Effects of housing systems and glucose oxidase on growth performance and intestinal health of Beijing You Chickens

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ABSTRACT We investigated the effects of housing systems and dietary glucose oxidase (**GOD**) on the growth performance and intestinal health of Beijing You chickens (**BYC**). The experiment was designed as a factorial arrangement of 2 housing systems × 2 dietary treatments. Chickens were fed a basal diet or a diet with 200 U/kg GOD and were reared on the floor with deep litter or in the cages. Compared with the litter floor groups, the decreased average daily feed intake of 1 to 42 d, decreased feed conversion ratio (**FCR**), improved average daily gain of 42 to 77 d, and the whole period were identified in the cage rearing groups ($P < 0.05$). The FCR of 42 to 77 d and the whole period, the 42-d ileal pH, and 77-d jejunal and ileal pH decreased with the supplement of GOD ($P < 0.05$). Additionally, 16S rRNA gene of ileum contents was sequenced by high-throughput sequencing. Sequencing data indicated that the *Firmicutes* phylum of 42 d and the *Bacteroidetes* phylum were significantly higher in the litter group with GOD supplement ($P < 0.05$). The jejunal *Occludin*, *Mucin-2* mRNA expression levels were higher in the litter floor groups than those in the cage rearing groups on 42 d ($P < 0.05$). The *Mucin-2* and *TNF-α* mRNA expression levels increased with cage rearing on 77 d ($P < 0.05$). The *Occludin* and *TLR-4* mRNA expression levels increased with the supplementation of GOD on 77 d ($P < 0.05$). Moreover, the upregulation effects of *Occludin* and *ZO-1* mRNA expression levels were more obvious in the litter floor group fed with GOD diet on 77 d ($P < 0.05$). The serum endotoxin content of 42-day-old cage rearing groups were higher than that of the litter floor groups, and the serum endotoxin content significantly decreased with the supplement of GOD on 77 d. The results indicated that the litter floor systems were beneficial to the development of intestinal barrier junction in the early stage, but the cage systems were more conducive to the growth performance of BYC. The dietary GOD could inhibit the harmful bacteria and promote the beneficial bacteria, which might be related to the improvement of the growth performance and intestinal barrier function.

Key words: Beijing You chicken, housing system, glucose oxidase, intestinal health

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INTRODUCTION

Glucose oxidase (**GOD**) is an aerobic dehydrogenase fermented by *Aspergillus niger*, *Penicillium notatum*, or other fungi, which could catalyze the oxidation of β-D-glucose to gluconic acid by utilizing molecular oxygen as an electron acceptor with simultaneous production of hydrogen peroxide ($\mathbf{H_2O_2}$) (Bankar et al., 2009). Gluconic acid is a kind of organic acid, which acts as an acidifier in the intestine (Rafacz-Livingston et al., 2005). Related studies have shown that gluconic acid can reduce intestinal pH, enhance digestive ability, increase the beneficial bacteria, and produce short-chain fatty acids by fermentation of specific bacteria in the hindgut (Femia et al., 2002; Kameue et al., 2004), mainly butyric acid. Butyric acid plays a certain role in maintaining the morphology of intestinal mucosa and antiinflammatory (Peng et al., 2009). The anaerobic environment produced by catalytic reaction of GOD is conducive to the proliferation of anaerobic beneficial bacteria, and the accumulation of H_2O_2 produced at the same time can be sterilized and is antibacterial (Dobbenie et al., 1995). Therefore, GOD is considered as a kind of green additive that could both keep the intestinal health and stimulate the growth potential of chickens (Wu et al., 2019). In addition, some studies have shown that dietary GOD could improve intestinal immune function and antioxidant capacity (Hou et al., 2017; Wang et al., 2018).

Housing systems are closely related to the growth performance and intestinal health of poultry (Li et al., 2017). The

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floor rearing with deep litter and the cage housing systems respectively represent the traditional free-range housing system and the modern large-scale housing system, which have great differences in growth performance, nutrient digestion and absorption, immune system development, and intestinal microbiota with respect to diversity, composition, and community structure of chickens. The litter floor rearing refers to a mode of rearing on the floor with deep litter. The litter-excreta mixture contains complex and dynamic microbiota. The early environment of the litter floor is relatively simple, which may be beneficial to the establishment and proliferation of intestinal microbiota (Li et al., 2017). There is almost no microbial colonization resistance in the intestine of young chickens, and exposure to the microbial environment could greatly affect the development of their intestinal microbiota (Wang et al., 2016). The nonstarch polysaccharides component in litter could also be used as a potential source of prebiotics to promote the competitive inhibition of pathogenic microbiota in the intestine (Santos et al., 2008; Hou et al., 2020). However, when the intestinal microbiota becomes complex and diversified over time and environmental conditions, long-term exposure to feces and high air pollution environments has a higher risk of infection with pathogenic bacteria. The cage housing system was developed under the pressure of environmental protection and the limitation of land resources. The cage systems could greatly improve the rearing efficiency and increase the body weight gain (Li et al., 2017). Chickens reared in the cage have less contact with feces, which reduces the risk of intestinal diseases such as coccidia and pathogen infection (Bogosavljevic-Boskovic et al., 2012), but it also reduces the diversity of intestinal microbiota (Chen et al., 2019).

Most of the studies and production practices on GOD in poultry were carried out under cage systems. Compared with cage systems, the environment of litter floor is more complex and has a more significant effect on intestinal health. The role of GOD is mainly based on the improvement of intestinal environment. It is necessary to compare the differences between 2 housing systems, so that could provide reference for the establishment of a suitable rearing model. In this experiment, the effects of housing systems and the GOD supplementation on growth performance and intestinal health of Beijing You chickens (**BYC**) were investigated.

Beijing You chickens, a Chinese indigenous poultry breed, with both excellent egg laying performance and meat production performance, occupies an important position in the yellow feather broiler breed. We desired to help people better understand broiler management practices and provide a reference for further studies and may improve the growth performance and decrease incidence of diseases.

