

METABOLISM AND NUTRITION

Yeast culture promotes the production of aged laying hens by improving intestinal digestive enzyme activities and the intestinal health status

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ABSTRACT Yeast culture (YC) positively affects the performance of laying hens. The purpose of the present study was to explore the underlying mechanism for the YC-mediated performance improvement. Sixty 67-week-old Hy-Line Brown laying hens were randomly allocated into 2 experimental groups with 5 replicates of 6 birds each. One group was fed a control diet, whereas the other received the control diet supplemented with YC at 3.0 g/kg; treatment lasted for 8 wk. The results showed that dietary YC supplementation increased ($P < 0.05$) the total egg weight (11.2–13.6%) and egg-laying rate (13.0–13.5%) but decreased ($P < 0.05$) the feed/egg ratio by 9.3 to 11.0% during weeks 5 to 6 and 7 to 8 compared with the control. However, egg quality, including eggshell strength, eggshell thickness, egg weight, albumen height,

egg yolk color, and Haugh unit, was not affected ($P > 0.05$) by YC supplementation. Furthermore, dietary YC supplementation increased ($P < 0.05$) chymotrypsin and α -amylase activities by 54.8 to 62.5% in the duodenal chyme and reduced ($P < 0.05$) plasma endotoxin by 44.1%. YC dietary supplementation also upregulated ($P < 0.05$) the mRNA levels of intestinal barrier-related genes (occludin and claudin 1) and antimicrobial peptides genes (β -defensin 1 and 7 and cathelicidin 1 and 3) in the duodenum or jejunum compared with the control. In conclusion, dietary YC supplementation improved the performance of aged laying hens, potentially through the upregulation of intestinal digestive enzyme activities and intestinal health-related gene expression.

Key words: yeast culture, aged laying hen, performance, egg quality, intestinal health

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INTRODUCTION

Older laying hens present several problems for the poultry industry, including decreased performance and egg quality (Bar et al., 1999). The number of broken eggs from aged laying hens increases by 7.5%, an amount that leads to great economic losses (Roland, 1988). A previous study showed that reduced intestinal health in aged laying hens, which causes digestion, absorption, and immune problems, is one of the major reasons for

altered laying performance (Jing et al., 2014). Therefore, poultry producers are constantly pursuing solutions to enhance intestinal health, improve laying performance, and extend the laying period in aged laying hens. Feeding poultry various microorganisms to enhance intestinal health and thus improve performance is one strategy that has attracted prominent attention (Shamsi et al., 2015; Forte et al., 2018; Özsoy et al., 2018; Adetunji and Adejumo, 2019).

Yeast culture (YC) is a natural yeast fermentation product that comprises a variety of biologically active substances, including yeast cells, vitamins, peptides, amino acids, proteins, peptides, organic acids, and oligosaccharides (Jensena et al., 2008). YC improves the performance of monogastric and ruminant animals (Bontempo et al., 2006; Desnoyers et al., 2009; Özsoy et al., 2018). However, few studies have focused on the effects of YC on the performance of laying hens.

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Previous studies reported that yeast cell wall and yeast autolysate supplied to laying hens increase egg production and egg weight and improve feed efficiency (Yalcin et al., 2010; Hashim et al., 2013). These findings are consistent with our previous large-scale production study with 20,400 laying hens, which showed that dietary YC supplementation increases the performance of aged laying hens (Li et al., 2016). Specifically, dietary supplementation of 0.2% YC decreases the feed/egg ratio (1.8%) and mortality rate (20.6%), while increasing eggshell strength (1.2%), egg weight (3.4%), yolk weight (4.0%), albumen height (7.4%), and Haugh unit (4.5%; Li et al., 2016). However, the mechanism behind the positive effects of YC on laying hens' performance remains unclear.

