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Dietary supplementation of micro-encapsulated essential oils on the performance of white leghorn layers

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Abstract
To evaluate the effect of microencapsulated essential oil supplementation on percent hen day egg production, feed intake, feed conversion ratio, egg weight, egg mass, egg quality and serum parameters of laying hens. For this purpose, a total of 240 Bovans White laying hens at 22 weeks old were randomly assigned to 3 treatments with 20 replicates of 4 birds each. The birds were fed with Control diet (CD), CD + micro-encapsulated essential oil (MEEO-I: thymol, carvacrol and menthol) and CD + micro-encapsulated essential oil (MEEO-II: cinnamaldehyde, eugenol and piperine) respectively. The results revealed that percent hen day egg production was significantly ($P<0.05$) improved, whereas feed intake was reduced ($P<0.05$) in birds fed with MEEO-I and MEEO-II diets. Significantly better feed conversion ratio was observed in microencapsulated essential oil supplemented groups when compared to control group. Among egg quality parameters Haugh unit, albumen index, eggshell thickness were significantly ($P<0.05$) influenced in MEEO-I and MEEO-II supplemented groups. Supplementation of essential oil groups significantly reduced the serum cholesterol ($P<0.05$) concentration compared to control at 37th week of age. Finally, it can be concluded that supplementation of microencapsulated essential oils in the layer diet has beneficial for improving overall performance of layers.

Keywords: micro-encapsulated essential oils, egg production, egg quality, laying hens

1. Introduction
As many countries across the world make ban on the usage of antibiotics as growth promoting agents in animal feeds, it gave an opportunity for the researchers to exploit the usage of alternative feed supplements in animal production. Among them essential oils (EO's) acquiring much more importance as they are plant derived products. EO's are complex mixture of aromatic oily liquids derived from different plant parts namely buds, seeds, flowers twigs, bark, fruits, roots (Gopi *et al*. 2014) [11]. EO's have some biological properties such as antimicrobial (Gopi *et al*. 2014; Patil *et al*. 2016) [11, 16], antioxidant (Yu *et al*. 2018), enzymatic stimulation (Migliorini *et al*. 2019), digestion stimulating (Alcicek *et al*. 2003) [2], growth enhancer (Srinivasan *et al*. 2006), anti- inflammatory (Feisst *et al*. 2005) [9], Immunomodulatory effect (Mahima *et al*. 2012) [12]. Inclusion of the EO's in poultry diets will also improve animal health and productivity (Gerzilov *et al*. 2015) [10], reduce serum cholesterol levels (Case *et al*. 1995; Bulbul *et al*. 2014) [7, 5]. As these compounds are lipophilic nature it limits there efficient delivery of these compounds to the gut. It can be resolved by micro-encapsulation techniques (Yang *et al*. 2015) [20]. Microencapsulation techniques of EOs will increase the bioavailability of active compounds, as well as their application in the animal feed additive industry (Stevanovic *et al*. 2018) [19].
The study was designed to evaluate the impact of microencapsulated oil supplementation (MEEO) on the laying performance, egg quality characteristics and some blood parameters in white leghorn laying hens.

2. Materials and Methods
The present study was conducted at the Department of Poultry Science, College of Veterinary Science, Hyderabad (India). As a whole, 240 commercial white leghorn layers (Bovans White), 22 week old with uniform body weight, were attributed to 3 equal groups replicated 20 times with 4 birds per replicate. The experiment was conducted from December 2020 to March 2021 in a well-ventilated platform 2 tier cage layer house. Birds were housed by placing 4

birds in a colony cage (18× 17× 15 inch). A common linear feeder is provided for each replicate and water is provided with nipple drinkers fitted on the top of the cages. LED bulbs are used to provide 16h light period daily, including daylight. The birds were given *adlib* feed and water during the entire experimental period (4 periods of each 28 days from 22 to 37 weeks). Temperature and relative humidity were within the normal range of birds.

The control diet (CD) consisted of a corn-soyabean based basal diet without adding the additive. Table 1: Ingredient and the nutrient composition of the basal diet (%) fed to layer birds from 22-37 wks. The remaining 2 treatments were given the same basal diet supplemented with MEEO-I and MEEO-II both at 100g/ton of feed. Microencapsulated essential oil-I (MEEO-I) contains thymol, carvacrol and menthol. Microencapsulated essential oil-II (MEEO-II) contains cinnamaldehyde, eugenol and piperine as active ingredients. All the diets were isonitrogenous and isocaloric. Birds were provided with standard layer mash at 110g/day. The test diets were formulated to comply with nutrient requirements for layer hens according to the recommendations of the breeder (Bovans White Management Guide, Skylark Hatcheries Pvt. Ltd, Jind (Haryana), India).

Table 1: Ingredient and the nutrient composition of the basal diet (%) fed to commercial layers from 22-37 wks.

