Long-term effects of dietary supplementation with an essential oil mixture on the growth and laying performance of two layer strains

Mehmet Bozkurt,1 Kamil Küçükylmaz,1 Mehmet Pamukçu,2 Metin Çabuk,3 Ahmet Alçiçek,4 Abdullah U. Çatlı1
1Erbeýli Tavukçuluk Araðtırma Enstitüsü, Aydin, Turkey
2Batı Akdeniz Tarımsal Araştırma Enstitüsü, Erbeyli, Antalya, Turkey
3Kanatlı Bölümü, Celal Bayar Üniversitesi, Manisa, Turkey
4Zootekni Bölümü, Ege Üniversitesi, İzmir, Turkey

Abstract

One thousand two hundred 1-day-old Lohmann LSL white and Lohmann Brown layer chickens were fed diets supplemented with either an antibiotic growth promoter (AGP) or an herbal essential oil mixture (EOM) till 58 wk of age to reveal the long-term effects of those additives on growth, performance and wholesome egg quality parameters. The study was arranged in a 2×3 factorial design with two layer strains and three feed additive regimens. Thus, the layer birds of both strains were randomly assigned to the three dietary treatments, i.e., standard basal diet (control), control with AGP (specifically, avilamycin, 10 mg/kg diet) and control with EOM (24 mg/kg diet). The data regarding egg production were recorded between 22 to 58 weeks of age. Neither the dietary treatments nor the bird strain influenced the body weight and mortality of the birds in both the growing and laying period. AGP or EOM supplementation to the laying hen diet significantly increased the egg production rate and egg weight as compared to the control diet alone, but egg mass output, feed consumption, and feed conversion ratio were not affected by the dietary treatments. Neither dietary treatment created any statistically significantly differences in egg quality parameters. The research findings have confirmed the beneficial effects of supplementation with feed-grade EOM on the laying rate and egg weight of both white and brown layers.

Introduction

Consumer concern and governmental regulations continue to limit or prohibit the use of antibiotics growth promoters (AGP) at subtherapeutic levels as growth promoters in the feed of food animals since resistance to pathogenic microorganisms and some residues in food products associated with AGP have been evidenced (Rosen, 1996; Gustafson and Bowen, 1997; Hayes and Jensen, 2003). For example, such a ban was implemented in the European Union (EU) on 1 January 2006; indeed, these new strict rules forbid the use of all feed-grade AGP in poultry diets (Cervantes, 2006; Michaud, 2008). Consequently, this resulted an urgent industrial demand for potential AGP replacements, such as probiotics, prebiotics, organic acids, and botanicals. One alternative to antimicrobial feed additives is the use of essential oils derived from herbs and spices as additives. Today, this practice is receiving a great deal of attention, particularly in terms of its use in the diets of broiler chickens. Several reports in the scientific literature discuss administration the laying hen diet with essential oils as dietary supplements on the performance of laying hens, the responses of laying pullets and hens to treatments with AGP, but their efficacy in achieving the same effects remains open to question. Although an understanding of their mode of action would be a prerequisite for their optimal application in terms of efficacy, a full understanding of these aspects in farm animals is not yet achieved (Steiner, 2009; Wallace et al., 2010).

Indeed, a series of scientific reports and field trials have demonstrated that the addition of essential oils, either individual oils or in combination, resulted in beneficial effects in terms of the bird’s zootecnological performance, including body weight gain, feed efficiency, and viability (Williams and Losa, 2001; Alçiçek et al., 2009a; Bozkurt et al., 2009b). Conversely, the responses of laying pullets and hens to dietary essential oil supplementation are seemingly less pronounced than that of broiler chickens. Several reports in the scientific literature discuss administration the laying hen diet with extract and essential oils of aromatic herbs. According to those few reports available, supplementation of layer diets with herbal essential oils may affect performance parameters such as egg production rate, egg weight, feed intake and feed conversion ratio (Bölükbaş et al., 2007, 2008, 2009; Orhan and Eren, 2011). Effects on egg quality traits, such as yolk and albumen ratio, shell thickness, and shell weight, were reported in few studies only (Bölükbaş et al., 2008, 2010). In a recent pioneer work, beneficial effects on egg production, egg output, feed conversion ratio, and cracked-broken egg ratio were observed when an essential oil combination and AGP were added to the diets of laying hens (Çabuk et al., 2006). The same essential oil combination also showed comparable results to that of an AGP in terms of settable egg yield and reproductive performance (Bozkurt et al., 2009a).

