

Effects of Dietary Supplementation of Yeast Oligosaccharides on Production Performance, Egg Quality, Serum Biochemical Indices, and Intestinal Health of Laying Hens under High-Density Stocking Conditions: Postprint

Authors: Wang Yu, Li Enkai, Meng Yanmiao, Ding Xiangwen, YANG Jichang, Hu Xiyi, Zhang Bo, Song Zhigang

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Abstract

This study aimed to investigate the effects of dietary yeast oligosaccharides (YOS) on production performance, egg quality, intestinal mucosal morphology and microbial flora distribution, serum biochemical indices, and intestinal function-related gene expression in laying hens under high-density farming conditions. A total of 864 12-week-old Hy-Line Brown laying hens were randomly divided into 6 groups, with 12 replicates per group, 3 cages per replicate, and each cage containing 3 birds (low density) or 5 birds (high density). The control group was fed a basal diet, while the experimental groups were fed the basal diet supplemented with 250 and 500 g/t YOS, respectively, for an experimental period of 18 weeks. The results showed that: 1) dietary YOS supplementation significantly improved eggshell color uniformity ($P < 0.05$). 2) dietary YOS supplementation increased the intestinal villus height/crypt depth ratio in laying hens ($P = 0.059$), with the 250 g/t YOS group being significantly higher than the control group ($P < 0.05$). 3) dietary YOS supplementation significantly increased the number of *Lactobacillus* in the jejunum ($P < 0.05$) and significantly decreased the number of *Escherichia coli* ($P < 0.05$), with the 250 g/t YOS group showing more pronounced changes in flora. 4) dietary YOS supplementation significantly reduced serum endotoxin content ($P < 0.05$) and increased immunoglobulin A content ($P = 0.090$) in laying hens, with the 250 g/t YOS group showing a significant increase compared to the control group ($P < 0.05$). In conclusion, dietary YOS supplementation can improve intestinal function in laying hens under high-density farming conditions, with a supplementation level of 250 g/t showing the best overall effects.

Full Text

Effects of Dietary Yeast Oligosaccharides Supplementation on Production Performance, Egg Quality, Serum Biochemical Indices and Intestinal Health of Laying Hens under High-Density Cultivation

WANG Yu¹, LI Enkai¹, MENG Yanmiao², DING Xiangwen¹, YANG Jiachang¹, HU Xiyi¹, ZHANG Bo³, SONG Zhigang¹

¹College of Animal Science and Technology, Shandong Agricultural University, Tai'an 271018, China

²Animal Husbandry Bureau of Xinji, Xinji 052360, China

³Phileo-Lesaffre Animal Nutrition and Health Division, Shanghai 200235, China

Abstract

This trial was conducted to investigate the effects of dietary yeast oligosaccharides (YOS) supplementation on production performance, egg quality, intestinal mucosal morphology and microflora distribution, serum biochemical indices, and intestinal function-related gene expression in laying hens under high-density cultivation conditions. A total of 864 twelve-week-old Hy-Line Brown hens were randomly allocated to six groups with 12 replicates per group. Each replicate consisted of three cages, with three birds per cage (low density) or five birds per cage (high density). The control group received a basal diet, while experimental groups were fed the basal diet supplemented with 250 or 500 g/t YOS for an 18-week experimental period. The results demonstrated that: (1) dietary YOS supplementation significantly improved eggshell color uniformity ($P < 0.05$); (2) YOS supplementation increased the villus height-to-crypt depth ratio of intestinal mucosa ($P = 0.059$), with the 250 g/t YOS group showing significantly higher values than the control ($P < 0.05$); (3) YOS supplementation significantly increased jejunal *Lactobacillus* populations ($P < 0.05$) while decreasing *Escherichia coli* populations ($P < 0.05$), with the 250 g/t level showing more pronounced effects; and (4) dietary YOS significantly reduced serum endotoxin content ($P < 0.05$) and increased immunoglobulin A content ($P = 0.090$), with the 250 g/t YOS group showing significantly higher IgA levels than the control ($P < 0.05$). These findings indicate that dietary YOS supplementation, particularly at 250 g/t, can enhance intestinal function in laying hens under high-density cultivation conditions.

Keywords: yeast oligosaccharides; laying hens; production performance; egg quality; serum biochemical indices; intestinal health

Stress refers to the non-specific response of animals to environmental stimuli. In poultry production, numerous factors can act as stressors, with stocking density becoming the most prevalent stress factor under modern intensive production

systems. High-density cultivation-induced stress not only affects production performance but also significantly impacts intestinal health. The intestine serves as the primary site for digestion and absorption in livestock and poultry, and intestinal health is essential for optimal production performance and immune function. While antibiotics have proven effective in maintaining animal health, promoting growth, and improving feed utilization, their use disrupts intestinal microecological balance and compromises gut health. Consequently, the application of antibiotics as growth promoters should be gradually restricted until completely banned, necessitating the adoption of novel additives that can comprehensively replace antibiotics without any toxic side effects.

