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Pre- and Post-Hatch Effects of Eucalyptol Supplements to Water-Based Humidifiers in Broilers' Incubators

Research Article

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ABSTRACT

Direct abiotic environmental factors may influence embryonic development, chick quality and adult phenotype. Supplementing various levels of 1, 8-cineol (eucalyptol) to water as humidity source in broiler's incubator and investigating its pre- and post-hatch effects to determine the appropriate application protocol were the aims of this study. Four levels (0, 0.2, 0.4, 0.6%) of pure eucalyptol with 3 replicates were used on 1440 Ross 308 broiler chicks. During incubation, moisture loss on 18th day of incubation, hatchability, and weights of hatched chicks were measured. Post-hatch, various performance factors and carcass traits were determined through 42 d rearing period. Eucalyptol at 0.2% resulted in the greatest moisture loss at 18 d of incubation (12.8%) in addition to the highest hatchability (91.8%) and the highest hatched weight (45.9 g) ($P<0.05$). The same treatment (0.2% eucalyptol) significantly improved average daily gain (ADG), feed conversion ratio (FCR), and the percentage of breast, thigh and back-neck ($P<0.05$). The positive effects of 0.2% eucalyptol on pre- and post-hatch traits indicate that this new protocol of incubation may be of benefit to the world broiler industry. The potential links between eucalyptol as a source of humidity for incubation and epigenetic modification and/or other modification mechanisms require further investigation.

KEY WORDS broiler, eucalyptol, humidity, incubation, pre- and post-hatch traits.

INTRODUCTION

In addition to environmental factors after hatching, the incubation environment (e.g., incubation temperature and humidity) can affect embryonic and hence influence hatching fitness and subsequent growth (Ho *et al.* 2011; Reed and Clark, 2011; Oliveira *et al.* 2015). Epigenetic processes are good candidates for mediating these mechanisms (Nichelmann *et al.* 1999; Tzschentke and Basta, 2002; Piestun *et al.* 2008; Shinder *et al.* 2011; Frésard *et al.* 2013; Huth and Archer, 2015).

About 80% of Eucalyptus essential oils contain 1, 8-cineole, which is an anti-spasmodic, respiratory stimulator, anti-microbial and anti-fungal agent (Worth *et al.* 2009). Inhalation of cineole by rabbits has been reported to have an effect similar to that of surfactants by reducing surface tension (Worth *et al.* 2009). Cineole has positive effects on the frequency of respiratory cilia of mucous membrane and it also has bronchial dilation and anti-inflammatory effects (Juergens *et al.* 2003). The ability to replace antibiotics with Eucalyptol for producing organic chickens has been confirmed (Diaz-Sanchez *et al.* 2015).

Incubator's humidity is an essential component of incubation. Moisture (humidity) does enter and leave the egg via pores in the eggshell. It is possible that certain agents might enter the egg through its pores in an incubator and may affect embryogenesis and growth of developing chicks. For that reason, identifying a suitable medicinal plant extract and its optimal dosage to supply incubator's humidity was the innovative hypothesis of this study. Thus, based on the antiseptic and bronchial dilation properties of eucalyptol, this study examined the effects of using Eucalyptol in the incubator on hatchability, performance and carcass traits of Ross 308 broiler chicks.

MATERIALS AND METHODS

Incubation period

Incubation hall and incubators were washed and disinfected with Persidin and then, the second step of disinfection was carried out using formaldehyde gas after 48 h. A total of 1440 eggs were obtained, with an average weight of about 65 ± 2.5 g, from Ross 308 strain of broiler breeder flock (36 weeks of age). Pure eucalyptus essential oil (1, 8-cineol or eucalyptol; Barij Essence Co. Kashan-Iran) was used as a source of incubator humidity and randomly applied at four doses (0, 0.2, 0.4, and 0.6%) over the course of a 21 day incubation period. Twelve EIG126 incubators (AndishehSabz Co. Urmia-Iran) were randomly assigned to each treatment resulting in 3 replicate incubators per treatment. The eggs were randomly allocated to the incubators at 120 eggs/incubator. The incubation temperature was set at 37.8 ˚C until the 18th day, and humidity of 60% was supplied depending on the type of treatment either with pure water or specified concentration of diluted eucalyptol, which was filled every 24 hours. Rotation time was set every 120 min. From 18 d until hatch, the incubator temperature was decreased to 37.5 ˚C and incubator humidity increased to 70%. During the incubation period, traits were measured including 18 day-moisture loss of eggs, percent fertile eggs hatched, percent embryo mortality, and the average weight of hatched chicks.

