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Effect of dietary supplementation and *in ovo* feeding of menthol on the growth performance, development of lymphoid and digestive organs and carcass quality traits of broilers

B.B. BONDAR, P.K. SHUKLA, A. BHATTACHARYYA*, A.R. KHERDE, M. PATEL, P.S. GULHANE AND M. SHARMA

Department of Poultry Science, College of Veterinary Science and Animal Husbandry, DUVASU, Mathura, India

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ABSTRACT

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This study was carried out to evaluate the effect of *in ovo* feeding and dietary supplementation of menthol on the growth performance, digestive and lymphoid organs, and carcass yield of broiler chickens. Fertile eggs (n = 372) of CARIBRO VISHAL birds were set for incubation. On the 18th day, *in ovo* feeding was done at the broad end of the egg. The eggs were divided into three groups: un-injected control, sham control (injected with 0.5 ml of 5% ethanol), and *in ovo* injected with menthol (0.5 ml of 1% menthol solution). After hatching, 180 one-day-old chicks were reared for feeding trial. Chicks from these three groups were further divided into two subgroups, each with three replicates and ten chicks per replicate. One subgroup received a basal diet, while the other received a basal diet with menthol supplementation (250 mg/kg diet). Day old body weight of *in ovo* menthol group birds was significantly higher ($P \leq 0.01$) compared to un-injected control. Day old and 1st week body weight of basal diet along with menthol supplementation group birds were significantly higher ($P=0.04$, $P=0.003$) than basal diet. No significant differences were recorded in digestive and lymphoid organs, carcass quality traits, yield of giblets and cut-up-parts after *in ovo* feeding and/or dietary supplementation of menthol. Thus, *in ovo* feeding of 1% menthol as well as dietary supplementation of menthol @ 250 mg/ kg diet resulted in significant positive effects on early body weight.

Keywords: Carcass quality, In ovo, Menthol, Growth, Broiler

INTRODUCTION

Phytogenic feed additives are plant-based feed additives that are used in natural substances in poultry nutrition. These substances are derived from herbs, spices, other plants and their extracts like essential oils. Being natural, less toxic and residue free, they are ideal feed additives for poultry when compared to synthetic antibiotics. The benefits of using phytogenic feed additives in poultry nutrition are increased feed intake, stimulation of digestion, increased growth performance, reduced incidence of disease, improved reproductive parameters, feed efficiency, profitability and reduced poultry house emissions (Bhattacharyya *et al*., 2013; Mishra *et al*., 2016; Singh *et al*., 2019; Rai *et al*., 2022; Raghav *et al*., 2023). Further, these feed additives positively influence gut morphology in broilers and significantly increase nutrient digestibility. Moreover, it stimulates the production of antioxidant enzymes (Roofchaee *et al*., 2011). So phytogenic feed additives have been widely used to elicit the performance of animals and are now used in poultry feeding practices extensively (Collington *et al*., 1990; Khan *et al*., 2007) not only to stimulate the growth and feed efficiency but also to improve the health and performance of birds (Fadlalla *et al*., 2010; Abouelfetouh and Moussa, 2012). Mint is one such phytogenic feed additive used in broilers in recent years.

Menthol (also known as menthol camphor) is a cyclic monoterpene alcohol that is a major component of Mentha. Peppermint leaves contain about 0.5 to 4% essential oils that are composed of 25 to 78% menthol, 14 to 36% menthone, 1.5 to 10% isomenthone, 2.8 to 10% menthyl acetate, and 3.5 to 14% cineol (Grigoleit and Grigoleit, 2005; Bupesh *et al*., 2007; Aziz *et al*., 2011; Beigi *et al*., 2018). There are many *in vitro* and *in vivo* publications stating that peppermint leaves and their essential oil and particularly menthol have many desirable effects, including; moderate antibacterial effect on pathogenic bacteria (Sharifi *et al*., 2013), antiviral and fungicidal activity (Schlez *et al*., 2006; Bupesh *et al*., 2007) appetizing, digestion stimulating and antimicrobial properties (Alcicek *et al*., 2004), reduces stress by decreasing heterophil lymphocytes ratio (Sultan *et al*., 2017). Menthol can be used as an effective feed additive to improve growth performance of broilers (Abdel-Wareth *et al*., 2019). Also, the antioxidant properties of menthol have been reported in many studies (Kamkar *et al*., 2010).

