

## EFFECT OF DIETARY SUPPLEMENTATION OF CLOVE, PEPPERMINT, CINNAMON OILS AND THEIR BLENDS ON GROWTH PERFORMANCE, CARCASS CHARACTERISTICS, BLOOD BIOCHEMICAL PARAMETERS AND ANTIOXIDANT STATUS OF BROILER CHICKS

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### SUMMARY

The present study was conducted to evaluate the effect of dietary supplementation of clove, peppermint, cinnamon oils and their blends on growth performance, some blood biochemical constituents, antioxidants activities and economic efficiency. A total of 300 unsexed (Ross – 308) broiler chicks, one-day old were fed from 1-35 days on this specific dietary supplementation. Birds were divided randomly into five dietary treatment groups composed of 60 chicks per group, each in 3 replicates. The 1<sup>st</sup> group (control) received basal diet without additions, the 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> groups received basal diet supplemented with clove, peppermint and cinnamon oils at 0.3 g/kg diet of each, respectively and, the 5<sup>th</sup> group received basal diet supplemented with blend of clove, peppermint and cinnamon oils at 0.1 g/kg diet of each. The results indicated that essential oils blend dietary supplementation significantly improved body weight, gain, feed conversion ratio, performance index in different periods of the experiment. Dressing, giblets and some immune organ percentages had higher values in the essential oil supplemented chicks than the control, while abdominal fat percentage had lower values in the essential oil supplemented chicks than the control. Broiler chicks fed the essential oil diet had greater serum total protein, glutathione (GPx) activities and high density lipoprotein (HDL) than those fed the control, however, significantly decreased serum cholesterol, triglycerides, low density lipoprotein (LDL) and lipid peroxidation (MDA) concentrations. In conclusion, beneficial effects of using blends of clove, peppermint and cinnamon oils at 0.1 g/kg diet had been obtained on performance, blood biochemical and economical efficiency with no harm effect on health under experimental conditions.

**Keywords:** broiler performance, blood parameters, clove, cinnamon and peppermint oils

### INTRODUCTION

After removal of antibiotic growth promoters as feed additives in poultry nutrition from the market in many regions around the world, scientists have searched for suitable alternative supplements of vital importance for broiler production. Probiotics, prebiotics, enzymes, organic acids, herbs, immune-stimulants and essential oils have been found as unconventional and safe alternatives feed additives in poultry production. Essential oils have a wide variety of uses aromatherapy, beauty treatments, herbal medicines and perfumery applications (Alagawany *et al.*, 2020).

Essential oils (EOs) are volatile aromatic oil liquids that are distilled from plants, with a unique aroma and composition. They are complex mixtures of compounds such as terpenes, terpenoids, aldehydes, alcohols, phenols, methoxy derivatives and some other more common compounds. A detailed classification can be found in other reviews (Szczerbinska, 2018) usually, EOs will contain several components with two or three major ones at large concentrations ranging from 20% to 70%. Although potentially differing in their constituents, properties that are common to most essential oils being vaporized with steam, lipophilic nature, liquid state at 18°C, optically active (ability of EOs

components to rotate the plane of plane polarized light which is an indicator of quality and purity), and solubility in ethanol and lipids (Adaszynska and Skwirzynska, 2018). Major aromatic oils that may have potential in poultry production and processing include nutmeg, lime, mandarine, orange, rosewood, oregano, mountain savory, fennel, turmeric, rosemary, sage, cinnamon, thyme, ginger, eucalyptus, garlic, pimenta, lemongrass, peppermint and clove (Dewi *et al.*, 2018).

The use of essential oils in poultry feeding has been practiced for their role as antibacterial, antiviral, antifungal, antioxidant, digestive stimulants, immunomodulators, hypolipidemic agents, and heat stress alleviators (Ruff *et al.*, 2021). In this regard, there are several studies referred that some herbal substances has a positive effect on the growth performance of broiler chickens, their antioxidant status, and the oxidative stability of meat. As well enhance oxidation stability of poultry meat during storage. Cinnamon is a good medicinal plant, it has some properties, astringent, warming, stimulating, carminative, antiseptic, antifungal, antiviral, blood purifying, and aiding digestion. Also, Yun-feng *et al.* (2019) indicated that dietary supplementation with cinnamon essential oil, an alternative to aureomycin, improved the immune status, antioxidative ability and

cecal microbiota of broilers. The clove oil possess superoxide anion radicals scavenging activity (Gulcin et al., 2012), also it possesses antioxidant and antimicrobial properties. In addition, Abdel-Wareth et al. (2019) illustrated that peppermint leaves can be used as an effective novel nutritional bio- agent up to 15 g/kg to improve the performance of broiler chicks, mainly due to its active components. Peppermint has been used extensively in herbal medicine and is believed to be particularly helpful in building the immune system. It has powerful antimicrobial and antioxidant properties, as well as its ability to boost appetite, mainly due to its active ingredients (Yalcin et al., 2012). Essential oil of peppermint leaves 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 (Beigi et al., 2018). Therefore, the objective of this research was to investigate the effects of supplementation of clove, peppermint, cinnamon oils and their blends on performance, carcass traits, antioxidant activity and some blood biochemical constituents of broiler chicks and economics of production.