Rearing period

In this stage of the study, 20 mixed sex chicks from each incubator (n=240 total) were randomly selected and assigned to each of the 12 pens. All chicks were fed on corn-soybean meal diets including starter (CP=21.56% and ME=2950 kcal/kg feed from1 to 21 days) and grower (CP=18.75% and ME=3000 kcal/kg feed from 22 to 42 days) diets based on 9th revised edition of Nutrient Requirements of Poultry (NRC, 1994). Water and feed were supplied *ad-libitum* under 23 h lighting. Rearing temperature was 32 ˚C during the first week and was reduced to 22 ˚C on a weekly basis. An optical program, which includes 24 h of light in the first three days and 23 h of light in the remaining days, was initiated. Light intensity was set at 20 lux. All pens were fed the same diet and managed in similar conditions.

During the rearing period, feed intake (FI), average daily gain (ADG), feed conversion ratio (FCR), mortality percentage and European production efficiency factor (EPEF, calculated as below formula) were determined. FI and ADG were calculated weekly for all chicks in a pen. FCR (including mortality) was determined weekly, for each rearing period, and for the entire experiment. At the end of the experiment, one male and one female from each pen, with body weight (BW) closest to the average for the pen, were selected. After a 9-12 h fasting, they were processed for carcass and carcass part determination on a digital scale (±1 g).

EPEF= (viability (%)×BW (kg)) / (age (d)×FCR) × 100

Statistical analyses

Data were subjected to analysis of variance using the general linear model (GLM) procedure in the Statistical Analysis System (SAS, 2009) and followed by the Duncan's multiple range tests. The level of statistical significance was considered as 0.05.

RESULTS AND DISCUSSION

Traits related to incubation

The effects of eucalyptol on traits related to incubation are given in Table 1. The highest egg weight loss at 18 d incubation was observed in treatment 2 (0.2%), but the difference with treatment 3 (0.4%) was not significant (P>0.05) and treatment 2 has a significant difference with other treatments (P<0.0001). Moisture loss in treatment 1 (control) was less than all treatments and had a significant difference with other treatments. Eucalyptol had a significant effect on hatchability so that the results indicated that treatment 2 (0.2% of eucalyptol) with 91.8% was assigned the maximum hatchability, which had a significant difference with other treatments (P<0.0001). Treatment 4, with 80.2% demonstrated lowest hatchability. The results in Table 1 for hatching weight demonstrate a significant difference of treatment 2 (45.9 g) and it has the highest average among all treatments (P<0.05).

Traits of growing period performances

According to the results presented in Table 2, treatments 2 and 4 had the maximum and minimum ADG and the best and worst FCR in the starter period, respectively (P<0.05). No numerical difference was found between treatments for feed intake in the starter period (P>0.05). For grower period (Table 3), treatment 2 with 68.00 g daily gain was significantly different from other treatments (P<0.0001). Treatment 4 with 58.42 g daily gain has the minimum value among treatments and has a level of significant difference with other treatments (P<0.0001). For feed intake, no statistically significant difference was found between groups (P>0.05).

Table 1 Incubation used eucalyptol effects on hatching traits of 308 Ross broilers

Treatments*	Moisture loss at 18 d incubation (%)	Hatchability (%)	Hatching weight mean (g)
1	9.27^{c}	83.39^{b}	43.92^{b}
2	12.1^{a}	91.78^{a}	45.92^{a}
3	11.8^{ab}	84.04^{b}	44.33^{b}
4	10.6^{b}	80.22^{c}	43.33^{b}
SEM	0.004	0.840	0.340
P-value	< 0.0001	< 0.0001	0.0038

* Treatments were arranged 1: (without eucalyptol; control); 2: (0.2% eucalyptol); 3: (0.4% eucalyptol) and 4: (0.6% Eucalyptol).
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 2 Incubation used Eucalyptol effects on 0-21 days' performance

Treatments*	Average daily gain (g/chick/day)	Feed intake (g/chick/day)	Feed conversion ratio
1	42.5^{b}	60.66	1.43^{b}
2	43.1^{a}	60.70	1.41^{c}
3	42.4^{b}	60.70	1.44^{b}
4	41.9^{c}	60.67	1.45^{a}
SEM	0.137	0.027	0.013
P-value	0.0017	0.6861	0.0009

* Treatments were arranged 1: (without eucalyptol; control); 2: (0.2% eucalyptol); 3: (0.4% eucalyptol) and 4: (0.6% Eucalyptol).
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 3 Incubation used eucalyptol effects on grower period traits (21-42 days) of 308 Ross broilers in grower period

Treatments*	Average daily gain (g/chick/day)	Feed intake (g/chick/day)	Feed conversion ratio
1	61.10^{c}	148	2.43^{b}
2	68.00^{a}	145	2.13^{d}
3	64.97^{b}	147	2.27^{c}
4	58.42^{d}	149	2.55^{a}
SEM	0.533	2.050	0.031
P-value	< 0.0001	0.5741	< 0.0001

* Treatments were arranged 1: (without eucalyptol; control); 2: (0.2% eucalyptol); 3: (0.4% eucalyptol) and 4: (0.6% Eucalyptol).
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

For FCR like the starter period, significant differences were observed among the treatments.