Ingredient	**Composition (%)**
Maize	58
Soyabean meal	24.7
De-oiled rice bran	5.37
Stone grit	9.30
Dicalcium Phosphate	1.70
Salt	0.40
DL-Methionine	0.17
L-Lysine HCL	0.04
Trace Mineral Mixture*	0.12
Choline chloride	0.05
Toxin Binder	0.10
Vitamin Premix**	0.05
Nutrient composition	
Metabolic energy(kcal/kg)	2625
Crude protein (%)	16.62
Available phosphorus (%)	0.41
Calcium (%)	3.53
Sodium (%)	0.17
Digestible lysine (%)	0.85
Digestible methionine (%)	0.40

* Trace mineral provided per kg diet: Manganese 120mg, Zinc 80mg, Iron 25mg, Copper 10mg,
Iodine 1mg and Selenium 0.1mg.
** Vitamin premix provided per kg diet: Vitamin A 200000 IU, Vitamin B225 mg, Vitamin D3 3000IU, Vitamin K 2mg, Riboflavin 25mg, Vitamin B1 1mg, Vitamin B6 2mg, Vitamin B12 40mg and Niacin 15mg.

During the whole experimental period, hen day egg production (%) (HDEP), mortality was recorded daily. Feed intake (FI), feed conversion ratio (FCR), egg weight (EW), egg mass (EM), were measured at 28-day intervals and during the entire trial period. Bodyweight (BW) of hens was recorded at the beginning and end of the trial by weighing the hens using a 1g precision scale. Hen day egg production in per cent was calculated by dividing the total number of eggs laid every day by number of hens survived during each day. Feed consumption of each replicate was recorded at periodic intervals, on cumulative basis and the feed intake/bird/day was arrived at the end. The feed conversion ratio (FCR) was calculated as feed intake in kg/dozen eggs produced. Eggs were collected at the end of each period for three consecutive days and the average egg weights were recorded to the nearest 0.1 g accuracy. The egg mass (EM) was calculated by EM = HDEP (%) × average EW.

At the end of each period (28 days) 1 egg (20 eggs per treatment) were collected from each replicate at 28th, 56th, 84th, 112th days to evaluate egg quality indexes like haugh unit, albumen index, yolk index, eggshell weight (with shell membrane), eggshell thickness was measured as a mean value by using a Mitutoyo dial gauge meter (Model no.7301, Japan) to an accuracy of 0.01 mm.. Haugh unit was determined by the following formula: HU = 100 log (h – 1.7w 0.37 + 7.57) (Singh, R.A. 2006) [17]. Albumen index was calculated after the measurement of height and diameter of thick albumen with the help of spherometer and digital vernier calipers respectively. The yolk index was calculated after the measurement of height and diameter of yolk with the help of by a tripod micrometer (Mitutoyo, 0.01 mm, Japan) and vernier calipers, respectively. Yolk colour was measured using the Roche yolk colour fan (DSM). The shell weight (g) was recorded after air drying and the observations were measured by using digital balance nearest to 0.1 mg accuracy. The eggshell thickness was measured at three locations on the egg (broad, middle, narrow end)
using Mitutoya dial gauge meter (Model no.7301, Japan). The eggshell percentage was calculated by dividing the shell weight with egg weight and then multiplied by 100 and eggshell breaking strength measured by shell force gauge (static compressor) expressed in Newton’s/ kg2.

At the end of the experiment (37th week) in layers about 2ml of blood sample was collected from one representative bird from each replicate. Blood samples were collected aseptically from
wing vein with the help of sterilized needles and placed in a clean sterilized vacutainers and kept in incubator at room temperature for serum collection. Further, blood samples were centrifuged at 3000rpm for 5minutes to separate the serum and serum was transferred to labeled 5ml eppendorf tubes which were stored at -200C until analysis. The serum is used for estimation of cholesterol and total protein by using spectrophotometer with commercially available kits (ERBA diagnostic Mannheim- GmbH transasia bio-medicals Limited). The data were analyzed using General Linear Model procedure of Statistical Package for Social Sciences (SPSS) 20th version and comparison of means was done using Duncan’s multiple range test (Duncan, 1955) [8] and significance was considered at $P<0.05$.

3. Results and Discussion

The results revealed that supplementation of MEEO-I and MEEO-II groups significantly ($P<0.05$) increased the percent hen day egg production when compared to control group. The increased performance in test diets might be due to the active principles in the essential oils which improved the health status of birds. The above result was in agreement with Abo ghanima *et al*. (2020) [1] who concluded that percent hen day egg production was significantly ($P<0.001$) higher in thymol supplemented group. There was significantly ($P<0.05$) lowered feed intake among MEEO-I and MEEO-II supplemented group. As the essential oils have stimulating effects on the secretion of digestive enzymes, this may be responsible for lowered feed consumption. Similar results have been obtained by Bolukbasi *et al*. (2008) [3] with the supplementation of thyme oil to the laying birds. Inclusion of MEEO-I had significantly (*P*<0.05) improved FCR/dozen eggs from 1.45 to 1.39. Similar results have also been obtained by Bolukbasi *et al*. (2008) [3] in laying birds by supplementation of thyme oil and Marume *et al*. (2020) [13] by inclusion of *Citrullus lanatus* essential oil in layer diet.