Numerous studies have demonstrated that yeast oligosaccharides (YOS), as a new type of microecological additive, can promote the proliferation of beneficial bacteria, inhibit harmful bacteria, enhance intestinal absorption function and integrity, and improve animal growth and feed utilization. This study investigated the preventive effects and mechanisms of YOS on stress induced by high-density cultivation in laying hens by examining its impacts on production performance, egg quality, intestinal mucosal morphology and microflora distribution, serum biochemical indices, and intestinal function-related gene expression, thereby providing a theoretical basis for the rational use of YOS in laying hens.

1. Materials and Methods

1.1 Experimental Material The YOS product used was Safmannan E (containing 21% YOS) manufactured by Phileo-Lesaffre, France.

1.2 Experimental Design A total of 864 twelve-week-old Hy-Line Brown laying hens were randomly divided into six groups with 12 replicates per group. Each replicate comprised three cages, with each cage housing three birds (low density) or five birds (high density). The control group received a basal diet, while experimental groups were fed the basal diet supplemented with 250 or 500 g/t YOS. The experimental period lasted 18 weeks. The basal diet was formulated according to the *Feeding Standard of Chickens* (NY/T 33–2004). The composition and nutrient levels of the basal diet are presented in Table 1.

1.3 Management Practices Experimental hens were housed in a semi-open chicken house with three-tier cage systems. Natural daylight was supplemented with artificial lighting to maintain a 16-hour photoperiod at 20 lx intensity. Relative humidity in the house ranged from 50% to 90%, with ventilation provided through natural and negative-pressure cross-ventilation systems. Birds were fed dry mash three times daily with four redistributions. Water was provided ad libitum via nipple drinkers. The house was managed by dedicated personnel with egg collection twice daily per replicate, weekly whole-house disinfection, and twice-daily manure removal.

1.4 Measurements 1.4.1 Production Performance and Egg Quality

At the end of week 18, feed intake and laying rate were recorded per replicate. Twenty-four eggs per group were collected to determine egg weight and quality parameters including egg shape index, shell thickness and strength, yolk color, Haugh units, and shell color. Feed conversion ratio was subsequently calculated.

1.4.2 Serum Biochemical Indices

At the end of week 18, 12 hens per group were selected for blood collection from the wing vein. Serum was separated and stored at -20°C . Serum glucose (GLU), total cholesterol (TC), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) concentrations were measured using an automatic biochemical analyzer (7170A, Hitachi, Japan). Immunoglobulin A (IgA) and endotoxin concentrations were determined by enzyme-linked immunosorbent assay using kits purchased from Nanjing Jiancheng Bioengineering Institute.

1.4.3 Intestinal Mucosal Morphology

Jejunal tissue sections were prepared to measure villus height and crypt depth, from which the villus height-to-crypt depth ratio was calculated.

1.4.4 Intestinal Microflora Counts

Jejunal contents were collected to enumerate *Lactobacillus* and *Escherichia coli* populations using methods described in references [3-4].

1.4.5 Intestinal Function-Related Gene Expression

Total RNA was extracted from jejunal samples using an RNA extraction kit (CW0584, CoWin Biotech). RNA integrity was verified by agarose gel electrophoresis. Complementary DNA was synthesized from total RNA using the Transcriptor First Strand cDNA Synthesis Kit (Roche, 4897030001). Real-time quantitative PCR was performed using the FastStart Universal SYBR Green Master kit (Roche, 4913914001). The mRNA expression levels of Toll-like receptor 4 (TLR4), nutrient transporter glucose transporter 2 (GLUT2), and fatty acid binding protein 1 (FABP1) were analyzed using the $2^{-\Delta\Delta\text{Ct}}$ method.

1.5 Statistical Analysis Data were analyzed using the ANOVA procedure of SAS 9.13, with Duncan's multiple range test used for post-hoc comparisons. Significance was declared at $P < 0.05$, and trends were noted at $P < 0.10$. Results are expressed as means \pm standard error.

2. Results

2.1 Effects of Dietary YOS Supplementation on Production Performance As shown in Table 2, high-density cultivation significantly reduced feed intake and feed conversion ratio ($P < 0.01$) but did not significantly affect laying rate ($P > 0.05$). Dietary YOS supplementation did not significantly influence production performance parameters ($P > 0.05$). However, a significant interaction between YOS supplementation and stocking density was observed

for feed intake ($P < 0.01$), though no interactions were detected for feed conversion ratio or laying rate ($P > 0.05$).