Thus, treatment 4 with conversion ratio of 2.55 (the highest among all treatments) was the poorest and had a significant level of other treatments (P<0.05). Treatment 2, like the starter period, had the best yield in FCR and a significant difference with other treatments with 2.13 (P<0.0001). By investigating performance traits of total period (Table 4), the efficiency of eucalyptol in the incubator was confirmed. According to the results presented in Table 4, all eucalyptol levels had a significant difference with each other in ADG (P<0.0001); and eucalyptol levels of 0.2 and 0.6%, with 55.5 and 49.9 g were the highest and lowest values, respectively. Feed intake, like other previous periods demonstrated no significant difference (P<0.05). FCR in the total period as the most important studied trait recorded the maximum difference. Treatment 2 with 1.85 had the best FCR (P<0.05). Treatments 3, 1 and 4, with 1.93, 2.01 and 2.07 had next FCR ordinal values (P<0.05). Mortality percentage in the total period of the study, however, was not significantly different between groups, but in numerical terms, the lowest mortality is related to treatment 2 (P>0.05).

European production efficiency factor (EPEF) calculated for different treated groups, as a managerial and economic index for broilers' rearing, demonstrated that treatment 2 had the best EPEF value (292) and a significant difference compared to other treatments (P<0.05).

Carcass traits

Carcass percentage demonstrated the superiority of treatment 2 in comparison to other treatments except treatment 3 that has a significant difference with other treatments (P<0.05); and also the poorest yield in this trait belongs to treatment 4 with no significant difference with treatment 1 (P>0.05). Percentage of abdominal fat and heart did not demonstrate any significant difference between the treatments (P>0.05). In the percentage of gizzard and liver, treatment 4 had the highest percentage and in comparison to other treatments demonstrated significant difference (P<0.05). Breast, thigh, back and neck percentages are the most important traits of internal organs that are taken into consideration economically. In this regard, treatment 2 by recording the highest percentage was rated as the best among all treatments. The significant observable difference in the 3 traits was seen only in Treatment 2 (P<0.05).

Table 4 Incubation used eucalyptol effects on 0-42 days' post-hatch traits

Treatments*	Average daily gain (g/chick/day)	Feed intake (g/chick/day)	Feed conversion ratio	Mortality (%)	European production efficiency factor
1	51.63^{c}	103.5	2.01^{b}	11.67	235^{bc}
2	55.49^{a}	102.6	1.85^{d}	5.00	292^{a}
3	53.48^{b}	103.4	1.93^{c}	8.33	259^{b}
4	49.89^{d}	103.3	2.07^{a}	15.00	215^{c}
SEM	0.285	0.639	0.016	2.36	8.17
P-value	< 0.0001	0.7065	< 0.0001	0.077	0.0009

* Treatments were arranged 1: (without eucalyptol; control); 2: (0.2% eucalyptol); 3: (0.4% eucalyptol) and 4: (0.6% Eucalyptol).
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 5 Incubation used eucalyptol effects on carcass traits (carcass has been measured in percent per live weight and other traits measured in percent per carcass weight) of 308 Ross broilers at the end of grower period (42 d)

Treatments*	Carcass (%)	Abdominal Fat (%)	Gizzard (%)	Liver (%)	Heart (%)	Breast (%)	Drumstick (%)	Back and neck (%)
1	72.97^{b}	2.68	2.46^{b}	3.068^{b}	0.816	33.7^{b}	25. 9^{b}	25.7^{b}
2	75.92^{a}	2.62	2.47^{b}	3.11^{b}	0.786	35.77^{a}	27.4^{a}	26.9^{a}
3	74.58^{a}	2.63	2.53^{b}	3.00^{b}	0.786	34.07^{b}	25.8^{b}	25.4^{b}
4	72.95^{b}	2.71	2.62^{a}	3.26^{a}	0.737	34.27^{b}	25.7^{b}	25.8^{b}
SEM	0.498	0.084	0.026	0.041	0.0261	0.325	0.1919	0.187
P-value	0.0009	0.8726	0.0006	0.0022	0.2244	0.0012	< 0.0001	< 0.0001

* Treatments were arranged 1: (without eucalyptol; control); 2: (0.2% eucalyptol); 3: (0.4% eucalyptol) and 4: (0.6% Eucalyptol).
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Finally, treatment 4 containing the maximum amount of Eucalyptol revealed the poorest result that was even poorer than control treatment (Table 5).

