Effects of Essential Oil Blend on Production Performance, Egg Quality, Follicular Development, and Serum Reproductive Hormone Indices in Hy-Line Brown Laying Hens (Postprint)

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Abstract

This experiment was conducted to investigate the effects of a plant essential oil mixture on production performance, egg quality, follicle development, and serum reproductive hormone parameters in Hy-Line Brown laying hens. A total of 432 healthy 25-week-old Hy-Line Brown laying hens with similar body weight and laying rate were randomly allocated into 4 groups, each consisting of 6 replicates of 18 hens. The control group received a corn-soybean meal basal diet, while the experimental groups received diets supplemented with 75, 150, or 225 mg/kg of the plant essential oil mixture. The study consisted of a 3-week preliminary period followed by a 12-week formal experimental period. The results indicated: 1) Egg weight in the 150 mg/kg group was significantly higher than in the control group ($P<0.05$), with no significant differences observed among groups in laying rate, feed intake, egg production, or feed-to-egg ratio ($P>0.05$). 2) Haugh units in the 75 and 150 mg/kg groups were significantly higher than in the 225 mg/kg group ($P<0.05$), albumen height in the 150 mg/kg group was significantly higher than in the 225 mg/kg group ($P<0.05$), and shell percentage in the 225 mg/kg group was significantly higher than in all other groups ($P<0.05$). No significant differences were detected among groups in egg shape index, shell color, shell thickness, shell strength, yolk color, yolk percentage, or albumen percentage ($P>0.05$). 3) No significant differences in follicle development indices were observed among groups ($P>0.05$). 4) Serum progesterone concentration in the 225 mg/kg group was significantly higher than in the other groups ($P<0.05$), with no significant differences in other serum reproductive hormone indices among groups ($P>0.05$). In summary, dietary supplementation with 150 mg/kg of the plant essential oil mixture improved egg weight and albumen quality in laying hens while exerting minimal effects on follicle development.

Full Text

Effects of Essential Oil Mixture on Performance, Egg Quality, Follicular Development and Serum Reproductive Hormone Indices of Laying Hens

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Abstract

This study investigated the effects of an essential oil mixture (EOM) on production performance, egg quality, follicular development, and serum reproductive hormone indices in Hy-Line Brown laying hens. A total of 432 healthy 25-week-old hens with similar body weight and laying rate were randomly allocated to 4 groups, each consisting of 6 replicates of 18 birds. The control group received a corn-soybean meal basal diet, while the experimental groups received the basal diet supplemented with 75, 150, or 225 mg/kg of EOM. The study included a 3-week adaptation period followed by a 12-week formal experimental period.

The results showed: (1) Egg weight in the 150 mg/kg EOM group was significantly higher than in the control group ($P<0.05$), though no significant differences were observed among groups in laying rate, feed intake, egg production, or feed-to-egg ratio ($P>0.05$). (2) The Haugh unit in both the 75 and 150 mg/kg EOM groups was significantly higher than in the 225 mg/kg group ($P<0.05$). Albumen height in the 150 mg/kg group was significantly higher than in the 225 mg/kg group ($P<0.05$), while the eggshell ratio in the 225 mg/kg group was significantly higher than in all other groups ($P<0.05$). No significant differences were detected among groups in shape index, eggshell color, thickness, strength, yolk color, yolk ratio, or albumen ratio ($P>0.05$). (3) No significant differences were found among groups in any follicular development parameters ($P>0.05$). (4) Serum progesterone content in the 225 mg/kg EOM group was significantly higher than in other groups ($P<0.05$), though no significant differences were observed in other serum reproductive hormone indices ($P>0.05$). In conclusion, dietary supplementation with 150 mg/kg EOM improved egg weight and albumen quality while having minimal effects on follicular development.

Keywords: laying hens; essential oil mixture; egg quality; follicular development; reproductive hormone

Introduction

Antibiotics have been widely used as growth promoters in livestock production worldwide, contributing significantly to the rapid development of the poultry industry. However, growing concerns about food safety and public health have drawn increasing attention to the problems associated with antibiotic use, including bacterial resistance and antibiotic residues. Following the EU' s complete ban on antibiotic growth promoters in 2006, the search for safe and effective alternatives has intensified. Potential substitutes include herbal essential oils, prebiotics, probiotics, and organic acids. Plant essential oils have become a research focus due to their antimicrobial, antioxidant, immunomodulatory, growth-promoting, and gut environment-modulating properties.

International research on the biological activities of plant essential oils began earlier and has achieved substantial progress, with investigations extending to cellular and molecular mechanisms of action. In contrast, domestic research remains relatively limited, focusing primarily on the effects of essential oils on growth performance and intestinal function in pigs and broilers. This study aimed to evaluate the effects of dietary supplementation with different levels of an essential oil mixture (EOM) on production performance, egg quality, follicular development, and serum reproductive hormone indices in Hy-Line Brown laying hens, providing a theoretical basis for the application of EOM in layer production.

