



Effect of cinnamaldehyde and 1,8-cineole on performance, egg quality and some blood parameters of laying hens

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ABSTRACT

This study was aimed at determining the effects of the addition of cinnamaldehyde and 1,8-cineole to laying hen rations on performance, egg quality and some blood parameters. For this purpose, 96 (48-week-old and initial body weight average 1,773.19 g) laying hens of the Bovans White lineage were used. Birds were fed on a standard basal ration (PC) and basal rations were added with 500 mg/kg of antibiotic (NC); 100 mg/kg of cinnamaldehyde (T1); and 100 mg/kg of 1,8-cineole (T2) for 60 days. While the feed consumption levels of groups T1 and T2 were significantly lower than that of group PC, their egg production was significantly lower than that of groups PC and NC. When compared to groups PC and NC, it was observed that the feed additive given to group T2 had significantly reduced the feed conversion rate. All of the feed additives used in this study were determined to have decreased egg weight, eggshell strength and eggshell thickness, in comparison to the measurements of group PC. Furthermore, when compared to groups PC and NC, groups T1 and T2 were ascertained to have lower serum glucose and cholesterol levels. However, when compared to the other 3 groups, group T1 presented with significantly higher serum aspartate aminotransferase (AST) levels and group T2 displayed a significantly higher rate of defective eggs. In result, the addition of cinnamaldehyde and 1,8-cineole to the ration was observed to show a positive impact on serum glucose and cholesterol levels, and a negative impact on other performance parameters and eggshell quality. It was concluded that further more detailed investigation is required in this added to laying hen rations.

Key words: 1,8-cineole, Cinnamaldehyde, Egg quality, Laying performance, Serum parameters

The increasing demand for natural products has increased the importance of medicinal and aromatic plants in the livestock sector. These plants, which are known to have a rather large potential in terms of their chemical constituents, contain two or three main components at high levels (Karakullukçu *et al.* 2016). These main components can be found at high levels in more than one plant. For instance, while 1,8-cineole has been detected at a rate of approximately 50% in daphne, 50% in sage, and 45% in rosemary; cinnamaldehyde is found at a significantly high level of almost 90% in cinnamon (Guler and Dalkilic 2005). The levels of active substances in plants vary with species, variety, time of harvest, type of processing, and the environmental and ecological conditions of the growth environment (Basyigit and Baydar 2017). Both the level and scope of the effectiveness of plants may vary with the properties and amount of active substances they contain (Toroglu and Cenet 2006, Malayoglu 2010, Turan and Ozturk 2012, Andrade *et al.* 2014). The amount of active substances found in plants showing such variances accounts for the different results achieved with the use of the same plants in different studies (Malayoglu 2010). Recent

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research has shown that cinnamon not only has strong antibacterial, antioxidant, anti-inflammatory, anti-allergic, and hypocholesterolaemic effects, but also regulates lipogenesis (Ciftci *et al.* 2010, Bingol and Akbulut 2012, Torki *et al.* 2015). Furthermore, in an investigation on its antidiabetic components, the active substance cinnamaldehyde reduces plasma glucose and HbA1C levels and affects cellular glucose metabolism (Subash Babu *et al.* 2007, Bingol and Akbulut 2012). Literature indicated that while eucalyptus, which contains a high level of 1,8-cineole, is effective in the treatment of malaria and upper respiratory infections (Akagunduz 2016), daphne, sage and rosemary have antibacterial, antioxidant and immunostimulatory effects (Gachkar *et al.* 2007, Kaya and Turgut 2012, Cimrin and Demirel 2016b). To date, in animal research, neither the specific effects of the several active substances found in plant products nor whether these substances have synergistic or antagonistic effect, have been determined. This study was aimed at finding an answer to some questions not yet replied. For this purpose, a group fed on a standard basal ration, and another group given long-term antibiotic in feed to increase performance, were used as controls. This study was designed to determine the impact of rations, containing 99% of cinnamaldehyde in 100 mg/

kg substance and 98.63% of 1,8-cineole in 100 mg/kg substance, on layer hen performance, egg quality criteria and some blood parameters.

