

Effects of High-Temperature Heat Treatment of Powdered Feed on Production Performance, Egg Quality, and Egg Hygiene Indicators in Laying Hens (Postprint)

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Abstract

This experiment was conducted to investigate the effects of high-temperature heat-treated powder feed on laying hen performance, egg quality, and egg hygiene indicators. A total of 1,080 40-week-old Hy-Line Brown laying hens were selected and randomly divided into 4 groups, with 6 replicates per group and 45 hens per replicate. The control group was fed conventional powdered layer feed, while the experimental groups were fed powdered layer feed subjected to conditioning temperatures of 70, 80, and 90 °C. The pre-trial period lasted 1 week, and the formal trial period lasted 12 weeks. The results showed: 1) The total bacterial count and coliform count in the experimental group feeds were significantly lower than those in the control group ($P < 0.05$); as the conditioning temperature increased, the total bacterial count in the experimental group feeds decreased significantly ($P < 0.05$), while the coliform count also gradually decreased, but the difference was not significant ($P > 0.05$). 2) There were no significant differences in laying hen performance indicators among all groups during weeks 1-12 ($P > 0.05$). 3) No significant differences were observed among groups in eggshell strength, eggshell thickness, egg shape index, yolk ratio, and Haugh unit at week 12 ($P > 0.05$); the brightness, redness, and yellowness values of yolk in the 80 °C high-temperature heat-treated powder feed group were significantly higher than those in the control group ($P < 0.05$). 4) At weeks 4, 8, and 12, the total bacterial count on eggshell surfaces in the experimental groups was lower than that in the control group, but the difference was not significant ($P > 0.05$); the coliform count on eggshell surfaces was lower than that in the control group. 5) The relative weights of gizzard, jejunum, and ileum in the experimental groups were lower than those in the control group, but the differences were not significant ($P > 0.05$); as the conditioning temperature increased, the relative weights of proventriculus, gizzard, duodenum, jejunum, and ileum

in the experimental groups gradually increased, but the differences were not significant ($P>0.05$). The results suggest that high-temperature heat treatment can improve the hygiene indicators of feed and eggs, but has no significant effect on laying hen performance, egg quality, and digestive tract indicators.

Full Text

Abstract

This experiment was conducted to investigate the effects of high-temperature heat treatment of mash feed on the production performance, egg quality, and egg hygiene indicators of laying hens. A total of 1,080 healthy 40-week-old Hy-Line Brown laying hens with normal feed intake, similar body weight, and consistent laying rate were selected and randomly allocated into 4 groups with 6 replicates per group and 45 hens per replicate. The control group was fed a conventional mash layer diet, while the experimental groups were fed mash diets conditioned at 70, 80, and 90°C, respectively. The adaptation period lasted for 1 week, followed by a 12-week formal experimental period. The results showed: (1) The total bacterial count and coliform bacteria number in the experimental group feeds were significantly lower than those in the control group ($P<0.05$). As the conditioning temperature increased, the total bacterial count in the experimental group feeds decreased significantly ($P<0.05$), while the coliform bacteria number also decreased but without significant difference ($P>0.05$). (2) No significant differences were observed in laying hen performance indices among all groups during weeks 1-12 ($P>0.05$). (3) At week 12, no significant differences were found in eggshell strength, eggshell thickness, egg shape index, yolk ratio, or Haugh unit among groups ($P>0.05$). However, the lightness (L), redness (a), and yellowness (b^*) values of yolk in the 80°C heat treatment group were significantly higher than those in the control group ($P<0.05$). (4) At weeks 4, 8, and 12, the total bacterial count on eggshell surfaces in the experimental groups was lower than that in the control group, but the difference was not significant ($P>0.05$), while the coliform bacteria numbers on eggshell surfaces were all lower than those in the control group. (5) The relative weights of gizzard, jejunum, and ileum in the experimental groups were lower than those in the control group, but without significant difference ($P>0.05$). As the conditioning temperature increased, the relative weights of proventriculus, gizzard, duodenum, jejunum, and ileum in the experimental groups gradually increased, but the differences were not significant ($P>0.05$). These results indicate that high-temperature heat treatment can improve the hygiene indicators of feed and eggs, but has no significant effects on the production performance, egg quality, or digestive tract indices of laying hens.