Materials and Methods

1.1 Test Materials

The essential oil mixture was provided by Menon Animal Nutrition Technology Co., Ltd. (Shanghai, China). The product contained 18% cinnamaldehyde, 3% carvacrol, and 1% thymol, microencapsulated with palm oil using silicon dioxide as a carrier.

1.2 Experimental Design

A total of 432 healthy 25-week-old Hy-Line Brown laying hens with similar body weight and laying rate were randomly divided into 4 groups, with 6 replicates per group and 18 birds per replicate. The control group received a corn-soybean meal basal diet, while the experimental groups received the basal diet supplemented with 75, 150, or 225 mg/kg of EOM. The study consisted of a 3-week adaptation period followed by a 12-week formal experimental period. The basal diet was formulated according to NRC (1994) poultry nutrient requirements and the Chinese Feeding Standard for Chickens (2004). Diet composition and nutrient levels are presented in Table 1 .

The premix provided per kilogram of diet: VA 7,715 IU, VD 2,755 IU, VE 8.81 IU, VK 2.2 mg, VB 0.55 mg, nicotinic acid 19.8 mg, folic acid 0.28 mg, Mn 50 mg, Fe 25 mg, Cu 2.5 mg, Zn 50 mg, I 1.0 mg, Se 0.15 mg.

1.3 Housing and Management

The experiment was conducted at the test base of the Poultry Institute, Chinese Academy of Agricultural Sciences. Hens were housed in a three-tier cage system and fed twice daily at 08:00 and 15:00. Feed and water were provided ad libitum under conventional management practices. All groups were maintained under identical housing and management conditions according to Hy-Line' s recommended management guidelines.

1.4 Measurement Indices and Methods

1.4.1 Production Performance During the experimental period, daily records were maintained for egg number, egg weight, and mortality on a replicate basis. Feed consumption was recorded weekly. Laying rate, feed intake, egg production, egg weight, and feed-to-egg ratio were calculated accordingly.

1.4.2 Egg Quality At the end of the experiment, 6 eggs were randomly selected from each replicate for quality assessment. Parameters measured included shape index, eggshell color, eggshell thickness, eggshell strength, albumen height, yolk color, Haugh unit, yolk ratio, eggshell ratio, and albumen ratio. The Haugh unit was calculated using the formula: Haugh unit = $100 \times \log(H - 1.7W^{\cdot 3} + 7.57)$, where H represents thick albumen height (mm) and W represents egg weight (g). Equipment used included a CM-2300d spectrophotometer (Minolta, Japan), digital calipers (Guanglu Digital Measurement & Control Co., Ltd.), and multifunctional egg quality analyzer, eggshell thickness gauge, and eggshell strength tester (ORKA, Israel).

1.4.3 Follicular Development At the end of the experiment, 2 hens from each replicate were randomly selected, weighed, and blood samples were collected via wing vein. Following slaughter, hierarchical yellow follicles, atretic follicles, small yellow follicles, and large white follicles were visually counted and recorded. Oviduct length was measured, and relative oviduct length was calculated as: Relative oviduct length (cm/kg) = oviduct length (cm) / live body weight (kg).

1.4.4 Serum Reproductive Hormones Blood samples collected at the end of the experiment were centrifuged to obtain serum for determination of reproductive hormone concentrations, including prolactin, progesterone, androgen, follicle-stimulating hormone, luteinizing hormone, and estradiol. All serum reproductive hormone indices were measured using radioimmunoassay (RIA) with kits purchased from North Biotechnology Company, following the manufacturer' s instructions.

1.5 Data Processing and Statistical Analysis

All data were initially processed using Excel 2010 and then analyzed using SPSS 20.0 software. Results are expressed as "mean ± standard deviation." One-way ANOVA was used to test for significant differences among groups, followed by LSD multiple comparisons. Differences were considered significant at $P<0.05$.

Results

2.1 Effects of Essential Oil Mixture on Production Performance

The effects of EOM on production performance are presented in Table 2 . Egg weight in the 150 mg/kg EOM group was significantly higher than in the control group ($P<0.05$). No significant differences were observed among groups in laying rate, feed intake, egg production, or feed-to-egg ratio ($P>0.05$). The 150 mg/kg group exhibited the highest laying rate and feed intake, while the 225 mg/kg group showed the lowest feed-to-egg ratio.

2.2 Effects of Essential Oil Mixture on Egg Quality

The effects of EOM on egg quality are shown in Table 3 . No significant differences were detected among groups in shape index, eggshell color, thickness, strength, yolk color, yolk ratio, or albumen ratio ($P>0.05$). The Haugh unit in both the 75 and 150 mg/kg EOM groups was significantly higher than in the 225 mg/kg group ($P<0.05$). Albumen height was highest in the 150 mg/kg group, which was significantly higher than in the 225 mg/kg group ($P<0.05$). The eggshell ratio in the 225 mg/kg group was significantly higher than in all other groups ($P<0.05$).