MATERIALS AND METHODS

Bovans White laying hens (96), aged 48 weeks, were used in this study. The hens were randomly allocated to positive and negative control groups and 2 treatment groups, each including 24 animals. The average initial and final body weight of the hens were 1,773.19–1,709.71 g. Each group was divided into 4 replicates as subgroups, comprising 6 hens each. The groups were housed in cages (30 cm × 44 cm × 44 cm) in a windowed poultry house under a 16/8 h light/dark regimen. Feed in mash form and water were provided *ad lib.* during the 60 day-study period. The rations provided to the groups were formulated according to the nutrient requirements set by the National Research Council (NRC 1994) for laying hens. The ingredients and chemical composition of the rations are shown in Table 1. While no additives were given to the positive control group (PC), the basal ration was added 500 mg/kg of antibiotic (chlortetracycline) in the negative control group (NC), 100 mg/kg of cinnamaldehyde (99%)

Table 1. Ingredients and chemical composition of the basal ration

Parameter	%
<i>Ingredient</i>	
Corn	63.8
Soybean meal	16.1
Sunflower meal	4.5
Full fat soybean	2.9
Vegetable oil	0.5
Meat bone meal	2.5
Limestone	8.2
Dicalcium phosphate	1
Salt	0.25
*Vitamin mineral premix	0.25
Total	100
<i>Chemical composition (analyzed)</i>	
Dry matter (%)	89.91
Crude protein (%)	16.26
Ether extract (%)	3.62
Crude fiber (%)	3.27
Ash (%)	11.06
Calcium (%)	2.78
Total Phosphorus (%)	0.49
**Metabolizable energy (MJ/kg)	11.70 MJ/kg

*The following mineral-vitamin premix was provided per kg of ration: 480,000 IU vitamin A, 100,000 IU vitamin D₃, 1,200 mg vitamin E, 160 mg vitamin K₃, 120 mg vitamin B₁, 280 mg vitamin B₂, 1,200 mg Niacin, 200 mg vitamin B₆, 0.6 mg vitamin B₁₂, 2,000 mg vitamin C, 400 mg Ca-D-Pantothenate, 1.8 mg D-biotin, 40 mg folic acid, 8,000 mg choline chloride, 240 mg Zn, 40 mg Cu, 180 mg Mn, 47,520 mg DL-methionine, 32,400 mg P, 40 mg I, 6 mg Se and 200,000 mg NuPro, 1800 SPU 3 phytase, 3,600 mg glucan, 2,400 mg mannan, 75,000 HUT protease, 500 CMCU cellulase, 140 mg canthaxanthin.** Calculated value.

in group treatment 1 (T1), and 100 mg/kg of 1,8-cineole (98.63%) in group treatment 2 (T2). The nutrient composition of the rations was analysed according to the method of the AOAC (2000). The samples were ashed in a muffle furnace prior to the analysis of calcium (Farese *et al.* 1967) and total phosphorus (ADAS 1981) levels. Crude fibre was determined as per Crampton and Maynard (1938). The metabolizable energy levels of the samples were estimated using the equation of Carpenter and Clegg (1956).

The analysis results and suppliers of the feed additives are presented in Table 2. The active substance analyses were performed at the Centre for Research and Development and Laboratories for Pharmacokinetic Applications and Environmental and Food Analyses of Ege University [ARGEFAR/L-ÇEG-AY-17 (U.S. Pharmacopeia National Formulary USP 23 NF 18, p 1755 (1995))] using a gas chromatography-mass spectrometry device (GC-MSD).

Hens were individually weighed at both the beginning and end of the experiment. Mortality cases were recorded. Eggs were collected daily and egg production (EP) was expressed on a per hen per day basis. The defective eggs of the groups were recorded daily (broken, cracked, crustless, etc). All eggs were collected daily and weighed individually to determine egg weight. Feed intake was also recorded biweekly and calculated as g feed per day per hen. The feed conversion ratio was calculated as g feed per g egg.

Egg quality was determined biweekly by examining 12 eggs randomly collected from 09:30 to 12:30 h from each replicate on two consecutive days. A total of 768 eggs were analyzed during the study period. Each egg was weighed and its shape index was measured with a special instrument (B.V. Apparatenfabriek Van Doorn, No: 75 135/2, De Bilt, Holland). Eggshell strength was measured using an egg breaking tester (static compression device, Dr.-Ing. Georg Wazau Mess-+Prüftechnik, Berlin, Germany).