Numerous studies showed that YC affects intestinal mucosal morphology and ileal villus development in broilers (Santin et al., 2001; Zhang et al., 2005). These studies evidenced the positive influences of YC on the intestinal health of chickens. Intestinal health problems are a major reason for reduced laying performance in aged laying hens, and thus, we hypothesized that YC may augment the performance of aged laying hens because of improved intestinal health. Intestinal barrier function and digestive capacity play key roles in intestinal health (Katherine et al., 2009). In general, intestinal permeability-related genes, including tight junction protein, that is, occludin (*OCLN*), claudin (*CLDN*), and zonula occludens (*ZO*; Shin et al., 2018), and antimicrobial peptides, that is, avian β -defensins (*AvBD1-14*; Lynn et al., 2007) and cathelicidins (*CAHT1-3*, *B1*; Achanta et al., 2012), play important roles in the intestinal barrier function. Furthermore, enzymes related to the digestive capacity, including amylase, chymotrypsin, and lipase, play pivotal roles in feed digestion. However, it is still unclear whether YC improves the performance of laying hens by regulating these genes. Therefore, we selected aged laying hens to determine whether dietary YC supplementation could alleviate the reduction in performance and egg quality; and YC improved performance of aged laying hens by regulating these intestinal barrier function and digestive capacity genes.

MATERIALS AND METHODS

Birds, Treatment, Growth Performance, and Sample Collection

The Institutional Animal Care and Use Committee of Huazhong Agricultural University, China, supervised and approved the experimental protocol of this study. In total, sixty 67-week-old Hy-Line Brown laying hens were randomly divided into 2 groups with 5 replicates of 6 birds each. The control group received a basal diet (BD, Table 1) with nutrients that met the recommendations provided by the National Research Council (NRC, 1994). The dietary treatment group was prepared by supplementing the same BD with 3.0 g/kg YC as a dry powder (1.8×10^{18} cfu/kg *Saccharomyces cerevisiae*;

Table 1. Composition and nutrient content of basal diet.

Ingredients	Contents (%)
Corn	53.5
Soybean meal	23.5
Wheat bran	6.5
Soybean oil	5.0
Limestone	8.5
Salt	0.3
DL-methionine	0.11
Dicalcium phosphate	1.59
Premix ¹	1.0
Total	100
Nutrient levels ²	
Metabolizable energy MJ·kg ⁻¹	14.59
Crude protein	15.2
Calcium	3.42
Total phosphorus	0.62
Available phosphorus	0.39
Lysine	0.91
Methionine	0.42

¹Contained the following per kilogram of diet: vitamin A, 12,000 IU; vitamin D₃, 4000 IU; vitamin E, 35 IU; vitamin K, 5 mg; thiamine, 2 mg; riboflavin, 8 mg; vitamin B₆, 5 mg; vitamin B₁₂, 50 μ g; D-biotin, 200 μ g; pantothenic acid, 15 mg; nicotinic acid, 50 mg; choline, 500 mg; folic acid, 1.5 mg; Mn, (MnSO₄·H₂O), 120 mg; Zn (ZnO), 80 mg; Fe (FeSO₄·H₂O), 120 mg; Cu (CuSO₄·5H₂O), 15 mg; I (KI), 1 mg and Se (Na₂SeO₃), 0.3 mg.

²Nutrient Levels were a calculated value.

Beijing Enhalar Biotechnology Co. Ltd., Beijing, China). The 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*. The nutrient composition of the YC powder is listed in the Supplemental Table 1 (Zhang et al., 2018). All birds were allowed *ad libitum* access to the mash diets and distilled water for 8 wk. Egg weight, feed intake, and mortality were recorded daily (Hashim et al., 2013). The feed/egg ratio and egg production rate were calculated biweekly. Interior egg and eggshell quality were measured from the eggs laid on the last day of weeks 4 and 8. At the end of the feeding trial, 10 hens (2 birds/cage) from each group were slaughtered to collect blood samples, duodenum, jejunum, and the chyme from the duodenum and jejunum. The mesentery was cut to uncoil the intestine, and then the entire duodenum and jejunum were removed. The chyme was collected from one end of the intestine by massaging the other end. A small segment of duodenum and jejunum tissue was collected after collection of the chyme. The intestinal and chyme samples were divided into aliquots, snap-frozen in liquid nitrogen, and stored at -80°C until further analysis.