MATERIALS AND METHODS

Animal Feeding and Management

All experimental procedures and sample collection methods were approved by the Animal Care and Use Committee of China Agricultural University. The study was conducted at Zhuozhou Experimental Base of China Agricultural University. Based on a 2 × 2 factorial arrangement with 2 housing systems (litter floor or cage) and 2 dietary GOD levels (0 or 200 U/kg of diet), a total of four hundred 1-day-old male BYC were randomly assigned to 4 groups: the litter floor group fed with basal diet (**FB**), the cage group fed with basal group (**CB**), the litter floor group fed with GOD diet (**FG**), and the cage group fed with GOD diet (**CG**). Each group included 8 replications, 15 chickens per replication in the litter floor groups and 10 chickens per replication in the cage rearing groups. The trial lasted 77 d, divided into 2 stages: 1 to 42 d and 42 to 77 d. All chickens were kept in the same poultry house with litter floor cages or double-floor cages. The padding was about 10 cm of new soft rice hulls in the litter floor cages. The chickens had free access to feed and water and were maintained on a 23 h constant-lighting program. The temperature was maintained at 33°C to 35°C in the first wk and decreased by 2°C to 3°C per wk until it reached 18°C to 21°C in the 6th wk. The humidity remained at 60 to 65% in the first wk, then decreased by 55% per wk until it reached 45% in the 4th wk. The unmedicated corn–soybean meal diets were prepared according to the BYC feeding and management technical regulations. The GOD expressed by *A. niger* was provided by Jinan Bestzyme Biological Engineering Co., Ltd. (Jinan, China), 200 U/kg is the application amount of GOD recommended by the company on BYC. Table 1 presents the composition and nutrient levels of basal diet.

Sample Collection

On d 42 and d 77, eight chickens were randomly selected per treatment and weighed. Blood samples were collected into vacuum tubes after 8-h fasting by wing vein puncture. Serum was separated by centrifugation at 3000 rpm for 10 min at 4°C and then stored at −20°C for the determination of serum endotoxin assay. Then, the chickens were killed by jugular exsanguination. The midregions of the jejunum (approximately 1 cm) were collected in RNA-free centrifuge tube, rapidly frozen in liquid nitrogen, and stored at −80°C for mRNA analysis. Duodenal, jejunal, and ileal content were collected, rapidly frozen with liquid nitrogen, and stored at −80°C for the determination of pH and ileal microbiota.

Growth Performance and Intestinal pH

On d 42 and d 77, all chickens of each replication were weighed, and the feed consumption was recorded. Mortality was recorded as it occurred. The body weight, average daily gain (**ADG**), average daily feed intake (**ADFI**), and feed conversion ratio (**FCR**) were calculated for the periods from d 1 to d 42, from d 42 to d 77, and from d 1 to d 77. The pH values of duodenal, jejunal and ileal contents were determined by pH meter (Mettler Toledo, USA).

Table 1. Composition and nutrient levels of basal diet (%, air-dry basis).

Items	1–42 d	42–77 d
Ingredients		
Corn	62.58	69.47
Soybean meal	33.38	26.17
Soy oil	0.48	1.21
Calcium hydrogen phosphate	1.87	1.61
Limestone	0.76	0.75
Sodium chloride	0.34	0.30
Mineral premix[1]	0.20	0.20
Choline chloride	0.20	0.16
DL-Met	0.14	0.08
Antioxidant	0.03	0.03
Vitamin premix[2]	0.02	0.02
Total	100.00	100.00
Nutrient levels[3]		
ME (Mcal/kg)	2.90	3.00
CP	20.50	18.00
Lys	1.11	0.94
Met	0.45	0.36
Met + Cys	0.79	0.68
Thr	0.79	0.69
Ca	0.95	0.85
AP	0.45	0.40

[1]Provide per kilogram of diet: copper, 2 mg; iron, 132 mg; zinc, 126 mg; manganese, 129 mg; iodine, 1.8 mg; selenium, 0.6 mg.
[2]Provide per kilogram of diet: vitamin A, 13,500 IU; vitamin D3, 3600 IU; vitamin E, 36 IU; vitamin K3, 4.5 mg; vitamin B1, 3.6 mg; vitamin B2, 11.25 mg; vitamin B6, 6 mg; vitamin B12, 0.039 mg; niacin, 39 mg; D-pantothenic acid, 16.5 mg; folic acid, 2.1 mg; biotin, 0.24 mg.
[3]Calculated values.

Real-Time Quantitative PCR

Total RNA was extracted from intestinal tissues using Trizol reagent (TaKaRa Bio, Kusatsu, Japan) according to the manufacturer's protocol. The concentration and purity of RNA were determined using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA). In brief, 1 μg of total RNA from each sample was reverse-transcribed into cDNA using a PrimeScript RT reagent kit with cDNA eraser (RR036 A; TaKaRa Bio). One-step real-time PCR instrument was used in accordance with the manufacturer's guidelines. Table 2 lists the quantitative real-time PCR primers used in our study. The relative mRNA expression levels of each target gene were calculated based on the expression of the house-keeping gene GAPDH using the $2^{-\triangle\triangle Ct}$ method (Livak and Schmittgen, 2001).

Bacterial DNA Extraction and Sequencing of 16S rRNA

The ileal content of chickens under litter floor system was collected for the determination of ileal microbiota. Ileal content DNA was extracted using PowerSoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA) according to the manufacturer's protocol. The concentration and purity of total DNA were detected by NanoDrop 2000 (Thermo Scientific, Waltham, MA, USA) and 1.5% agarose gel electrophoresis. To construct 16S rDNA sequencing libraries, the V3-V4 region of the 16S rDNA gene was amplified from the DNA samples by PCR using primer set of 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). The PCR products were purified, quantified, and homogenized to form a sequencing library. After being confirmed qualified, machine sequencing was used on the Novaseq PE250 sequencing platform. Sequence dereplication and denoising were done to generate amplicon sequence variants, and Qiime2-2019.7 (Bolyen, 2019, Nature Biotechnology) was used to generate species abundance tables at different classification levels. The Alpha diversity index (Shannon) and Beta diversity of samples (Bray-Curtis, principal coordinate analysis were analyzed using QIIME 2-2019.7) were analyzed.