In recent years, studies on *in ovo* feeding of essentials have been carried out to elicit growth and

*Corresponding author E mail: amitav6@gmail.com

immunity in broilers (Ma *et al*., 2022; Toosi *et al*., 2016). *In ovo* technology has been defined as "the direct inoculation of bioactive substances to the developing embryo to elicit superior lifelong effects, while considering the dynamic physiology of the chicken embryo" (Oladokun and Adewole, 2020). In recent years, *in ovo* feeding has gained attention as it provides beneficial biochemical and physiological balances, including improved oxidative protection, during incubation (Malheiros *et al*., 2012). The technique offers a cost-effective way to deliver nutrients directly to embryos, surpassing the need for dietary additives (Kadam *et al*., 2013). Additionally, *in ovo* injection technology has shown potential to improve hatching quality and subsequent growth of broiler chickens (Ipek *et al*., 2004), and may also be used to deliver bioactive compounds with antioxidant properties to developing embryos. Several nutrients, including L-carnitine, carbohydrates, amino acids, creatine, nucleotides, egg white, peptides, electrolytes, vitamins, and plant extracts, have been investigated *in ovo* (Kucharska-Gaca *et al*., 2017). Several plant extracts, such as moringa, tomato, garlic, savory, and thyme extracts, have been used (Fazli *et al*., 2015; Saki and Salary, 2015; N'nanle *et al*., 2017). Interestingly, research on *in ovo* delivery of menthol in broilers is relatively scarce in the literature.

Studies on dietary supplementation vis-à-vis *in ovo* feeding of menthol on the growth performance and carcass quality traits are limited in commercial broilers. Hence, the present study aimed at investigating the effect of dietary supplementation vis-a-vis *in ovo* feeding of menthol on the performance of commercial broilers.

MATERIALS AND METHODS

Eggs

Three hundred and seventy-five fertile eggs of CARIBRO VISHAL birds were procured from ICAR Central Avian Research Institute, Izatnagar, Bareilly. The eggs were set for incubation for first 18 days in the setter and last 3 days in the hatcher. On the 18^{th} day, eggs were candled, and dead-in-shell eggs were discarded. Further, remaining fertile eggs were divided into three treatment groups - uninjected control, sham control and *in ovo* injected with menthol.

Preparation of menthol solution for in ovo feeding

Menthol was procured from SRL Pvt. Ltd. Menthol is a white crystalline substance, which is poorly soluble in water-based preparations, but it is readily dissolvable at high concentrations in ethanol. So, 5% ethanol (prepared in double distilled water) was used as a solvent for preparing a 1% solution of menthol to be used for *in ovo* injection. 1% menthol solution was prepared by adding 1g of menthol in 100ml of 5% ethanol.

In ovo feeding

In ovo feeding was done on 18^{th} day of incubation at the broad end of incubated egg. Adherence to the prescribed biosafety and good laboratory practice (GLP) protocols were diligently maintained throughout the *in ovo* feeding process.

Birds

Out of the total chicks obtained after hatching, 180 one-day-old CARIBRO VISHAL chicks were reared for feeding trials. The chicks obtained from three *in ovo* treated groups were further subdivided into 2 subgroups each comprising of three replicates and ten chicks in each replicate. The chicks were wing banded, weighed individually and distributed randomly on uniform body weight basis in the treatment groups. The chicks were housed in deep litter system. Water was offered *ad libitum*. The experiment was conducted at Poultry Farm of Department of Poultry Science, U.P. Pandit Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwa Vidyalaya Evam Go-Anusandhan Sansthan (DUVASU), Mathura after due approval of the Institutional Animal Ethics Committee (IAEC) (138/IAEC/22/2/30) (28/12/22).

