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Title Page

Yeast Culture on Production of Hens

Yeast culture promotes production of aged laying hens by upregulating intestinal digestive enzyme activities and intestinal health-related genes

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ABSTRACT Yeast culture (**YC**) plays positive roles in improving the performance of laying hens. The purpose of the present study was designed to explore the underlying mechanism in this regard. Sixty 67-week-old Hy-Line Brown aged laying hens were randomly allocated into 2 experimental groups with 5 replicates of 6 birds each, which were fed a control diet and control diet supplemented with YC at 3.0 g/kg for 8 weeks. The results showed that dietary supplementation of YC increased ($P < 0.05$) egg-laying rate by 13.0-13.5% but decreased ($P < 0.05$) feed/egg ratio by 9.3-11.0% during the weeks 5-6 and 7-8 compared with the control. However, egg quality including eggshell strength, eggshell thickness, albumen height, egg yolk color, and Haugh unit were not affected ($P > 0.05$) by YC supplementation. Furthermore, dietary YC supplementation increased ($P < 0.05$) the chymotrypsin and α-amylase activities by 54.8-62.5% in the duodenal chime and reduced ($P < 0.05$) plasma endotoxin by 44.1%. Moreover, dietary supplementation of YC upregulated ($P < 0.05$) mRNA expression of intestinal barrier-related genes (occludin and claudin1) 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 the intestinal digestive enzyme activities and intestinal health-related genes expression.

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

INTRODUCTION

The main common problems facing the poultry industry in older laying hens are the decrease in the performance and egg quality (Bar et al., 1999). Increasing the number of broken eggs in aged laying hens accounted for 7.5%, which leads to great economic losses (Roland et al., 1988). Therefore, poultry producers were constantly pursuing the improvement in laying performance and extension of the laying period.

Yeast culture (**YC**) is a natural fermentation product of yeast including a variety of biologically active substances, such as yeast cell, vitamins, peptides, amino acids, proteins, peptides ,organic acids, and oligosaccharides (Jensena et al., 2008). YC has shown positive effects in improving the performance of monogastric and ruminant animals in previous studies (Bontempo et al., 2006; Desnoyers et al., 2009; Wohlt et al., 1991). However, few studies have focused on the effects of YC on the performance of laying hens. A previous study reported that yeast autolysate supplied to laying hens increased egg production, egg weight, and improved feed efficiency (Yalcin et al., 2010). These findings are consistent with our previous large-scale production study with 20,400 laying hens, which showed that dietary supplementation of YC increased the performance of aged laying hens (Li et al., 2016). However, the mechanism behind the positive effects of YC on laying hens' performance was unclear.

Numerous studies showed that YC affected intestinal mucosal morphology and ileal villus development of broilers (Santin et al., 2001; Zhang et al., 2005). These studies provided evidence that YC displayed positive influences on the intestinal health of chickens. In general, the small intestine is the largest section of the digestive tract, where the digestion and absorption of nutrients take place. Improvement in the intestinal health of chicken is conducive to maintain birds' performance (Forte et al., 2018). Intestinal barrier function and digestive capacity play key roles in the intestinal health (Katherine et al., 2009). In general, intestinal permeability related genes including tight junction protein, i.e., occludin (***OCLN***), claudin (***CLDN***), and zonula occludens (***ZO***) (Shin et al., 2018) and antimicrobial peptides, i.e., 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. As well as, digestive capacity related enzymes including amylase, chymotrypsin and lipase play pivotal roles in feed digestion. However, it is still unclear whether YC improved the performance of laying hens through regulating these genes. Therefore, we selected aged laying hens to determine whether dietary supplementation of YC could alleviate the reduction in performance and egg quality; and YC improved performance of aged laying hens through 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 aged 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 met the recommendations given by the National Research Council (NRC, 1994). The dietary treatment group was prepared by supplementing the same BD with 3.0 g/kg YC (1.8×10^{18} cfu/kg *Saccharomyces cerevisiae* and 55% crude protein; Beijing Enhalor Biotechnology Co. Ltd.). All birds were allowed free access the mash diets and distilled water ad libitum for 8 weeks. Egg weight, feed intake, and mortality of birds were recorded daily (Zhao et al., 2018). Feed/egg ratio and egg production rate were calculated biweekly. Interior egg quality and eggshell quality were measured from the eggs laid on the last day of the fourth and eighth weeks. 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 duodenum and jejunum. The intestinal and chyme samples were divided into aliquots, snap-frozen in liquid nitrogen, and stored at −80 °C until further analysis.

