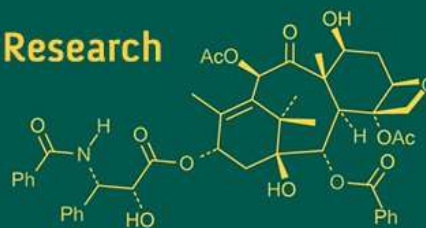
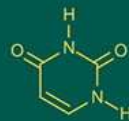
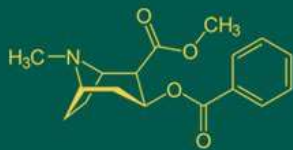


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## Influence of dietary inclusion of peppermint and eucalyptus essential oils on haematological parameters of Japanese quails

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### Abstract

Since several decades antibiotics are used as a feed supplement in the ration of farm animals because of their metaphylactic, prophylactic and growth promotion action which eventually has resulted in antimicrobial resistance (AMR) in livestock and humans. With an intention of resolving this issue, some potential replacements have been studied. Essential oils belong to those categories of supplements which have antimicrobial, anti-inflammatory, and antioxidant properties. Keeping these facts in view, this study was undertaken to investigate the influence of dietary inclusion of peppermint (PEO) and eucalyptus essential oils (EEO) on haematological parameters of the Japanese quails. For this, 180 Japanese quail chicks of 6-days old were randomly grouped into 5 treatments (3 replicates having 12 chicks each). The experimental treatments were T<sub>0</sub> (control), T<sub>1</sub> (0.2% PEO), T<sub>2</sub> (0.2% EEO), T<sub>3</sub> (0.1% each of PEO+EEO) and T<sub>4</sub> (0.05% each of PEO+EEO). By the end of the 5<sup>th</sup> week, blood samples of 2 birds from each replicate were collected and their haematological parameters were determined. The study revealed that the values of TEC, TLC, PCV, haemoglobin, MCHC, basophil + eosinophil and lymphocytes were significantly ( $p < 0.05$ ) increased in all the essential oil supplemented groups especially T<sub>3</sub>. The MCV, heterophils and H: L ratio was significantly ( $p < 0.05$ ) decreased in the group T<sub>3</sub> (0.1% of PEO+EEO).

However, MCH and monocytes did not vary significantly ( $p > 0.05$ ). Thus, it can be concluded that the supplementation of 0.1% of PEO and EEO in combination improved the physiological and health status of Japanese quails preventing the occurrence of pathological conditions.

**Keywords:** Japanese quails, haematological parameters peppermint and eucalyptus essential oils

### 1. Introduction

The existing problem of antimicrobial resistance (AMR) in the livestock due to the use of antibiotics as the feed additives has also severely impacted the human health because of consumption of animal products. The International and National organisations are working towards this direction in order to avoid over use or misuse of antibiotics in livestock industry (Walia *et al.*, 2019) [1]. In this concern, some potential alternatives are being tested which not only provides protection to the animals against the diseases but also helps to increase production parameters (Alagawany *et al.*, 2020) [1]. Nutraceuticals like vitamins, minerals, amino-acids, fatty acids, essential oils, polyphenols, herbs, spices etc. have been studied out of which essential oils have proven to be one of the good replacement. Essential oils are the purest and concentrated extracts obtained from spices and herbs. These oils are extracted by different methods like distillation, CO<sub>2</sub> extraction, Maceration, Enfleurage, Cold Press Extraction, Solvent extraction etc. Different essentials oils like oregano, cinnamon, peppermint, turmeric, thyme, lemon etc. have been used and tested for its medicinal properties. These essential oils when used in poultry have also produced better growth promotion, FCR, carcass and egg production (Krishan and Narang, 2014) [6]. Here in this experiment peppermint and eucalyptus essential oils were used as supplements. The antibacterial and antioxidant properties of Peppermint (*Mentha piperita*) is being well documented (Singh *et al.*, 2015) [8]. Similar effects of Eucalyptus (*Eucalyptus globulus*) have also been recorded along with its anti-inflammatory effect (Al-Snafi, 2017) [3]. Both these essential oils have been studied in poultry especially chicken.

