Research Article

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Yeast culture promotes albumen quality by improving magnum protein secretion and intestinal microbiota in aged laying hens

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Abstract

BACKGROUND: The supplementation of yeast culture (YC) has the potential to enhance egg quality in laying hens. However, most studies focus on eggshell quality. This study aimed to investigate the effects of dietary YC supplementation on the production performance, albumen quality, protein synthesis or secretion of the magnum and the cecal microbiota content of laying hens.

RESULTS: The results showed that dietary YC supplementation increased albumen height and Haugh unit ($P \leq 0.05$). Besides, dietary 100 g kg^{-1} YC addition increased significantly the ridge width of the magnum and the relative expression of SEC23A in the magnum and decreased significantly the relative expression of OVOB in the magnum ($P \leq 0.05$). Furthermore, the abundances of *Butyricicoccus*, *Alistipes* and *Flavonifractor* were increased significantly by 100 g kg^{-1} YC supplementation ($P \leq 0.05$). The diet supplemented with 100 g kg^{-1} YC significantly increased the butyric acid and isobutyric acid of the cecum.

CONCLUSION: Dietary supplementation with YC improved protein secretion in the magnum and enhanced the beneficial cecal microbiota, thus improving the albumen quality of laying hens.

Supporting information may be found in the online version of this article.

Keywords: yeast culture; egg quality; cecal microbiota; magnum; hens

INTRODUCTION

Extending the laying cycles is an effective way to improve the sustainability of laying hen production. Numerous studies have shown that the reproductive tract and intestinal health of aged laying hens are critical factors contributing to the decline in immunity and nutrient absorption, which negatively affects production performance.[1,2] Furthermore, the deterioration of egg quality may be attributed to alterations in the health of the layers.[3-5] As the age of hens increases, a decline in albumen quality is observed, which is linked to decreases in DNA repair, cell proliferation and protein synthesis in the magnum tissues of laying hens.[6] Additionally, structural changes in the magnum, such as the reduction in mucosal folds, epithelial height and glandular width, may further contribute to the thinning of albumen, reflecting a loss of both structural and functional integrity in the magnum mucosa.[7] Alongside these changes in the magnum, intestinal health also plays a significant role in egg quality. Studies have demonstrated that modulating the composition of the intestinal microbiota in hens during the late laying period can improve egg quality.[8]

Gut microbes are essential for maintaining the intestinal barrier, nutrient absorption and protein synthesis.[9] In addition, gut microbes also control the homeostasis of a host by participating in energy metabolism, immune system modification and tissue morphology improvement.[10] The intestinal microbes can spread to the oviduct through the cloaca.[11] Study has shown that *Salmonella* could negatively impact egg quality by altering the expression of genes related to Toll-like receptors, NOD-like receptors and avian β-defensins in the oviduct.[9] Moreover, short-chain fatty acids (SCFAs), which are significant byproducts of gut microbes, play a crucial role in maintaining bone metabolic balance and improving eggshell quality in aged laying hens.[12] Therefore, gut microbiota and their metabolites could be promising targets for improving egg quality. The composition changes of gut

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microbiota during the laying phase in aged hens mainly depend on the most effective nutritional management strategies.

Yeast culture (YC) is a kind of granular ecological product produced by yeast fermentation and culture medium under a specific process. It contains yeast and its culture, rich in proteins, peptides, polysaccharides, vitamins and other substances.[13] YC and its derivatives have a positive impact on egg-laying rate and feed–egg ratio.[14,15] Yeast supplementation can prevent pathogenic bacteria from attaching to the intestinal lining and support the health of the gut microbiome.[16] In studies involving laying hens, Zhang *et al.*[17] found that the addition of YC enhances the egg-laying rate and total egg weight of laying hens by enhancing intestinal enzyme activity and related gene expression. Another study reported that YC increased the height of the control field villus and the content of immunoglobulin A in breeder eggs when facing *Eimeria* challenges.[18] Furthermore, adding YC to the diet can reduce the incidence of enteritis in laying hens by decreasing *Salmonella* levels in the cecum and ovary.[19] In addition, many studies have shown that yeast and its derivatives in diet can enhance the quality of albumen and eggshells.[20,21] Probiotic and yeast supplementation at levels of 1500–2000 g kg^{-1} has been shown to increase egg weight, eggshell strength and albumen height.[22] However, it is still unclear whether the role of YC in enhancing albumen quality and enlarging tissues during the egg production of aged laying hens is linked to its regulatory effects on gut microbiota.

Therefore, the study reported here investigated the impact of dietary YC supplementation on albumen quality, morphology of the magnum, protein secretion of the magnum, cecal content microbial community and SCFAs of laying hens in the late phase of production. The study sought to improve albumen quality by exploring the potential effects of gut microbiota and SCFAs on oviduct functionality.

MATERIALS AND METHODS

Birds and experimental design

The animal protocol for this study was conducted under the management of the Animal Care and Use Committee of the Institute of Feed Research of the Chinese Academy of Agricultural Sciences (ACE-CAAS-20230415). A total of 384 52-week-old Hy-line brown laying hens were randomly divided into 4 groups with 8 replicates of 12 birds each. Egg production and egg quality were assessed and found to be similar across all the replicates before the experiment. The dietary groups were supplemented with YC (the contents of nutrients are listed in Table S1) at 0, 100, 200 and 300 g kg^{-1}. YC was produced by fermentation of a substrate that contained corn germ meal, wheat bran, sugar cane molasses, yeast extract and inorganic salts by *S. cerevisiae* (1.8×10^{18} CFU kg^{-1}) and dried to dry powder.[23] Birds were allocated to 1 cage (45 cm × 45 cm × 45 cm) of 3 birds each and exposed to 16 h of light (20 lx) with temperature maintained between 22 and 25 °C. The basal diet was formulated according to National Research Council (1994) guidelines and the Hy-line layer nutriment requirements (2021) (Table 1). The hens were fed twice a day at 8:30 and 14:00 and had unrestricted access to water. Throughout the study, all hens remained healthy.