Despite the lack of information regarding the effects of essential oils as dietary supplements on the performance of laying hens, the initial results related to their effect on broiler performance are encouraging (Isabel et al., 2009; Brenes and Roura, 2010). Therefore, this research was designed to more clearly define the mechanisms by which essential oils affect growth, laying performance, and egg quality parameters for layers. For that purpose, a long-term experiment (from 0 to 58 weeks of age, which is throughout the growing and laying phases) was conducted using commercial layer breeds, specifically Lohmann white and brown chickens.

Materials and methods

Animals and housing

Pullet trial

One-day-old Lohmann LSL-Classic White and
Lohmann Brown layer chickens (600 white and 600 brown layer chickens) were obtained from a commercial hatchery. The experiment was arranged in a 2×3 factorial design with two layer strains and three feed additive treatments. Accordingly, birds were weighed individually and then randomly distributed to three dietary treatments. Each treatment consisted of four replications (50 birds per floor pen) amounting to 200 birds per treatment group. From hatching to 16 weeks of age, all birds were housed in a 24-pen growing house. Floor pens, each occupying a space of 4.8 m² with pine shavings as litter material, were used. Birds were kept in an open-sided, naturally ventilated growing house and exposed to natural day length. Feed and water were provided ad libitum. Feed was provided in circular hanging feeders, and water was available in hanging bell drinkers. Each pen was equipped with two feeders and one drinker. The ingredients, nutrient composition, dietary inclusion levels, and content of the AGP and essential oil mixture (EOM) additives were explained details below, in the explanations of pullet and layer diets.

The individual body weight of each bird and the feed consumption on a pen basis was measured at 8 and 16 weeks of age. Mortality was recorded daily. After weighing pullets individually at the end of the 16 weeks of age, the average body weight was calculated immediately for each dietary treatment of both strains of birds. Meanwhile, the heaviest and smallest birds (the outliers) were eliminated from the treatments without creating a deviation from the original average data. Then, at the beginning of 17 weeks of age, the adjusted number of pullets (96 pullets from both strains for each dietary treatment) was moved to the laying house where the pullets continued on the dietary treatment without creating a deviation from the laying phase. Hens were at 5% and 50% egg production rate on week 19 and 21, respectively.

Pullet and layer diets

The ingredients and the nutrient composition of the experimental diets are presented in Table 1. Three different feed additive regimens were applied to both of the layer strains used in the present study. Commercial chick starter, grower, and layer hen diets were offered to layers from one-day-old to 8 weeks of age, 9 to 16 weeks of age, and 17 to 58 weeks of age, respectively. In the control treatment, birds were fed a basal diet with corn, wheat, and soybean as the main ingredients. The remaining two groups were given the same basal diet and supplements of an additional AGP or EOM. The three dietary treatments used in this study were as follows: i) basal diet with no performance enhancer feed additives (Control); ii) AGP (i.e., 10 mg avilamycin/kg basal diet); iii) EOM (i.e., 24 mg EOM/kg basal diet).

