59.53 ± 0.49	0.711
d21 BW, g	947.86 ± 49.82	981.21 ± 73.68	991.64 ± 96.37	0.590
d0-d21 ADG, g	42.29 ± 2.37	43.89 ± 3.50	45.47 ± 3.73	0.269
d0-d21 ADFI, g	84.07 ± 4.55	85.72 ± 6.01	78.45 ± 6.39	0.101
d0-d21 FCR	1.99 ± 0.16[a]	1.96 ± 0.08[a]	1.73 ± 0.13[b]	0.006
d42 BW, g	2898.29 ± 115.69	2935.80 ± 64.28	2912.57 ± 107.52	0.804
d0-d42 ADG, g	67.58 ± 2.75	68.48 ± 1.53	67.93 ± 2.55	0.800
d0-d42 ADFI, g	185.89 ± 14.41	179.17 ± 7.22	177.00 ± 9.56	0.358
d0-d42 FCR	2.75 ± 0.15	2.62 ± 0.10	2.61 ± 0.09	0.091

Abbreviations: BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio. CON (the corn-soybean basic diet), LSC (basic diet added with 600 mg/kg SC), HSC (basic diet added with 1200 mg/kg SC).

The different superscript small letters were judged as a significant difference($P < 0.05$), same letters indicated no difference ($P > 0.05$).

Table 4. The results about length of different intestinal segments (n = 12).

Items, cm		CON	LSC	HSC	*P*-value
d21	duodenum	14.03 ± 0.93	15.25 ± 1.39	14.75 ± 1.39	0.175
	jejunum	73.46 ± 3.42	77.64 ± 3.85	78.05 ± 7.93	0.202
	ileum	72.41 ± 2.92[b]	78.50 ± 4.28[a]	79.95 ± 4.02[a]	0.002
d42	duodenum	35.45 ± 1.73	36.09 ± 1.42	37.18 ± 2.01	0.158
	jejunum	84.56 ± 3.95	88.86 ± 5.51	88.63 ± 3.53	0.115
	ileum	86.45 ± 4.10[b]	91.46 ± 4.21[a]	91.08 ± 3.63[a]	0.036

Abbreviations: CON (the corn-soybean basic diet), LSC (basic diet added with 600 mg/kg SC), HSC (basic diet added with 1200 mg/kg SC).

The different superscript small letters were judged as a significant difference($P < 0.05$), same letters indicated no difference ($P > 0.05$).

(Figure 1C, D). We hold it might be related to the improvement of dietary SC on the intestinal flora.

Cecal Microbiota

Just as expected, we found that the α-diversity of the cecal chyme microbiota was improved with two doses of SC treated (Figure 2A). In addition, the structures of cecal flora in the two SC supplemented groups were significantly different from those in the control group (Figure 2B, C). Specifically, dietary SC supplementation tended to increase the RA of *Bacteroidetes* ($P = 0.071$). Beyond that, the RA of *Proteobacteria* and *Cyanobacteria* were down-regulated with two levels of SC added. Besides, the RA of *Ruminococcaceae_ UCG-014* were raised in the LSC group, as well as the RA of *Clostridiales_ vadinBB60* and *Alistipes* in the HSC group ($P < 0.05$) (Figure 2F). Based on the Pearson correlation analysis results, we also found that the RA of *Proteobacteria* was significantly negatively correlated with the mRNA levels of claudin *3*, occludin, and *i-FABP*, the RA of *Cyanobacteria* was also negatively correlated with the mRNA levels of *MUC 2*, while the RA of *Bacteroidetes* and *Ruminococcaceae_ UCG-014* were positively correlated with the protein expression of claudin1 ($P < 0.05$) (Figure 3).

DISCUSSION

SC is rich in protein and functional amino acids, which provide animals with nutrients needed for growth. The study suggested that dietary SC improved feed intake, daily gain, and FCR in broilers, and mitigated the negative effects of aflatoxin on broiler growth performance (Afsharmanesh et al., 2010). Some scholars have also confirmed that SC could help improve production performance in meat duck study (Bidura et al., 2019). In the present study, SC contributed to the FCR of Pekin ducks, which was consistent with previous studies on broilers (Afsharmanesh et al., 2010; Pizzolitto et al., 2013). We also found SC helped to raise the length of the ileum in this study. Previous study on pigs indicated that an increase in small intestine lengths within a certain range was significantly positively associated with an improvement in growth performance. It is possible that the longer small intestine could offer animal a better nutrient digestion and absorption capacity (Farre et al., 2020; Wang et al. 2020). We hold SC might improve the feed conversion efficiency via promoting intestinal development.

Table 5. The results of serum diamine oxidase and D-lactate (n = 8).