Table 1. Composition of the basal diet (air-dry basis, g kg^{-1})

Ingredient	Contents
Corn	610
Soybean meal	256
Soybean oil	18.0
Limestone	87.0
Dicalcium phosphate	17.8
Salt	3.00
Montmorillonite	4.00
Premix[a]	1.50
Phytase[b]	0.300
Choline chloride	1.20
DL-Methionine	1.20
Nutrient levels	
Apparent metabolizable energy (MJ kg^{-1}, calculated)	11.44
Crude protein (analyzed)	16.9
Calcium (analyzed)	3.52
Available phosphorus (calculated)	0.420
Methionine (calculated)	0.340
Methionine + cysteine (calculated)	0.680

[a] Supplied per kilogram of diet: vitamin A, 12 500 IU; vitamin D_3, 4125 IU; vitamin E, 15.0 IU; vitamin K, 2.00 mg; vitamin B_1, 1.00 mg; vitamin B_2, 8.50 mg; vitamin B_6, 8.00 mg; vitamin B_{12}, 5.00 mg; pantothenic acid, 50.0 mg; niacin, 32.5 mg; biotin, 2.00 mg; folic acid, 5.00 mg; choline, 500 mg; manganese, 65.0 mg; iodine, 1.00 mg; iron, 60.0 mg; copper, 8.00 mg; zinc, 66.0 mg.
[b] Unit of phytase is 10 000 IU g^{-1}.

Sample collection

For 3 consecutive days, 5 eggs per replicate were collected and albumen quality tested within 24 h at the end of weeks 6, 9 and 12. In addition, we evaluated the laying performance and egg quality parameters. Based on the results, the dietary supplementation of 100 g kg^{-1} YC demonstrated the most significant improvements. Consequently, the hens from both the control group and those supplemented with 100 g kg^{-1} YC were euthanized and dissected at the end of week 12. The middle portion of the magnum was fixed in 3.33 mol L^{-1} formaldehyde solution. Then, mucosa samples of magnum were collected and stored at −80 °C for mRNA analysis. The cecal content from each bird was gathered and placed into liquid nitrogen, then stored at −80 °C for analysis of microbial composition and SCFA analysis. At the end of week 12 of the experiment, five eggs from each replicate were collected for egg storage analysis. These eggs were subsequently evaluated after being stored at 25 °C for durations of 7, 14 and 21 days.

Laying performance

Daily records were maintained for egg production (quantity and weight) per replicate. Feed intake was measured weekly for each replicate. Average egg weight, egg production, average daily feed intake and feed conversion ratio were computed for the intervals of weeks 1–4, weeks 5–8, weeks 9–12 and the overall period of weeks 1–12.

Albumen quality

Albumen height, yolk color and Haugh unit were measured using an egg analyzer (Israel Orka Food Technology Ltd, Ramat Hasharon, Israel).[6] The yolk was isolated and measured directly. The albumen weight was egg weight minus yolk weight and eggshell weight. The albumen pH was measured with a pH-STAR (Matthäus GmbH & Co., Eckelsheim, Germany).

Table 2. Sequences for RT-PCR primers

Gene	Primer sequence[a]	Product size (bp)
β-Actin	F: GAGAAATTGTGCGTGACATCA	152
	R: CCTGAACCTCTCATTGCCA	
OVOA	F: TGTGTTTCTGTCTGCGGTTG	182
	R: ATTTTGCGTACGAAGCCCTC	
OVOB	F: TGCGTCTACCAAGCCTGTAA	177
	R: TGCCTGAGTGTTGTAGCTGA	
OVALX	F: AGTGTACCTGCCCCAAATGA	192
	R: CCTGCCATCTCAATGCCATC	
OVAL	F: CCCCATTGCCATCATGTCAG	225
	R: AGTCTACTGGCAAGGCTGAA	
Sec13	F: CTGGACATGCGATGATGCCTCTG	238
	R: CTGCTCATTCTGCTGCCCTTCTG	
Sec23A	F: TGGACCCTGCGTATCTCTGAACTC	357
	R: CCCTCTTCTGTCTCCGCCCTATAC	
POMT1	F: CTGGTCCACGGCATCACAACTC	327
	R: TTCCACATTCCACACCATGCTCTG	
POMT2	F: GCCTCATCTTGCTTCCGCTCAC	375
	R: CTCCATGCCTCACAAACTCCACAG	

[a] F, forward; R, reverse.
OVOA, ovomucin, alpha subunit; *OVOB*, ovomucin, beta subunit; *OVALX*, ovalbumin-related protein X; *OVAL*, ovalbumin; *SEC13*, SEC13 homolog, nuclear pore and COPII coat complex component; *SEC23A*, SEC23 homolog A, COPII coat complex component; *POMT1*, protein-O-mannosyltransferase 1; *POMT2*, protein-O-mannosyltransferase 2.

Magnum histomorphology

Each magnum sample was sectioned into 5 μm slices and subsequently subjected to staining with hematoxylin–eosin for the purpose of histological examination.[6] Ridge height, ridge width and diameter of the tubular gland of oviducal magnum were observed and measured using an optical microscope (Nikon Co. Ltd, Tokyo, Japan).

Magnum gene expression analysis

Total mRNA was extracted using an EasyPure® RNA Kit (Trans Gen Biotech Co. Ltd, Beijing, China) from frozen magnum mucosa (20 mg tissue; $n = 8$ hens per group). The purity and concentration of the total RNA were measured with a NanoDrop™ One/OneC (Thermo Fisher Scientific Inc., MA, USA). The cDNA samples were obtained by reverse transcription of the total RNA using a first-strand synthesis kit (TransGen Biotech Co. Ltd, Beijing, China).[24] The relative expression of the genes was assessed utilizing an iCycler iQ multicolor RT-PCR system (Bio-Rad Laboratories, Hercules, CA, USA). *β*-Actin was used as the internal reference gene. Primer sequences are presented in Table 2. The reaction conditions were as follows: 50 °C for 2 min, 95 °C for 10 min; 40 cycles of 95 °C for 15 s, 60 °C for 1 min. For the calculation of the relative expression value of genes, the $2^{-\Delta\Delta Ct}$ method was used.[25]

Sequencing of cecal content microbiota

The methodology employed in this study was derived from and consistent with prior research.[26] Microbial DNA was extracted from cecal content (20 mg of chyme; $n = 8$ hens per group) using an E.Z.N.A. Soil Kit (Omega Bio-tek, Norcross, GA, USA). DNA concentrations were measured with a NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA) and 10 g L^{-1} agarose gel electrophoresis. Using the obtained DNA as a template, the v3–v4 variable region of the 16S rRNA gene was amplified by PCR. The PCR was performed using the following cycle conditions: 2 min at 95 °C (denaturation), 25 cycles at 95 °C for 30 s (denaturation), 30 s for annealing at 55 °C, 30 s of extension at 72 °C, with a final extension at 72 °C for 5 min. PCR products were extracted from a

Table 3. Effects of dietary yeast culture on production performance of laying hens[a]