Plasma Immunoglobulin and Endotoxin Levels and Chyme Digestive Enzyme Activity Analysis

The plasma samples were prepared by centrifuging the collected blood samples at $1000 \times g$ at 4°C for 10 min (Sun et al., 2016). The concentrations of immunoglobulin (Ig) A, IgG, and IgM and endotoxin in plasma were measured with ELISA kits (A40199-S, A04022-S, A02721-S, A049165-S; Shanghai Jining Shiye Co. Ltd., Shanghai, China), according to the manufacturer's

Table 2. Effects of dietary YC supplementation on laying performance of laying hens.¹

Item	Weeks 1 to 2		Weeks 3 to 4		Weeks 5 to 6		Weeks 7 to 8	
	Control	YC	Control	YC	Control	YC	Control	YC
Total feed intake, kg/hen	1.38 ± 0.06	1.34 ± 0.07	1.61 ± 0.07	1.59 ± 0.11	1.66 ± 0.07	1.67 ± 0.10	1.75 ± 0.04	1.77 ± 0.04
Total egg weight, kg/hen	0.56 ± 0.05	0.56 ± 0.08	0.60 ± 0.08	0.64 ± 0.12	0.59 ± 0.04 ^a	0.67 ± 0.02 ^b	0.60 ± 0.03 ^a	0.67 ± 0.05 ^b
Feed/egg ratio, g/g	2.49 ± 0.25	2.48 ± 0.50	2.71 ± 0.37	2.52 ± 0.31	2.81 ± 0.07 ^b	2.50 ± 0.12 ^a	2.91 ± 0.11 ^b	2.64 ± 0.15 ^a
Egg-laying rate, %	66.7 ± 7.7	66.7 ± 15.6	69.3 ± 10.6	75.9 ± 6.2	66.9 ± 5.6 ^a	75.9 ± 2.0 ^b	66.9 ± 4.4 ^a	75.6 ± 5.4 ^b

^{a,b}Means within a row with different superscripts differ significantly ($P < 0.05$).

¹Results are reported as the mean ± SD, n = 5; YC = yeast culture.

instructions. The activities of α -amylase, lipase, and chymotrypsin in chyme from the duodenum and jejunum were determined by a colorimetric method using specific assay kits (C016-1, A054-1, A080-1; Nanjing Jiancheng Bioengineering Institute of China, Nanjing, China).

Real-Time Quantitative Polymerase Chain Reaction Analyses

Total RNA was isolated from the duodenum and jejunum (20 mg tissue; n = 10 hens per group). The RNA sample preparation, q-PCR procedure, and relative RNA abundance quantification were conducted as previously described (Luo et al., 2019; Zhao et al., 2019). Briefly, total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA), according to the manufacturer's instructions. The RNA quality and concentration were determined using a NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA) at 260 and 280 nm. Reverse transcription to synthesize the complementary DNA library was performed using the PrimeScript™ RT reagent kit (TaKaRa, Kusatsu, Japan). The mRNA levels of the genes were analyzed using a q-PCR machine (CFX384; Bio-Rad, Hercules, CA) with SYBR Green Dye (Bio-Rad), following the manufacturer's instructions. Primers for the intestinal barrier-related genes, including *OCN*, *CLDN1*, and *ZO-1*, antimicrobial peptide genes, including *AvBD1*, *4*, and *7* and *CAHT1* and *3*, and the reference gene β -actin were designed using Primer Express 3.0 (Applied Biosystems, Foster City, CA) and are presented in Supplemental Table 2. The primer quality was verified by amplification plots and dissociation curves. The 2^{-ddCt} method was used for the quantification with β -actin as a reference gene, and the relative abundance was normalized to the control group.