Serum Endotoxin

The content of serum endotoxin was determined by End-point Chromogenic Tachypleus Amebocyte Lysate (Xiamen Bioendo Technology Co., Ltd., Xiamen, China) according to the manufacturer's protocol. In brief, 100 μl serum was added to the 0.9 ml sample treatment solution and mixed to make a 10-ford dilution. Heat the 10-fold diluent in a dry heat thermostat at 70°C for 10 min, then cool it in an ice water bath for 3 min. Add 100 μl sample dilution to 100 μl tachypleus amebocyte lysate in a warm bath at 37°C. After adding chromogenic matrix, take a warm bath at 37°C, add azo reagent, mix and rest, and read the absorbance value at 545 nm wavelength. The level of serum endotoxin was calculated according to the standard curve.

Correlation Analysis

To investigate the relationship of growth performance, health parameters, and intestinal microbiota composition, Pearson's correlation analysis was applied to evaluate the potential link between them. After pairing the variables, the Pearson correlation coefficients were obtained by correlation analysis. Furthermore, the positive values denote positive linear correlation between 2 variables.

Statistical Analysis

The data were analyzed using the General Linear Model procedure in SPSS, version 23.0 (SPSS Inc., Chicago, IL), and subjected to 2-way ANOVA in a 2 × 2 factorial arrangement to analyze the main effects of raising systems and GOD and their interaction. One-way ANOVA and Duncan's multiple comparisons were used when a significant interaction was observed. To test microbial community compositional differences, a 2-tailed nonparametric Mann-Whitney U test was performed to compare the variables of the 2 groups (FB and FG) with non-normally distributed data. We used analysis of variance of permutational analysis of covariance from R's package vegan to compare the effects of GOD on microbial community structures of chickens reared on litter floor. Results are presented as the means with standard error of the mean. Statistical

Table 2. Primer sequences of qPCR.

Gene name	Primer sequence (5′ to 3′)	Accession number
Occludin	F:ACGGCAGCACCTACCTCAA R:GGGCGAAGAAGCAGATGAG	NM_205128.1
Claudin-1	F:CATACTCCTGGGTCTGGTTGGT R:GACAGCCATCCGCATCTTCT	AY750897.1
ZO-1	F:CTTCAGGTGTTTCTCTTCCTCCTC R:CTGTGGTTTCATGGCTGGATC	XM_413773
TLR-4	F:GGATCTTTCAAGGTGCCACA R:CAAGTGTCCGATGGGTAGGT	AY064697
IL-1β	F: ACTGGGCATCAAGGGCTA R: GGTAGAAGATGAAGCGGGTC	NM_204524.1
TNF-α	F:GAGCGTTGACTTGGCTGTC R:AAGCAACAACCAGCTATGCAC	NM_204267.1
IFN-γ	F: AGCTGACGGTGGACCTATTATT R: GGCTTTGCGCTGGATTC	NM_205149.1
IL-4	F:GCTCTCAGTGCCGCTGATG R:GAAACCTCTCCCTGGATGTCAT	NM_001007079.1
Mucin-2	F:TTCATGATGCCTGCTCTTGTG R:CCTGAGCCTTGGTACATTCTTGT	XM_421035
GADPH	F:TGCTGCCCAGAACATCATCC R: ACGGCAGGTCAGGTCAACAA	NM_204305.1

Abbreviations: F, forward; R, reverse.
Primers were synthesized by Biotech(shanghai)Co., Ltd.

differences were considered significant at $P < 0.05$, and $0.05 < P < 0.10$ was viewed as a trend.

RESULTS

Growth Performance and Intestinal pH

The effects of GOD on growth performance of BYC during the experimental period of 77 d were all shown in Table 3. From 1 to 42 d, it was observed that different housing systems and supplementation of GOD had no significant differences in the ADG among the 4 groups ($P > 0.05$). Compared with the litter floor groups, the ADFI and FCR of the BYC from the cage rearing groups were significantly reduced ($P < 0.05$). There were no significant differences among the basal diet groups and GOD supplementation groups ($P > 0.05$). Additionally, there was a significant interaction between housing systems and GOD supplementation on FCR ($P < 0.05$); The CB group had the lower FCR than FB group ($P < 0.05$), but there were no differences in FCR between FG group and CG group ($P > 0.05$). There were no significant differences in mortality among the 4 treatments ($P > 0.05$), and the data were not shown.

From 42 d to 77 d, cage systems significantly improved ADG and decreased FCR of chickens ($P < 0.05$). The dietary GOD significantly decreased the FCR ($P < 0.05$) but had no influence on the ADG and ADFI ($P > 0.05$). During the whole feeding period, the improvement of ADG and decrease of FCR were identified in the cage rearing groups ($P < 0.05$), and the supplementation of GOD significantly decreased the FCR of BYC ($P < 0.05$).

Intestinal pH is closely related to intestinal digestion and could be affected by factors such as diets and housing systems. As shown in Table 4, we found that the supplementation of GOD could significantly reduce the ileal pH of 42-day-old BYC and the ileal pH of 77-day-old BYC ($P < 0.05$). Meanwhile, we also found that the litter floor significantly reduced the ileal pH of 77-day-old BYC, when compared with the cage rearing groups ($P < 0.05$).

The mRNA Expression Levels of Tight Junction and Inflammatory Cytokines in Jejunum

The results were presented in Table 5. In 42-day-old BYC, compared with the cage rearing, the litter floor rearing significantly elevated the mRNA expression levels of jejunal *Occludin* and *Mucin-2* ($P < 0.05$), and there were no significant differences in other mRNA expression levels among the 4 groups ($P > 0.05$). The dietary GOD had no significant effects on the expression levels of tight junctions and inflammatory cytokines in BYC ($P > 0.05$).

In 77-day-old BYC, the mRNA expression levels of jejunal *Mucin-2* and *TNF-α* reared in the cages were higher than those reared on the litter floor ($P < 0.05$). The dietary GOD has significantly increased the mRNA expression levels of *TLR-4* ($P < 0.05$). There were significant interactions between housing systems and dietary treatments on the mRNA expression levels of jejunal *Occludin* and *ZO-1* ($P < 0.05$). In the basal diet groups, the expression levels of BYC reared in cage were significantly higher than that reared on the litter floor ($P < 0.05$). In the GOD supplementation groups, there were no significant differences between the expression levels of BYC reared in cage and those reared on the litter floor.