The EOM (Heryumix® Herba Ltd. Co., Izmir, Turkey) included carvacrol, thymol, 1,8-cineole, p-cymene, and limonene as active components and also contained six different essential oils [Oregano oil (Origanum sp.), Laurel leaf oil (Laurus nobilis), Sage leaf oil (Salvia triloba), Myrtle leaf oil (Myrtus communis), Fennel seeds oil (Foeniculum vulgare), and Citrus peel oil (Citrus sp.)]. The EOM used 976 g of zeolite as a carrier for 24 g of essential oil. An AGP preparation containing 10,000 mg of avilamycin per kg of premix (Kavlialamycin®, Kartal Kimya- BASF, Gebze, Turkey) was also investigated as a supplement. The AGP and EOM premixes were added as supplements to the basal diet (1 kg of supplement/1000 kg of feed) was added in place of antibiotics and was applied to both of the layer strains used in the present study.

Table 1. Ingredients and chemical composition of the basal layer diets, as fed.

| Ingredients, % | Chicken, 1-8 weeks | Grower, 9-16 weeks | Layer, 17-58 weeks |
|---------------|--------------------|--------------------|-------------------|
| Corn          | 53.54              | 50.47              | 46.73             |
| Wheat         | 10.00              | 22.00              | 12.00             |
| Soybean meal, CP 46% | 20.00              | 13.20              | 19.31             |
| Sunflower meal, CP 31% | 10.00              | 10.00              | 7.00              |
| Fish meal, CP 64% | 2.88               | -                  | -                 |
| Bone and meat meal, CP 32% | -                  | -                  | 5.71              |
| Fish oil      | -                  | -                  | -                 |
| Ground limestone | 1.60              | 2.00               | 7.15              |
| Dicalcium phosphate | 1.08              | 1.53               | -                 |
| NaCl          | 0.25               | 0.25               | 0.25              |
| Vitamin premix† | 0.25               | 0.25               | 0.25              |
| Mineral premix† | 0.10               | 0.10               | 0.10              |
| DL-methionine | 0.10               | 0.06               | 0.10              |
| Lysine        | 0.10               | 0.10               | -                 |
| Sawdust§      | 0.10               | 0.10               | 0.10              |
| Total         | 100.00             | 100.00             | 100.00            |

Analysed chemical composition

| Dry matter, % | 88.94              | 89.26              | 89.88             |
| Crude protein, % | 19.63              | 15.84              | 16.23             |
| Ether extract, % | 3.28               | 3.53               | 4.16              |
| Crude fibre, % | 4.15               | 5.22               | 3.65              |
| Crude ash, %   | 4.94               | 5.74               | 10.55             |
| Starch, %      | 38.72              | 40.75              | 40.18             |
| Sugar, %       | 2.97               | 3.32               | 3.23              |
| Calcium, %     | 1.09               | 1.13               | 3.23              |
| Total phosphorus, % | 0.70               | 0.63               | 0.69              |
| Lysine †, %    | 0.85               | 0.86               | 0.85              |
| Methionine+cystein †, % | 0.68               | 0.60               | 0.69              |
| AME †, kcal/kg | 2839               | 2789               | 2836              |

CP, crude protein; Vitamin premix (kg diet): vitamin A 12,000 U; vitamin D3, 2400 U; vitamin E, 30 mg; vitamin Kc, 2.5 mg; vitamin B6, 2.5 mg; vitamin B12, 12 mg; niacin amid, 55 mg; calcium D-pantothenate, 8 mg; vitamin B1, 6.25 mg; vitamin B2, 0.03 mg; folic acid, 15 mg; D-biotine 0.045 mg; vitamin C, 50 mg; choline chloride, 150 mg; *Mesorial premix (mg/kg diet): Mn, 80; Fe, 80; Zn, 60; Cu, 8; Co, 0.2; I, 0.5; Se, 0.15; †Sawdust was substituted by antibiotic growth promoter or essential oil mixture preparations; †calculated composition.
of the sawdust that is included in the basal diet as inert filler. All diets were isocaloric and isonitrogenous and were formulated to meet or exceed the NRC’s (National Research Council, 1994) recommendations for layer hens. Feed was prepared four-weekly. The standard techniques for proximate analysis were used to determine the nutrient concentrations in the diets (Naumann and Bassler, 1993). The experimental diets were also analyzed for starch, sucrose, total calcium, and phosphorus (Table 1) using the Association of German Agricultural Analysis and Research Institutes (VDLUFA) method (Naumann and Bassler, 1993). The metabolizable energy content of the diets was calculated based on chemical composition (Turkish Standards Institute, 1991).