2.2 Effects of Dietary YOS Supplementation on Egg Quality Table 3 shows that neither dietary YOS supplementation nor high-density cultivation significantly affected egg weight, egg shape index, shell thickness, or Haugh units ($P > 0.05$). High-density groups exhibited significantly greater yolk color values ($P < 0.05$) and significantly lower shell strength ($P < 0.05$) compared to low-density groups. The 250 g/t YOS group showed significantly lower eggshell color values than the control group ($P < 0.05$). Significant interactions between YOS supplementation and stocking density were observed for shell thickness, yolk color, and eggshell color ($P < 0.05$), though no interactions were found for other egg quality parameters ($P > 0.05$).

2.3 Effects of Dietary YOS Supplementation on Jejunal Mucosal Morphology Table 4 indicates that high-density cultivation did not significantly affect jejunal mucosal morphology ($P > 0.05$). Compared with the control group, the 250 g/t YOS group significantly reduced crypt depth ($P < 0.05$) and significantly increased the villus height-to-crypt depth ratio ($P < 0.05$), while the 500 g/t YOS group significantly increased villus height ($P < 0.05$). A significant interaction between YOS supplementation and stocking density was observed for the villus height-to-crypt depth ratio ($P < 0.05$), suggesting that YOS supplementation ameliorated the effects of high-density cultivation on intestinal morphology.

2.4 Effects of Dietary YOS Supplementation on Jejunal Microflora Counts As presented in Table 5, dietary YOS supplementation significantly increased *Lactobacillus* populations ($P < 0.05$) and decreased *Escherichia coli* populations ($P < 0.05$) in the jejunum of hens under high-density conditions. No significant interaction between stocking density and YOS supplementation was observed for jejunal microflora counts ($P > 0.05$).

2.5 Effects of Dietary YOS Supplementation on Serum Biochemical Indices Table 6 reveals that high-density cultivation did not significantly affect serum biochemical indices ($P > 0.05$). Dietary YOS supplementation significantly reduced serum endotoxin content ($P < 0.05$) and increased serum IgA content ($P = 0.090$), with the 250 g/t YOS group showing significantly higher IgA levels than the control group ($P < 0.05$). No significant interactions between YOS supplementation and stocking density were observed for serum biochemical indices ($P > 0.05$).

2.6 Effects of Dietary YOS Supplementation on Intestinal Function-Related Gene Expression Table 7 shows that dietary YOS supplementation significantly reduced TLR4 mRNA expression in the jejunum ($P < 0.05$). Compared with the control group, the 250 g/t YOS group exhibited increased

GLUT2 and FABP1 mRNA expression levels, though these differences were not statistically significant ($P > 0.05$). A significant interaction between YOS supplementation and stocking density was observed for FABP1 mRNA expression ($P < 0.05$).

3. Discussion

3.1 Effects of Dietary YOS Supplementation on Production Performance under High-Density Conditions Previous research has demonstrated that increased stocking density reduces feed intake and laying rate in flocks. As stocking density increases, reduced activity space places birds in a state of stress, resulting in decreased feed intake and impaired laying rate and feed conversion efficiency. The current findings of reduced feed intake and feed conversion ratio in high-density groups align with these reports. Dietary mannan oligosaccharide supplementation has been shown to significantly improve feed efficiency in laying hens, while other studies indicate that YOS supplementation can increase feed intake. In this experiment, YOS supplementation tended to increase feed intake, though the observed reduction in laying rate may be attributed to ambient temperature, as heat stress is known to markedly decrease laying performance.

3.2 Effects of Dietary YOS Supplementation on Egg Quality under High-Density Conditions Under high-density stress, a substantial portion of dietary nutrients is diverted toward coping with stress responses, potentially reducing egg weight. Studies have reported that high cage density decreases feed intake and egg weight in Japanese quail. In this trial, egg weight was 0.93 g lower in high-density groups compared to low-density groups, indicating that high-density cultivation tends to reduce egg weight. Previous research has shown that dietary mannan oligosaccharide supplementation in quail significantly reduces yolk diameter, while supplementation in laying hens decreases egg weight but increases shell ratio. In the present study, YOS supplementation significantly reduced eggshell color values without significantly affecting other egg quality parameters, suggesting minimal impact on overall egg quality.