A variety of poultry species are raised in response to the growing demand for animal protein; among these, the rearing of Japanese quails has shown to be a lucrative endeavour due to its low resource requirements, low investment, and favourable returns that can be gained within a short time frame. They are also hardy birds when compared to poultry. Thus, this experiment was conducted to study the effect of peppermint and eucalyptus essential oils on the haematological parameters in the Japanese quails.

## 2. Material and Methods

The experiment was conducted at Instructional Poultry Farm (I.P.F), Govind Ballabh Pant University of Agriculture and Technology, Pantnagar (U.S. Nagar), Uttarakhand for 5 weeks with CRD (Completely Randomized Design). For this study, 180, six-day old, Japanese quail chicks were divided randomly into five treatment groups each consisting of 36 birds. This treatment groups were further allocated into three replicates each having 12 quail chicks. These birds were reared in battery cages with 24 hrs. Light supply and were given ad libitum access to water. Feeding was done according to different phases: Starter diet (I-II week) and Finisher diet (III-V week) which were composed according to the specifications of ICAR (2013). The peppermint and eucalyptus essential oils were provided through feed. The treatments groups were T<sub>0</sub> (control) fed with basal diet without essential oils, T<sub>1</sub> fed with basal diet containing 0.2% peppermint essential oil, T<sub>2</sub> fed with basal diet containing 0.2% eucalyptus essential oil, T<sub>3</sub> fed with basal diet containing combination of 0.1% peppermint essential oil and 0.1% eucalyptus essential oil and T<sub>4</sub> fed with basal diet containing combination of 0.05% peppermint essential oil and 0.05% eucalyptus essential oil. As essential oils are potent, they were incorporated into feed after mixing it with food grade coconut oil which acted as a carrier.

The birds were reared for 5 weeks after which 2 birds from each replicate were bled by puncturing the jugular vein. From the total blood obtained, a part of the blood was put into vials containing ethylene diamine tetra acetate (EDTA) and used to Estimate Hemoglobin (Hb), Red Blood Cells (RBC), Packed Cell Volume (PCV), and White Blood Cells (WBC). The results were recorded, analyzed, and assessed according to Snedecor and Cochran (1994) [9].

## 3. Results and Discussions

The results thus obtained were presented in the Table 1 and Table 2.

### Hematological parameters

A significant ( $p < 0.05$ ) increase in the total erythrocyte count, total leucocyte count, packed cell volume and haemoglobin of the Japanese quails were noted by the

supplementation of peppermint and eucalyptus essential oils. The TEC and TLC was significantly ( $p < 0.05$ ) high in the birds belonging to the T<sub>3</sub> group while the group T<sub>0</sub> (control) showed significant lower count.

The PCV value was recorded significantly ( $p < 0.05$ ) higher in birds belonging to groups T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> whereas the group T<sub>0</sub> (control) and T<sub>4</sub> showed a significantly ( $p < 0.05$ ) lower packed cell volume. The birds belonging to the group T<sub>1</sub> and T<sub>2</sub> showed significantly ( $p < 0.05$ ) higher concentration of haemoglobin when compared the group T<sub>0</sub> (control).

These results are substantiated by Witkowska *et al.* (2019) [12] who observed a significantly higher TEC, TLC, haematocrit and Haemoglobin value in the broilers misted with peppermint oil compared to control. Bello (2015) [4] also found that supplementation of *Eucalyptus globulus* dried leaves to the Japanese quails resulted in significantly higher TEC, TLC, haematocrit and Haemoglobin value. However, the above results are contradicted by Al-Kassie (2010) [2] who recorded that supplementation of dried peppermint to broilers did not result a significant variation in TEC, TLC, haematocrit and Haemoglobin value. Similarly, Toghyani *et al.* (2010) [10] found no significant difference in the TEC, TLC, haematocrit and Haemoglobin value in the broiler chickens fed with dried peppermint leaves when compared to control. Petrolli *et al.* (2019) [7] also did not find any significant variation in the TEC, TLC, haematocrit and Haemoglobin value in broilers when eucalyptus oil was supplemented in water or sprayed as mist.