Experimental parameters measured

All birds were weighed individually throughout the laying period at 24, 40, and 56 weeks of age to determine average body weight. Hen-day egg production (in percentage) was recorded daily and is described as egg production rate hereafter. The feed consumption and feed conversion ratio (FCR) were determined on a replicate basis at weekly intervals. As previously stated, mortality was recorded daily. The magnitude of production variables, such as feed intake, feed conversion ratio, and egg production were adjusted for hen mortality. During the laying period, a random sample of 8 eggs/replicate/day were collected on two consecutive days per week. Hence, a total of 2304 eggs were individually weighed for each treatment to determine average egg weight throughout the laying stage. Egg mass was calculated by multiplying egg production by the average egg weight on replicate basis and determined at weekly intervals. External egg quality characteristics including egg specific gravity, egg shell breaking strength, egg shell weight, egg shell thickness; and internal egg quality indices including albumen height, were monitored at the end of every 28 day period. The egg quality characteristics were evaluated using random samples of 6 eggs from each replicate, thereby totaling 24 eggs from each treatment. When determining egg quality characteristics, the sample of eggs were individually weighed at initiation, and then the egg specific gravity was measured using a serious of graded salt solutions according to method described by Hamilton (1982). Egg shell breaking strength was measured using egg shell tester equipment (Egg Force Reader, SANONO Technology AS, Odense NV, Denmark), and expressed as unit of compression force exposed to unit egg shell surface area (kg/cm²). Then, eggs were cracked and carefully separated from the egg shell. After removing shell membranes manually, egg shell thickness (without the inner and outer shell membranes) was measured at three different points (top, middle, and bottom) using a micrometer (model IT-014UT, Mitutoya, Kawasaki, Japan). An average of three different thickness measurements from each egg was used to describe egg shell thickness. The shell without the membranes was weighted and the relative proportion of shell was determined. Albumen height (±0.1 mm) was measured as indicated by Keener et al. (2006) using an electronic height gauge (QCH-Systems, TSS). The experiment used a completely randomized design, and each experimental unit was a replicate consisting of six groups of adequately caged layer hens fed as one group. The experimental unit was a group of 50 layer pullets in grower period. The data was analyzed on two-way ANOVA using the general linear models procedure found in SAS software (SAS, 1991). The main effects of diet, strain, and diet-strain interaction were tested. Duncan’s multiple range test was carried out to detect differences among treatments. All differences were considered significant at P<0.05.

Results and discussion

The effects of dietary treatments and bird strain on body weight, feed intake, and mortality of layer pullets are shown in Table 2. Dietary additives had no statistically significant effect on body weight and feed intake of pullets at 8 and 16 weeks of age. As expected, brown pullets were heavier than white pullets and consumed more feed due to their genetic characteristics. The mortality of pullets was not significantly influenced by the dietary additives or by the different bird strains throughout the growing period. No statistically significant diet by strain interaction was observed for any of the traits examined.

The body weight of hens determined at 24, 40, and 58 weeks of age was not affected by the dietary treatments. Naturally, the brown strain was significantly heavier than the white strain throughout the test period. Mortality did not differ significantly among the treatments throughout the laying period. Unfortunately, there has been only one scientific evident that indicated that the positive effects of essential oils on mortality and body weight of laying hens (Çabuk et al., 2006). The effects of the dietary treatments on the laying hen performance of the two strains are presented in Table 3. The egg production rate of hens fed diets supplemented with AGP and EOM was 2.25% and 2.19% higher, respectively, than that of hens receiving the standard control diet (P<0.01). Egg production did not differ for hens on the AGP supplemented diet as compared to those on the EOM supplemented diet. Additionally, egg production for the white layer strain was similar to that of the browns. However, a significant interaction between diets and strains was observed (P<0.05). While