Keywords: laying hens; mash feed; high-temperature heat treatment; production performance; egg quality; hygiene indicators

Introduction

China has traditionally used conventional processing technology for layer feed production, typically resulting in coarsely ground mash compound feeds. With improving living standards and heightened attention to food safety, feed safety has attracted increasing concern. However, conventionally produced mash layer feed undergoes no maturation treatment and suffers from high bacterial contamination rates, which may lead to harmful microorganisms being carried both internally and externally in eggs, making it difficult to meet the requirements for safe breeding and production [1-4]. Due to these safety concerns, high-temperature heat-treated mash feed has gradually gained application in layer production because of its safety, energy efficiency, environmental benefits, and high feed utilization efficiency, achieving favorable results. High-temperature heat-treated mash feed has several characteristics: First, after hydrothermal treatment, the feed effectively inactivates pathogens such as Salmonella and sufficiently destroys and deactivates harmful factors like protease inhibitors, while simultaneously gelatinizing starch and effectively denaturing proteins. Second, starch gelatinization and protein denaturation make feed particles rough and sticky on the surface, allowing larger starch and protein particles to adsorb smaller micronutrients and vitamins during mixing, with good uniformity and flowability, reducing dust and segregation phenomena in subsequent processing [5]. Current research on high-temperature heat-treated mash feed has primarily focused on feed hygiene indicators and layer digestion and utilization rates, while its effects on layer production performance, egg quality, and digestive tract indices have not been reported. Therefore, this experiment used mash layer feed conditioned at three temperatures (70, 80, and 90°C) to investigate the effects of high-temperature heat treatment on layer production performance, egg quality, egg hygiene indicators, and digestive tract indices, which holds practical significance for improving feed quality and achieving better economic and social benefits.

1.1 Experimental Design

A single-factor experimental design was employed. A total of 1,080 healthy 40-week-old Hy-Line Brown laying hens with normal feed intake, similar body weight, and consistent laying rate were selected and randomly divided into 4 groups with 6 replicates per group and 45 hens per replicate. The basal diet was formulated according to NRC (1994) and China's "Feeding Standard of Chicken" (NY/T 33-2004), combined with the Hy-Line Brown layer management manual. The composition and nutrient levels of the basal diet are shown in Table 1. The conventional mash group (control group, Group A) was fed a conventional mash layer diet (basal diet), while the high-temperature heat-treated mash groups (Groups B, C, and D) were fed mash diets conditioned at 70, 80, and 90°C, respectively (using a double-layer conditioner of an SZLH25 pellet mill, steam pressure 0.2 MPa, conditioning time 30 s). The adaptation period lasted for 1 week, and the formal experimental period lasted for 12 weeks.

1.2 Management

The experiment was conducted in a closed chicken house with three-tier step-cage systems, with 3 hens per cage. Conventional management methods were followed. The house used automatic artificial lighting with 16 h light and 8 h dark at an intensity of 20 lx. Room temperature was maintained at $(25\pm 5)^{\circ}\text{C}$ with relative humidity of 40%-70%. Hens had free access to feed, which was provided at 08:00 and 14:00 daily with 4 times of feed distribution. Nipple drinkers provided free access to water. Weekly disinfection with chickens present and manure removal were performed once per week.

1.3.1 Production Performance

During the experimental period, daily records were kept for each replicate regarding feed intake, egg number, number of defective eggs (broken, misshapen, cracked, soft-shelled, or shell-less), number of culled and dead hens, time of death, and body (carcass) weight. Average daily feed intake, average egg weight, laying rate, feed-to-egg ratio, and broken egg rate were calculated for the experimental period.