Serum Endotoxin

As shown in Table 6, the serum endotoxin content of 42-day-old BYC reared in cage was higher than those reared on the litter floor ($P < 0.05$). However, GOD

Table 3. The growth performance of Beijing You Chickens.

	Group					Main effect				*P* value		
Items	FB	FG	CB	CG	SEM	Basal	GOD	Litter floor	Cage	GOD	Housing systems	Housing systems × GOD
1–42 d												
ADG, g/d	14.60	14.90	14.72	14.30	0.167	14.66	14.60	14.75	14.51	0.865	0.494	0.307
ADFI, g/d	37.35	36.90	35.07	34.75	0.372	36.21	35.82	37.12[a]	34.91[b]	0.564	0.002	0.921
FCR, g/g	2.56[a]	2.47[b]	2.38[b]	2.43[b]	0.018	2.47	2.46	2.52	2.41	0.512	0.001	0.034
42–77 d												
ADG, g/d	26.48	28.13	30.09	29.79	0.391	28.28	28.96	27.30[b]	29.94[a]	0.282	<0.001	0.122
ADFI, g/d	77.74	78.11	80.14	75.96	0.614	78.86	77.04	77.93	77.91	0.115	0.917	0.062
FCR, g/g	2.94	2.78	2.67	2.55	0.034	2.82[a]	2.67[b]	2.86[a]	2.61[b]	0.008	<0.001	0.678
1–77d												
ADG, g/d	20.00	20.91	21.71	21.34	0.224	20.85	21.13	20.46[b]	21.52[a]	0.505	0.014	0.127
ADFI, g/d	55.71	55.63	55.56	53.48	0.399	55.63	54.56	55.67	54.52	0.168	0.141	0.199
FCR, g/g	2.78	2.66	2.56	2.51	0.024	2.67[a]	2.59[b]	2.72[a]	2.54[b]	0.011	<0.001	0.305

[a–b]Means in the same row without the same superscript differ significantly ($P < 0.05$). "+" means add. SEM means standard error of the mean. (n = 8).
Abbreviations: ADG, average daily gain; ADFI, average daily feed intake; CB, rear in the cage fed with basal dietary; CG, rear in the cage fed with GOD; FCR, feed conversion ratio; FB, rear on the litter floor fed with basal dietary; FG, rear on the litter floor fed with GOD dietary; GOD, glucose oxidase.

had no significant effects on serum endotoxin content ($P > 0.05$). On d 77, there were no significant differences in serum endotoxin content between 2 housing systems ($P > 0.05$). The dietary GOD significantly reduced the content of serum endotoxin ($P < 0.05$).

Ileal Microbiota

Based on the growth performance and the mRNA expression levels change induced by GOD supplement, we found that it was more obvious in the litter floor groups, so that we further identified the possible roles of dietary GOD in the litter floor groups by measuring the ileal microbiota. A total of 16 ileal luminal content samples were used for 16S rRNA gene sequencing. We obtained 5,593,079 tags. After filtering, denoising, and removing chimeras, 323,394 effect tags were obtained.

Shannon diversity indexes were used to evaluate the alpha diversity of ileal microbiota of the FB and FG groups. At 42 d of age, there were no differences observed in ileum between the 2 groups, but the supplementation of GOD showed a tendency to increase Shannon index ($P = 0.093$) (Figure 1). Subsequently, the bacterial compositions of the ileum with the 2 dietary treatments were compared based on Bray–Curtis distance. Analysis of variance of permutational analysis of covariance was used to examine whether the matrix of major PCoA axes was dependent on the dietary GOD. According to the results of PCoA, the ileal microbial communities were clustered tightly in the 2 groups ($P > 0.05$) (Figure 2).

The effects of the supplementation of GOD on ileal microbiota in BYC under litter floor systems were analyzed at phyla and genus levels. At the phylum level (Table 7), *Firmicutes*, *Cyanobacteria,* and *Proteobacteria* were enriched in the ileum of 42-day-old FB group, and *Firmicutes* were more abundant in the 42-day-old FG group ($P < 0.05$), meanwhile, *Proteobacteria* were less abundant ($P = 0.064$). The relative abundance of *Bacteroidetes* significantly increased in the 77-day-old FG group ($P < 0.05$). At the genus level (Table 8), the top 10 bacteria were *Lactobacillus, Romboutsia, Uncultured_bacteria_f_Peptostreptococcaceae, Uncultured_bacteria, Candidatus Arthromitus, Enterococcus, Turicibacter, Uncultured_bacteria_o_Chloroplast, Faecalibacterium,* and *Escherichia_Shigella* in the 42-day-old BYC. *Enterococcus* were more abundant in the FG group ($P = 0.061$), whereas *Escherichia_Shigella* showed a decreased trend ($P = 0.064$). There were no significant differences between the 2 treatments for other strains. At 77 d of age, the top 10 bacteria were *Lactobacillus, Uncultured_bacteria, Romboutsia, Faecalibacterium, Turicibacter, Enterococcus, Helicobacter, Ruminococcus torques group, Bacteroides,* and *Streptococcus.* The relative abundance of *Bacteroides* in the FG group was significantly higher than that in the FB group ($P < 0.05$), and *Faecalibacterium* showed a trend of increase ($P = 0.064$), and there were no significant differences in other species from the 2 treatments ($P > 0.05$).

Correlation Between Alterations in Intestinal Microbiota Composition and Growth Performance and Intestinal Health Parameters

Using Pearson correlation analysis to explore the relationship between the bacteria composition and the other health parameters (Figure 3). On d 42, ileal *Escherichia_Shigella* with a decreasing trend ($P = 0.064$) was positively related with ADFI, FCR, and the mRNA expression levels of *TLR-4* and *IFN-γ* ($P < 0.05$) and, meanwhile, was significant negatively correlated with the mRNA expression levels of *Mucin-2* ($P < 0.05$). However, the ileal *Enterococcus* with an increasing trend ($P = 0.061$) had no significant correlation with other health parameters. On d 77, ileal *Faecalibacterium* was positively related with the mRNA expression levels of *Occludin* ($P = 0.093$) and ADG ($P = 0.085$). *Bacteroides* was positively correlated with the mRNA expression levels of *Occludin* ($P < 0.05$), *ZO-1* ($P = 0.087$), and *IFN-γ* ($P < 0.05$).