Statistical Analysis

Statistical analysis was performed with SPSS Statistics 20 (SPSS Inc., IBM, Chicago, IL). Data are presented as mean ± SD. Dietary effects were determined by one-way ANOVA with a significance level of $P < 0.05$. The Tukey–Kramer method was used for multiple mean comparisons.

RESULTS

Laying Performance and Egg Quality

Laying performance results are presented in Table 2. There were no differences ($P > 0.05$) in the initial egg production rate between the 2 groups (data not shown). Dietary YC supplementation did not affect ($P > 0.05$) the total egg weight, feed/egg ratio, and laying rate during weeks 1 to 2 and 3 to 4 and total feed intake throughout the entire experimental period. However, it increased ($P < 0.05$) total egg weight (11.7–13.6%) and egg-laying rate (13.0–13.5%) but decreased ($P < 0.05$) the feed/egg ratio (9.3–11.0%) compared with the control during weeks 5 to 6 and 7 to 8. Egg quality results are presented in Table 3. Eggshell strength, eggshell thickness, egg weight, albumen height, egg yolk color, and Haugh unit were not affected ($P > 0.05$) by dietary YC supplementation at weeks 4 and 8.

Plasma Immunoglobulin and Endotoxin Levels and Chyme Digestive Enzyme Activity

Plasma immunoglobulin and endotoxin concentration results are presented in Table 4. After 8 wk of experimental treatment, dietary YC supplementation decreased ($P < 0.05$) plasma endotoxin by 44.1%

Table 3. Effects of dietary YC supplementation on egg quality¹.

Item	Week 4		Week 8	
	Control	YC	Control	YC
Eggshell strength, N	28.9 ± 5.6	32.1 ± 4.1	30.8 ± 5.1	31.2 ± 4.5
Eggshell thickness, mm	0.353 ± 0.022	0.349 ± 0.036	0.351 ± 0.029	0.372 ± 0.031
Egg weight, g	62.4 ± 2.4	60.2 ± 5.6	64.0 ± 3.3	63.6 ± 2.1
Albumen height, mm	8.42 ± 0.78	8.68 ± 0.88	8.76 ± 1.07	8.94 ± 0.87
Egg yolk color	6.05 ± 0.82	6.01 ± 0.57	6.02 ± 0.91	5.83 ± 0.64
Haugh unit	91.3 ± 3.7	92.7 ± 5.4	92.1 ± 5.4	94.0 ± 4.2

¹Results are reported as the mean ± SD, n = 5; YC = yeast culture.

Table 4. Effects of YC supplied in diets on concentration of immunoglobulin and endotoxin in plasma¹.

Item	Control	YC
IgM, µg/mL	327.5 ± 134.9	318.3 ± 97.1
IgA, µg/mL	184.9 ± 74.0	174.1 ± 37.7
IgG, µg/mL	1,192.8 ± 443.5	1,149.1 ± 405.5
Endotoxin, µg/mL	53.6 ± 5.5 ^a	29.9 ± 10.6 ^b

^{a,b}Means within a row with different superscripts differ significantly ($P < 0.05$).

Abbreviation: Ig, immunoglobulin.

¹Results are reported as the mean ± SD, n = 5; YC = yeast culture.

compared with the control. However, the concentrations of plasma immunoglobulins, including IgA, IgG, and IgM, were not affected ($P > 0.05$) by YC supplementation. Chyme digestive enzyme activity results are presented in Table 5. Although dietary YC supplementation did not affect ($P > 0.05$) α -amylase and chymotrypsin in the jejunum or lipase in the duodenum and jejunum, it increased ($P < 0.05$) α -amylase and chymotrypsin activities in the duodenum by 54.8 and 62.5%, respectively, compared with the control.

Expression of Intestinal Barrier-Related and Antimicrobial Peptide Genes

The mRNA levels of the examined gene results are presented in Figure 1. In the duodenum, dietary YC supplementation enhanced ($P < 0.05$) *OCN* and *CLDN1* (intestinal barrier-related genes) and *AvBD1*, *CATH1*, and *CATH3* (antimicrobial peptide genes) mRNA levels at week 8 compared with the control (Figure 1A). Dietary YC supplementation had a minimal effect in the jejunum; it only increased ($P < 0.05$) the *AvBD7* mRNA level compared with the control (Figure 1B).