Items		CON	LSC	HSC	*P*-value
DAO, U/mL	d21	5.65 ± 0.21	5.46 ± 0.24	5.69 ± 0.40	0.318
	d42	5.87 ± 0.52	5.66 ± 0.17	6.07 ± 0.07	0.075
D-LA, nmol/L	d21	20.41 ± 1.39	20.31 ± 1.44	20.78 ± 1.44	0.794
	d42	20.45 ± 1.38	20.22 ± 0.97	20.75 ± 0.45	0.623

Abbreviations: DAO: diamine oxidase; D-LA: D-lactate. CON (the corn-soybean basic diet), LSC (basic diet added with 600 mg/kg SC), HSC (basic diet added with 1200 mg/kg SC).

The different superscript small letters were judged as a significant difference ($P < 0.05$), same letters indicated no difference ($P > 0.05$).

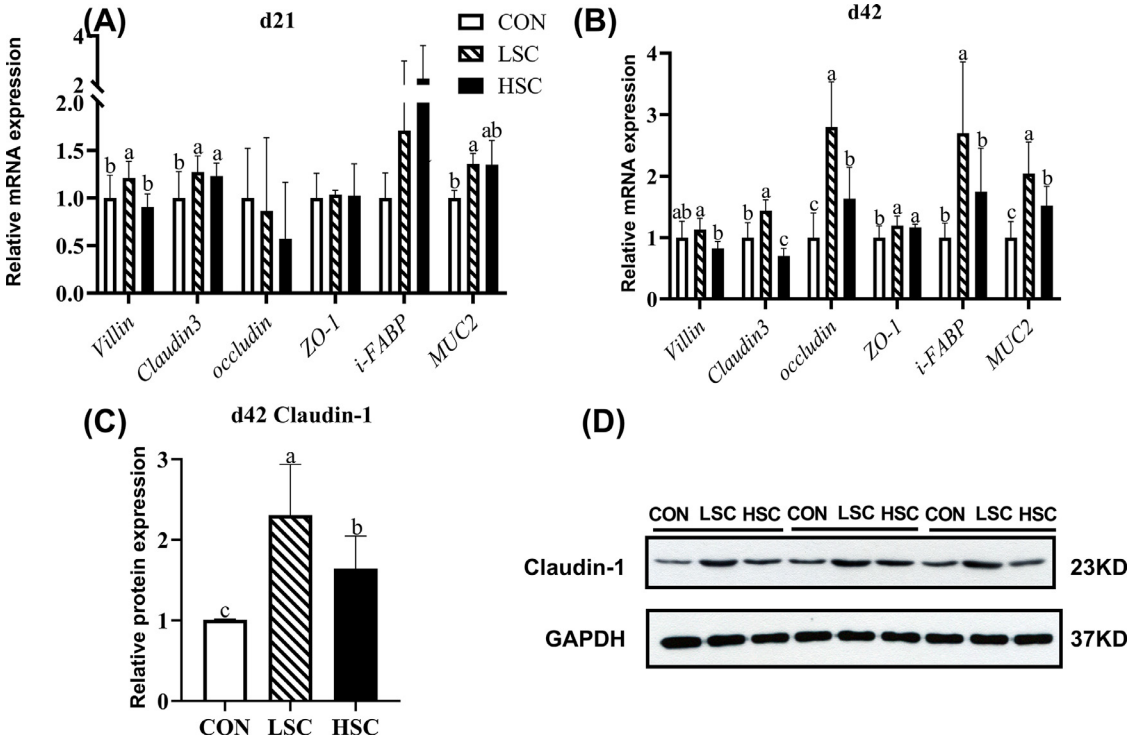

Figure 1. The effect of SC on the mRNA expression of TJP in ileum of Pekin ducks. A and B showed the TJP mRNA expression at the end of d21 and d42, respectively (n = 8). C and D represented the western blots and band density analysis of claudin1in the ileum of Pekin ducks at d42, respectively. The different superscript small letters were judged as a significant difference ($P < 0.05$), the same letters indicated no difference ($P > 0.05$).

