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

Authors: Zhang An, Laidi, Peng Yan, Shi Shourong

Date: 2018-12-24T00:00:00+00:00

Abstract

This study aimed to investigate the effects of plant essential oil mixture 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 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 hens per replicate. The control group was fed a corn-soybean meal basal diet, while the experimental groups were fed experimental diets supplemented with 75, 150, and 225 mg/kg plant essential oil mixture in the basal diet, respectively. The pre-trial period lasted for 3 weeks, and the formal trial period lasted for 12 weeks. The results showed: 1) The egg weight in the 150 mg/kg plant essential oil mixture group was significantly higher than that in the control group ($P<0.05$). There were no significant differences in laying rate, feed intake, egg production, and feed-to-egg ratio among all groups ($P>0.05$). 2) The Haugh unit in the 75 and 150 mg/kg plant essential oil mixture groups was significantly higher than that in the 225 mg/kg plant essential oil mixture group ($P<0.05$), the albumen height in the 150 mg/kg plant essential oil mixture group was significantly higher than that in the 225 mg/kg plant essential oil mixture group ($P<0.05$), and the eggshell ratio in the 225 mg/kg plant essential oil mixture group was significantly higher than that in the other groups ($P<0.05$). There were no significant differences in egg shape index, eggshell color, eggshell thickness, eggshell strength, yolk color, yolk ratio, and albumen ratio among all groups ($P>0.05$). 3) There were no significant differences in follicular development indices of laying hens among all groups ($P>0.05$). 4) The serum progesterone content in the 225 mg/kg plant essential oil mixture group was significantly higher than that in the other groups ($P<0.05$). There were no significant differences in other serum reproductive hormone indices among all groups (P>0.05). In conclusion, dietary supplementation with 150 mg/kg plant essential oil mixture can improve egg weight and albumen quality in laying hens, with minimal effects on follicular development.

Full Text

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

ZHANG An[1], WANG Laidi[1], PENG Yan[2], SHI Shourong[1]*

[1]Poultry Institute, Chinese Academy of Agricultural Sciences, Yangzhou 225125, China

[2]Menon Animal Nutrition Technology Co., Ltd., Shanghai 201807, China

Abstract

This study investigated the effects of essential oil mixture (EOM) on performance, egg quality, follicular development, and serum reproductive hormone indices in 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 to 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 EOM. The adaptation period lasted 3 weeks, 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), while 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 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, eggshell thickness, eggshell strength, yolk color, yolk ratio, or albumen ratio (P>0.05). (3) No significant differences were found among groups in follicular development indices (P>0.05). (4) Serum progesterone content in the 225 mg/kg EOM group was significantly higher than in all other groups (P<0.05), while 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 in laying hens with minimal effects on follicular development.

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

Introduction

In livestock and poultry production, antibiotics have been widely used worldwide as growth promoters, ensuring rapid development of the animal industry. However, growing concerns about food safety and health have drawn increasing attention to problems associated with antibiotic use, such as bacterial resistance and antibiotic residues. The European Union banned all antibiotic usage in 2006, prompting the search for safe and effective alternatives, including 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-improving properties. International research on the biological activities of plant essential oils began earlier and has achieved certain results, progressively advancing to cellular and molecular mechanistic studies. In contrast, domestic research remains limited, focusing primarily on the effects of plant essential oils on growth performance and intestinal function in pigs and broilers. This study aimed to investigate the effects of dietary supplementation with different levels of essential oil mixture (EOM) on performance, egg quality, follicular development, and serum reproductive hormone indices in Hy-Line Brown laying hens, providing a theoretical reference for its application in egg production.

1.1 Experimental Materials

The essential oil mixture was provided by Menon Animal Nutrition Technology Co., Ltd., Shanghai, containing primarily 18% cinnamaldehyde, 3% carvacrol, and 1% thymol, coated with palm oil using silica 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 adaptation period lasted 3 weeks, followed by a 12-week formal experimental period. 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 EOM. The basal diet was formulated according to NRC (1994) poultry nutrient requirements and China' s "Feeding Standard of Chickens" (2004). Its composition and nutrient levels are shown in Table 1 .