3.3 Effects of Dietary YOS Supplementation on Intestinal Microecology under High-Density Conditions Research has demonstrated that mannan oligosaccharide supplementation modulates intestinal microbial communities by promoting *Lactobacillus* adhesion to the intestinal wall through increased mucin expression, thereby stabilizing the mucosal barrier and preventing pathogen overgrowth while enhancing intestinal integrity. This study confirmed that dietary YOS supplementation significantly reduced jejunal *Escherichia coli* populations, with 250 g/t YOS significantly increasing *Lactobacillus* populations, consistent with previous findings. High-density cultivation significantly reduced both *Escherichia coli* and *Lactobacillus* populations in the jejunum, indicating that high-density conditions disrupt intestinal microflora balance, while

supplementation with 250 g/t YOS effectively regulates intestinal microbial communities.

3.4 Effects of Dietary YOS Supplementation on Intestinal Mucosal Morphology under High-Density Conditions Intestinal villi are the primary sites for nutrient absorption. Dietary mannan oligosaccharide supplementation has been shown to significantly increase ileal villus height in fattening rabbits and enhance villus height without affecting crypt depth in other studies, while some research indicates reduced crypt depth without changes in villus length. These findings collectively demonstrate that mannan oligosaccharide supplementation increases the villus height-to-crypt depth ratio, thereby expanding intestinal absorptive surface area. In this experiment, compared with the control group, the 250 and 500 g/t YOS groups exhibited 3.00% and 10.21% increases in villus height, 12.67% and 7.93% reductions in crypt depth, and 15.4% and 13.2% increases in the villus height-to-crypt depth ratio, respectively. These results indicate that YOS exerts pronounced effects on intestinal function by effectively increasing absorptive surface area and improving intestinal absorption capacity. The significant interaction between YOS supplementation and stocking density for the villus height-to-crypt depth ratio suggests that YOS ameliorates the negative effects of high-density cultivation on intestinal absorptive function.

3.5 Effects of Dietary YOS Supplementation on Serum Biochemical Indices under High-Density Conditions In this study, changes in serum GLU content corresponded with feed intake patterns, as YOS supplementation increased serum GLU levels, potentially mitigating stress-induced hypoglycemia associated with high-density cultivation. Mannan oligosaccharides promote the growth of beneficial bacteria such as *Lactobacillus* and *Bifidobacterium*, which can assimilate cholesterol and reduce serum cholesterol concentrations. Compared with the control group, the 500 g/t YOS group exhibited a 10.06% reduction in serum TC content. HDL and LDL play crucial roles in cholesterol transport, with LDL delivering hepatically synthesized cholesterol to extrahepatic tissues and HDL transporting cholesterol from peripheral tissues to the liver for metabolism. The observed increase in serum HDL and decrease in LDL concentrations following YOS supplementation further confirm its cholesterol-modulating effects.

Mannan oligosaccharides possess immunogenic properties that stimulate immune responses by binding to specific receptors on intestinal villi, thereby enhancing cellular and humoral immunity. In this study, the 250 g/t YOS group showed significantly increased serum IgA levels, consistent with previous reports of increased bile IgA and plasma IgG in turkeys fed mannan oligosaccharides. Additionally, mannan oligosaccharides can reduce intestinal pH, and the acidic environment inhibits abnormal fermentation by putrefactive bacteria, thereby reducing endotoxin production. The significant reduction in serum endotoxin content observed with YOS supplementation demonstrates its beneficial effects

on immune function in laying hens.

3.6 Effects of Dietary YOS Supplementation on Intestinal Function-Related Gene Expression under High-Density Conditions Toll-like receptors (TLRs) are key pattern recognition receptors that initiate innate immune responses in poultry intestines. TLRs recognize microbial lipopolysaccharides and activate genes associated with adaptive immunity, releasing immune effectors to trigger immune reactions. Elevated TLR4 expression has been shown to improve gastrointestinal barrier function and enhance resistance to pathogen invasion and infection. However, Lu et al. reported increased TLR4 expression in broilers infected with *Clostridium perfringens*. In this study, dietary YOS supplementation significantly reduced TLR4 expression in the jejunum, suggesting that YOS inhibits pathogen proliferation and consequently reduces TLR4 expression.

GLUT2 is the fundamental glucose transporter in intestinal epithelial cells, and its expression increases with insulin secretion. The observed changes in GLUT2 expression corresponded closely with serum GLU concentrations in this study. Recent research indicates that FABP1 facilitates fatty acid transport and metabolism and participates in inflammatory immune responses, enhancing disease resistance. Dietary YOS supplementation increased FABP1 mRNA expression in this study, though the difference was not statistically significant, which aligns with the observed changes in serum TC content.

In conclusion, high-density cultivation stress reduces feed intake, eggshell strength, and intestinal microflora populations in laying hens. Dietary YOS supplementation increases intestinal absorptive surface area, modulates intestinal microecology, and enhances immune function, thereby alleviating stress induced by high-density cultivation.

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