1.3.2 Egg Quality

At the ends of weeks 4, 8, and 12 of the formal experiment, 10 eggs were randomly selected from each replicate for egg quality determination (completed within 24 h). Measured indices included egg weight, egg shape index, eggshell strength, eggshell thickness, yolk ratio, Haugh unit, and yolk color. Haugh unit was calculated as: $\text{HU} = 100 \times \log(\text{H} - 1.7\text{W}^{0.37} + 7.57)$, where H is thick albumen height (mm) and W is egg weight (g). Yolk color was measured using a LabScan XE colorimeter, including lightness (L), *redness* (a), and yellowness (b*) values.

1.3.3 Hygiene Indicators

Feed hygiene indicators: Total bacterial count was determined according to national standard GB/T 13093-2006, coliform bacteria number according to GB/T 18869-2002, and Salmonella number according to GB/T 13091-2002.

Egg hygiene indicators: At the ends of weeks 4, 8, and 12 of the experimental period, 10 eggs were randomly selected from each replicate for hygiene indicator determination. Total bacterial count and numbers of *Escherichia coli* and *Salmonella* on eggshell surfaces and in egg liquid were measured. Eggs were sequentially placed in sterilized beakers, and the eggshell surfaces were moistened with sterile physiological saline. Sterile cotton balls were used to thoroughly wipe the eggshell surfaces, which were then repeatedly rinsed in the beakers. Wiping solutions from 10 eggs in the same replicate were combined and thoroughly mixed. Finally, the eggshell wiping solution was gradient-diluted 10, 100, 1,000, and 10,000 times with physiological saline, and total bacterial count

and coliform bacteria number were determined according to national standard GB 4789-2010.

1.3.4 Digestive Tract Indices

At the end of week 12 of the experimental period, 2 hens with good health, medium body weight, and normal egg production were selected from each replicate and slaughtered by neck bleeding. The proventriculus, gizzard, duodenum, jejunum, and ileum were removed and separated. The weight of each digestive organ was measured for each hen, and the relative weight of each digestive organ part was calculated.

1.4 Statistical Analysis

Experimental data were analyzed using SPSS 17.0 for one-way ANOVA and expressed as “mean \pm standard deviation.” Duncan’s multiple comparison test was used for significance testing among group means, with $P < 0.05$ as the criterion for significant difference.

2.1 Effects of High-Temperature Heat Treatment on Feed Hygiene Indicators

The effects of high-temperature heat treatment on feed hygiene indicators are shown in Table 2 . The results show that both total bacterial count and coliform bacteria number in the heat-treated mash groups were significantly lower than those in the conventional mash group ($P < 0.05$), with no Salmonella detected in any group. As the conditioning temperature increased, the total bacterial count in the heat-treated mash groups decreased significantly ($P < 0.05$), while the coliform bacteria number also decreased gradually but without significant difference ($P > 0.05$). Compared with the conventional mash group (Group A), Groups B, C, and D showed reductions in total bacterial count of 60.81%, 84.86%, and 98.20%, respectively, and reductions in coliform bacteria number of 99.60%, 99.75%, and 99.79%, respectively. These results demonstrate that higher conditioning temperatures yield better feed hygiene indicators.

2.2 Effects of High-Temperature Heat-Treated Mash Feed on Layer Production Performance

The effects of high-temperature heat-treated mash feed on layer production performance are presented in Table 3 . During weeks 1-4, the laying rate of heat-treated mash groups was higher than that of the conventional mash group, with lower feed-to-egg ratios. As conditioning temperature increased, average daily feed intake and average egg weight showed increasing trends, though differences were not significant ($P > 0.05$). During weeks 5-8, average daily feed intake in heat-treated mash groups was lower than in the conventional mash group, but the difference was not significant ($P > 0.05$). During weeks 9-12, average daily

feed intake, average egg weight, and laying rate in the conventional mash group were higher than those in heat-treated mash groups, with laying rate showing an increasing trend as conditioning temperature rose. Over the entire 1-12 week period, no significant differences were observed among groups in average daily feed intake, average egg weight, laying rate, or feed-to-egg ratio ($P>0.05$). Additionally, as feeding duration extended, average daily feed intake, average egg weight, and laying rate showed increasing trends across all groups, while feed-to-egg ratio first increased then decreased. These data indicate that while high-temperature heat-treated mash feed affects layer production performance differently at various stages, its overall impact during the entire experimental period was not significant.