DISCUSSION

The current study showed that dietary supplementation of 0.3% YC improved the performance of aged laying hens. Specifically, dietary YC supplementation reduced the feed/egg ratio and increased the total egg weight and egg-laying rate during weeks 5 to 8. These outcomes were similar to previous studies that revealed YC supplementation for 1 to 4 wk is necessary to detect positive effects on the performance of livestock and poultry (Mathew et al., 1998; Lesmeister et al., 2004; Gao et al., 2008). However, in the present study, interior egg and eggshell quality were not affected by

dietary YC supplementation, data that are consistent with previous studies (Yalcin et al., 2008; Li et al., 2016). This divergence may be because of the differences in the domestic animal age, duration, doses, and varieties of yeast.

Activities of intestinal digestive enzymes, including lipase, chymotrypsin, and α -amylase, play pivotal roles in nutrient digestion and have been described as valuable parameters for feed utilization efficiency and performance of domestic animals (Yi et al., 2013). Interestingly, dietary YC supplementation significantly increased the activities of chymotrypsin and α -amylase in duodenal chyme. These findings are similar to a previous study that showed yeast products—autolyzed whole yeast and yeast cell wall—can improve pancreatic function and increase digestive enzyme synthesis of broilers (Ahiwe et al., 2020). These results indicated that YC supplementation improved the digestibility of the protein and starch components of the feed. Furthermore, these data explain the improved feed conversion efficiency observed in hens supplemented with YC. These outcomes were consistent with previous studies, which provided evidence that dietary YC supplementation can improve nutrient digestibility in dairy cows, sheep, and lambs (Chademana and Offer, 1990; Haddad and Goussous, 2005; Dias et al., 2017).

YC-mediated enhancement of intestinal health and immune function are recognized as pivotal factors for improved performance of domestic animals (Gao et al., 2008; Shen et al., 2009; Lee et al., 2018). Endotoxin, a component of the cell wall of gram-negative bacteria, is released by cell lysis. Once released, it exerts toxic effects on the host that cause severe intestinal damage (Hutcheson et al., 1990). Plasma endotoxin concentration has been well documented as a valuable parameter of intestinal permeability and health (Liu et al., 2018). In the current study, dietary YC supplementation reduced plasma endotoxin in laying hens, concomitant with improved intestinal barrier function. *OCN* and *CLDN1*, which code tight junction proteins that play pivotal roles in maintaining intestinal barrier function (Pinton et al., 2009; Zhao et al., 2011), were upregulated by YC in the duodenum. These novel findings might explain the lower plasma endotoxin observed in the YC treated laying hens. Moreover, the YC-mediated improvement in gut integrity may be associated with several mechanisms: (1) increased abundance of *Lactobacillus* (Wu et al., 2018), which can improve intestinal barrier

Table 5. Effects of dietary YC supplementation on activities of digestive enzymes in chyme from duodenum and jejunum of aged laying hens¹.

Item	Chyme in duodenum		Chyme in jejunum	
	Control	YC	Control	YC
Lipase, U/mgprot	44.3 ± 21.8	39.0 ± 16.8	101.3 ± 45.6	101.5 ± 86.7
Chymotrypsin, U/mgprot	1.04 ± 0.42 ^b	1.61 ± 0.21 ^a	4.71 ± 2.12	4.01 ± 1.06
α -amylase, U/mgprot	0.24 ± 0.06 ^b	0.39 ± 0.07 ^a	0.58 ± 0.23	0.56 ± 0.17

^{a,b}Means within a row with different superscripts differ significantly ($P < 0.05$).

¹Results are reported as the mean ± SD, n = 5; YC = yeast culture.