Table 4. Effects of GOD on intestinal pH of Beijing You Chickens under different housing systems.

		pH					
		42 d			77 d		
Items		Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum
FB[1]		5.56	5.26	6.17	5.42	5.39	6.64
FG		5.47	5.19	5.41	5.47	5.11	5.90
CB		5.55	5.31	6.07	5.48	5.43	7.26
CG		5.60	5.30	5.50	5.42	5.36	6.86
SEM		0.021	0.026	0.148	0.016	0.044	0.115
Main effect	Litter floor	5.51	5.23	5.79	5.44	5.25	6.27[b]
	Cage	5.58	5.30	5.78	5.45	5.39	7.06[a]
	Basal	5.56	5.28	6.12[a]	5.45	5.41[a]	6.95[a]
	GOD	5.54	5.25	5.45[b]	5.44	5.24[b]	6.38[b]
P value	Housing systems	0.123	0.146	0.983	0.889	0.091	<0.001
	GOD	0.629	0.480	0.028	0.826	0.041	0.001
	Housing systems × GOD	0.098	0.573	0.749	0.061	0.189	0.302

[a–b]Means in the same column without the same superscript differ significantly ($P < 0.05$). "+" means add. SEM means standard error of the mean. (n = 8).

Abbreviations: CB, rear in the cage fed with basal dietary; CG, rear in the cage fed with GOD; FG, rear on the litter floor fed with GOD dietary; GOD, glucose oxidase.

[1]FB, rear on the litter floor fed with basal dietary.

DISCUSSION

The recent studies focus on the effects of GOD on the growth performance, immune function, and intestinal barrier function (Hou et al., 2017; Wu et al., 2019); however, most related studies were carried out under cage systems (Pang et al., 2013; Wu et al., 2019). Meanwhile, the effects of dietary GOD were not consistent because of the influence of the amount of supplementation, stage of use, and environmental systems. In addition, it has been well recognized that the effects of housing systems on growth performance were different (Li et al., 2017). Compared with cage systems, the litter floor systems, which contained a mixture of feces and rice hulls, were more complex and had a more significant impact on intestinal health (Hubert et al., 2019). The GOD played a growth-promoting role mainly based on the improvement of intestinal environment, so that may have different effects on the growth performance of chickens under 2 housing systems. In the present work, different from the litter floor systems, the decreased ADFI of 1 to 42 d decreased FCR and improved ADG of 42 to 77 d, and the whole period was identified in the cage rearing groups ($P < 0.05$). The finding of the early stage was different from Li et al. (2017), who reported that the litter floor systems improved the ADG of Arbor Acre broilers than the cage systems from 1 to 21d. This suggested that the effects of housing systems on growth performance might be affected by the breed and the rearing periods. Wu et al. (2019) proved that supplementation

Table 5. The mRNA expression levels of tight junctions and inflammatory cytokines in jejunum of Beijing You Chickens.

	Group					Main effect				*P*-value		
Items	FB	FG	CB	CG	SEM	Basal	GOD	Litter floor	Cage	GOD	Housing systems	Housing systems × GOD
42d												
Occludin	1.00	1.18	0.89	0.81	0.050	0.95	1.00	1.09[a]	0.85[b]	0.598	0.016	0.169
Claudin-1	1.00	0.99	1.13	0.98	0.092	1.06	0.99	0.99	1.06	0.691	0.753	0.729
ZO-1	1.00	0.99	1.12	1.03	0.067	1.06	1.01	1.00	1.07	0.716	0.588	0.749
Mucin-2	1.00	1.02	0.66	0.58	0.056	0.83	0.80	1.01[a]	0.62[b]	0.692	<0.001	0.587
IL-1β	1.00	1.27	1.00	1.32	0.105	1.00	1.30	1.13	1.16	0.173	0.903	0.884
IL-4	1.00	0.88	0.98	1.29	0.065	0.99	1.09	0.94	1.13	0.438	0.142	0.101
TLR-4	1.00	0.94	1.13	1.06	0.075	1.07	1.00	0.97	1.09	0.677	0.423	0.984
IFN-γ	1.00	1.14	1.21	0.97	0.132	1.10	1.05	1.07	1.09	0.851	0.954	0.498
TNF-α	1.00	1.00	0.93	0.88	0.037	0.96	0.94	1.00	0.90	0.790	0.195	0.752
77d												
Occludin	1.00[b]	1.68[a]	1.65[a]	1.43[a]	0.073	1.32	1.55	1.34	1.54	0.050	0.095	0.001
ZO-1	1.00[b]	1.68[a]	1.89[a]	1.42[a,b]	0.123	1.45	1.55	1.34	1.66	0.642	0.173	0.017
Mucin-2	1.00	0.91	1.61	1.30	0.077	1.31	1.10	0.96[b]	1.45[a]	0.115	<0.001	0.373
IL-1β	1.00	1.47	0.91	1.04	0.113	0.96	1.25	1.23	0.97	0.195	0.251	0.447
IL-4	1.00	1.20	1.39	0.95	0.089	1.20	1.07	1.10	1.17	0.479	0.686	0.073
TLR-4	1.00	1.49	1.09	1.43	0.065	1.05[b]	1.46[a]	1.25	1.26	0.001	0.892	0.505
IFN-γ	1.00	1.75	1.21	1.15	0.134	1.10	1.45	1.38	1.18	0.199	0.454	0.132
TNF-α	1.00	0.95	1.64	1.55	0.074	1.32	1.25	0.97[b]	1.60[a]	0.490	<0.001	0.852

[a–b]Means in the same row without the same superscript differ significantly ($P < 0.05$). "+" means add. SEM means standard error of the mean. (n = 8).

Abbreviations: CB, rear in the cage fed with basal dietary; CG, rear in the cage fed with GOD; FB, rear on the litter floor fed with basal dietary; FG, rear on the litter floor fed with GOD dietary; GOD, glucose oxidase.

Table 6. Serum endotoxin contents of Beijing You Chickens (EU/mL).