Experimental design

Birds were reared for 42 days (6 weeks) and kept on a basal or control diet [BIS, 2007; broiler starter diet till 3 weeks and thereafter broiler finisher diet till 6 weeks]. The experimental design was 3×2 CRD. The dietary treatments offered have been tabulated below:

Table 1: Experimental treatments showing *in ovo* and/or dietary menthol supplementation

Treatment	*In-Ovo* Injection	Diet
T1	Un-injected (Control)	Basal Diet
T2	Un-injected (Control)	Basal Diet + menthol @ 250mg/kg
T3	Injected with 0.5ml of 5% ethanol/egg (Sham Control)	Basal Diet
T4	Injected with 0.5ml of 5% ethanol/egg (Sham Control)	Basal Diet + menthol @ 250mg/kg
T5	Injected with 0.5 ml of 1% menthol solution/egg	Basal Diet
T6	Injected with 0.5 ml of 1%menthol solution/egg	Basal Diet + menthol @ 250mg/kg

Growth performance parameters

Weekly body weight, group feed consumption and mortality was recorded. Feed conversion ratio (feed intake: body weight gain) of 0 to 6 weeks were calculated at the end of the experiment.

Measurement of lymphoid and digestive organs and carcass quality traits

At the time of slaughter, 2 male and 2 female birds from each group i.e. total 24 birds were taken after 6 weeks of age for studying various slaughter traits *viz*: pre-slaughter fasting shrinkage in live weight (%), bled weight (%), defeathered weight (%), dressed weight (%), yield after evisceration loss or ready to cook yield (%), giblets yield (%), total ready-to-cook yield (%) and development of digestive organs. Further, percent yield of cut-up-parts (thighs, drumsticks, breast, back, neck and wing) on eviscerated carcass yield were determined.

The birds were starved for 12 hours before slaughter. However, drinking water was provided *ad lib*. During the starvation period, their body weights were recorded after starvation. The birds were sacrificed by improved Kosher method, bled for 1.5 to 2 minutes and defeathered. The birds were dressed by cutting the head at atlanto-occipital joint, leg at hock joint and oil gland located at the base of the tail and weighed. Evisceration was done by making a slit opening at the neck skin to remove oesophagus and trachea, vertical cut below the tip of breast bone to remove viscera. The heart, liver and gizzard were separated and cleaned. The internal lining of gizzard and pericardium of heart were removed before weighing them. Further, the length and weight of different digestive organs (proventriculus, small intestine, large intestine and caecum) were measured separately at 6 weeks of age.

Statistical Analysis

The data pertaining to various parameters were analyzed statistically as per the standard procedure (Snedecor and Cochran, 1989) and difference between the treatment means were obtained by using Duncan multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Body weight

There were no significant differences in the average weekly body weight after *in ovo* feeding, except at day old body weight and 1stweek body weight (Table 2). Day old body weight of *in ovo* menthol group birds was significantly higher (P≤0.01) as compared to un-injected control; and during 1st week, un-injected control had significantly and numerically lower body weight as compared to sham control and *in ovo* menthol birds, respectively. In contrast, Toosi *et al*. (2016) reported that *in ovo* injection of blend of essential oils and organic acids did not affect body weight at day old and throughout the experiment. Oladokun *et al*. (2021) also reported that there was no significant difference in day old chick weight and average weekly body weight after *in ovo* feeding of 0.2ml of blend of essential oils. However, our findings suggest that *in ovo* feeding of menthol

Table 2: Effect of *in ovo* feeding and dietary supplementation of menthol on average weekly body weight (g) of commercial broilers during 0-6 weeks of age

Group	Age in weeks						
	Day-old	1st	2nd	3rd	4th	5th	6th
Un-injected Control + Basal Diet	38.00^{abc}	115.53^{b}	290.43	596.36	840.04	1159.0	1540.0
Un-injected Control + Menthol supplementation	37.53^{ab}	116.20^{b}	291.88	614.13	874.20	1196.8	1603.8
Sham Control + Basal Diet	37.27^{a}	98.44^{a}	259.51	585.19	830.08	1152.5	1542.5
Sham Control + Menthol supplementation	39.13^{cd}	119.04^{b}	293.29	618.14	881.07	1198.8	1588.5
In ovo Menthol + Basal Diet	38.87^{bcd}	112.80^{b}	280.90	608.93	834.87	1160.8	1549.1
In ovo Menthol + Menthol Supplementation	39.93^{d}	115.82^{b}	279.53	599.32	853.09	1183.3	1570.8
Treatment							
Un-injected Control	37.77^{a}	108.74^{a}	291.16	605.24	857.12	1177.91	1571.94
Sham Control	38.20^{a}	115.87^{b}	276.4000	601.67	855.57	1175.69	1565.46
In ovo Menthol	39.40^{b}	114.31^{ab}	280.2139	604.13	843.98	1172.07	1559.93
Diet							
Basal Diet	38.04^{a}	108.92^{a}	276.95	596.83	834.99	1157.46	1543.86
Menthol Supplementation	38.87^{b}	117.02^{b}	288.23	610.53	869.45	1192.99	1587.69
Pooled SEM	0.27	1.88	3.90	6.38	8.76	9.28	11.62
Sig Level							
Treatment	P ≤ 0.01	P ≤ 0.05	NS	NS	NS	NS	NS
Diet	P = 0.04	P = 0.003	NS	NS	NS	NS	NS
Treatment × Diet	P ≤ 0.01	P = 0.002	NS	NS	NS	NS	NS