Table 2. Body weight, feed intake and mortality of white and brown line layer pullets through grower period fed on diets with antibiotic growth promoter or essential oil mixture.

| Items          | Diet          | Body weight, g | Feed intake, g/pullet | Mortality, % |
|----------------|---------------|----------------|-----------------------|--------------|
|                | As hatched    | 8 weeks        | 16 weeks              | 0 to 8 weeks | 0 to 16 weeks | 0 to 16 weeks |
| White          | Control       | 34.04          | 589a                  | 1128b       | 1784         | 5313         | 3.80          |
|                | EOM           | 34.00          | 601b                  | 1157b       | 1735         | 5213         | 2.88          |
|                | AGP           | 34.18          | 683a                  | 1289a       | 1885         | 5597         | 1.92          |
| Brown          | Control       | 34.08          | 704a                  | 1330a       | 1812         | 5529         | 0.96          |
|                | EOM           | 34.11          | 636                   | 1208        | 1834         | 5455         | 2.86          |
|                | AGP           | 34.17          | 640                   | 1243        | 1778         | 5413         | 1.92          |
|                | EOM           | 34.04          | 652                   | 1243        | 1773         | 5371         | 1.88          |
|                | Control       | 34.04          | 592b                  | 1144b       | 1749         | 5262a        | 2.84          |
| Strain         | White         | 34.16          | 690b                  | 1318b       | 1841         | 5583a        | 1.60          |
|                | Brown         | 0.29           | 4.38                  | 6.32        | 32.13        | 42.96        | 0.56          |

| Source of variation | Probability |
|---------------------|-------------|
| Diet                | 0.7562      | 0.0128        | 0.0193         | 0.0193       | 0.0271       | 0.0671       | 0.0398       | 0.0204       |
| Strain              | 0.8521      | 0.0001        | 0.0001         | 0.0001       | 0.0001       | 0.0001       | 0.0001       | 0.0001       |
| Diet × strain       | 0.8803      | 0.0279        | 0.3886         | 0.6043       | 0.6451       | 0.5645       | 0.5645       | 0.5645       |

AGP, antibiotic growth promoter; EOM, essential oil mixture; a,b means within columns, within main effects, with different superscripts are different at P<0.05; data are means of 4 replicate pens of 50 pullets each per treatment.
In EOM treated birds in brown strain. In accordance with the results of the present experiment, similar improvements on laying performance of brown (Çabuk et al., 2006) and white (Bölükbaşı et al., 2007) layers were observed in previous works. Specifically, the dietary supplementation of mixed essential oils and thyme oil improved egg production rate by 5.43% and 6.20%, respectively, in the former and latter works. These results were also confirmed by our group focusing on broiler breeders (Bozkurt et al., 2009b). A similar observation was reported by Ather (2000), who determined that the addition of an essential oil combination to the broiler breeder diet resulted in remarkable improvements on egg production. Our findings are also in agreement with those of several authors (Miles et al., 1985; Bessie, 1994; Jamroz et al., 1998), who reported that the supplementation of AGP to layer hen diet improved egg production rate or egg weight.

Egg weight was significantly affected by dietary treatments. Eggs from hens fed the EOM and AGP supplemented diets were heavier (0.75 g and 1.07 g, respectively) than that of hens fed a control diet (P<0.05). Conformingly, there have been some researches that reported the beneficial effects of dietary supplementation with essential oils of thyme at 0.01, 0.02, 0.03% (Bölükbaşı et al., 2007), and sage and rosemary at 0.02 % (Bölükbaşı et al., 2008) on egg weight in white layer strain. Egg weight was not affected by the strain. However, a significant diet by strain interaction was observed on egg weight (P<0.01).