2.3 Effects of High-Temperature Heat-Treated Mash Feed on Egg Quality

The effects of high-temperature heat-treated mash feed on egg quality are shown in Table 4. At week 4, no significant differences were observed among groups in eggshell strength, egg shape index, yolk ratio, or Haugh unit ($P>0.05$). Eggshell strength and thickness in heat-treated mash groups showed increasing trends with higher conditioning temperatures, exceeding those of the conventional mash group, with Group D showing significantly greater eggshell thickness than other groups ($P<0.05$). Haugh unit in heat-treated mash groups showed a decreasing trend with higher conditioning temperatures, falling below that of the conventional mash group ($P>0.05$). The yolk L^* value of the conventional mash group was significantly higher than that of heat-treated mash groups ($P<0.05$). The yolk b^* value of the conventional mash group was significantly lower than Groups C and D ($P<0.05$) but significantly higher than Group B ($P<0.05$), with no significant differences in yolk a^* values among groups ($P>0.05$).

At week 8, no significant differences were found among groups in eggshell strength, eggshell thickness, egg shape index, or yolk ratio ($P>0.05$), but Haugh unit in heat-treated mash groups was significantly lower than in the conventional mash group ($P<0.05$). The yolk L^* and a^* values of the conventional mash group were significantly lower than those of heat-treated mash groups ($P<0.05$), while yolk b^* value was higher than in heat-treated mash groups but without significant difference ($P>0.05$).

At week 12, no significant differences were observed among groups in eggshell strength, eggshell thickness, egg shape index, yolk ratio, or Haugh unit ($P>0.05$), but Group C showed significantly higher yolk L , a , and b^* values than the conventional mash group ($P<0.05$). As feeding duration extended, eggshell strength, eggshell thickness, and yolk ratio showed increasing trends, while Haugh unit showed a decreasing trend. The yolk L^* values of Groups A and B tended to decrease, while those of Groups C and D tended to increase. Yolk a^* and b^* values showed increasing trends across all groups, indicating that feeding diets conditioned at higher temperatures had greater effects on yolk color a^* and b^* values. These data demonstrate that high-temperature heat-treated

mash feed had no significant effects on eggshell strength, eggshell thickness, egg shape index, yolk ratio, or Haugh unit, but significantly affected yolk color.

2.4 Effects of High-Temperature Heat-Treated Mash Feed on Egg Hygiene Indicators

The effects of high-temperature heat-treated mash feed on egg hygiene indicators are presented in Table 5 . At weeks 4, 8, and 12, neither Salmonella nor coliform bacteria were detected in eggshell surfaces or egg liquid from either heat-treated or conventional mash groups, with total bacterial counts in egg liquid <10 CFU/mL and coliform bacteria <0.3 MPN/mL. The total bacterial count on eggshell surfaces in heat-treated mash groups was lower than in the conventional mash group, but the difference was not significant ($P>0.05$). As conditioning temperature increased, the total bacterial count gradually decreased in heat-treated mash groups, though differences were not significant ($P>0.05$). At week 4, coliform bacteria numbers on eggshell surfaces in Groups C and D were significantly lower than in the conventional mash group ($P<0.05$). At week 8, Group D showed significantly lower coliform bacteria numbers on eggshell surfaces than the conventional mash group ($P<0.05$). At week 12, Groups B, C, and D had lower coliform bacteria numbers on eggshell surfaces than the conventional mash group, but differences were not significant ($P>0.05$). These data indicate that higher conditioning temperatures yield better egg hygiene indicators.