Item	Time	Control	Yeast culture			SEM[b]	*P*[c]		
			100 g kg^{-1}	200 g kg^{-1}	300 g kg^{-1}		A	L	Q
AEW (g)	1–4 wk	62.4	62.7	62.5	62.7	0.171	0.904	0.648	0.895
	5–8 wk	61.9	61.7	61.4	62.0	0.187	0.765	0.928	0.657
	9–12 wk	61.5	61.1	61.0	61.2	0.210	0.869	0.588	0.696
	1–12 wk	62.0	61.9	61.9	62.0	0.158	0.994	0.888	0.962
EP (%)	1–4 wk	91.3	91.9	90.5	91.3	0.512	0.846	0.787	0.961
	5–8 wk	88.5	91.7	88.0	90.7	0.643	0.123	0.603	0.862
	9–12 wk	83.1	86.9	83.6	86.7	0.811	0.211	0.325	0.604
	1–12 wk	87.6	90.2	87.8	89.8	0.524	0.197	0.393	0.674
ADFI (g)	1–4 wk	116	117	115	117	0.414	0.256	0.735	0.710
	5–8 wk	109	111	108	109	0.625	0.263	0.559	0.822
	9–12 wk	103	103	102	101	0.827	0.791	0.308	0.593
	1–12 wk	110	110	107	107	0.563	0.196	0.068	0.185
FCR (g g^{-1})	1–4 wk	2.03	2.04	2.01	2.05	0.010	0.525	0.715	0.637
	5–8 wk	2.00	1.96	1.99	1.95	0.013	0.639	0.338	0.636
	9–12 wk	2.04	1.97	2.02	1.93	0.020	0.229	0.137	0.337
	1–12 wk	2.01	2.00	1.98	1.99	0.010	0.684	0.374	0.502

[a] Eight replicates were set for each treatment.
[b] SEM is standard error of the mean.
[c] A represents one-way ANOVA and Duncan's multiple comparisons; L and Q represent linear and quadratic analysis using regression analysis, respectively.
AEW, average egg weight; EP, egg production; ADFI, average daily feed intake of hen per day; FCR, feed conversion ratio.

Table 4. Effects of dietary yeast culture on albumen quality of laying hens[a]

Item	Time	Control	Yeast culture			SEM[b]	*P*[c]		
			100 g kg^{-1}	200 g kg^{-1}	300 g kg^{-1}		A	L	Q
Egg weight (g)	wk 6	60.4	61.1	61.5	61.0	0.209	0.382	0.248	0.216
	wk 9	61.1	61.4	60.6	60.5	0.253	0.588	0.305	0.549
	wk 12	60.5	60.3	60.2	61.2	0.311	0.721	0.530	0.535
Albumen height (mm)	wk 6	6.52	6.79	7.16	6.67	0.088	0.055	0.302	0.051
	wk 9	6.75^{b}	7.60^{a}	7.59^{a}	7.43^{a}	0.119	0.025	0.057	0.012
	wk 12	7.21^{b}	7.79^{a}	7.55^{ab}	7.54^{ab}	0.068	0.018	0.230	0.037
Haugh unit	wk 6	79.3^{b}	80.7^{ab}	83.2^{a}	78.7^{b}	0.613	0.035	0.899	0.050
	wk 9	80.6^{b}	85.7^{a}	84.9^{a}	85.0^{a}	0.701	0.029	0.043	0.023
	wk 12	83.3^{b}	86.4^{a}	85.2^{a}	85.7^{a}	0.374	0.017	0.073	0.037

Mean values with different lowercase letters in a row indicate significant differences ($P \leq 0.05$).
[a] Eight replicates were set for each treatment.
[b] SEM is standard error of the mean.
[c] A represents one-way ANOVA and Duncan's multiple comparisons; L and Q represent linear and quadratic analysis using regression analysis, respectively.

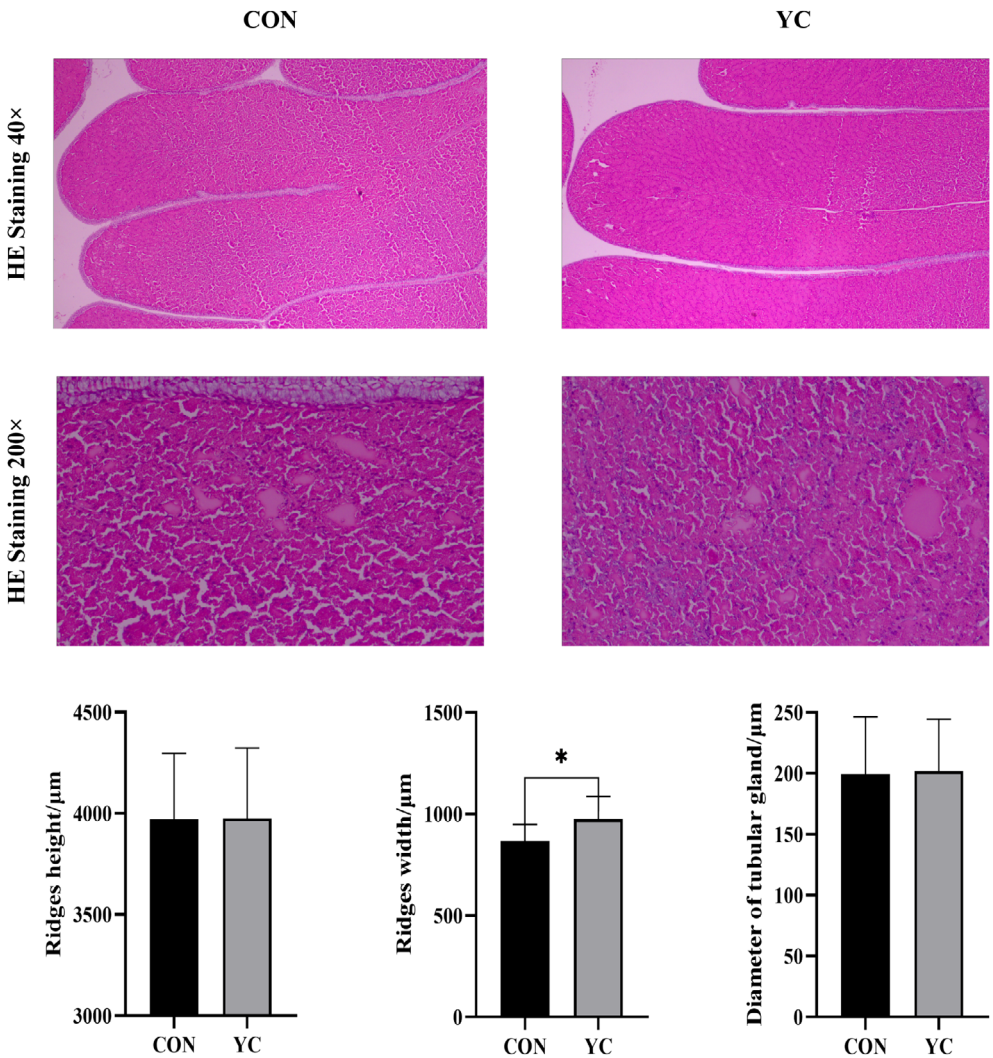

Figure 1. Effects of dietary YC on magnum morphology of laying hens ($n = 8$). CON, control; YC, yeast culture (control + yeast culture addition at 100 g kg^{-1}). *$P < 0.05$.