The egg was broken on to a glass-topped table. Eggshell thickness was measured in 3 different parts (upper and lower ends and middle) using a micrometre (Mitutoya, No. 2050-08, 0.01–20 mm; Kawasaki, Japan). The height of the albumen (Ah) and the yolk (Yh) was measured with a tripod micrometre (Mitutoyo, No. 2050S-19, 0.01–20 mm; Kawasaki, Japan). The length and width of the albumen and the diameter of the yolk were measured using a digital calliper. Using these values, the yolk index [(yolk height/yolk diameter) × 100], albumen index [(albumen height/average of albumen length and albumen width) × 100] and Haugh units [100 × log (H + 7.57–1.7W^{0.37}), where H is albumen height and W is egg weight] were calculated (Card and Nesheim 1972). Egg yolk colour was determined by means of comparison with the Roche colour fan.

Blood samples were collected at the end of the trial, from the brachial vein, under the wing, of 12 hens randomly chosen from each group and were centrifuged at 3,000 × g for 10 min. Serum was collected and stored at –20°C for the determination of glucose, total cholesterol, triglyceride, aspartate aminotransferase (AST) and alkaline phosphatase (ALP) levels with an auto analyser using commercial kits.

Table 2. Analysis results and suppliers of the feed additives used

Active Substance 1,8-cineole			Active Substance Cinnamaldehyde		
	Results (%)	NTS**/LOQ		Results (%)	NTS**/LOQ
1,8-cineole	98.63	—	Cinnamaldehyde	99.00	—
Limonene	0.48	—	Benzaldehyde	0.44	—
Cinnamaldehyde	0.43	—	Thymol	0.19	—
Paracymene	0.17	—	Undefined	0.37	—
Alpha-phellandrene	0.11	—			
Beta-myrcene	0.09	—			
Alpha-pinene	0.07	—			

**NTS/LOQ: Limit of Quantitation. *Source*: Synthesized compound; Company Agromiks Feed Additives, Livestock Industry and Trade Limited Company; Antibiotic (Chlortetracycline) Vimar Food, Agriculture and Livestock Joint-Stock Company.

The biochemical parameters were analyzed by Architect c8000 system (Abbott Laboratories, USA)

Statistical analyses: Data obtained from the present study were analysed using the SPSS 21.0 programme (SPSS Inc., Chicago, IL, USA, 2012). One-way ANOVA was performed to examine the differences between the groups. The significances of the mean differences among the groups were tested by Duncan's test (Dawson and Trapp 2001).

RESULTS AND DISCUSSION

The differences detected between the groups for the mean lives weights measured at the beginning and end of the trial were statistically insignificant ($P > 0.05$; Table 3). This was attributed to the feed provided to the animals being isocaloric and isonitrogenic. The findings obtained in the present study are in agreement with those reported in previous research on the use of cinnamon (Jamroz *et al.* 2005, Kollanoor-Johny *et al.* 2012, Tonbak and Ciftci 2012, Naderi *et al.* 2014, Torki *et al.* 2015) and essential oil additives containing the active substance 1,8-cineole (Hajiazizi *et al.* 2016).

The daily feed consumption levels of groups T1 and T2 were significantly lower than that of the positive control group ($P < 0.01$, Table 3). The animals, which were fed on rations that were added cinnamaldehyde and 1,8-cineole, having been determined to consume less feed was considered to be related to either these active substances having a strong odour or to the particular levels they were

added to the ration. These results were similar to those reported to have been obtained with the use of the active substance 1,8-cineole (Cimrin and Demirel 2016a), but differed from those obtained with the use of cinnamon powder and oil (Abdalla *et al.* 2011, Tonbak and Ciftci 2012, Naderi *et al.* 2014, Simsek *et al.* 2015, Torki *et al.* 2015) and mixtures of plant extracts containing 1,8-cineole (Sizmaz *et al.* 2014, Simsek *et al.* 2015, Hajiazizi *et al.* 2016), which demonstrated no effect to have occurred on feed consumption. To date, the majority of related research has been conducted with the use of the dry powder, extracts or essential oils of plants, and the focus has not been the isolation of plant active substances. However, in the present study, the active substances cinnamaldehyde, found in cinnamon, and 1,8 cineole found in various plants such as eucalyptus, daphne, sage and rosemary, were used in almost pure form, at concentrations of 99% and 98.63%, respectively. This is considered the main reason of the differences observed between the findings of the present study and the previous research referred to above, as the use of plants containing several active substances together differs from the use of plant active substances in pure form. For example, in addition to 1,8-cineole the rosemary plant contains other active substances such as alpha-pinene, trans-caryophyllene and camphor, which may have synergistic and antagonistic interactions resulting in positive or negative effects. This is also valid for the cinnamon plant.