In the present study egg weight, egg mass, body weight were not influenced by the supplementation of either MEEO-I (or) MEEO-II. The results are supported by Yu *et al*. (2018) for both egg weight, egg mass with inclusion of star anise essential oil in layer diet, Marume *et al*. (2020) [13] for egg weight and Olgun *et al*. (2016) [15] in laying hens, Bozkurt *et al*. (2009) [4] in Ross 308 broiler breeders for body weight.

Table 2: Effects of microencapsulated essential oils in diets on production performance.

	Treatments				
Parameter	**CD**	**MEEO-I**	**MEEO-II**	**SEM**	**P-value**
HDEP	90.15b	92.12a	91.34a	0.217	0.001
FI	108.2a	106.5c	107.3b	0.178	0.003
FCR	1.454a	1.391c	1.415b	0.039	0.001
EW	58.54	57.35	57.88	0.184	0.280
EM	52.76	52.86	52.93	0.219	0.950
Initial BW	1257	1253	1254	7.423	0.975
Final BW	1382	1386	1391	9.198	0.917

HDEP: Hen day egg production (%), FI: Feed intake (g/hen/day), FCR: Feed conversion ratio (kg feed/dozen eggs), EW: Egg weight (g), EM: Egg mass (g), BW: Body weight (g).
Means in the same row with different superscripts (a,b,c) are significantly (*P*<0.05) different.

Among the internal egg quality parameters Haugh unit and Albumen index were significantly (*P*<0.05) improved by the supplementation of MEEO-I. Similar results were also reported by Bolukbasi *et al*. (2010) who revealed inclusion of bergamot oil in layer diet had improved Haugh unit value. Marume *et al*. (2020) [13] also reported supplementation of *Citrullus lanatus* essential oil to laying hens had improved Haugh unit score of eggs. Yesilbag *et al*. (2013) [21] noted that supplementation of rosemary oil to the quails had improved albumen index of the eggs. Yolk index and Yolk color values in all treatments have no significant difference (P>0.05). Similar results were also observed for the yolk index and yolk color of eggs by Marume *et al*. (2020) [13] and Abo ghanima *et al*. (2020) [1] for yolk index. With respect to external egg quality parameters significant (*P*<0.05) difference was obtained for eggshell thickness in MEEO-I and MEEO-II supplemented groups. Olgun *et al*. (2016) [15] noted that supplementation of EOM (thyme oil, black cumin oil, fennel oil, anise oil, rosemary oil) to laying hens had positively influenced eggshell thickness. Shell weight, shell percent, shell breaking strength were not influenced by the supplementation of MEEO-I and MEEO-II in the layer diet.

The results obtained further showed that serum total protein concentration was not influenced by the supplementation of MEEO-I and MEEO-II in layer diet at 37th week of age. These findings are in agreement with those reported by Abo ghanima *et al*. (2020) [1], who concluded that supplementation of thymol, carvacrol and eugenol essential oils in the layer diet did not influenced serum total protein concentration. Supplementation of layer ration with MEEO-I (or)
MEEO-II significantly (*P*<0.05) decreased serum cholesterol levels in comparison with the control group. Similar findings were also observed by Abo ghanima *et al*. (2020) [1] who studied the effect of thymol, carvacrol and eugenol essential oils on the performance of the brown layers and Bulbul *et al*. (2014) [5] with myrtle oil supplementation on the serum total cholesterol levels in laying quails.

Table 3: Effects of microencapsulated essential oils in diets on egg quality and serum parameters.

	Treatments				
Parameter	**CD**	**MEEO-I**	**MEEO-II**	**SEM**	**P-value**
HU	88.43b	90.30a	89.37ab	0.264	0.013
AI	0.071b	0.076a	0.073b	0.051	0.001
YI	0.468	0.480	0.473	0.252	0.114
YC	6.15	6.30	6.29	0.056	0.508
SW	6.007	5.962	5.957	0.014	0.293
SP	10.22	10.23	10.19	0.035	0.916
SBS	20.41	21.37	20.68	0.277	0.350
ST	0.401c	0.413a	0.407b	0.001	0.001
TP	4.44	4.28	4.33	0.833	0.735
CHOL	208.07a	179.75c	187.82b	1.993	0.001

Parameters- HU: Haugh unit, AI: Albumen index, YI: Yolk index, YC: Yolk color, SW: Shell weight, SP: Shell percent, SBS: Shell breaking strength, ST: Shell thickness, TP: Total protein (g/dL), CHOL: Cholesterol (mg/dL).
Means in the same row with different superscripts (a,b,c) are significantly (*P*<0.05) different.

4. Conclusion

Finally, It can be concluded that microencapsulated essential oil supplementation (100 g/ton of feed) had improved% hen day egg production, feed intake, feed conversion ratio/ dozen eggs, albumen quality, shell thickness and reduced serum cholesterol levels.

5. Acknowledgement

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6. Conflict of Interest Statement
The authors declare that they have no conflict of interest

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