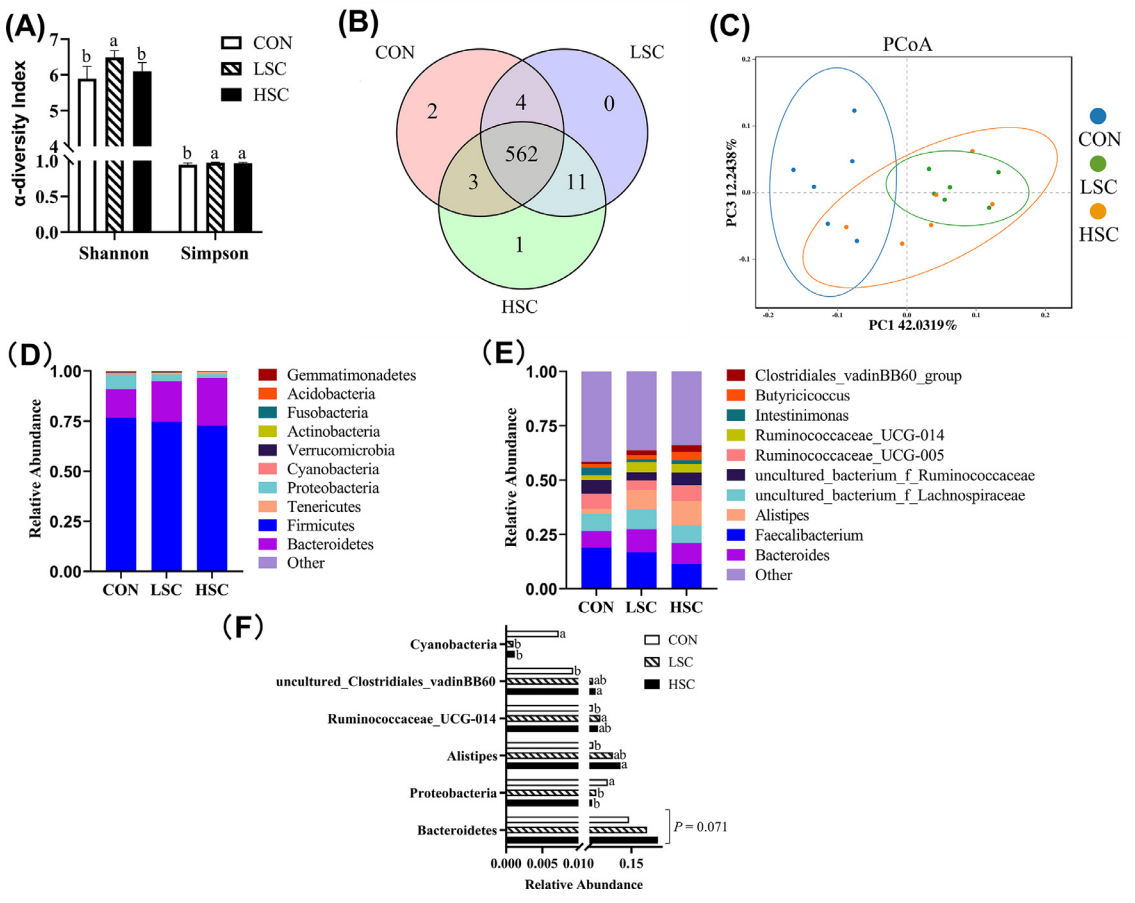

Figure 2. The effect of SC on the microbial structure of the Pekin ducks in the ileum at the end of d42. A, B, C, D, and E respectively showed the α-diversity of the microbiota, the Ven-n diagram, the β-diversity (PCoA), and the relative abundance of bacteria at the phylum and genus levels. There was a significant difference in the relative abundance of bacteria shown in FigF.The different superscript small letters were judged as a significant difference ($P < 0.05$), same letters indicated no difference ($P > 0.05$).

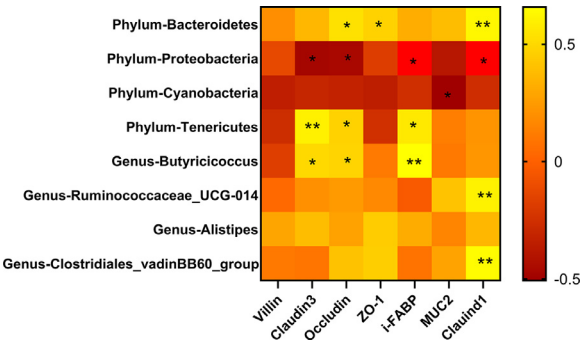

Figure 3. Pearson correlation analysis between the RA of the d42 ducks' cecal flora with TJP mRNA expression and claudin1 protein expression. The number of "0.5", "0", and "-0.5" mean the Pearson correlation coefficient, and positive number (yellow) means positive correlations, while negative number (red) means negative correlations. The shade of these color corresponded roughly to the Pearson correlation coefficient. * indicated a significant difference ($0.01 < P < 0.05$), and ** indicated an extremely significant difference ($P < 0.01$).