Significant differences existed between the groups with

Table 3. Effects of the experimental treatments on the performance parameters of laying hens

Parameter	Treatment group					P value
	PC	NC	T1	T2	SEM	
Initial body weight (g)	1781	1771	1764	1773	0.01	0.942
Final body weight (g)	1695	1741	1686	1705	0.013	0.524
Feed intake (g/day)	115.67 ^a	112.94 ^{ab}	110.97 ^b	109.75 ^b	0.622	0.008
Feed conversion ratio (kg feed/kg egg)	1.80 ^b	1.83 ^b	1.88 ^{ab}	1.95 ^a	0.015	0.003
Egg production (%)	97.90 ^a	96.69 ^a	91.36 ^b	87.66 ^b	0.007	0.000
Egg weight (g)	65.56 ^a	63.72 ^d	64.94 ^b	64.37 ^c	0.069	0.000
Defective eggs (%)	1.17 ^b	2.23 ^b	1.87 ^b	8.59 ^a	0.605	0.003

Means in the same row with different superscripts differ significantly ($P < 0.05$; $P < 0.01$); SEM, standard error of mean; P-value, significance level ($\alpha = 0.05$).

respect to the feed conversion ratio ($P < 0.01$; Table 3). In group T2, the addition of 1,8-cineole to the ration was determined to have negatively affected the feed conversion ratio of the hens, when compared to the groups PC and NC. This result was attributed to the egg production of group T2 being significantly low with respect to the level of feed intake as well as the percentage of defective eggs being significantly higher than those of the other groups. This result contradicts with the report indicating rosemary essential oil not having any effect on the feed conversion ratio group T1, which received cinnamaldehyde in feed, did not show any statistically significant difference from the other groups. Furthermore, reports indicated that cinnamon powder and oil, containing the active substance cinnamaldehyde, have no effect on the feed conversion ratio in poultry (Tonbak and Ciftci 2012, Naderi *et al.* 2014) and do not positively affect the feed conversion ratio (Abdalla *et al.* 2011, Torki *et al.* 2015, Simsek *et al.* 2015).

Egg production was observed to have significantly decreased in Groups T1 and T2, when compared to groups PC and NC ($P < 0.001$; Table 3). While this result of the present study contradicted with the report of Simsek *et al.* (2015) indicating cinnamon oil to significantly increase egg production, it supported the report of the same researchers suggesting rosemary essential oil to reduce egg production. The present study disagrees with previous research, which report plant essential oils and extract additives containing cinnamaldehyde and 1,8-cineole to not have any effect on egg production (Sizmaz *et al.* 2014, Cimrin and Demirel 2016a, Hajiazizi *et al.* 2016) and to increase egg production (Abdalla *et al.* 2011, Torki *et al.* 2015).

In the present study, egg weight was ascertained to have been negatively affected by all of the additives used, and when compared to the positive control group, group T1 showed the greatest decrease followed by groups T2 and NC ($P < 0.001$; Table 3). While some researchers have reported plant additives containing the active substances cinnamaldehyde and 1,8-cineole do not have any effect on egg weight (Sizmaz *et al.* 2014, Hajiazizi *et al.* 2016, Simsek *et al.* 2015), some other researchers indicated that these active substances increase egg weight (Abdalla *et al.* 2011, Torki *et al.* 2015).