Specifically, the egg weight of white layers fed an EOM supplemented diet was 1.06 g heavier than that of untreated birds, whereas less benefit (0.48 g) was obtained in brown strain. However, these initial determinations in the scientific literature have not point out yet a significant promotion in terms of their food consumption by dietary essential oils based on their ad libitum feed intake. Additionally, the feed conversion ratio (FCR) was not affected (P>0.05) by the different feed additive regimens or different strains of hen (Table 3). Similar reports from Florou-Paneri et al., (2005) and Bölükbaşı et al., (2007) found that the addition of oregano oil and thyme oil, respectively, to layer hen diet had no significant effect on FCR. Some other earlier works revealed similar levels of improvements in FCR as was reported in the present study regarding with essential oil mixture supplementation to the diet (Çabuk et al., 2006, Bölükbaşı et al., 2008).

The effect of diet and strain on some egg quality characteristics are shown in Table 5.

### Table 3. Influence of dietary administration with antibiotic growth promoter or essential oil mixture on performance of white and brown laying hens from 22 to 58 weeks of age.

| Items          | Diet  | Egg production rate, % | Egg weight, g | Egg mass, g/day | Feed intake, g/day | FCR, g feed/g egg |
|----------------|-------|-------------------------|--------------|----------------|-------------------|------------------|
| White          | Control | 80.77       | 60.58       | 48.93           | 108.13            | 2.21             |
|                | EOM    | 84.34       | 61.07       | 51.50           | 111.24            | 2.16             |
|                | Control | 81.28       | 60.54       | 49.20           | 111.68            | 2.27             |
|                | AGP    | 82.21       | 61.56       | 50.60           | 111.32            | 2.20             |
| Brown          | Control | 83.37       | 61.02       | 50.87           | 109.87            | 2.16             |
|                | EOM    | 81.02       | 60.56       | 49.06           | 109.90            | 2.24             |
|                | AGP    | 83.27       | 61.31       | 51.05           | 111.28            | 2.18             |
|                | EOM    | 83.21       | 61.63       | 51.28           | 109.98            | 2.14             |
|                | White  | 82.72       | 61.29       | 50.70           | 109.82            | 2.16             |
|                | Brown  | 82.28       | 61.04       | 50.22           | 110.95            | 2.21             |
| Pooled SEM°    |        | 0.49        | 0.16        | 0.48            | 2.24              | 0.05             |

Source of variation Probability

| Diet | 0.0001 | 0.0001 | 0.0785 | 0.8739 | 0.2111 |
|------|--------|--------|--------|--------|--------|
| Strain | 0.2796 | 0.0536 | 0.8725 | 0.3105 | 0.2986 |
| Diet x strain | 0.0125 | 0.0001 | 0.7750 | 0.7953 | 0.9898 |

FCR, feed conversion ratio; AGP, antibiotic growth promoter; EOM, essential oil mixture; °data are means of 4 replicate of six adjacent cages with 24 hens each per treatment.

### Table 4. Effects of dietary supplementation with antibiotic growth promoter or essential oil mixture on body weight and mortality of two layer hen strains during the laying period.

| Items          | Diet  | Body weight, g | Mortality, % |
|----------------|-------|----------------|--------------|
|                | 20 weeks | 24 weeks | 40 weeks | 58 weeks | 22 to 58 weeks |
| White          | Control | 1314        | 1521       | 1581     | 1722       | 6.12          |
|                | EOM    | 1308        | 1512       | 1605     | 1705       | 5.28          |
|                | Control | 1394        | 1693       | 1921     | 2110       | 3.12          |
|                | AGP    | 1506        | 1732       | 1928     | 2112       | 4.16          |
|                | EOM    | 1495        | 1719       | 1905     | 2095       | 3.12          |
|                | Control | 1399        | 1597       | 1753     | 1912       | 4.68          |
|                | AGP    | 1410        | 1626       | 1754     | 1917       | 3.64          |
|                | EOM    | 1401        | 1615       | 1755     | 1895       | 4.20          |
| Brown          | Control | 1305        | 1512       | 1590     | 1712       | 4.88          |
|                | AGP    | 1501        | 1715       | 1918     | 2104       | 3.46          |
| Pooled SEM°    |        | 9.32        | 11.93      | 14.02    | 15.59      | 1.12          |