20 g L^{-1} agarose gel and purified using an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) in accordance with the guidelines provided by the manufacturer. Purified amplicons were assessed for quality and subsequently subjected to paired-end sequencing (2 × 300 bp) using the Illumina MiSeq platform (Illumina, San Diego, USA).

SCFSs of cecal content

A total of 500 mg of cecal content samples ($n = 8$ hens per group) was diluted with 2.5 mL of distilled water, followed by centrifugation at 13 800 × *g* for 10 min. Subsequently, 1 mL of supernatant was transferred to a new centrifuge tube, to which 50 µL of perchloric acid was added. The mixture was then subjected to centrifugation under identical conditions for a duration of 3 h.[27] The supernatant was subsequently discarded and subjected to filtration using a membrane, followed by extraction with ethanol. Acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid and valeric acid were detected using an ultra-performance liquid chromatography I-class system and mass spectrometer (Waters, Milford, MA, USA). The assessment of SCFAs was carried out following previously established methods.[28]

Statistical and bioinformatics analyses

The laying performance, egg quality, SCFAs of cecal digesta and abundances of cecal content microbiota were assessed using SPSS 19.0 (IBM SPSS Statistics 20; SPSS Inc., Chicago, IL, USA). The performance metrics related to the date of laying performance and egg quality were evaluated through one-way analysis

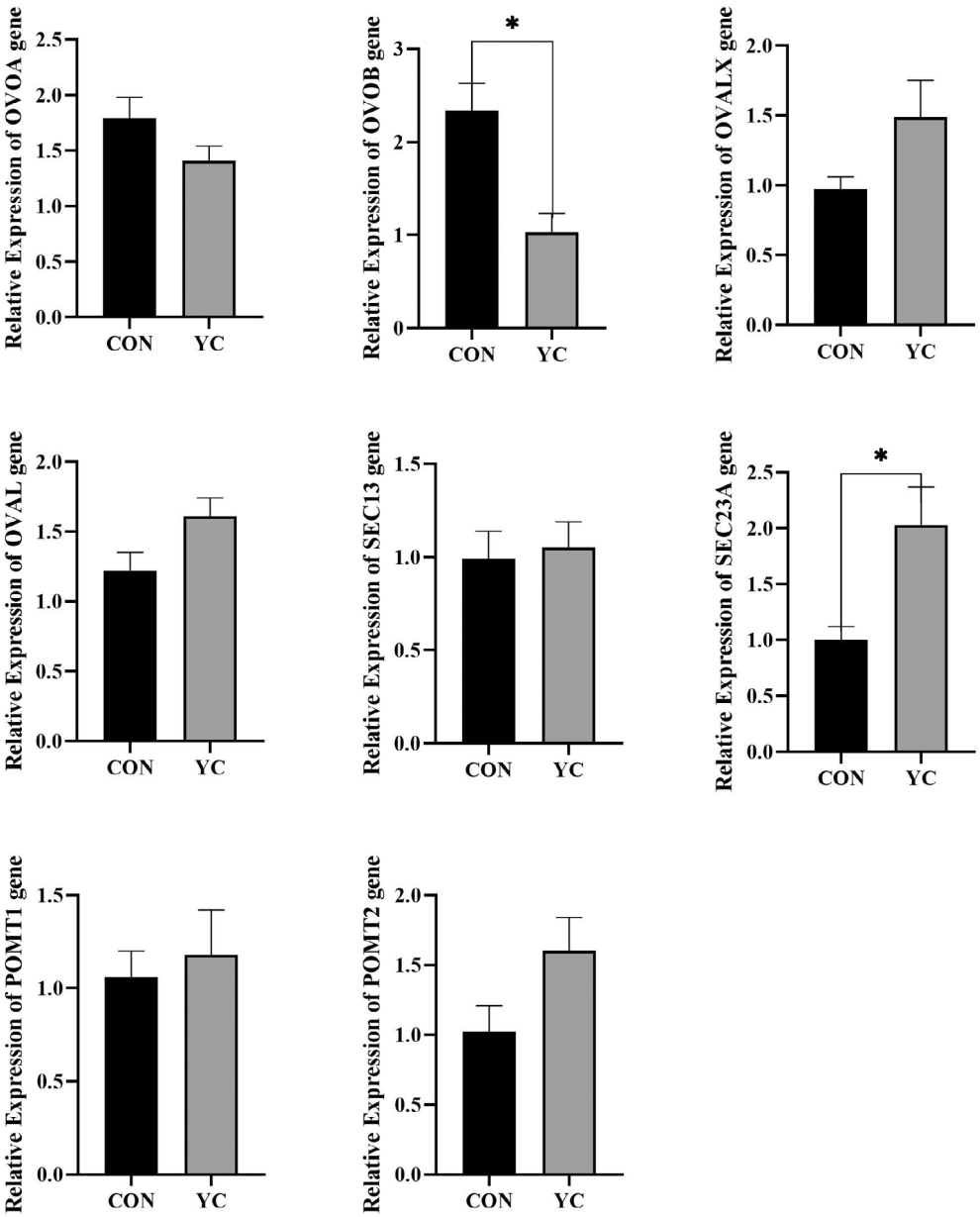

Figure 2. Effects of dietary YC on the relative expression of different genes in the magnum of laying hens ($n = 8$). CON, control; YC, yeast culture (control + yeast culture addition at 100 g kg^{-1}). *OVOA*, ovomucin, alpha subunit; *OVOB*, ovomucin, beta subunit; *OVALX*, ovalbumin-related protein X; *OVAL*, ovalbumin; *SEC13*, SEC13 homolog, nuclear pore and COPII coat complex component; *SEC23A*, SEC23 homolog A, COPII coat complex component; *POMT1*, protein-O-mannosyltransferase 1; *POMT2*, protein-O-mannosyltransferase 2. *$P < 0.05$.