In the present study, the percentage of defective eggs was determined to have significantly increased in group T2, which was fed on a ration having 1,8-cineole ($P < 0.001$; Table 3). The present study showed that in terms of performance parameters, none of the additives used had a positive effect on these parameters, other than having reduced the feed consumption of laying hens. Some literature reports suggested that certain feed additives may negatively affect performance parameters in poultry (Salgueiro *et al.* 2010, Tajodini *et al.* 2015). In the present study, the increase observed in the percentage of defective eggs with the long-term use of 1,8-cineole at the indicated dose, was attributed to the disruption of mineral absorption in the hens. Furthermore, as 1,8-cineole has not been added to laying hen rations at such a high percentage before in

previous studies, such a negative impact may not have been encountered. Thus, the results of the present study contradict with the findings of Hajiazizi *et al.* (2016), who suggested the addition of rosemary essential oil to laying hen rations do not have any effect on the percentage of defective eggs.

The findings obtained in the present study demonstrated that the addition of cinnamaldehyde and 1,8-cineole to the basal ration did not cause any statistically significant effect on the egg shape index ($P > 0.05$; Table 4). These results are in agreement with the results of some earlier studies conducted with the use of plant extracts containing the same active substances (Sizmaz *et al.* 2014, Torki *et al.* 2015). Differently, Hajiazizi *et al.* (2016) reported the egg shape index to increase with the use of rosemary essential oil.

The present study showed that the differences between the groups for eggshell weight were statistically insignificant ($P > 0.05$; Table 4). Our results were in agreement with previous research suggesting that cinnamon (Torki *et al.* 2015) and rosemary essential oil (Hajiazizi *et al.* 2016) do not have any influence on eggshell weight, they contradicted with the report of Simsek *et al.* (2015) suggesting a positive impact of cinnamon on eggshell weight and quality. According to Bozkurt *et al.* (2012), an essential oil mixture provided increments in eggshell weight.

Each of the additives used in the present study significantly reduced the eggshell strength, when compared to group PC ($P < 0.01$, Table 4). The decrease in the eggshell strength of the eggs laid by the animals that received additives in their feed, in comparison to those fed on a standard basal ration, was considered to be due to either the increased passage rate of nutrients through the digestive tract resulting in the disrupt of their absorption or an antagonistic effect with other nutrients. Contrary to our results, some literature reports suggested that similar other additives do not affect eggshell strength (Sizmaz *et al.* 2014, Torki *et al.* 2015).

In this study, the additives negatively affected and significantly decreased eggshell thickness ($P < 0.05$, Table 4). These results disagreed with reports suggesting plant extracts containing cinnamon (Torki *et al.* 2015) and 1,8-cineole (Sizmaz *et al.* 2014, Hajiazizi *et al.* 2016) do not to alter eggshell thickness, and that cinnamon oil increased eggshell thickness, compared to rosemary essential oil (Simsek *et al.* 2015). Our results were attributed to the additives given to groups T1 and T2 having increased the passage rate of nutrients through the digestive tract, and thus, having decreased the feed conversion rate (Table 3).

It was ascertained that the groups did not significantly differ from each other for the egg, albumen index, yolk index, Haugh unit or yolk colour ($P > 0.05$, Table 4). Our result showed similarity to the results reported in earlier research (Torki *et al.* 2015, Hajiazizi *et al.* 2016, Ding *et al.* 2017).

Serum glucose levels were significantly lower in groups NC, T1 and T2, in comparison to group PC ($P < 0.001$,

Table 4. Mean values of the internal and external quality traits of the eggs

Parameters	Treatment group					P
	PC	NC	T1	T2	SEM	
Shape index (%)	76.11	76.12	75.87	76.76	0.202	0.446
Shell weight (g)	7.29	6.94	7.15	7.09	0.047	0.060
Shell breaking strength (kg/cm ²)	5.16 ^a	4.65 ^b	4.43 ^b	4.74 ^b	0.076	0.005
Shell thickness (µm)	384.95 ^a	369.27 ^b	367.86 ^b	365.46 ^b	2.518	0.029
Albumen index (%)	7.41	7.45	7.58	7.88	0.089	0.271
Yolk index (%)	39.84	39.18	40.01	39.65	0.169	0.355
Haugh unit (HU)	78.51	79.48	81.94	80.15	0.453	0.058
Yolk colour	11.25	11.47	11.69	11.47	0.062	0.101

Means in the same row with different superscripts differ significantly ($P<0.05$; $P<0.01$); SEM: standard error of mean; P value, significance level ($\alpha=0.05$).