Plasma biochemical and chyme digestive enzyme activity analysis

The plasma samples were prepared by centrifuge the collected blood samples at 1000×g at 4 °C for 10 min (Sun et al., 2016). The concentrations of immunoglobulin (**Ig**) A, G, and M 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 instructions. The activities of α-amylase, lipase, and chymotrypsin of chyme in the duodenum and jejunum were determined by a colorimetric method using specific assay kits (C016-1, A054-1, A080-1) from the Nanjing Jiancheng Bioengineering Institute of China.

Real-time q-PCR analyses

Total RNA was isolated from the duodenum and jejunum (20 mg tissue) of 10 hens from each group. The RNA sample preparation, q-PCR procedure, and relative RNA abundance qualification were conducted as previously described (Luo et al., 2019; Zhao et al., 2019). Primers for the intestinal barrier-related genes, including *OCLN*, *CLDN1*, and *ZO-1*; antimicrobial peptides genes, including *AvBD*1, 4, and 7 and *CAHT*1 and 3; and the reference gene β-actin were designed using Primer Express 3.0 (Applied Biosystems) and are presented in the **Supplemental Table 1**. 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 by SPSS Statistics 20 (SPSS Inc., IBM, USA). Data are presented as mean ± standard deviation (SD). Dietary effects were determined by one-way ANOVA with a significance level of $P < 0.05$, and the Tukey-Kramer method was used for multiple mean comparisons.

RESULTS

Laying performance and egg quality

Laying performance results are presented in **Table 2**. Non-significant differences ($P > 0.05$) in the initial egg production rate were observed among the two groups (data not shown). Although dietary YC supplementation did not affect ($P > 0.05$) the egg weight, feed/egg ratio, and laying rate during weeks 1-2 and 3-4 and feed intake throughout the whole experimental period, it increased ($P < 0.05$) egg-laying rate (13.0-13.5%) but decreased ($P < 0.05$) feed/egg ratio (9.3-11.0%) during weeks 5-6 and 7-8. Egg quality results are presented in **Table 3**. However, the eggshell strength, eggshell thickness, albumen height, egg yolk color, and Haugh unit were not affected ($P > 0.05$) by dietary YC supplementation at week 4 and 8.

Plasma biochemistry and chyme digestive enzyme activity

Plasma biochemistry results are presented in **Table 4**. After 8 weeks of experimental treatment, dietary supplementation of YC decreased ($P < 0.05$) plasma endotoxin by 44.1% 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 ($P > 0.05$) the α-amylase and chymotrypsin in the jejunum and lipase in both duodenum and jejunum, it increased ($P < 0.05$) activities of α-amylase and chymotrypsin in the duodenum by 54.8% and 62.5%, respectively, compared with the control.

Expression of the intestinal barrier-related and antimicrobial peptides genes

The mRNA levels of the pertaining genes results are presented in **Figure 1**. In the duodenum, compared with the control, dietary supplementation of YC enhanced ($P < 0.05$) mRNA abundance of 2 intestinal barrier-related genes (*OCLN* and *CLDN1*) and 3 antimicrobial peptides genes (*AvBD1*, *CATH1*, and *CATH3*) at week 8 **(Figure 1A)**. In the jejunum, the effect of dietary YC supplementation on the intestinal barrier-related and antimicrobial peptides genes was limited, as it only increased ($P < 0.05$) mRNA abundance of antimicrobial peptides gene *AvBD7* compared with the control **(Figure 1B)**.