Eucalyptol (1, 8-cineole) is a natural organic compound, which has attracted increased interest for clinical use in humans, animals and poultries because of its mucolytic, antimicrobial, anti-inflammatory, antioxidant and spasmolytic properties. It is also added in food preparations to enhance the aroma and taste (Bhowal and Gopal, 2015; Juergens *et al.* 2017). Extensive studies disclosed that eucalyptol can be considered a safe chemical when taken in normal doses. In higher doses, eucalyptol is unsafe via ingestion, skin contact or inhalation (Bhowal and Gopal, 2015). Eucalyptol does not show mutagenicity, genotoxicity or carcinogenicity, but it may be toxic to the reproductive system. Subacute hepatotoxic and nephrotoxic effects have been reported in animal experiments after the application of high doses (Kirsch and Buettener, 2013).

The best egg moisture loss is 10-12% of total weight and this leads to maximum hatchability according to Ross broiler management manual (Aviagen, 2009). Therefore, by relying on disinfectant properties and avoiding clogging of the pores in the eggshell, it can be interpreted that the use of low concentrations of eucalyptol causes the most desirable weight loss (about 12%) and a positive effect on improving incubation process. However, increasing the concentration of eucalyptol may cause choking or clogging of the pores in the eggshell and finally preventing moisture from leaving inside the egg.

Low rate of hatchability and low hatching weight mean in groups 3 (0.4% eucalyptol) and 4 (0.6% eucalyptol) may be caused by toxic properties of eucalyptol in high dose application in the incubator. For example, orally administered eucalyptol in rats at 1560 mg/kg is reported to produce rapid cyanosis, stupor, irregular breathing, and utmost sensitivity to noise, convulsions, and death from respiratory failure (Bhowal and Gopal, 2015).

Weights of hatched chicks have always been of great importance in the selection of chicks for rearing. This weight is directly correlated with the final body weight of rearing period. It may be interpreted that low levels of eucalyptus essential oil increased 1st day body weight by increasing embryo bronchial volume and facilitating respiratory system and then increased metabolism and accelerated yolk sac use.

Santos and Rao (2000) studied effects of eucalyptol on male Swiss mice and Wistar rats and showed that eucalyptol exhibits anti-nociceptive properties thereby having a potential calmative and depressant action on the central nervous system. They also concluded that eucalyptol enhances blood circulation and causes vasodilation and bronchodilation. This may be the reason of superiority of treatment 2 (0.2% as optimal dosage of eucalyptol) in our study on broiler chicks.

The results of all studied traits in incubation period may possibly be attributed to disinfection, cooling and thermal balance, and respiratory tract stimulation caused by eucalyptol.

This implies that eucalyptol inhibits the growth and proliferation of pathogens by disinfection which finally leads to low loss of treatments containing eucalyptol.

Cooling and thermal balance prevents temperature fluctuations and causes increase in hatchability. Moreover, owing to its mucolytic and respiratory tract stimulation properties, eucalyptol facilitates respiratory and carbon dioxide excretion of eggs and this factor can influence the rate of hatchability (Juergens *et al.* 2003).

Eucalyptol may affect functional traits of rearing period and carcass traits by possible epigenetic effects in the incubation period and also a direct effect on bronchial volume thereby increasing breathing strength, facilitating and accelerating metabolism, and improving feed intake efficiency, ADG and finally feed conversion ratio. Hepatotoxic effect of high acute oral administration of eucalyptol (2.5 g/kg) has been reported in rats (Kirsch and Buettener, 2013). It is in accordance with our results that liver percent in treatment 4 (0.6% eucalyptol) was highest percentage and differed significantly with other treatments. High percent of liver may be caused of hepatotoxic effects of high level of eucalyptol application at incubator.

CONCLUSION

Several efforts have been made to increase incubation efficiency and produce resistant and high yield chicks. But no new source and process of incubator's humidity have been reported that increases chicks' production efficiency. This study identified a new applicable medicinal plant extract and its optimal dosage as a new source of incubator's humidity. According to the results of this study, the use of low levels of eucalyptol (0.2%) as a source of incubator's humidity, had beneficial effects on several economically important traits in broilers such as hatchability, embryo mortality and FCR. The effects may be attributed to eucalyptol epigenetic effects on embryogenesis and / or other modification mechanisms. Further studies are required to identify the mechanisms leading to the mentioned improvements.

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