Items		Serum endotoxin 42 d	Serum endotoxin 77 d
FB		0.08	0.28
FG		0.10	0.20
CB		0.13	0.47
CG		0.13	0.19
SEM		0.007	0.040
Main effect	Basal	0.11	0.37[a]
	GOD	0.12	0.20[b]
	Litter floor	0.09[b]	0.24
	Cage	0.13[a]	0.33
P-value	GOD	0.545	0.026
	Housing systems	0.008	0.248
	Housing systems × GOD	0.231	0.190

[a–b]Means in the same column without the same superscript differ significantly ($P < 0.05$). SEM means standard error of the mean. (n = 8).

Abbreviations: CB, rear in the cage fed with basal dietary; CG, rear in the cage fed with GOD; FB, rear on the litter floor fed with basal dietary; FG, rear on the litter floor fed with GOD dietary; GOD, glucose oxidase.

of GOD, especially the supplement of 60 U/kg GOD, could improve the growth performance of Arbor Acres broilers by influencing the digestibility and absorption of nutrients. In our study, from 1 to 42 d, the chickens reared in cages had the lower FCR than those reared on the litter floor, but the dietary GOD made no difference in FCR between these housing systems, which indicated that GOD had better effects on the chickens under litter floor systems. The dietary GOD could also improve the FCR in the late rearing period and the whole period regardless of different housing systems.

To explore the relationship between the growth performance and intestinal status changes, we analyzed the intestinal pH. According to the functional mechanism of GOD, gluconic acid is the key metabolites of GOD, which has been widely proved to be involved in improving growth performance and benefit to intestinal

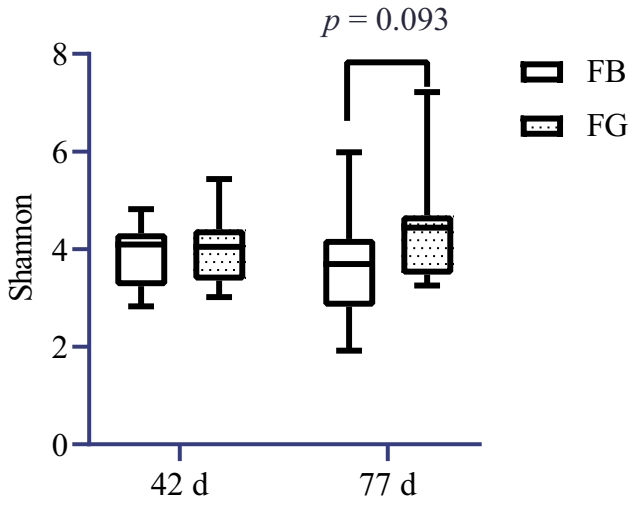

Figure 1. Effects of GOD on ileal microbiota Shannon index of Beijing You Chickens reared on litter floor (CFU/kg). Abbreviations: FB, rear on the litter floor fed with basal dietary; FG, rear on the litter floor fed with GOD dietary; GOD, glucose oxidase.

health as organic acid and prebiotics (Kameue et al., 2004; Biggs and Parsons, 2008). Gluconic acid itself as an organic acidifier could decrease intestinal pH, about 70% of which will be fermented by specific bacteria to produce short-chain fatty acids after achieving the hindgut, including acetic acid, butyric acid, and so on (Tsukahara et al., 2002). These weak acids could also play a role in decreasing intestinal pH. Acidic environment is conducive for improving the activity of intestinal digestive enzymes, promoting digestion and absorption, and increasing the community of beneficial bacteria so that could promote the growth performance and maintain the intestinal environmental homeostasis. In our study, the dietary GOD significantly decreased the 42-d ileal pH and 77-d jejunal and ileal pH of BYC but had no significant effects on duodenal pH. It may be because of the short residence time of content in duodenum and gluconic acid accumulation in the posterior segment of small intestine, which was consistent with the results of Zhao et al. (2009). At the age of 77 d, the ileal pH of BYC reared on the litter floor was significantly lower than those reared in the cages, which may be because of environmental differences in intestinal microbiota and different metabolites, which had effects on intestinal pH.

Intestinal barrier is the sum of structures and functions that prevent luminal noxious molecules, such as pathogens, toxins, and antigens, which are made up of 4 main components: the physical, chemical, immunological, and microbiological barriers (Yegani and Korver, 2008). The intercellular tight junction structures are an important structural basis of physical barrier. Occludin is one of the components of intestinal tight junction protein. Previous studies have shown that Occludin protein plays an essential role in tight junction of intestinal epithelium(Suzuki, 2013), whose loss will lead to the increase of paracellular permeability to macromolecules(Al-Sadi et al., 2011). ZO-1 and the other ZO proteins provide intracellular scaffold for intestinal tight junction proteins, which are necessary to regulate and maintain intestinal tight junction structure, and play a role in tight junction assembly and regulation. The chemical barrier is primarily the layer of mucus that covers the intestinal epithelium. The Mucin-2 forms the skeleton of the intestinal mucus and covers and protects the intestinal tract from self-digestion and numerous microorganisms. Related studies have shown that the lack of Mucin-2 may lead to an increase in inflammatory response and the abnormal colonization of symbiotic bacteria (Johansson et al., 2008; Wei et al., 2012). At the age of 42 d, the mRNA expression levels of *Occludin* and *Mucin-2* in BYC reared on the deep litter were higher than those reared in cages, indicating that the intestinal barrier of BYC on the litter floor was better than those under cage systems in the early stage. On d 77, the mRNA expression levels of *Mucin-2* of BYC reared in cage were higher than those under the litter floor systems, which indicated that the later cage systems were beneficial to the maintenance of intestinal barrier function, and the long-term contact with