DISCUSSION

Dietary supplementation of 0.3% YC was shown to improve the performance of aged laying hens in the current study. Although dietary YC supplementation did not affect feed intake, egg weight, feed/egg ratio, and egg-laying rate during weeks 1-4, it reduced feed/egg ratio and increased egg-laying rate during weeks 5-8. These outcomes were similar with previous studies, which revealed that supplementation of YC for 1-4 weeks 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, egg quality including eggshell strength, eggshell thickness, albumen height, egg yolk color, and Haugh unit were not affected by the dietary YC supplementation, which is consistent with a previous study (Yalcin et al., 2008).

Activities of intestinal digestive enzymes such as lipase, chymotrypsin and α-amylase play pivotal roles in nutrients digestion and have been described as valuable parameters of feed utilization efficiency and performance of domestic animals (Yi et al., 2013). Interestingly, dietary supplementation of YC significantly increased the activities of chymotrypsin and α-amylase in duodenal chyme. These results indicated that YC supplementation could improve the digestibility of protein and starch of the feed, which explain the improvement in feed conversion efficiency because of YC supplementation. These outcomes were consistent with previous studies, which provided evidence that dietary supplementation of YC can improve the nutrients digestibility in dairy cow, sheep, and lamb (Chademana et al., 1990; Haddad and Goussous, 2005; Dias et al., 2017).

Enhancement of the intestinal health and immune function by YC has been recognized as the pivotal factors for the improvement of the performance of domestic animals (Gao et al., 2008; Shen et al., 2009; Lee et al., 2018). Endotoxin is a component of the cell wall of gram-negative bacteria and released by the lysis of cells, with toxic properties to the host causing severe intestinal damage (Hutcheson et al., 1990). Plasma endotoxin concentration has been well documented as a valuable parameter of the intestinal permeability and health (Liu et al., 2018). Dietary YC supplementation sharply reduced plasma endotoxin in laying hens in the current study, which revealed that YC improved the intestinal barrier function. Interestingly, *OCLN* and *CLDN1* coding tight junction proteins that play pivotal roles in maintaining the intestinal barrier function (Pinton et al., 2009; Zhao et al., 2011) were upregulated by YC in the duodenum, which might explain the lower plasma endotoxin observed in the YC treated laying hens. Meanwhile, *AvBD1* and *AvBD7* coding β-defense peptides that exhibit stronger activity against gram-negative strains (Derache et al., 2009) and *CATH1*and *CATH3* coding antimicrobial peptides with the capability of killing a broad range of gram-negative and gram-positive bacteria (Xiao et al., 2006) were upregulated by dietary YC supplementation. Upregulation of these β-defense and antimicrobial peptide genes in the duodenum and jejunum of laying hens treated by YC could attribute to the improvement in the intestinal immune function. However, inconsistent with the previous study (Fathi et al., 2012), dietary YC supplementation did not affect the plasma IgA, IgG, and IgM. This discrepancy may be due to the differences in the domestic animal species, age, and yeast varieties (Zhang et al., 2018; Gao et al., 2008).

In summary, the present study successfully confirmed that dietary supplementation of 0.3% YC could be adopted to improve the performance of aged laying hens. Furthermore, the positive effects of YC on the performance of laying hens were associated with the enhancement of the intestinal digestive enzymes 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 of the mechanisms of the positive effects of YC on laying hens, thus 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.

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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 $MJ \cdot kg^{-1}$	14.59
Crude protein	15.2
Calcium	3.42
Total phosphorus	0.62
Available phosphorus	0.39
Lysine	0.91
Methionine	0.42

[1] Contained the following per kilogram of diet: vitamin A, 12,000 IU; vitamin D_3, 4000 IU; vitamin E, 35 IU; vitamin K, 5 mg; thiamine, 2 mg; riboflavin, 8 mg; vitamin B_6, 5 mg; vitamin B_{12}, 50 μg; D-biotin, 200 μg; pantothenic acid, 15 mg; nicotinic acid, 50 mg; choline, 500 mg; folic acid,1.5 mg; Mn, ($MnSO_4$, H_2O), 120 mg; Zn (ZnO), 80 mg; Fe ($FeSO_4$, H_2O), 120 mg; Cu ($CuSO_4 \cdot 5H_2O$), 15 mg; I (KI), 1 mg and Se (Na_2SeO_3), 0.3 mg.