Source of variation Probability

| Diet | 0.8327 | 0.5466 | 0.9955 | 0.3118 | 0.781 |
|------|--------|--------|--------|--------|-------|
| Strain | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.468 |
| Diet x strain | 0.3105 | 0.4894 | 0.2125 | 0.6803 | 0.523 |

AGP, antibiotic growth promoter; EOM, essential oil mixture; °data are means of 4 replicate of six adjacent cages with 24 hens each per treatment.

The daily feed intake of hens was not affected (P>0.05) by the strain of the hens or by the supplementation of EOM and AGP to the diet (Table 4). While this data was in accordance with that of several preliminary studies (Florou-Paneri et al., 2005; Çabuk et al., 2006; Nichol and Steiner, 2008; Bölükbaşı et al., 2009, 2010), some others with reduced feed intake showed contrast to our results (Bölükbaşı et al., 2007, 2008). However, those initial determinations in the scientific literature have not point out yet a significant promotion in terms of their food consumption by dietary essential oils based on their ad libitum feed intake. Additionally, the feed conversion ratio (FCR) was not affected (P>0.05) by the different feed additive regimens or different strains of hen (Table 3).
Conclusions

The results from the present study clearly demonstrate that the use of a simple mixture of six herbal essential oils as individual supplement create some noticeable benefits on egg production and egg weight of white and brown laying hens when compared to unsupplemented control diet; it is an effective performance enhancer. In conclusion, these results also suggest that the dietary addition of an herbal EOM as natural origin is a viable alternative to AGP in layer hen nutrition.

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Table 5. Effects of antibiotic growth promoter or essential oil mixture supplementation to diet on egg quality traits of two layer strains.

| Items     | Diet   | Specific gravity, g/cm³ | Shell breaking strength, kg/cm² | Shell weight, % | Shell thickness, µm | Albumen height, mm |
|-----------|--------|------------------------|---------------------------------|----------------|---------------------|-------------------|
| Strain    |        |                        |                                 |                |                     |                   |
|           | White  | 1.092                  | 2.49                            | 10.43          | 357                 | 8.33              |
|           | AGP    | 1.092                  | 2.69                            | 10.42          | 361                 | 8.06              |
|           | EOM    | 1.089                  | 2.53                            | 10.22          | 356                 | 8.20              |
|           | Control| 1.078                  | 2.68                            | 9.95           | 371                 | 7.17              |
|           | Brown  | 1.073                  | 2.58                            | 10.06          | 375                 | 7.03              |
|           | AGP    | 1.077                  | 2.67                            | 10.05          | 370                 | 7.18              |
|           | EOM    | 1.085                  | 2.58                            | 10.19          | 364                 | 7.75              |
|           | Control| 1.083                  | 2.61                            | 10.24          | 368                 | 7.54              |
| Diet      | White  | 1.091                 | 2.57                            | 10.35          | 355                 | 8.19              |
|           | Brown  | 1.070                 | 2.64                            | 10.02          | 372                 | 7.12              |
| Pooled SEM |       | 0.0027                | 0.069                           | 0.089          | 2.49                | 0.116             |
| Diet      |        | 0.5176                | 0.665                           | 0.5888         | 0.1528              | 0.0535            |
| Strain    |        | 0.0001                | 0.1440                          | 0.0001         | 0.0001              | 0.0001            |
| Diet × strain | 0.3875 | 0.0688               | 0.2697                          | 2.49           | 0.116               | 0.0535            |

AGP, antibiotic growth promoter; EOM, essential oil mixture; a,b means within columns, within main effects, with different superscripts are different at P<0.05; °data are means of 4 replicate of six adjacent cages with 24 hens each per treatment.
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