Table 5. Effects of dietary yeast culture on storage period albumen quality of laying hens[a]

Item	Time	Control	Yeast culture			SEM[b]	*P*[c]		
			100 g kg^{-1}	200 g kg^{-1}	300 g kg^{-1}		A	L	Q
Albumen height (mm)	D7	4.52	4.81	4.61	4.58	0.091	0.722	0.965	0.701
	D14	2.67^{b}	2.97^{ab}	3.23^{a}	2.71^{b}	0.081	0.044	0.618	0.031
	D21	2.45	2.51	2.64	2.61	0.066	0.758	0.329	0.595
Haugh unit	D7	61.7	62.8	61.9	61.7	0.640	0.915	0.848	0.859
	D14	38.5	40.2	44.8	35.3	1.34	0.079	0.689	0.100
	D21	36.1	37.6	37.6	35.1	1.13	0.845	0.767	0.663
Albumen ratio, 100 g g^{-1}	D7	63.0	63.0	64.3	64.1	0.274	0.205	0.069	0.193
	D14	61.1	62.3	61.8	62.2	0.237	0.233	0.184	0.304
	D21	59.3	61.4	60.6	59.5	0.380	0.164	0.940	0.109
Albumen pH	D7	8.63	8.55	8.49	8.56	0.019	0.081	0.098	0.010
	D14	8.75	8.56	8.61	8.70	0.033	0.167	0.863	0.137
	D21	8.73	8.73	8.78	8.73	0.021	0.774	0.832	0.841

Mean values with different lowercase letters in a row indicate significant differences ($P \leq 0.05$).
[a] Eight replicates were set for each treatment.
[b] SEM is standard error of the mean.
[c] A represents one-way ANOVA and Duncan's multiple comparisons; L and Q represent linear and quadratic analysis using regression analysis, respectively.

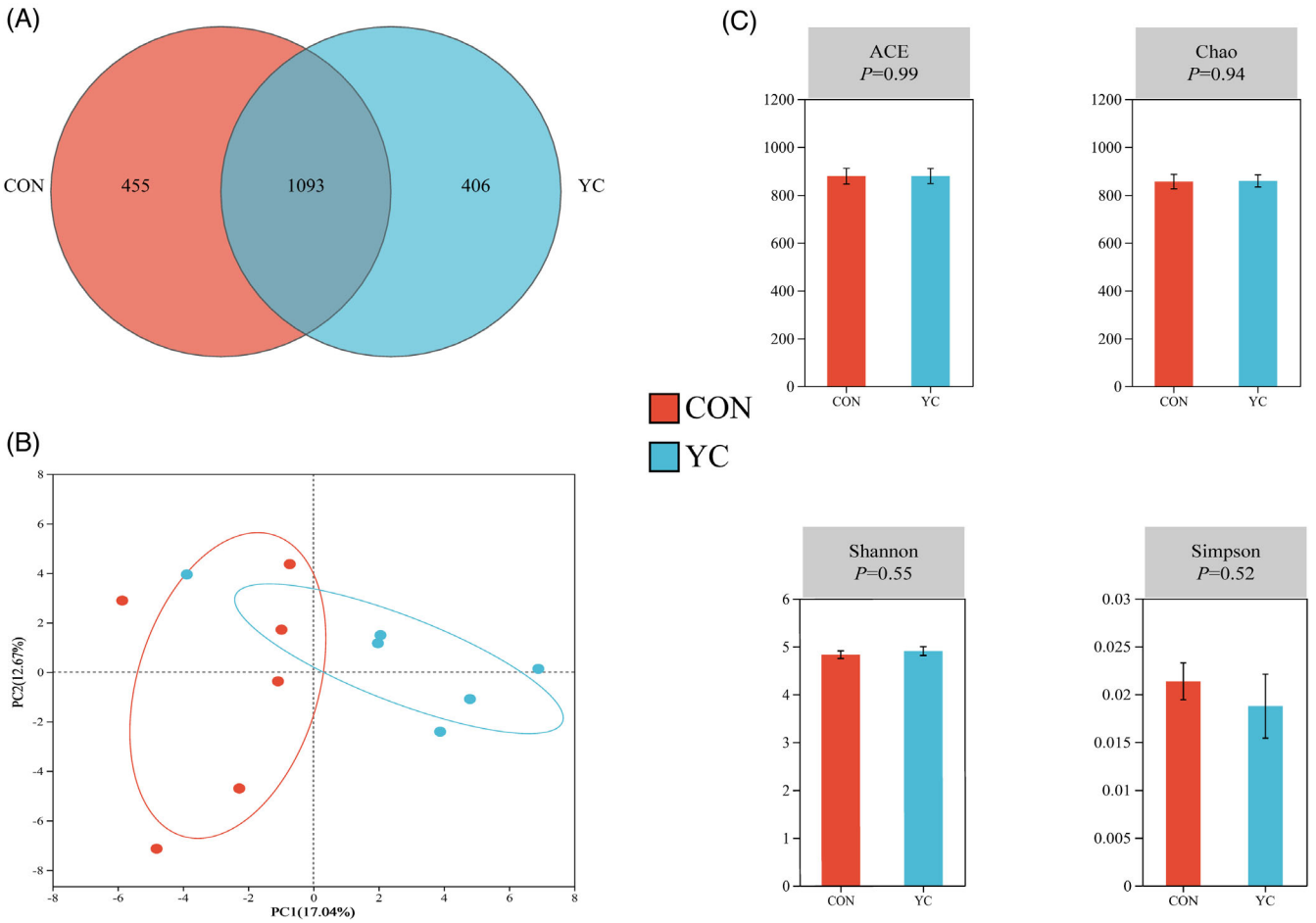

Figure 3. Venn graph of cecal microbial community (A). Alpha (α) and Beta (β) diversity analysis of cecal digesta microbiota from laying hens (B, C). CON, control; YC, yeast culture (control + yeast culture addition at 100 g kg^{-1}).