Means bearing different superscripts within a column differ significantly (P≤0.05) NS: Not Significant (P≥0.05) SEM: Standard Error of Means significantly increased body weight of day-old chicks which is in line with Uni *et al*. (2005) who concluded that *in ovo* feeding of a solution of maltose, sucrose, dextrin and hydroxymethylbutyrate (HMB) to the late term embryos increased the hatching weight by 5-6% over control. Similarly, Ohta *et al*. (2001) as well as Bhanja and Mandal (2005) reported that *in ovo* administration of all 20 amino acids increased the chick weight by 3.6% and 2% respectively. Further, Bhanja *et al*. (2006) reported that though there was no difference in chick weight to egg weight ratio, but the ratio was higher in 0.25 IU vitamin E and 25 mg linoleic acid injected chicks than un-injected control. Interestingly, researches on in ovo feeding of menthol in broilers are limited. The present study indicated that *in ovo* feeding of menthol increased day-old chick weight and the effect continued till 1st week. However, thereafter the positive effect of *in ovo* feeding in weekly body weight was not carried forward in the experiment.

There were no significant differences in average weekly body weight in dietary supplementation throughout the experiment except day old and 1st week body weight. Basal diet with menthol supplementation significantly increased day old body weight (P=0.04) and 1st week body weight (P=0.003) than basal diet. Abdel-Wareth *et al.* (2019) reported that peppermint leaves or menthol in different concentrations to broiler diet significantly increased body weight compared to control groups. Akbari and Torki (2014) reported that dietary supplementation of peppermint essential oil (250 mg/kg diet) had no significant effect on body weight but numerically peppermint supplemented group has higher body weight as compared to control. In our study, significant increase was observed only on body weight at day-old and during the first week, with all menthol-supplemented dietary groups showing higher body weight compared to basal diet. The significantly higher body weight at day-old and week one may be due to the *in ovo* feeding of menthol, which was combined with dietary supplementation. However, despite the positive effect of *in ovo* feeding, this benefit did not carry forward to a significant level beyond the first week. After first week, dietary supplementation of menthol resulted in numerical higher body weight compared to the basal diet fed group.

Similarly, interaction of *in ovo* feeding and dietary supplementation of menthol did not impart any significant effect on body weight throughout the experiment except day old body weight. Day-old body weight of *in ovo* menthol along with dietary menthol supplementation group birds were significantly higher (P≤0.01) than un-injected control with basal diet, un-injected with menthol supplementation in basal diet and sham control along with basal diet group birds. First week body weight of sham control with basal diet was significantly lower (P=0.002) than body weight of chicks of other interaction groups. This clearly reflects the positive effect of *in ovo* feeding rather than dietary supplementation of menthol to the broilers.

Body weight gain

There were no significant differences observed in weekly body weight gain due to *in ovo* feeding during the experiment except 1st week of age (Table 3). The weekly body weight gain at first week of un-injected control birds was significantly higher (P≤0.05) than sham control. Oladokun *et al*. (2021) also observed that at the grower phase (≤15–28) and for the entire length of study (≤0–28), there was no treatment effect of *in ovo* essential oil on growth performance parameters.

Dietary supplementation of menthol caused significantly higher (P≤0.01) 1st week body weight gain as compared to basal diet group. Further, data of weekly body weight gain showed no significant difference throughout the experiment due to dietary supplementation. This agrees with the studies of Ayman *et al.* (2016) and Akbari and Torki (2014). Akbari and Torki (2014) reported that dietary supplementation of peppermint essential oil (250 mg/kg diet) had no significant effect on body weight gain but numerically peppermint supplemented group has higher body weight as compared to control which is in line with our present study.