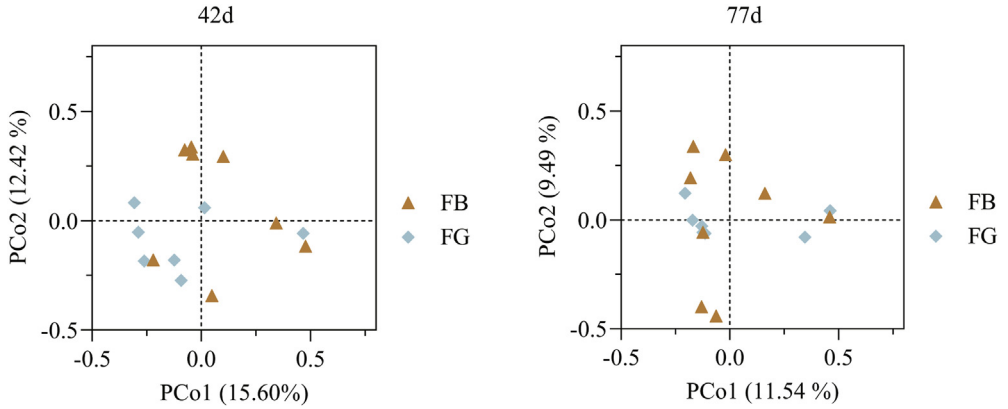

Figure 2. Principal coordinate analysis of Bray–Curtis distances of the ileum samples under litter floor conditions. Abbreviations: FB, rear on the litter floor fed with basal dietary; FG, rear on the litter floor fed with GOD dietary; GOD, glucose oxidase.

feces of chickens reared on the litter floor resulted in a higher risk of infection of pathogens and damage to intestinal barrier function. There was a significant interaction between different housing systems and the supplementation of GOD on the mRNA expression levels of *Occludin* and *ZO-1*. The dietary GOD significantly increased the expression of the 2 genes of chickens under deep litter systems, whereas it had no significant effects on them under cage systems. The results showed that the supplementation of GOD was beneficial for maintenance of intestinal barrier junction of BYC, especially the chickens reared on the litter floor.

The content of serum endotoxin is an important index to evaluate the integrity of intestinal barrier. The increase of serum endotoxin content is a prominent manifestation permeability and mucosal barrier dysfunction (Magnotti and Deitch, 2005; Singleton and Wischmeyer, 2006). In our study, the content of serum endotoxin in 42-day-old BYC reared on the litter floor was lower than those reared in cage, indicating that the litter floor systems may be beneficial to the intestinal development in the early age, which is consistent with the results of mRNA expression levels of *Occludin* and *Mucin-2*. The supplementation of GOD in the CG and FG groups significantly decreased the content of serum endotoxin at 77-day-old of age, which indicated that GOD was beneficial to the maintenance of intestinal integrity of BYC in the later growth stage, and the increase of mRNA expression levels of *Occludin* supported this view.

GOD could produce H_2O_2 during the reaction, which is widely used in medicine to induce oxidative stress in medicine in cell (Jacobson et al., 2018). Oxidative stress can lead to oxidative damage of biomolecules, causing endogenous damage-associated molecular patterns and release of cytokines. Damage-associated molecular patterns can mediate chronic aseptic inflammation through PRRs, like Toll-like receptors, and nonpattern recognition receptors(Yu et al., 2013). At 77-day-old age, GOD significantly increased the mRNA expression levels of *TLR-4*. TLR-4 could initiate inflammatory response. Moderate inflammation is beneficial to maintaining the integrity of the body and removing exogenous and self-mutated antigens. Combined with the beneficial effects of GOD on growth performance and the upregulation of intestinal tight junction protein of chicken reared on the litter floor at the 77-day-old age, we considered that the supplementation of 200 U/kg GOD in dietary was appropriate considering the production of H_2O_2. Meanwhile, the mRNA expression levels of *TNF-α* of BYC under cage systems were higher than those under litter floor systems. This may contribute to a normal low level inflammation of the intestine as defense.

Intestinal microorganisms play an important role in the digestion and absorption of nutrients and the development of immune system. The abundance and diversity of bacteria in the gastrointestinal tract are regulated by pH, oxygen content, and other factors. The GOD can play a certain role in improving intestinal microbiota by consuming oxygen to produce gluconic acid and H_2O_2, which has also been confirmed by many studies (Tang et al., 2016; Wu et al., 2019). In the present experiment, GOD could significantly improve the growth performance and intestinal barrier function of BYC reared on the litter floor, so the 16S rRNA sequence analysis was used to explore the effects of GOD on ileal microbiota of BYC under litter floor systems. The results showed that the

Table 7. Effects of GOD on relative abundance of phyla level in ileal microbiota of Beijing You Chickens reared on litter floor (%).

Items	FB	FG	SEM	*P*-value
42 d				
Firmicutes	86.53[b]	99.98[a]	2.956	0.049
Uncultured_bacterium	10.92	0.02	2.564	0.064
Cyanobacteria	1.37	0.00	0.570	0.144
Proteobacteria	0.76	0.00	0.222	0.064
77 d				
Firmicutes	77.12	65.44	6.500	0.345
Uncultured_bacterium	14.00	22.11	5.570	0.380
Bacteroidetes	0.84[b]	7.03[a]	1.370	0.025
Proteobacteria	2.71	3.11	0.711	0.590
Epsilonbacteraeota	4.63	1.00	1.579	0.831

[a–b]Means in the same row without the same superscript differ significantly ($P < 0.05$). SEM means standard error of the mean. (n = 8).

Abbreviations: FB, rear on the litter floor fed with basal dietary; FG, rear on the litter floor fed with GOD dietary; GOD, glucose oxidase.

Table 8. Effects of GOD on relative abundance of genus level in ileal microbiota of Beijing You Chickens reared on litter floor (%).

Items	FB	FG	SEM	*P*-value
42d				
Lactobacillus	62.34	74.65	4.979	0.248
Romboutsia	10.75	9.18	2.467	0.915
Uncultured_bacteria_f_Peptostreptococcaceae	8.83	3.69	2.178	0.501
Uncultured_bacteria	10.91	0.02	2.565	0.064
Candidatus Arthromitus	1.88	7.28	2.104	0.189
Enterococcus	0.44	2.05	0.556	0.061
Turicibacter	0.80	1.12	0.506	0.469
Uncultured_bacteria_o_Chloroplast	1.37	0.00	0.570	0.144
Faecalibacterium	0.27	0.84	0.364	0.952
Escherichia_Shigella	0.54	0.00	0.201	0.064
77 d				
Lactobacillus	34.35	25.87	7.475	0.345
Uncultured_bacteria	14.00	22.11	5.570	0.380
Romboutsia	18.47	7.19	3.663	0.141
Faecalibacterium	0.66	9.53	2.311	0.064
Turicibacter	5.47	0.80	1.973	1.000
Enterococcus	4.62	1.28	1.222	0.746
Helicobacter	4.62	0.69	1.590	0.666
Ruminococcus torques group	2.25	2.19	0.835	0.466
Bacteroides	0.51[b]	2.85[a]	0.618	0.028
Streptococcus	2.75	0.45	1.266	0.763

[a–b]Means in the same row without the same superscript differ significantly ($P < 0.05$). SEM means standard error of the mean. (n = 8).