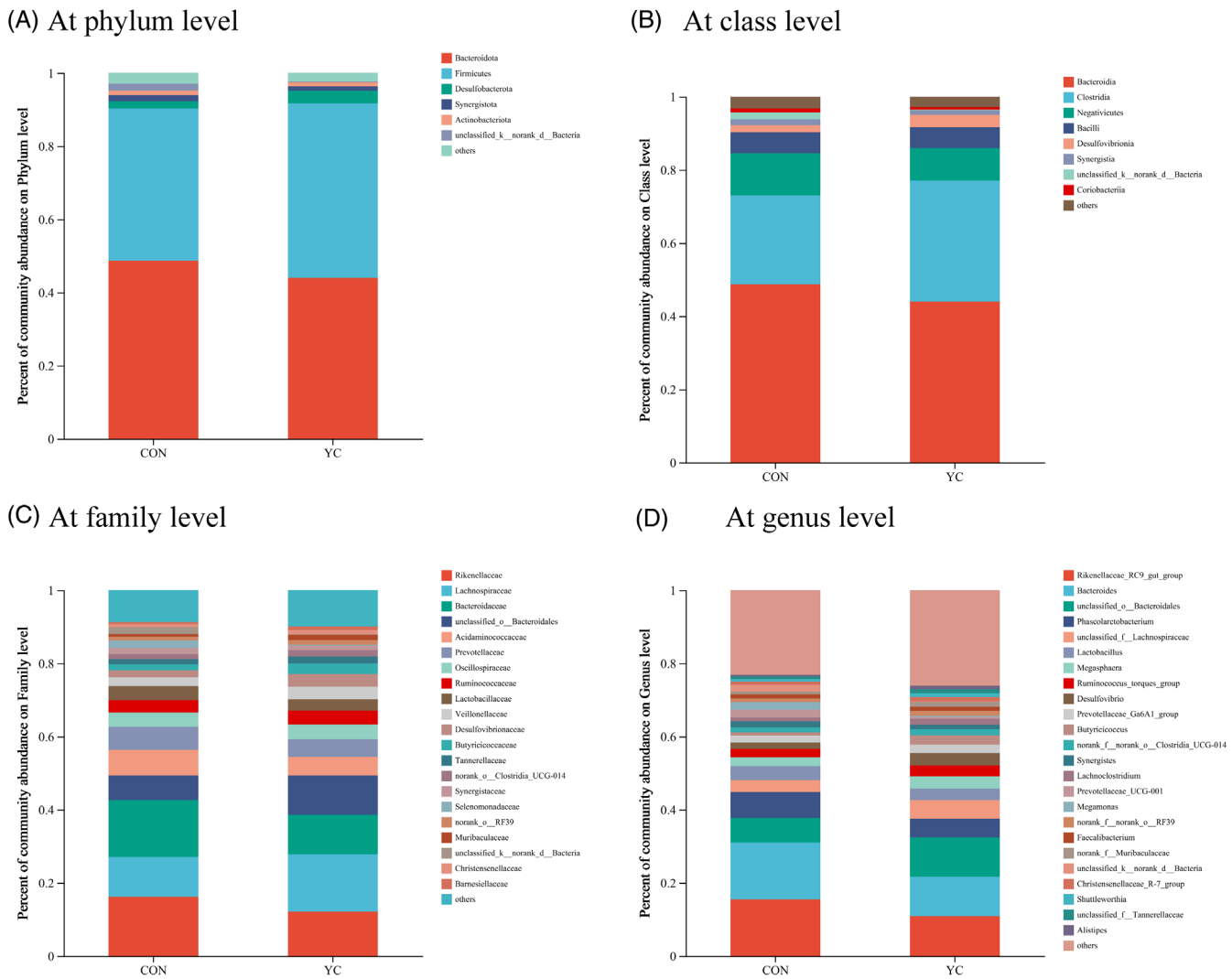

Figure 4. Relative abundance of cecal digesta microbiota from laying hens at phylum level (A), at class level (B), at family level (C) and at genus level (D). CON, control; YC, yeast culture (control + yeast culture addition at 100 g kg^{-1}).

of variance (ANOVA), with subsequent comparisons of differences made using Duncan's multiple range test. A significance level of $P \leq 0.05$ was established, while a range of $0.10 < P < 0.05$ was interpreted as indicative of a significant trend. Results are presented as means accompanied by the standard error of the mean. The magnum morphology, the relative expression of genes in the magnum, SCFAs of cecal content data and abundances of cecal content microbiota were analyzed by the *t*-test method, and the results were expressed as mean value and standard deviation.

Raw fastq files were demultiplexed and quality-filtered using Quantitative Insights into Microbial Ecology 2. Sequencing and bioinformatics analyses were conducted utilizing QIIME 2 (Version 2019.4), with the sequencing outcomes evaluated based on amplicon sequence variants. Two oblivious outliers in two groups were deemed potentially disruptive to the statistical analysis of the microbiota and were therefore excluded from subsequent analyses. Beta diversity was assessed through the application of principal component analysis (PCA). To measure microbial richness and evenness, various alpha diversity metrics were computed, including the Chao 1 estimator, the count of observed species, the Shannon index and the Simpson index. The Kruskal–Wallis test was employed to identify the biological differential taxa among groups and the *P* value for false discovery rate. The PCA was conducted for the relationship between Haugh unit, albumen height, SCFAs and microbial composition of the cecum (Origin Pro, Version 2021, Origin Lab Corporation, Northampton, MA, USA). In addition, Haugh unit, albumen height, magnum gene expression levels and SCFAs of the cecum were also analyzed.

RESULTS

Laying performance

There were no significant effects of dietary YC supplementation ($P > 0.05$) on the average egg weight, egg production and feed conversion ratio throughout all periods of the experiment (Table 3). During the whole experiment period, there was a linear decrease ($P = 0.07$) in average daily feed intake with the elevated levels of YC during the whole experiment period.

Albumen quality

With regard of albumen quality, there was a quadratic increase ($P \leq 0.05$; Table 4) in albumen height and Haugh unit with the increasing addition of YC during the whole experiment period. Compared with the control group, dietary YC supplementation at 100–300 g kg^{-1} increased ($P \leq 0.05$) albumen height and Haugh unit at the end of week 9, and increased Haugh unit at the end of week 12.

Magnum histomorphology

According to the production performance and egg quality results, the magnum of the control group and the group having diet supplemented with 100 g kg^{-1} YC were subjected to histomorphological investigation (Fig. 1). The mucosal folds of the magnum were arranged by the columnar cells. The mucosal folds were closely arranged and normal structure with the addition of YC (Fig. 1). Moreover, YC addition significantly deepened mucosal fold width in the magnum of laying hens when compared with the control group ($P \leq 0.05$).

Gene expression of the magnum

There were no significant effects of dietary YC addition on ovomucin (*OVOA*), ovalbumin-related protein X (*OVALX*), ovalbumin (*OVAL*), SEC13 homolog (*SEC13*), protein-O-mannosyltransferase 1 (*POMT1*) or protein-O-mannosyltransferase 2 (*POMT2*) in the magnum ($P > 0.05$, Fig. 2). Compared with the control group, the relative gene expression of beta subunit in ovomucin (*OVOB*) in the magnum of laying hens was decreased significantly for the diet supplemented with YC, but the relative gene expression of SEC23 homolog A (*SEC23A*) in the magnum increased significantly ($P \leq 0.05$).