Table 1 Composition and nutrient levels of the basal diet (air-dry basis)

Items	Content
Ingredients	

Items	Content
Corn	
Soybean meal	
Limestone	
DL-Met	
$CaHPO_4$	
Choline chloride (50%)	
NaCl	
Premix	
Total	
Nutrient levels	
ME (MJ/kg)	
CP	
AP	
Lys	
Met	

The premix provided the following per kg of diet: VA 7,715 IU, VD_3 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 Management

The experiment was conducted at the experimental base of the Poultry Institute, Chinese Academy of Agricultural Sciences. A three-tier step cage system was used with feeding at 08:00 and 15:00 daily. Birds had free access to feed and water under conventional rearing conditions. All groups were maintained under identical management conditions following Hy-Line Company' s recommended management practices.

1.4 Measurements

1.4.1 Performance During the experimental period, daily records were kept 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.

1.4.2 Egg Quality At the end of the experiment, 6 eggs per replicate were randomly selected for egg quality assessment, including shape index, eggshell color, eggshell thickness, eggshell strength, albumen height, yolk color, Haugh unit, yolk ratio, eggshell ratio, and albumen ratio.

Haugh unit = $100 \times \log(H - 1.7W^{0 \cdot 37} + 7.57)$, where H is thick albumen height (mm) and W is egg weight (g).

Primary equipment included: CM-2300d spectrophotometer (Minolta, Japan), digital calipers (Guanglu Digital Measurement & Control Co., Ltd.), and multi-functional 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 per replicate were randomly selected, weighed, and blood samples were collected via wing vein. After slaughter, hierarchical yellow follicles, atretic follicles, smaller 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 contents, including prolactin, progesterone, androgen, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estradiol. All serum reproductive hormone indices were measured by radioimmunoassay (RIA) using kits from Northern Bioengineering Company according to the manufacturer' s instructions.

1.5 Data Processing and Statistical Analysis

All data were initially processed using Excel 2010 and then analyzed by one-way ANOVA using SPSS 20.0. Results are expressed as "mean ± standard deviation." Differences among groups were tested by one-way ANOVA followed by LSD multiple comparison, with $P<0.05$ considered statistically significant.

Results

2.1 Effects of EOM on Performance

The effects of EOM on laying hen performance are shown 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 showed the highest laying rate and feed intake, while the 225 mg/kg group had the lowest feed-to-egg ratio.

Table 2 Effects of essential oil mixture on performance of laying hens

<table>
<tr><th>Items</th><th>Essential oil mixture supplemental level (mg/kg)</th><th>P-value</th></tr>
<tr><td></td><td>0</td><td>75</td></tr>
<tr><td>Laying rate (%)</td><td>95.68$±1.37|95.03±1.68|96.79±0.74|95.17±0.86|||Feedintake(kg)|121.24±3.18|121.52±0.70|122.81±</td><td></td></tr>
</table>

In the same row, values with different small letter superscripts differ significantly (P<0.05), while those with the same or no superscripts do not differ significantly (P>0.05). The same applies below.

2.2 Effects of EOM on Egg Quality

The effects of EOM on egg quality are presented in Table 3 . No significant differences were observed among groups in shape index, eggshell color, eggshell thickness, eggshell strength, yolk color, yolk ratio, or albumen ratio (P>0.05). The Haugh unit in 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, significantly exceeding that of the 225 mg/kg group (P<0.05). The eggshell ratio in the 225 mg/kg EOM group was significantly higher than in all other groups (P<0.05).

Table 3 Effects of essential oil mixture on egg quality of laying hens

Items	Essential oil mixture supplemental level (mg/kg)	P-value
	0	75
Shape index	1.31$±0.00|1.30±0.02|1.31±0.01|1.30±0.01|||$Eggshellcolor$|23.23±2.83|21.86±2.07|22.18±2.89|22.52	

2.3 Effects of EOM on Follicular Development

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

Table 4 Effects of essential oil mixture on follicle development of laying hens

Items	Essential oil mixture supplemental level (mg/kg)	P-value
	0	75
Hierarchical yellow follicles (n)	5.42$±0.38|5.58±0.58|5.50±0.77|5.92±1.16|||$Atreticfollicle(n)$|12.75±3.42|12.50±0.84|12.67±4.74|1	

2.4 Effects of EOM on Serum Reproductive Hormone Indices

The effects of EOM on serum reproductive hormone indices are presented 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 observed among groups in prolactin, androgen, FSH, LH, or estradiol contents (P>0.05).