Abbreviations: FB, rear on the litter floor fed with basal dietary; FG, rear on the litter floor fed with GOD dietary; GOD, glucose oxidase.

supplementation of GOD had no significant effects on the Shannon index of ileum at the age of 42 d but tended to increase the Shannon index at the age of 77 d. In addition, the representative ileal phylum was *Firmicutes*, which was consistent with the previous research results (Lin et al., 2016). The dietary GOD has a tendency to reduce *Proteobacteria*, which is a sign of intestinal microbiota imbalance (Shin et al., 2015). Many environmental factors and host factors could cause balance disorder in the body, which could induce the increase of *Proteobacteria*. On d 77, *Firmicutes*, *Bacteroidetes,* and *Proteobacteria* were the most abundant bacteria in ileum of BYC. *Bacteroidetes* in the FG group were significantly higher than those in FB group. *Bacteroidetes* are usually the dominant bacteria in animal intestines. At the levels of genus, the supplementation of GOD decreased the abundance of pathogenic bacteria *Escherichia_Shigella* and increased the abundance of *Enterococcus*. Some strains of *Enterococcus* are probiotics, such as *Enterococcus faecium*, which play a role in regulating metabolism, intestinal barrier function, and immune function, but genus *Enterococcus* also includes some

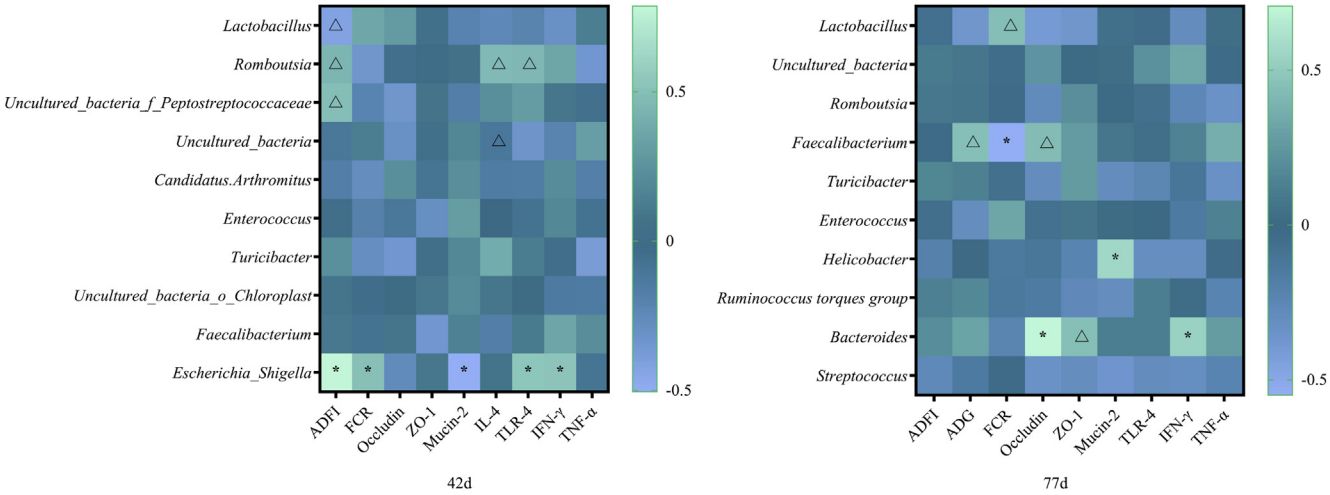

Figure 3. Heatmap of the Pearson rank correlations between the ileal microbiota and growth performance and health parameters of Beijing You Chickens. * means $P < 0.05$; **Δ** means $0.05 < P < 0.1$. Abbreviations: ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio.

opportunistic species of pathogenic bacteria, which usually induce infection in individuals with immunodeficiency (Morrison et al., 1997).

At 77 d of age, compared with the FB group, the abundance of *Bacteroidetes* genus significantly increased in the FG group. *Bacteroidetes* is considered to be one of the most hydrolytic genera of all known genera, which can effectively degrade indigestible carbohydrates and produce short-chain fatty acids(Stanley et al., 2014). Propionic acid produced by *Bacteroidetes* can disturb the intracellular acid–base balance of *Salmonella*, can inhibit its growth, and can mediate the colonization resistance of symbiotic bacteria to *Salmonella* infection(Jacobson et al., 2018). *Faecalibacterium* is one of the main components of intestinal microbiota, in which *Faecalibacterium prausnitzii* is an important butyric acid-producing bacteria in the colon (Ferreira-Halder et al., 2017). The relative abundance of *Faecalibacterium* in the FG group is also higher.

Meanwhile, the abundance of intestinal microorganisms is closely related to the changes of other indicators. The changes of relative abundance of ileal microbiota caused by the supplementation of GOD were closely related to growth performance and other health indexes. The supplementation of GOD decreased the relative abundance of *Escherichia_ Shigella*, which had negative effects on feed utilization and intestinal barrier function of 42-d-old BYC. The relative abundance of *Faecalibacterium* and *Bacteroidetes* increased after the supplementation of GOD, which were beneficial to weight gain, feed utilization, and intestinal barrier function of 77-day-old BYC. Therefore, it is considered that the changes of intestinal microbiota on BYC under litter floor systems caused by GOD are related to the growth performance and intestinal health and play a beneficial role.

CONCLUSIONS

In conclusion, the litter floor systems were beneficial to the development of intestinal barrier junction in the early stage, but the cage systems were more conducive to the growth performance of BYC. The dietary GOD had beneficial effects on the growth performance and the intestinal junction barrier of BYC, especially under the litter floor systems. The dietary GOD could also inhibit the harmful bacteria and promote the beneficial bacteria, which might be related to the improvement of the growth performance and intestinal barrier function.

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DISCLOSURES

The authors declare no conflicts of interest.

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