2.3 Effects of Essential Oil Mixture on Follicular Development

The effects of EOM on follicular development are presented in Table 4 . No significant differences were observed among groups in the numbers of hierarchical yellow follicles, atretic follicles, small yellow follicles, or large white follicles ($P>0.05$). Similarly, no significant differences were found in relative oviduct length among groups ($P>0.05$).

2.4 Effects of Essential Oil Mixture on Serum Reproductive Hormone Indices

The effects of EOM on serum reproductive hormone indices are shown in Table 5 . Serum progesterone content in the 225 mg/kg EOM group was significantly higher than in all other groups ($P<0.05$). No significant differences were detected among groups in prolactin, androgen, follicle-stimulating hormone, luteinizing hormone, or estradiol concentrations ($P>0.05$).

Discussion

3.1 Effects of Essential Oil Mixture on Production Performance

The results demonstrated that dietary supplementation with 150 mg/kg EOM significantly increased egg weight, consistent with findings reported by Bölükbasi et al. However, Metin et al. and Bozkurt et al. found no significant effect of dietary essential oils on egg weight. In the present study, EOM supplementation had no significant effects on laying rate, feed intake, egg production, or feed-to-egg ratio, which aligns with the results of Mao et al. Similarly, Bölükbasi et al. reported that dietary thyme oil had no significant effect on feed-to-egg ratio in laying hens, and Florou-Paneri et al. found that dietary oregano essential oil did not significantly affect feed intake. In contrast, Ma et al. and Metin et al. reported that essential oil supplementation significantly improved egg production and feed-to-egg ratio. These discrepancies may be attributed to differences in essential oil composition and concentration among studies, or possibly to the supplementation levels and rearing conditions employed in our experiment, which may not have been optimal for eliciting the full performance-enhancing effects of EOM.

3.2 Effects of Essential Oil Mixture on Egg Quality

Bozkurt et al. investigated the effects of essential oil mixtures on alleviating heat stress in laying hens and found that dietary supplementation significantly reduced mortality and increased eggshell weight. This is consistent with our finding that the 225 mg/kg EOM group exhibited a significantly higher eggshell ratio compared to other groups. The increased eggshell ratio may be attributed to enhanced proliferation of beneficial gut bacteria, which improves intestinal absorption of minerals, particularly magnesium and calcium ions, thereby promoting eggshell formation. Dietary EOM had no significant effects on yolk color or yolk ratio, which agrees with Bozkurt et al. However, other studies have suggested that carvacrol can increase yolk ratio by mediating hepatic regulatory mechanisms that promote transfer of yolk precursors from the liver to the ovary. These divergent results may stem from differences in essential oil composition; our EOM primarily contained cinnamaldehyde and thymol, whereas other studies utilized oils with higher carvacrol content. Albumen height and Haugh unit are critical indicators of egg quality. Our results showed that the 150 mg/kg EOM group had the highest albumen height, significantly greater than the 225 mg/kg group, and that both the 75 and 150 mg/kg groups had significantly higher Haugh units than the 225 mg/kg group. These findings indicate that dietary EOM can improve albumen quality, though excessive supplementation levels should be avoided. These results differ from those of Metin et al., possibly due to variations in essential oil composition. The underlying mechanism may involve effects of EOM on the composition and quantity of thick albumen secreted by oviduct glands during egg formation, warranting further investigation.

3.3 Effects of Essential Oil Mixture on Follicular Development and Serum Reproductive Hormone Indices

Our results showed no significant differences among groups in the numbers of hierarchical follicles, atretic follicles, small yellow follicles, or large white follicles, indicating minimal effects of EOM on follicular development. However, dietary EOM supplementation increased serum progesterone content without significantly affecting other reproductive hormones such as prolactin, androgen, follicle-stimulating hormone, luteinizing hormone, or estradiol, and had no significant effect on relative oviduct length. Shi et al. reported that perilla seed extract significantly increased both progesterone and estradiol levels in laying hens. Previous research has shown that progesterone can inhibit elevation of blood calcium levels while promoting calcium transport to the oviduct, thereby increasing eggshell thickness and strength. This is consistent with our findings that the 225 mg/kg EOM group exhibited significantly higher serum progesterone content, accompanied by the highest eggshell ratio, eggshell strength, and eggshell thickness values. The molecular mechanisms linking dietary EOM supplementation, serum progesterone content, and eggshell quality require further investigation.

Conclusion

Dietary supplementation with 150 mg/kg essential oil mixture improved egg weight and albumen quality in laying hens while exerting minimal effects on follicular development.

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