Storage period albumen quality

Compared with the control group, the diet supplemented with 200 g kg^{-1} YC significantly increased the albumen height for D14 ($P \leq 0.05$; Table 5). There was an increasing trend in the Haugh unit for D14 ($P = 0.08$) and the pH value of albumen stored for D7 ($P = 0.08$).

Diversity and composition of cecal microbiota

In microbiota analysis, 1093 common microorganisms from the control group and the group with diet supplemented with 100 g kg^{-1} YC were detected in the cecum (Fig. 3(A)). In addition, the number of unique microbiotas in the control group and YC group were 455 and 406, respectively. No change occurred in species richness (as reflected by ACE and Chao) or alpha diversity (as reflected by Shannon and Simpson indices; Fig. 3(C)). The PCA results showed the two groups occupied distinct positions (Fig. 3(B)), revealing the cecum microbial communities of laying hens fed diets with YC addition. Bacteroidota and Firmicutes were the dominant phyla in the control and YC groups, accounting for more than 90% of the whole phyle (Fig. 4(A)). At the genus level, *Butyricicoccus*, *Alistipes* and *Flavonifractor* were significantly increased in the YC group ($P \leq 0.05$; Table 6). Moreover, *Bacteroides* and *Fusobacterium* showed an increasing trend in the YC group compared with the control group ($0.10 < P < 0.05$).

SCFAs of cecal content

Compared to the control group, the dietary supplementation of 100 g kg^{-1} YC significantly elevated the concentrations of butyric acid and isobutyric acid in the cecal content ($P \leq 0.05$; Table 7). Acetic acid, propionic acid, valeric acid and isovaleric acid of cecal content were increased, but not significantly so, with 100 g kg^{-1} YC addition.

Correlation analysis

To explore the microbiota associated with albumen quality, gene expression and SCFAs, correlation analysis was conducted to assess the relationships between bacterial abundance and various phenotypic traits in this study (Fig. 5). The results revealed that albumen height and the mRNA expression of *SEC23A* exhibited a positive correlation with the abundances of *Butyricicoccus*, *Alistipes* and *Flavonifractor* ($P \leq 0.05$), and exhibited a positive correlation with the abundance of *Bacteroides* ($P \leq 0.05$). Additionally, the Haugh unit and the mRNA expression of *POMT2* were negatively correlated ($P \leq 0.05$) with the abundance of *Bacteroides* and positively correlated ($P \leq 0.05$) with the abundance of

Table 6. Differences of microbial distribution in cecal digesta between the control and yeast culture groups (percentage of total sequences)[a]

Item	Control	100 g kg^{-1} YC	SEM[b]	*P*
Phyla				
Bacteroidota	48.7	44.0	6.72	0.296
Firmicutes	41.6	47.7	6.08	0.231
Classes				
Bacteroidia	48.7	44.0	6.72	0.296
Clostridia	24.4	33.1	6.25	0.120
Fusobacteriia	0.038	0.062	0.011	0.083
Families				
Rikenellaceae	16.2	12.2	6.77	0.900
Lachnospiraceae	10.9	15.7	3.07	0.052
Bacteroidaceae	15.5	10.8	3.56	0.102
Muribaculaceae	0.750	1.372	0.337	0.034
Anaerovoracaceae	0.391	0.992	0.364	0.056
Clostridiaceae	0.002	0.022	0.010	0.038
Genera				
Bacteroides	15.5	10.8	3.56	0.120
Butyricicoccus	0.784	2.57	0.892	0.011
Alistipes	0.482	1.03	0.257	0.035
Flavonifractor	0.083	0.334	0.022	0.016
Fusobacterium	0.038	0.062	0.088	0.086

[a] Eight replicates were set for each treatment.
[b] SEM is standard error of the mean.

Table 7. Effects of dietary yeast culture on SCFAs in cecal chyme of laying hens (mg g^{-1})[a]

Item	Control	100 g kg^{-1} YC	SEM[b]	*P*
Acetic acid	1785	2198	202	0.060
Propionic acid	940	1259	159	0.066
Butyric acid	491	860	169	0.047
Isobutyric acid	136	205	29.5	0.043
Valeric acid	161	232	37.4	0.078
Isovaleric acid	82.2	124	20.7	0.059

[a] Eight replicates were set for each treatment.
[b] SEM is standard error of the mean.

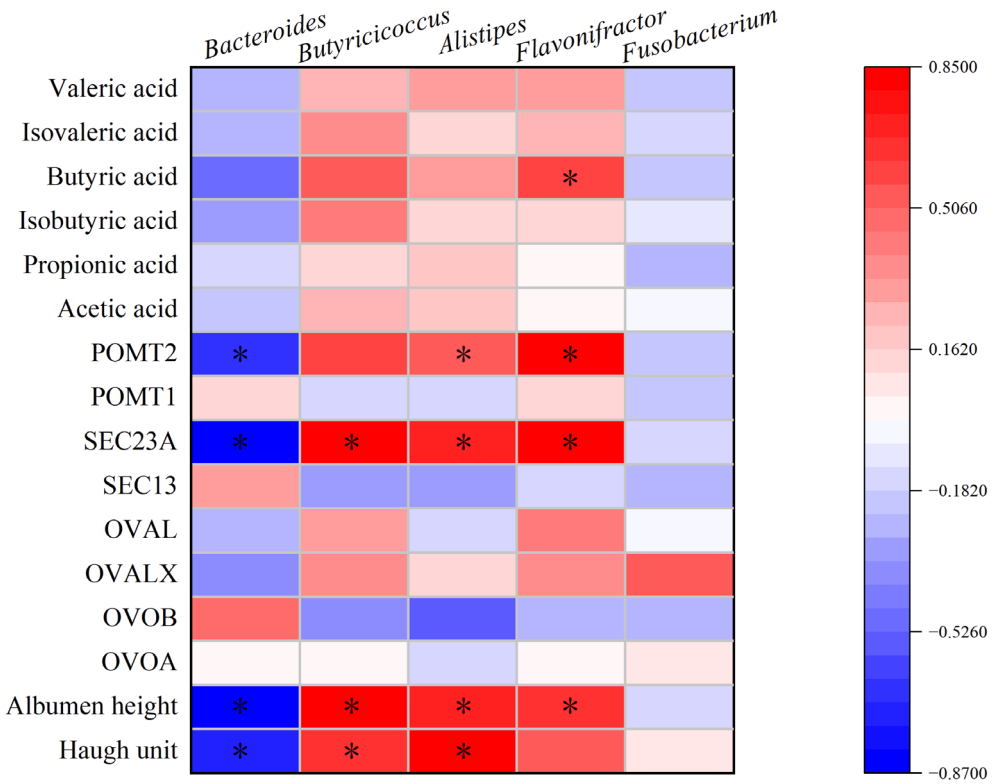

Figure 5. Pearson's correlation analysis between the abundances of cecal microbiota and albumen quality, magnum characteristics or SCFAs. The intensity of the colors represents the degree of association. *$P < 0.05$.