The birds belonging to the group T<sub>3</sub> showed significantly ( $p < 0.05$ ) lower MCV value than the control (T<sub>0</sub>) group. These results are in contradiction with Toghyani *et al.* (2010) [10] who found that when broilers were fed with dried peppermint leaves, no significant difference in the MCV when compared to control was noticed. Whereas, Bello (2015) [4] found that with the increase in the levels of dried *Eucalyptus globulus* leaves MCV increased significantly in the Japanese quails. No significance ( $p > 0.05$ ) difference was noticed in MCH value of the Japanese quails which were fed with peppermint and eucalyptus essential oils when compared to that of control. In contrast, Bello (2015) [4] found that the MCH was significantly increased in Japanese quails fed with dried *Eucalyptus globulus* leaves.

The group T<sub>1</sub> showed significantly ( $p < 0.05$ ) maximum mean corpuscular haemoglobin concentration while the control showed the minimum value of MCHC. The results of this study are in agreement with Bello (2015) [4] who found that with the increase in the levels of dried *Eucalyptus globulus* leaves the MCHC increased significantly in the Japanese quails. In contrast, Toghyani *et al.* (2010) [10] found that when broilers were fed with dried peppermint leaves, no significant difference in the MCHC was noticed.

**Table 1:** Impact of dietary inclusion of peppermint and eucalyptus essential oils on haematological profile of Japanese quails (Mean  $\pm$  S.E.)

Treatment	TEC* (10 <sup>6</sup> /μL)	TLC* (10 <sup>3</sup> / μL)	PCV* (%)	Hb* (g/dl)	MCV* (fl)	MCH (pg)	MCHC* (%)
T <sub>0</sub>	2.96 <sup>c</sup> ±0.02	20.59 <sup>b</sup> ±0.23	32.43 <sup>b</sup> ±0.36	10.88 <sup>c</sup> ±0.18	109.46 <sup>a</sup> ±0.34	36.71±0.43	33.54 <sup>b</sup> ±0.40
T <sub>1</sub>	3.47 <sup>ab</sup> ±0.21	21.95 <sup>a</sup> ±0.17	34.74 <sup>a</sup> ±0.14	12.75 <sup>a</sup> ±0.11	100.66 <sup>ab</sup> ±5.31	36.94±1.98	36.70 <sup>a</sup> ±0.25
T <sub>2</sub>	3.37 <sup>ab</sup> ±0.19	22.05 <sup>ab</sup> ±0.21	34.99 <sup>a</sup> ±0.01	12.43 <sup>a</sup> ±0.29	104.44 <sup>ab</sup> ±6.28	37.07±2.15	35.52 <sup>ab</sup> ±0.82
T <sub>3</sub>	3.70 <sup>a</sup> ±0.06	22.14 <sup>a</sup> ±0.25	34.63 <sup>a</sup> ±0.74	12.21 <sup>ab</sup> ±0.11	93.58 <sup>b</sup> ±1.23	33.02±0.54	35.29 <sup>ab</sup> ±0.54
T <sub>4</sub>	3.07 <sup>bc</sup> ±0.07	21.13 <sup>bc</sup> ±0.50	32.3 <sup>b</sup> ±0.36	11.48 <sup>bc</sup> ±0.43	105.16 <sup>ab</sup> ±1.44	37.34±1.15	35.53 <sup>ab</sup> ±1.28

Within the identical column, values with distinct superscripts differ significantly (\* $p < 0.05$ )

The effect of supplementation of peppermint and eucalyptus essential oils on the differential leukocyte count in Japanese quails is significant ( $p<0.05$ ) except for the monocytes. The essential oil supplemented groups i.e., T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> showed the significantly ( $p<0.05$ ) lower heterophils count while the control group T<sub>0</sub> showed the higher count. The birds belonging to the group T<sub>3</sub> showed a significantly ( $p<0.05$ ) higher basophils + eosinophils count than the group T<sub>0</sub>. The control group (T<sub>0</sub>) recorded the minimum lymphocytes counts while the group T<sub>1</sub> and T<sub>4</sub> showed the significantly higher lymphocytes count. The H: L ratio in birds belonging to the group T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> was significantly ( $p<0.05$ ) low in comparison to T<sub>0</sub>.