Table 5 Effects of essential oil mixture on serum reproductive hormone indices of laying hens

<table>
<tr><th>Items</th><th>Essential oil mixture supplemental level (mg/kg)</th><th>P-value</th></tr>
<tr><td></td><td>0</td><td>75</td></tr>
<tr><td>Prolactin (mIU/mL)</td><td>289.12$±52.95|221.25±62.38|257.86±61.06|232.61±44.05|||*Androgen(ng/mL)*|0.08±0.05|0.10±0.06|</td><td></td></tr>
</table>

Discussion

3.1 Effects of EOM on Performance

The results showed that dietary supplementation with 150 mg/kg EOM significantly increased egg weight, consistent with findings by Bölükbasi et al. However, Metin et al. and Bozkurt et al. reported no significant effects of dietary essential oils on egg weight. In this study, EOM supplementation had no significant effects on laying rate, feed intake, egg production, or feed-to-egg ratio, which aligns with results from Mao et al. Bölükbasi et al. also found that dietary thyme oil had no significant effect on feed-to-egg ratio, and Florou-Paneri et al. reported no significant effects of dietary oregano essential oil on feed intake. However, Ma et al. and Metin et al. demonstrated that dietary 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, as well as the supplementation level and rearing environment in this study, which may not have been optimal for eliciting maximal performance benefits.

3.2 Effects of EOM on Egg Quality

Bozkurt et al. investigated the effects of EOM 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 had a significantly higher eggshell ratio than other groups. The increased eggshell ratio may be due to enhanced intestinal absorption of minerals, particularly Mg^{2+} and Ca^{2+}, resulting from increased beneficial bacteria promoted by essential oil supplementation. Dietary EOM had no significant effects on yolk color or yolk ratio, consistent with Bozkurt et al., although some studies have shown that carvacrol can increase yolk ratio by mediating hepatic regulatory mechanisms to promote yolk precursor transfer from the liver. These discrepancies may stem from differences in EOM composition; our mixture primarily contained cinnamaldehyde and thymol, whereas other studies used higher carvacrol concentrations. Albumen height and Haugh unit are important indicators of egg quality. Our results showed that the 150 mg/kg group had the highest albumen height, significantly greater than the 225 mg/kg group, and 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, but excessive supplementation levels should be avoided. These results differ from Metin et al., possibly due to variations in essential oil composition. The underlying mechanism may involve effects of EOM on the content and composition of thick albumen secreted by oviduct glands during egg formation, requiring further investigation.

3.3 Effects of EOM on Follicular Development and Serum Reproductive Hormone Indices

This study found no significant differences among groups in the numbers of hierarchical yellow follicles, atretic follicles, smaller yellow follicles, or large white follicles. Dietary EOM increased serum progesterone content but had no significant effects on other reproductive hormones including prolactin, androgen, FSH, LH, or estradiol, and no significant effect on relative oviduct length. These results suggest that EOM has minimal impact on follicular development and oviduct length, while increasing progesterone content. Shi et al. reported that perilla seed extract significantly increased serum progesterone and estradiol levels in laying hens. Research has shown that progesterone can inhibit elevation of blood calcium levels and promote calcium transport to the oviduct, thereby increasing eggshell thickness and strength. This is consistent with our findings that the 225 mg/kg EOM group had significantly higher serum progesterone and eggshell ratio, with the highest values for eggshell strength and thickness. The molecular mechanisms underlying the effects of dietary EOM on 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.

References

[1] HERTRAMPF J W. Alternative antibacterial performance promoters[J]. Poultry International, 2001, 40(1): 50-52.

[2] HOOGE D M. Dietary alternatives for improving live performance of antibiotic-free poultry[J]. Zootecnica International, 2006(3): 42-51.

[3] METIN Ç, SERDAR E, AHMET A, et al. Effects of herbal essential oil mixture as a dietary supplement on egg production in quail[J/OL]. The Scientific World Journal, 2014, http://dx.doi.org/10.1155/2014/573470.