[2]Detected value

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

	Weeks 1-2		Weeks 3-4		Weeks 5-6		Weeks 7-8	
	Control	YC	Control	YC	Control	YC	Control	YC
Feed intake, g	98.2±3.9	95.6±4.4	115.2±4.3	115.0±7.2	118.6±4.5	119.8±6.8	124.7±2.6	126.6±2.7
Egg weight, g	59.8±2.7	60.0±1.2	62.4±2.4	60.2±5.6	63.6±4.5	63.1±1.9	64.0±3.3	63.6±2.1
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[a]	2.50±0.12[b]	2.91±0.11[a]	2.64±0.15[b]
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 ($P < 0.05$) .

[1]Results are reported as the mean ± SD; YC = yeast culture.

Table 3. Effects of dietary YC supplementation on egg quality[1]

	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
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

[a,b]Means within a row with different superscripts differ significantly ($P < 0.05$).

[1]Results are reported as the mean ± SD; YC = yeast culture.

328 **Table 4.** Effects of YC supplied in diets on concentration of immunoglobulin and endotoxin in
329 plasma[1]

	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	1192.8±443.5	1149.1±405.5
Endotoxin, µg/mL	53.6±5.5[a]	29.9±10.6[b]

330 [a,b]Means within a row with different superscripts differ significantly ($P < 0.05$).

331 [1]Results are reported as the mean ± SD; YC = yeast culture.

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Table 5. Effects of dietary YC supplementation on activities of digestive enzymes in chyme from duodenum and jejunum of aged laying hens[1]

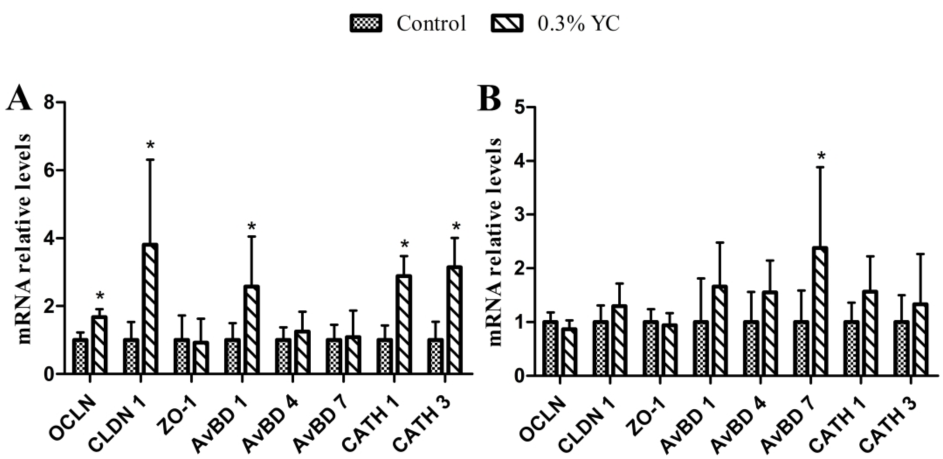
	Chyme in duodenum		Chyme in jejunum	
	Control	YC	Control	YC
Lipase, U/mgprot	44.3 ± 21.8	39. 0± 16.8	101.3 ± 45.6	101.5 ± 86.7
Chymotrypsin, U/mgprot	1.04±0.42[b]	1.61±0.21[a]	4.71±2.12	4.01±1.06
α-amylase, U/mgprot	0.24±0.06[b]	0.39±0.07[a]	0.58±0.23	0.56±0.17

[a,b]Means within a row with different superscripts differ significantly (*P* < 0.05).

[1]Results are reported as the mean ± SD; YC = yeast culture.

Figure legend

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 ± SD, n = 8. Means with * are different from the control, $P < 0.05$. YC = yeast culture.



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