2.5 Effects of High-Temperature Heat-Treated Mash Feed on Layer Digestive Tract Indices

The effects of high-temperature heat-treated mash feed on layer digestive tract indices are shown in Table 6 . The relative weights of gizzard, jejunum, and ileum in heat-treated mash groups were lower than those in the conventional mash group, but differences were not significant ($P>0.05$). As conditioning temperature increased, the relative weights of proventriculus, gizzard, duodenum, jejunum, and ileum all gradually increased in heat-treated mash groups, though differences were not significant ($P>0.05$). These data indicate that high-temperature heat-treated mash feed had no significant effects on layer digestive tract indices.

3.1 Effects of High-Temperature Heat Treatment on Feed Hygiene Indicators

The main evaluation indices for feed hygiene include total bacterial count, coliform bacteria number, mold count, and Salmonella number, with Salmonella posing the greatest hazard. China' s “Feed Hygiene Standard” stipulates that Salmonella must not be detected [6]. Since most harmful microorganisms in feed are primarily composed of proteins, their heat-induced denaturation can kill them, and the feed conditioning process—a high-temperature, high-moisture

procedure—can eliminate many harmful microorganisms [7-9]. Mccapes et al. [8] found that feed with 14.5% moisture conditioned at 85°C for 4.1 min could effectively eliminate *E. coli*. Yao [10] reported that *E. coli* could be killed after 15 min at 60°C. Ziggers [9] found that conditioning at 85°C with 14.5% moisture for 4 min was required to effectively reduce *Salmonella* numbers in feed. Huang et al. [11-12] demonstrated that conditioning processes could effectively reduce *E. coli* numbers in feed, with maximum and minimum effect values of 6.62 and 0.40 log reductions, respectively. Higher temperatures, longer durations, and greater moisture levels resulted in larger log reductions of *Salmonella*, with maximum and minimum effect values of 6.51 and 0.38, respectively. Pang and Feng [13] found that conditioning killed an average of 77.89% of microorganisms during feed processing, with kill rates of 95.70% for mold spores, 64.94% for bacterial spores, and 95.98% for yeast spores. From conditioning through pelleting and cooling to finished product, microbial numbers decreased by an average of 95.38%, with mold, bacterial, and yeast numbers decreasing by 97.99%, 99.78%, and 93.95%, respectively. In this study, conditioning conventional mash layer feed at 70, 80, and 90°C reduced total bacterial counts by 60.81%, 84.86%, and 98.20%, respectively, and coliform bacteria numbers by 99.60%, 99.75%, and 99.79%, respectively, with no *Salmonella* detected. These results demonstrate that conditioning can effectively kill microorganisms in feed and improve feed hygiene, consistent with the aforementioned studies.

3.2 Effects of High-Temperature Heat-Treated Mash Feed on Layer Production Performance

The main evaluation indices for layer production performance include average daily feed intake, laying rate, average egg weight, and feed-to-egg ratio. Currently, few studies have examined the effects of high-temperature heat-treated mash feed on layer production performance. Comparative data between domestic mash feeding and foreign heat-treated mash feeding show that domestic mash-fed layers have lower laying rates, smaller average egg weight, and higher feed-to-egg ratios (2.5:1.0), while foreign heat-treated mash-fed layers have higher laying rates, larger average egg weight, and lower feed-to-egg ratios (2:1) [1-4]. This study found no significant differences in average daily feed intake between conventional and heat-treated mash groups, possibly because while high-temperature heat treatment can gelatinize starch and degrade antinutritional factors, thereby improving nutrient properties and feed nutritional value, it can also inactivate enzymes and vitamins in feed and reduce protein and starch utilization [14-15]. Particularly for corn-based diets, high-temperature conditioning may cause Maillard reactions that reduce lysine and arginine utilization [16]. Additionally, this study found that high-temperature heat-treated mash feed affected layer production performance differently at various stages, but overall had no significant effect, which differs from domestic and foreign comparisons of raw versus heat-treated mash. This discrepancy may be due to differences in layer breeds, feed formulations, and feeding methods between domestic and foreign studies, all of which can influence final results.