[4] JIA C H, CHEN M Y, YANG C M, et al. Regulation of plant essential oils on production performance and health of monogastric animals[J]. Chinese Journal

of Animal Nutrition, 2015, 27(4): 1055-1060.

[5] JI S L, LI A H, JIANG J L. Effects of dietary plant extracts on growth performance and carcass quality of weaner-finisher pigs[J]. Chinese Journal of Animal Science, 2013, 49(8): 61-67.

[6] LIU Y, ZANG S M, LI T Z, et al. Effects of cinnamaldehyde on intestinal microflora, intestinal morphology, and nutrient digestibility in broilers[J]. Chinese Journal of Animal Science, 2013, 13: 65-68.

[7] WANG G Q, WU B C, CHENG Y F, et al. Effects of plant essential oils on growth performance and health status of growing pigs[J]. Journal of Domestic Animal Ecology, 2014, 8: 18-21.

[8] NRC. Nutrient requirements of poultry[S]. 9th ed. Washington D.C.: National Academy Press, 1994.

[9] BÖLÜKBASI S C, ERHAN M K, KAYNAR O. The effect of feeding thyme, sage and rosemary oil on laying hen performance, cholesterol and some proteins ratio of egg yolk and Escherichia coli count in feces[J]. Archiv für Geflugelkunde, 2008, 72(5): 231–237.

[10] BOZKURT M, KÜCÜKYILMAZ K, PAMUKCU M, et al. Long-term effects of dietary supplementation with an essential oil mixture on the growth and laying performance of two layer strains[J]. Italian Journal of Animal Science, 2012, 11(5): 23–28.

[11] MAO H X, WU S G, ZHANG H J, et al. Effects of plant extract essential oil mixture on growth performance, intestinal microflora, and intestinal mucosal morphology of broilers[J]. Chinese Journal of Animal Nutrition, 2011, 23(3): 433-439.

[12] BÖLÜKBASI S C, ERHAN M K, KAYNAR O. Effect of dietary thyme oil on laying hens performance, cholesterol ratio of egg yolk and Escherichia coli concentration in feces[J]. International Journal of Natural and Engineering Science, 2007(1): 55–58.

[13] FLOROU-PANERI P, NIKOLAKAKIS I, GIANNENAS I, et al. Hen performance and egg quality affected by dietary oregano essential oil and α-tocopheryl acetate supplementation[J]. International Journal of Poultry Science, 2005, 4(7): 449–454.

[14] MA D, SHAN A, CHEN Z, et al. Effect of Ligustrum lucidum and Schisandra chinensis on the egg production, antioxidant status and immunity of laying hens during heat stress[J]. Archives of Animal Nutrition, 2005, 59(6): 439–447.

[15] BOZKURT M, KÜCÜKYILMAZ K, CATLIAU M, et al. Performance, egg quality and immune response of laying hens fed diets supplemented with mannan-oligosaccharide or an essential oil mixture under moderate environmental conditions[J]. Poultry Science, 2012, 91(6): 1379-1386.

[16] ROBERFROID M B. Prebiotics and probiotics: are they functional foods?[J]. American Journal of Clinical Nutrition, 2000, 71(Suppl): 1682–1687.

[17] BOTSOGLOU N A, YANNAKOPOULOS A L, FLETOURIS D J, et al. Effect of dietary thyme on oxidative stability of egg yolk[J]. The Journal of Agricultural Chemistry, 1997, 45: 3711-3716.

[18] BOTSOGLOU N A, FLOROU-PANERI P, CHRISTAKI E, et al. Effect of dietary oregano essential oil on performance of chickens and on iron-induced lipid oxidation of breast, thigh and abdominal fat tissues[J]. British Poultry Science, 2002, 43: 223–230.

[19] SHI Y L, GU X H, HUANG Y, et al. Effects of perilla seed extract on production performance, reproductive hormones, and immune function of laying hens during late peak production[J]. Chinese Journal of Animal Nutrition, 2015, 27(5): 1519-1526.

[20] ZHAO H X. Effect of progesterone on eggshell calcification in laying hens[D]. PhD dissertation. Xinjiang: Shihezi University, 2013.

Note: Figure translations are in progress. See original paper for figures.