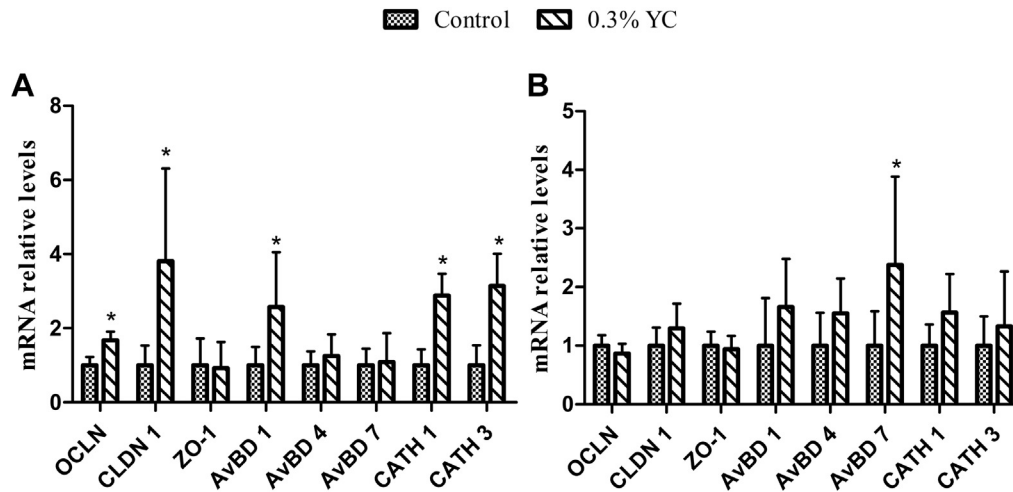


Figure 1. Effect of dietary YC supplementation on mRNA abundances of intestinal barrier-related genes and antimicrobial peptides genes relative to the control (set at 1.0) in the duodenum (A) and jejunum (B) of hens. Values are means \pm SD, $n = 5$. Means with * are different from the control, $P < 0.05$. YC, yeast culture.

function by modulating goblet cells and increase the tight junction-related genes expression in intestine (Anderson et al., 2010); (2) decrease in *Escherichia coli* and *Salmonella* colonization in the intestine (Shanmugasundaram et al., 2013), which can produce endotoxin and induce intestinal barrier dysfunction (Garber et al., 2012; Ren et al., 2017). Meanwhile, *AvBD1* and *AvBD7* code β -defense peptides that exhibit stronger activity against gram-negative bacterial strains (Derache et al., 2009); *CATH1* and *CATH3* code antimicrobial peptides that can kill a broad range of gram-negative and gram-positive bacteria (Xiao et al., 2006). Interestingly, these genes were upregulated by dietary YC supplementation, a novel finding in the current study. Upregulation of these β -defense and antimicrobial peptide genes in the duodenum and jejunum of YC-treated laying hens could mediate the improved intestinal immune function. However, dietary YC supplementation did not affect the plasma IgA, IgG, and IgM levels, data that are inconsistent with a previous study (Fathi et al., 2012). This discrepancy may be because of the differences in the domestic animal species, age, and yeast varieties (Gao et al., 2008; Zhang et al., 2018).

In conclusion, the present study successfully confirmed that dietary supplementation of 0.3% YC improved the performance of aged laying hens, including reducing the feed/egg ratio and improving total egg weight and egg-laying rate. Furthermore, the positive effects of YC on the performance of laying hens were associated with enhanced intestinal digestive enzyme activities and intestinal health. Moreover, the improvement in the intestinal health by dietary YC supplementation was related to the upregulation of intestinal barrier-related genes and antimicrobial peptides genes. Overall, these findings provide a potential explanation for the mechanisms that mediate the positive effects of YC on laying hens. Thus, these findings will be beneficial for the nutritional management of aged laying hens.

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Conflict of Interest: All authors have read and approved the final manuscript and declared that no competing interests exist.

SUPPLEMENTARY DATA

Supplementary data associated with this article can be found in the online version at <https://doi:10.1016/j.psj.2019.11.017>.

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