When the interaction of *in ovo* feeding and dietary supplementation was studied, body weight gain of sham control along with basal diet group was significantly lower (P≤0.01) as compared to other groups during 1st week of age. Further, there were no significant differences in body weight gain in birds among the different groups from 2nd week to 6th week.

Feed Intake

The results indicated that there were no significant differences in the average weekly feed intake of birds after *in ovo* feeding throughout the experimental period (Table 4). Similarly, Toosi *et al*. (2016) observed that *in ovo* injection of Biacid™ (blend of essential oil and volatile fatty acids) did not result in any significant effect on feed intake of broilers.

There were no significant differences in average weekly feed intake in dietary groups from 0-6 weeks, except at first week where birds in the menthol supplementation group had significantly higher (P=0.04) feed consumption compared to the basal diet. Although no significant differences were observed overall, all menthol-supplemented groups showed higher feed intake compared to the basal diet. Abdel-Wareth *et al*. (2019) observed that feed intake was increased with the increase in dietary menthol concentrations (linear, P≤0.05) during trial periods of 21 to 35, and 1 to 35 d of age. Mucha and Witkowska (2021) stated that essential oils may be used mainly as feed additives to improve feed palatability and

Table 3: Effect of *in ovo* feeding and dietary supplementation of menthol on average weekly body weight gain (g) of commercial broilers during 0-6 weeks of age

Group	Age in weeks					
	1st	2nd	3rd	4th	5th	6th
Un-injected Control + Basal Diet	77.53[b]	174.90	305.92	243.68	319.00	381.00
Un-injected Control + Menthol supplementation	78.67[b]	175.68	322.25	260.07	322.59	407.04
Sham Control + Basal Diet	61.17[a]	161.07	325.68	244.89	322.47	389.91
Sham Control + Menthol supplementation	79.91[b]	174.25	324.85	262.93	317.76	389.64
In ovo Menthol + Basal Diet	73.93[b]	168.10	328.03	225.93	325.93	388.30
In ovo Menthol + Menthol Supplementation	75.89[b]	163.71	319.79	253.77	330.25	387.41
Treatment						
Un-injected Control	78.10[b]	175.29	314.09	251.88	320.79	394.02
Sham Control	70.53[a]	167.66	325.27	253.91	320.12	389.78
In ovo Menthol	74.91[ab]	165.90	323.91	239.85	328.09	387.86
Diet						
Basal Diet	70.88[a]	168.02	319.88	238.17	322.47	386.41
Menthol Supplementation	78.15[b]	171.21	322.30	258.92	323.53	394.70
Pooled SEM	1.80	2.41	4.68	5.05	3.53	4.64
Sig Level						
Treatment	$P \leq 0.05$	NS	NS	NS	NS	NS
Diet	$P \leq 0.01$	NS	NS	NS	NS	NS
Treatment × Diet	$P \leq 0.01$	NS	NS	NS	NS	NS

Means bearing different superscripts within a column differ significantly ($P \leq 0.05$) NS: Not Significant ($P \geq 0.05$) SEM: Standard Error of Means

Table 4: Effect of *in ovo* feeding and dietary supplementation of menthol on average weekly feed intake (g) of commercial broilers during 0-6 weeks of age

Group	Age in weeks					
	1st	2nd	3rd	4th	5th	6th
Un-injected Control + Basal Diet	101.37[b]	221.16	439.52	548.57	670.67	908.62
Un-injected Control + Menthol supplementation	99.67[b]	238.61	424.25	521.07	644.41	838.74
Sham Control + Basal Diet	83.16[a]	250.41	467.64	556.86	690.74	880.46
Sham Control + Menthol supplementation	103.69[b]	243.81	444.12	540.68	686.59	888.55
In ovo Menthol + Basal Diet	96.13[b]	234.60	422.00	489.00	616.33	812.33
In ovo Menthol + Menthol Supplementation	96.92[b]	234.87	495.85	607.21	765.69	939.74
Treatment						
Un-injected Control	100.52	229.89	431.89	534.82	657.54	873.68
Sham Control	93.42	247.11	455.88	548.77	688.67	884.50
In ovo Menthol	96.53	234.73	458.93	548.10	691.01	876.04
Diet						
Basal Diet	93.56[a]	235.39	443.05	531.48	659.25	867.14
Menthol Supplementation	100.09[b]	239.09	454.74	556.32	698.90	889.01
Pooled SEM	1.99	6.32	12.70	16.25	19.94	25.12
Sig Level						
Treatment	NS	NS	NS	NS	NS	NS
Diet	$P = 0.04$	NS	NS	NS	NS	NS
Treatment × Diet	$P \leq 0.05$	NS	NS	NS	NS	NS