Table 5). It has been reported that cinnamaldehyde, which is the active substance of cinnamon, significantly reduces plasma glucose and HbA1C levels and affects the cellular glucose metabolism (Subash Babu *et al.* 2007, Ping *et al.* 2010, Bingol and Akbulut 2012). Cimrin and Demirel (2016b) determined that rosemary essential oil containing 45.04% of 1,8-cineole did not affect glucose levels.

These different results may be due to the purity or amount of the active substances or other active substances found in the plant extracts used in the indicated studies. In our study, neither of the additives had any effect on serum triglyceride levels ($P>0.05$; Table 5), and this result was similar to those reported earlier, suggesting neither cinnamon nor 1,8-cineole have such an effect (Torki *et al.* 2015, Cimrin and Demirel 2016b, Hajiazizi *et al.* 2016).

When compared to the control groups, groups T1 and T2 presented with significantly lower serum cholesterol levels ($P<0.05$; Table 5). Some reports suggested that serum total cholesterol levels decrease with the addition of rosemary and cinnamon powder to laying hen rations (Abdalla *et al.* 2011, Hajiazizi *et al.* 2016), while, some other reports suggested that neither cinnamon nor additives containing 1,8-cineole impacted serum cholesterol levels (Torki *et al.* 2015, Cimrin and Demirel 2016b). As mentioned above, the differences in the results of these studies are considered to be related to the active substances used.

In the present study, the serum aspartate aminotransferase (AST) levels of the animals included in group T1 were significantly higher than those of the animals included in

the other groups ($P<0.05$; Table 5). Altintas and Fidanci (1993) reported the standard range of AST values in poultry as 30–170 IU/l. However, in the present study, the AST level of group T1 was determined as 225.50 IU/l, and was above the reference range. On the other hand, the ALP level of group T1 was determined as 438.75 IU/l, which was although nonstatistically higher than the levels measured in the other groups ($P>0.05$). The ALP level of group T1 was within the reference range of 200–1060 IU/l reported by Altintas and Fidanci (1993).

The AST and ALP levels measured in the blood samples of group T1, being higher than those measured in the other groups suggested that the long-term continuous use of cinnamaldehyde in poultry at the indicated dose and purity level (99%) may have damaged tissue. As is known, liver damage is detected by means of the measurement of aminotransferase levels. Of these enzymes, AST is found in several tissues, including liver, heart, muscle, kidney and brain tissue. Damage to any of these tissues increases blood AST levels (Bulum and Mengi 2000). Therefore, it is considered that further more detailed investigation is required of the additive given to group T1, which eventually increased the AST levels of the animals.

In conclusion, it was determined that the addition of the active substances cinnamaldehyde (T1) and 1,8-cineole (T2) to the basal ration of laying hens had no positive impact on the performance parameters, other than decreasing feed consumption. In fact, it was ascertained that the feed conversion rate, egg weight, egg production and eggshell quality were adversely affected by the use of these additives.

Table 5. The effect of the treatments on some biological blood parameters

Parameter	Treatment group					P value
	PC	NC	T1	T2	SEM	
Glucose (mg/dL)	202.25 ^a	155.50 ^b	98.50 ^c	116.25 ^c	5.25	0.000
Triglyceride (mg/dL)	1125.00	1251.50	1043.50	976.75	54.74	0.363
Cholesterol (mg/dL)	208.75 ^a	234.50 ^a	124.00 ^b	130.50 ^b	12.07	0.015
AST (IU/L)	168.75 ^b	164.50 ^b	225.50 ^a	178.25 ^b	7.23	0.041
ALP (IU/L)	386.25	347.75	438.75	364.25	21.29	0.486

Means in the same row with different superscripts differ significantly ($P<0.05$; $P<0.001$); SEM: standard error of mean; P value: significance level ($\alpha=0.05$).

Furthermore, group T2, which was fed on a ration added with 1,8-cineole, presented with a percentage of defective eggs much higher than those of the other groups. While the 2 additives showed positive effects such as significantly decreasing serum glucose and cholesterol levels, it was also determined that, the level of AST an indicator of liver damage, had significantly increased in group T1, which was fed on a ration added with cinnamaldehyde. In this context, our results demonstrated the need for the conduct of more detailed investigations on the use of these active substances in laying hen rations to elucidate their exact effects.

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