Bello (2015) [4] found that supplementing dried *Eucalyptus globulus* leaves to the Japanese quails did not produce any significant changes in the heterophils count whereas basophils + eosinophils, lymphocytes count significantly increased while H: L ratio significantly reduced. Witkowska

*et al.* (2019) [12] found that the broilers misted with peppermint oil did not result in significant changes in heterophils, basophils + eosinophils, lymphocytes counts and H: L ratio when compared to control. Similarly, Petrolli *et al.* (2019) [7] noted no significant changes in the heterophil, basophils + eosinophils, lymphocyte counts and H: L ratio when broilers were supplemented with eucalyptus oil in water and when sprayed as mist.

Peppermint and eucalyptus essential oils supplementation did not produce any significant ( $p>0.05$ ) changes on the monocytes count among the Japanese quails. However, the group T<sub>0</sub> showed the quantitatively maximum monocyte count. Witkowska *et al.* (2019) [12] also found non-significant difference in monocyte counts in the broilers misted with peppermint oil compared to control. Likewise, Petrolli *et al.* (2019) [7] noted no significant change in the monocyte count when broilers were supplemented with eucalyptus oil in water or when sprayed as mist.

**Table 2:** Impact of dietary inclusion of peppermint and eucalyptus essential oils on differential leukocyte count of Japanese quails (Mean  $\pm$  S.E.)

Treatment	Heterophils* (%)	Basophils + Eosinophils* (%)	Lymphocytes* (%)	Monocytes (%)	Heterophils-Lymphocyte ratio*
T <sub>0</sub>	22.67 <sup>a</sup> $\pm$ 0.88	5.00 <sup>b</sup> $\pm$ 0.58	69.33 <sup>b</sup> $\pm$ 0.88	3.00 $\pm$ 0.58	0.33 <sup>a</sup> $\pm$ 0.01
T <sub>1</sub>	20.00 <sup>b</sup> $\pm$ 0.58	5.67 <sup>ab</sup> $\pm$ 0.67	72.67 <sup>a</sup> $\pm$ 0.33	2.00 $\pm$ 0.00	0.27 <sup>b</sup> $\pm$ 0.01
T <sub>2</sub>	20.33 <sup>b</sup> $\pm$ 0.67	6.33 <sup>ab</sup> $\pm$ 0.33	71.33 <sup>ab</sup> $\pm$ 0.88	2.00 $\pm$ 0.00	0.28 <sup>b</sup> $\pm$ 0.01
T <sub>3</sub>	20.33 <sup>b</sup> $\pm$ 0.88	6.67 <sup>a</sup> $\pm$ 0.33	71.00 <sup>ab</sup> $\pm$ 0.58	2.00 $\pm$ 0.58	0.28 <sup>b</sup> $\pm$ 0.01
T <sub>4</sub>	21.00 <sup>ab</sup> $\pm$ 0.00	5.33 <sup>ab</sup> $\pm$ 0.33	71.67 <sup>a</sup> $\pm$ 0.33	2.00 $\pm$ 0.00	0.29 <sup>ab</sup> $\pm$ 0.00

Within the identical column, values with distinct superscripts differ significantly (\* $p<0.05$ )

#### 4. Conclusion

- It is concluded from the present study that dietary addition of thyme and turmeric essential oils at 0.075% and 0.125% in combination improved most of the ha.
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It is concluded from the present study that dietary inclusion of 0.1% of peppermint essential oil and 0.1% of eucalyptus essential oils in combination improved most of the hematological parameters of Japanese quails which in turn will improve the health status of birds and helps provide immunity against diseases.

#### 5. Acknowledgement

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#### 6. Conflict of Interest

There is no conflict regarding this research

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