A complete intestinal barrier is not only the basis for nutrient absorption, but also the key to lowering the invasion of external pathogenic microorganisms (Di Tommaso et al., 2021). Some TJP such as claudin and occludin serve as the mechanical division by the "pore" and "leak" pathway, and among them claudin protein family plays a key role in regulating intestinal permeability (Günzel and Yu, 2013; Paradis et al., 2021. Claudin1 and claudin3, known as main members of sealing claudin protein, are down-regulated in inflammatory bowel disease and thus considered as the potential marker of intestinal barrier function (Weber et al. 2008; Shim et al. 2015). *i-FABP* is also regarded as an important indicator to measure intestinal barrier function. A lack of *i-FABP* expression may be a "leaky gut" phenotype including impaired intestinal morphology and decreased claudin expression (Lackey et al., 2020). It is confirmed that the increase in villin expression level is related to the improvement in intestinal villus development and regeneration and remodeling of epithelial cell (Braunstein et al., 2002). Some scholars regarded the contents of DAO and D-LA in serum as one of the indicators for evaluating intestinal permeability (Zhao et al., 2017). In the present study, we found there were no changes in serum DAO and D-LA contents with SC treated. It was worth mentioning that dietary 600 mg/kg SC contributed to up-regulating the mRNA levels of villin, *ZO-1*, claudin3, occludin, and *i-FABP* in the ileum. Additionally, the protein level of claudin in the ileum was also raised with SC supplemented. These findings were consistent with the studies which described that *Saccharomyces boulardii* improved TJP expression and intestinal ultrastructure (Rajput et al., 2013). Mucin is a mesh-like structure attached to the intestinal villi, and the intestinal flora stimulates goblet cells to secrete mucin. Mucins are thought to be the first barrier against pathogenic microbes and reduce the adhesion of bacteria on the mucosal surface (Amiri et al., 2021). In this study, the transcript levels of ileal *MUC 2* were elevated with two doses of SC treated. This evidence suggested that 600 mg/kg SC helped improve intestinal barrier function. We would like to attribute this to the improvement of SC on the intestinal flora structure.

Numerous studies suggested the intestinal microbiota play an important role in transferring dietary components to active metabolites to directly or indirectly influence the gut barrier functions and nutrient absorption (Muller et al., 2020; Ghosh et al., 2021). Some beneficial bacteria in the intestine such as most of *Lactobacillus* and *Clostridium butyricum* promote the growth of intestinal epithelial cells, and some harmful bacteria such as *Proteobacteria* negatively affect the gastrointestinal tract (Burrello et al., 2018; Dai et al., 2020). Previous studies suggested that SC and its metabolites helped improve intestinal flora (Muthusamy et al., 2011; Wang et al., 2016; Ahiwe et al., 2019). In the present study, dietary SC tended to increase *Bacteroidetes* ($P = 0.071$), the RA of *Ruminococcaceae_UCG-014* were raised in the LSC group, while the RA of *Proteobacteria* was descended with two levels of SC treated. It was reported that gut epithelial dysfunction is always associated with an increase in the abundance of *Proteobacteria* (Litvak et al., 2017). This also was confirmed in the present study which found that the RA of *Proteobacteria* was significantly negatively correlated with the mRNA levels of claudin3, occludin, and *i-FABP*. B vitamins, as a metabolite of SC, are essential nutrients for the growth of *Ruminococcaceae*. However, the genome sequence analysis of *Ruminococcaceae* was auxotrophic for B vitamins indicating that these bacteria depend on vitamins supplied from other members of gut microbiota (Soto-Martin et al., 2020). This might be one of the reasons why SC upregulated the RA of *Ruminococcaceae*. This was also consistent with the finding in other studies that yeast cell wall increased the RA of *Ruminococcaceae* in the intestine of broilers (Bi et al., 2020). The main metabolites of *Ruminococcaceae* and *Bacteroidetes* (Harrison et al., 2018; Wang C et al., 2021) in the intestine are Short-chain fatty acids, which help to promote the growth of intestinal epithelial cells and elevate the expression of tight junction proteins (Miao et al., 2016; Del et al., 2021). These findings were consistent with the positive correlation in our study between the protein level of claudin1 with *Ruminococcaceae* and *Bacteroidetes*. This might be the reason why SC raised the ileum length and improved the feed conversion efficiency of Pekin ducks. Our findings illuminated that the enhancement of intestinal barrier function of SC on Pekin duck was mainly attributed to its improvement of intestinal flora.

This study extended our knowledge of the effects of dietary SC on the intestinal barrier and the intestinal flora in the Pekin ducks. Although a detailed investigation of the mechanism was beyond the scope of this work, we acknowledge that administration of 600 mg/kg SC could exert optimal effect on improving the intestinal barrier and the cecal microbiota. This study also provided a theoretical basis for hunting for green and safe feed additives in the post-antibiotic era.

CONCLUSION

Under the condition of our study, dietary *Saccharomyces cerevisiae*, particularly at 600 mg/kg level, enhanced the intestinal barrier function via improving the structure of intestinal flora in Pekin ducks.

AUTHOR CONTRIBUTIONS

Wenwen Gao and Zhaofei Xia designed the study, Wenwen Gao wrote the paper, Wenwen Gao and Keying An helped collect samples and organize data. Peng Li participated in improving the manuscript. All authors checked the final layout of the manuscript.

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DISCLOSURES

All authors declared that they have on relevant interest relationships.

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