Means bearing different superscripts within a column differ significantly ($P \geq 0.05$) NS: Not Significant ($P \geq 0.05$) SEM: Standard Error of Means increase feed intake. Akbari and Torki (2014) reported that supplementation of peppermint essential oil (250 mg/ kg diet) had no significant effect on feed intake but numerically peppermint supplemented group had higher feed intake as compared to control which is in line with our present study.

There were no significant differences in effect of interaction of *in ovo* feeding and dietary supplementation on average weekly feed intake except at 1st week where, sham control along with basal diet had significantly lower ($P \leq 0.05$) feed intake as compared to other groups.

Feed Conversion Ratio

Results indicated that there were no significant differences in weekly FCR after *in ovo* feeding and/or dietary supplementation of menthol during the entire experimental period of 0-6 weeks of age (Table 5). This agrees with the study by Akbari and Torki (2014) as well as Toosi *et al*. (2016). In contrary, Abdel-Wareth *et al*. (2019) reported linear improvements ($P \leq 0.01$) in feed conversion values with increasing peppermint leaves or menthol levels in diets of broiler chickens. In our study, dietary supplementation of menthol was 250 mg menthol per kg diet and there were no graded levels of dietary supplementation carried out in the experiment. Toosi *et al*. (2016) observed that *in ovo* injection of Biacid™ (blend of essential oil and volatile fatty acids) did not have significant effect on FCR which is in line with the present study.

Development of digestive and lymphoid organs

There were no significant differences observed in development of digestive and lymphoid organs after *in ovo* feeding and/or dietary supplementation of menthol (Table 6 and 7). This is strongly supported by the studies of Abdel-Wareth *et al*. (2019), Ayman *et al*. (2016), Hashemipour *et al*. (2013) and Oladokun *et al*. (2021).

Carcass quality traits, yield of giblets and cut-up-parts

No significant differences were recorded in carcass quality traits, yield of giblet and cut up parts after *in ovo* feeding and/or dietary supplementation of menthol (Table 8, 9 and 10). This agrees with studies by Toosi *et al.* (2016), Abdel-Wareth *et al*. (2019) and Ayman *et al*. (2016). Toosi *et al.* (2016) observed that *in ovo* injection of Biacid™ (blend of essential oil and volatile fatty acids) did not have any significant effect on carcass yield. Ayman *et al.* (2016) observed that carcass traits didn't differ between treatments (1.5 g Peppermint Leaves, 3.0 g Peppermint Leaves, 125 mg Peppermint Oil, 250 mg Peppermint Oil), except gizzard percent that increased with all peppermint

Table 5: Effect of *in ovo* feeding and dietary supplementation of menthol on weekly feed conversion ratio (FCR) of commercial broilers during 0-6 weeks of age

Group	Age in weeks					
	1st	2nd	3rd	4th	5th	6th
Un-injected Control + Basal Diet	1.31	1.27	1.47	2.27	2.10	2.38
Un-injected Control + Menthol supplementation	1.27	1.36	1.32	2.01	2.00	2.07
Sham Control + Basal Diet	1.36	1.58	1.44	2.27	2.14	2.25
Sham Control + Menthol supplementation	1.30	1.40	1.36	2.06	2.16	2.29
In ovo Menthol + Basal Diet	1.30	1.40	1.29	2.19	1.90	2.09
In ovo Menthol + Menthol Supplementation	1.30	1.45	1.55	2.41	2.31	2.43
Treatment						
Un-injected Control	1.29	1.32	1.40	2.14	2.05	2.22
Sham Control	1.33	1.49	1.40	2.16	2.15	2.27
In ovo Menthol	1.30	1.42	1.42	2.30	2.11	2.26
Diet						
Basal Diet	1.33	1.41	1.40	2.24	2.05	2.24
Menthol Supplementation	1.29	1.40	1.41	2.16	2.16	2.26
Pooled SEM	0.03	0.05	0.05	0.07	0.05	0.06
Sig. Level						
Treatment	NS	NS	NS	NS	NS	NS
Diet	NS	NS	NS	NS	NS	NS
Treatment × Diet	NS	NS	NS	NS	NS	NS