3.3 Effects of High-Temperature Heat-Treated Mash Feed on Egg Quality

Egg quality is a crucial factor affecting layer production profitability, with main evaluation indices including eggshell strength, eggshell thickness, egg shape index, yolk ratio, Haugh unit, and yolk color. Currently, no studies have reported on the effects of high-temperature heat-treated mash feed on egg quality. This study found that high-temperature heat-treated mash feed had no significant effects on eggshell strength, eggshell thickness, egg shape index, yolk ratio, or Haugh unit, but significantly affected yolk color. This may be because high-temperature heat treatment during processing partially destroys carotenoids, reducing the carotenoid content available during egg formation and resulting in lighter yolk color. Additionally, high temperatures can cause Maillard reactions that darken feed color, thereby increasing yolk a^* values [17-19]. Furthermore, high-temperature conditions can cause vitamin losses in heat-treated mash feed, affecting calcium and phosphorus absorption and resulting in lower calcium and phosphorus utilization compared with conventional mash feed, which may explain the lower eggshell strength observed in heat-treated groups [17].

3.4 Effects of High-Temperature Heat-Treated Mash Feed on Egg Hygiene Indicators

Microbial contamination of eggshell surfaces and egg liquid originates from contamination by feces, feed, etc., during the laying process, with eggshell surfaces carrying large numbers of microorganisms that can penetrate into the egg through pores, causing spoilage [20]. If pathogenic bacteria contaminate the eggshell and invade through pores, they can also pose food poisoning risks [21]. Related studies have found that eggs from layers fed conventional mash feed have high bacterial counts on eggshell surfaces and in egg liquid, with high detection rates of microorganisms in egg liquid including *Streptococcus lactis*, paracoliform bacteria, *E. coli*, and cocci [1-4]. This study found that heat-treated mash groups had lower total bacterial counts and coliform bacteria numbers on eggshell surfaces than the conventional mash group, with better egg hygiene indicators at higher conditioning temperatures.

3.5 Effects of High-Temperature Heat-Treated Mash Feed on Layer Digestive Tract Indices

Currently, no studies have reported on the effects of high-temperature heat-treated mash feed on layer digestive tract indices. This study found that the relative weights of gizzard, jejunum, and ileum in heat-treated mash groups were lower than those in the conventional mash group, but differences were not significant. This may be because hydrothermal treatment of mash feed gelatinizes starch and denatures proteins, making them more easily digestible and absorbable, with faster passage through the digestive tract that fails to adequately promote digestive tract development. Additionally, as conditioning

temperature increased, the relative weights of proventriculus, gizzard, duodenum, jejunum, and ileum gradually increased, but differences were not significant. This may be because higher conditioning temperatures result in greater starch gelatinization and viscosity, causing feed particles to bind together and increasing particle size, thereby promoting digestive tract development.

4 Conclusion

- (1) The total bacterial count and coliform bacteria number in heat-treated mash groups were significantly lower than those in the conventional mash group. The total bacterial count on eggshell surfaces was lower than in the conventional mash group but without significant difference, while coliform bacteria numbers were significantly lower. High-temperature heat treatment can improve the hygiene indicators of feed and eggs, providing mold-resistant properties and ensuring bird health, with better hygiene indicators at higher conditioning temperatures.
- (2) High-temperature heat-treated mash feed had no significant effects on layer production performance. As conditioning temperature increased, average egg weight and laying rate in heat-treated groups showed increasing trends, but differences were not significant.
- (3) High-temperature heat-treated mash feed had no significant effects on egg quality, but significantly affected yolk color. As conditioning temperature increased, eggshell strength and thickness in heat-treated groups showed increasing trends, but differences were not significant.
- (4) The relative weights of gizzard, jejunum, and ileum in heat-treated mash groups were lower than those in the conventional mash group, but the effects were not significant.

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