Alistipes. There was a positive correlation ($P \leq 0.05$) between the abundances of *Butyricicoccus* and *Flavonifractor*, and Haugh unit and the mRNA expression of *POMT2*, respectively. Simultaneously, butyric acid demonstrated a positive correlation ($P \leq 0.05$) with the abundance of *Flavonifractor*.

DISCUSSION

As laying hens age, the egg albumen quality declines, which exacerbates the challenges during shelf life.[5] This is one of the key issues affecting both the economic efficiency of the egg industry and food security. Previous studies have indicated that YC, due to its specific components (such as protein and amino acids, vitamins and minerals), has the potential to improve egg albumen quality in young hens.[29-32] Consistent with previous studies, our findings show that dietary supplementation with YC also improves albumen quality in aged hens, as demonstrated by increased albumen height and Haugh unit. Albumen height is an important indicator of egg freshness, and it generally decreases with storage due to factors such as moisture migration and protein degradation.[33,34] Our results suggest that YC supplementation can help mitigate the deterioration of albumen quality during storage, as shown by the increased albumen height observed on day 7 of storage. This benefit may be attributed to the functional components in YC, such as polysaccharides, peptides and vitamins, which have been reported to enhance antioxidant status and protein stability.[29,32] By maintaining protein integrity and functional structure, YC supplementation may help slow the degradation process, thus mitigating albumen quality deterioration during storage. Taken together, dietary supplementation with YC could improve egg albumen quality while mitigating its quality decline during storage.

Albumen is primarily formed in the magnum, and the integrity of this tissue is a prerequisite for albumen protein synthesis and transport.[35] The histomorphology of the magnum was first determined to analyze the potential mechanism by which YC improves albumen quality. The mucosal folds and tubular gland morphology in the magnum play a crucial role in albumen protein secretion.[36] This study found that the inclusion of dietary YC improved the closely arranged and normal structure of the magnum, which may enhance the secretion and deposition of albumen proteins.[6] Additionally, the addition of YC enlarged the mucosal fold width of the magnum, which may provide more space for albumen protein secretion. Thus, dietary YC addition may improve magnum morphology to facilitate albumen protein synthesis and secretion, thereby contributing to enhanced albumen quality. Furthermore, this study investigated the mechanisms by which YC supplementation enhances albumen quality, focusing on gene expression related to protein synthesis (*OVOA*, *OVOB*, *OVALX*, *OVAL*), secretion (*SEC13*, *SEC23A*) and glycosylation (*POMT1*, *POMT2*) of the magnum.[37-39] The *SEC13* and *SEC23A* genes have been associated with the secretion of collagen and proteoglycans,[40,41] and the upregulation of the *SEC23A* gene in the magnum may suggest that dietary YC supplementation promotes protein secretion during albumen formation, thereby improving albumen height and Haugh unit. Consistently, Chang *et al.* also suggested that the increased expression of *SEC23A* could improve albumen quality by enhancing albumen height and Haugh unit, and this improvement may reduce the hardness, stickiness and chewiness of the albumen, thereby enhancing its

processing characteristics.[42] Additionally, dietary supplementation with YC decreased the expression level of the *OVOB* gene, which encodes *β*-ovomucin, a major component of albumen. However, it is important to note that this change did not impair albumen quality. This could be because albumen height not only is related to protein levels but also depends on the glycosylation levels of the proteins.[43-45] Glycosylation of albumen proteins could increase their hydrophobic surface, which promotes hydrophobically driven native-like aggregation, thereby affecting the internal structure of the albumen.[45] In this study, the addition of YC resulted in a numerical increase in the expression level of a glycosylation-related gene (*POMT2*). This may partially explain the improvement in albumen quality in the YC group, warranting further investigation. Therefore, YC may primarily enhance albumen quality by upregulating the *SEC23A* gene, which promotes protein secretion in the magnum, thus improving eggshell quality.[39,46]

Our results showed that dietary supplementation with YC improved albumen quality in aged laying hens. This improvement may be attributed to the modulation of gut microbiota composition, particularly the increased relative abundances of *Bacteroides*, *Butyricicoccus*, *Alistipes* and *Flavonifractor*. *Bacteroides* and Firmicutes are known to participate in nutrient metabolism and promote intestinal absorption, thereby supporting production performance and egg quality.[12,46,47] Firmicutes also play a critical role in regulating intestinal pH and increasing the bioavailability of nutrients via SCFA production.[48] In particular, the abundance of *Flavonifractor* was significantly increased in the YC group, which is notable because this genus metabolizes flavonoids into acetic acid and butyric acid.[49] In this study, the observed elevations in acetic acid, propionic acid and butyric acid levels indicate that YC supplementation enhanced microbial fermentation activity. These SCFAs are key regulators of intestinal health; for example, butyric acid has been shown to enhance intestinal barrier function by promoting epithelial cell proliferation and differentiation, maintaining tight junction integrity and facilitating nutrient absorption.[46,50,51] Improved nutrient absorption contributes to the overall metabolic status of laying hens and may enhance protein secretion in the magnum, which is responsible for albumen synthesis. Moreover, we observed positive correlations between *Flavonifractor* and *Alistipes* and the expression of protein secretion and glycosylation genes (*SEC23A*, *POMT2*), suggesting a possible link between gut microbiota-derived metabolites and protein secretion processes in the magnum. Therefore, YC supplementation may improve albumen quality not only through direct nutritional effects but also by modulating the intestinal microbiota and increasing SCFA production, which in turn supports intestinal health, nutrient utilization and reproductive tract function.

CONCLUSIONS

In summary, the addition of YC into the diet of aged laying hens has the potential to enhance albumen quality by enhancing the beneficial microbiota and promoting the production of SCFAs. Furthermore, it may play a role in maintaining the integrity of magnum tissues and optimizing protein secretion functions. These findings may contribute to the production of high-quality eggs through the YC, and provide new ideas for exploring the nutritional strategies aimed at improving albumen quality.

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CONFLICT OF INTEREST

The authors confirm that there is no any conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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