Means bearing different superscripts within a column differ significantly ($P \leq 0.05$) NS: Not Significant ($P \geq 0.05$) SEM: Standard Error of Means

Table 6: Effect of *in ovo* feeding and dietary supplementations of menthol on development of digestive organs of commercial broilers after 6 weeks of age

Group	Proventriculus (g/100g)	SI weight (g/100g)	LI weight (g/100g)	Caecal Wt. (g/100g)	SI length (cm/100g)	LI length (cm/100g)	Caecal length (cm/100g)
Un-injected Control + Basal Diet	0.58	4.40	1.20	0.77	10.61	1.84	1.25
Un-injected Control + Menthol supplementation	0.60	4.40	1.34	0.78	10.99	1.80	1.31
Sham Control + Basal Diet	0.56	4.39	1.81	1.20	11.42	2.19	1.51
Sham Control + Menthol supplementation	0.60	5.08	1.41	0.74	11.94	2.01	1.49
In ovo Menthol + Basal Diet	0.58	5.12	1.16	0.63	11.11	1.78	1.33
In ovo Menthol + Menthol Supplementation	0.54	4.74	1.44	0.77	11.99	2.31	1.45
Treatment							
Un-injected Control	0.59	4.40	1.27	0.78	10.80	1.82	1.28
Sham Control	0.58	4.73	1.61	0.97	11.68	2.10	1.50
In ovo Menthol	0.56	4.93	1.30	0.70	11.55	2.05	1.39
Diet							
Basal Diet	0.57	4.64	1.39	0.87	11.05	1.94	1.36
Menthol Supplementation	0.58	4.74	1.40	0.76	11.64	2.04	1.41
Pooled SEM	0.02	0.14	0.08	0.06	0.20	0.07	0.05
Sig. level							
Treatment	NS	NS	NS	NS	NS	NS	NS
Diet	NS	NS	NS	NS	NS	NS	NS
Treatment × Diet	NS	NS	NS	NS	NS	NS	NS

NS: Not Significant (P≥0.05) SEM: Standard Error of Means SI: Small Intestine LI: Large Intestine

Table 7: Effect of *in ovo* feeding and dietary supplementations of menthol on development of lymphoid organs of commercial broilers after 6 weeks of age (% live weight)

Group	Spleen weight %	Thymus weight %	Bursa weight %
Un-injected Control + Basal Diet	0.19	0.31	0.29
Un-injected Control + Menthol supplementation	0.18	0.39	0.31
Sham Control + Basal Diet	0.21	0.39	0.30
Sham Control + Menthol supplementation	0.21	0.32	0.28
In ovo Menthol + Basal Diet	0.18	0.40	0.28
In ovo Menthol + Menthol Supplementation	0.19	0.31	0.24
Treatment			
Un-injected Control	0.18	0.35	0.30
Sham Control	0.21	0.36	0.29
In ovo Menthol	0.19	0.36	0.26
Diet			
Basal Diet	0.19	0.37	0.29
Menthol Supplementation	0.19	0.34	0.27
Pooled SEM	0.01	0.03	0.01
Sig. level			
Treatment	NS	NS	NS
Diet	NS	NS	NS
Treatment × Diet	NS	NS	NS

NS: Not Significant (P≥0.05) SEM: Standard Error of Means

Table 8: Effect of *in ovo* feeding and dietary supplementations of menthol on carcass quality traits of commercial broilers after 6 weeks of age (% live weight)

Group	Shrinkage (%)	Bled wt (%)	Defeathered wt (%)	Dressing wt (%)	Ready to cook yield (%)
Un-injected Control + Basal Diet	9.42	96.38	80.35	71.32	52.83
Un-injected Control + Menthol supplementation	7.94	96.51	80.23	71.01	49.57
Sham Control + Basal Diet	6.52	95.77	80.06	72.38	51.89
Sham Control + Menthol Supplementation	9.70	95.50	79.70	69.74	50.87
In ovo Menthol + Basal Diet	9.05	96.08	80.02	70.41	52.48
In ovo Menthol + Menthol	9.39	95.48	79.90	70.35	51.38
Treatment					
Un-injected Control	8.68	96.44	80.29	71.16	51.20
Sham Control	8.11	95.64	79.88	71.06	51.38
In ovo Menthol	9.22	95.78	79.96	70.38	51.93
Diet					
Basal Diet	8.33	96.08	80.14	71.37	52.40
Menthol Supplementation	9.01	95.83	79.94	70.37	50.61
Pooled SEM	0.40	0.19	0.19	0.37	0.49
Sig. level					
Treatment	NS	NS	NS	NS	NS
Diet	NS	NS	NS	NS	NS
Treatment × Diet	NS	NS	NS	NS	NS

NS: Not Significant (P≥0.05) SEM: Standard Error of Means

Table 9: Effect of *in ovo* feeding and dietary supplementations of menthol on yield of giblets (heart, liver, gizzard) of commercial broilers after 6 weeks of age (% live weight)

Group	Heart %	Liver %	Gizzard %
Un-injected Control + Basal Diet	0.54	2.17	2.77
Un-injected Control + Menthol supplementation	0.55	2.35	2.65
Sham Control + Basal Diet	0.62	2.37	2.50
Sham Control + Menthol supplementation	0.54	2.36	3.08
In ovo Menthol + Basal Diet	0.62	2.27	2.54
In ovo Menthol + Menthol Supplementation	0.60	2.68	2.63
Treatment			
Un-injected Control	0.55	2.26	2.71
Sham Control	0.58	2.37	2.79
In ovo Menthol	0.61	2.47	2.58
Diet			
Basal Diet	0.59	2.27	2.60
Menthol Supplementation	0.56	2.46	2.79
Pooled SEM	0.01	0.07	0.07
Sig. level			
Treatment	NS	NS	NS
Diet	NS	NS	NS
Treatment × Diet	NS	NS	NS

NS: Not Significant (P≥0.05) SEM: Standard Error of Means

extracts. In the present study also, gizzard % of birds in menthol supplemented diet had higher values as compared to basal diet. The results of the present study clearly indicated that there was no adverse effect of menthol on the carcass quality characteristics, yield of giblets and cut up parts at 6 weeks of age.

Table 10: Effect of *in ovo* feeding and dietary supplementation of menthol on cut-up-parts of commercial broilers after 6 weeks of age

Group	Breast%	Back%	Wings%	Neck%	Drumstick%	Thigh %
Un-injected Control + Basal Diet	24.60	25.08	4.96	10.24	17.62	17.50
Un-injected Control + Menthol supplementation	24.41	24.99	5.62	8.71	17.98	18.29
Sham Control + Basal Diet	24.29	25.47	6.05	10.31	17.80	16.08
Sham Control + Menthol supplementation	25.01	23.29	5.97	11.09	17.60	17.05
In ovo Menthol + Basal Diet	24.02	25.12	5.69	9.78	18.46	16.94
In ovo Menthol + Menthol Supplementation	24.91	23.35	5.78	10.43	19.06	16.47
Treatment						
Un-injected Control	24.51	25.04	5.29	9.47	17.80	17.89
Sham Control	24.65	24.38	6.01	10.70	17.70	16.57
In ovo Menthol	24.46	24.24	5.74	10.10	18.76	16.71
Diet						
Basal Diet	24.30	25.22	5.57	10.11	17.96	16.84
Menthol Supplementation	24.78	23.88	5.79	10.07	18.21	17.27
Pooled SEM	0.32	0.39	0.13	0.28	0.20	0.25
Sig. level						
Treatment	NS	NS	NS	NS	NS	NS
Diet	NS	NS	NS	NS	NS	NS
Treatment x Diet	NS	NS	NS	NS	NS	NS

NS: Not Significant (P≥0.05) SEM: Standard Error of Means

CONCLUSION

Based on the results of the present study, *in ovo* feeding of 1% menthol along with dietary supplementation of menthol @ 250 mg/ kg diet resulted in better early growth performance as compared to other treatment groups. Further, it did not significantly impact carcass quality traits, digestive and lymphoid organs and yield of cut up parts However, further studies are required with *in ovo* feeding and dietary supplementation of menthol at different levels to assess the effect of *in ovo* feeding vis-à-vis dietary supplementation of menthol on the performance of broilers.

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