## MATERIALS AND METHODS

### *Birds and Experimental diets:*

A total of 300 unsexed (Ross – 308) broiler chicks, one-day old were divided randomly into five dietary treatment groups composed of 60 chicks per group with 3 replicates per each group and 20 chicks per each replicate in completely randomized design. The 1<sup>st</sup> group (control) received basal diet without supplementation, the 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> groups were received basal diet supplemented with clove, peppermint and cinnamon oils at a dose of 0.3 g/ kg diet of each, respectively, and the 5th group received

basal diet supplemented with combination of clove, peppermint and cinnamon oils at a dose of 0.1 g/ kg diet of each. Chicks were housed in floor pen furnished with wood shavings. The experimental period continued 5 wks. Experimental diets were formulated to ensure adequate supply of nutrients according to NRC (1994) and offered to as starter diet (1-21 days) and grower diet (22- 35 days). The control diet contained soybean oil only, while in the other supplemental experimental diets, either clove, peppermint and cinnamon oils or their combination were mixed with soybean oil and then added to the basal diet. The ingredient and chemical composition of the diets are presented in Table 1. All diets and water were provided *ad libitum*. This study was performed in accordance with local ethical guidelines and met the requirements of the institutional bird's care and use committee. The diets were prepared freshly each day. All birds were subjected to similar feeding and management practices (vaccination, lighting, feeding and watering) that suggested by guidebook of (Ross – 308) broiler chicks.

### *Growth performance:*

The broiler chicks were weighed individually at the beginning of the experimental period (initial body weight (IBW), at day 21 of age and at 35d of age (Final body weight, FBW) to calculate body weight gain (BWG) throughout the experimental periods. Feed intake was recorded at 21 and 35 days of age and whole the experiment periods and feed conversion ratio (FCR) (gain: feed ratio) was then calculated. Performance index (PI) was calculated according to North (1984) as follows:  $PI = \text{Live body weight gain (kg)} \times 100 / \text{feed conversion ratio}$ .

**Table1 . Composition and calculated analysis of the experimental broiler diets fed duringstarter period (1 –21) and grower period (22 – 35) days of age**

| Ingredients                                     | Starter diet (1-21 day) | Finisher diet (22-35 day) |
|---|-------------------------|---------------------------|
| Ground yellow corn (8.5%)                       | 53.50                   | 56.50                     |
| Soybean meal (44% CP)                           | 33.00                   | 32.82                     |
| Corn gluten meal, (60%)                         | 5.60                    | 3.00                      |
| Vegetable oil (8800kcal)                        | 4.30                    | 4.50                      |
| Di calcium phosphate                            | 1.60                    | 1.25                      |
| Limestone, ground                               | 1.40                    | 1.33                      |
| Vitamins & minerals mixture <sup>1</sup>        | 0.30                    | 0.30                      |
| Sodium chloride (salt)                          | 0.30                    | 0.30                      |
| <b>Total</b>                                    | <b>100</b>              | <b>100</b>                |
| <b>Calculated chemical analysis<sup>2</sup></b> |                         |                           |
| Crude protein, (CP, %)                          | 22.11                   | 20.65                     |
| ME kcal/ kg diet                                | 3095                    | 3112                      |
| C/P ratio                                       | 139.80                  | 150.70                    |
| Ca, %   | 1.00                    | 0.89                      |
| Av. P, %  | 0.45                    | 0.39                      |
| Lysine, %                                       | 1.16                    | 1.13                      |
| Methionine, %                                   | 0.42                    | 0.38                      |
| SAA, %  | 0.79                    | 0.72                      |
| Crude Fiber, %                                  | 3.75                    | 3.76                      |

<sup>1</sup>Vitamin and Mineral mixture at 0.30% of the diet supplies the following per kilogram of the diet: Vitamin A, 12,000 IU; vitamin D<sub>3</sub>, 3,000 IU; vitamin E, 40 mg; vitamin K<sub>3</sub>, 3 mg; vitamin B<sub>1</sub>, 2 mg; vitamin B<sub>2</sub>, 6 mg; vitamin B<sub>6</sub>, 5 mg; vitamin B<sub>12</sub>, 0.02 mg; niacin, 45 mg; biotin, 0.075 mg; folic acid, 2 mg; pantothenic acid, 12 mg; manganese, 100 mg; zinc, 50 mg; iron, 30 mg; copper, 10 mg; iodine, 1 mg; selenium, 0.2 mg; and cobalt, 0.1 mg, <sup>2</sup>Calculate according to NRC (1994).

**Carcass measurements and immune organs:**

At the end of the study (35 day), six birds from each treatment with a body weight near the group average were fasted for 10 hours prior to slaughter and then slaughtered to determine weights of edible inner organs for carcass characteristics. The carcass parts were weighed separately, liver, heart, gizzard, giblet and abdominal fat and calculated related to the live body weight and expressed in percentage. Carcass% = (carcass weight/LBW) x100.

Immune organs (spleen, bursa and thymus glands), viscera (gizzard empty, liver and heart) were individually weighed and calculated in relation to LBW. The abdominal fat was removed from the parts around the viscera and gizzard and was weighed to the nearest one gram. Dressing percentage was calculated as follows: Dressing% = (carcass weight + giblets)/LBW) x100.

**Blood Serum parameters and antioxidant activity:**

Blood samples were collected at 35 days of age from the slaughtered chickens during their exsanguination. Blood samples were collected in dry clean centrifuge tube without anti-coagulant for serum separation and immediately centrifuged at 3000 rpm for 15 minutes. The clear serum samples were carefully drawn and transferred to epindorf tubes and stored at -20°C in the deep freezer until the time of chemical determinations. Serum total protein (TP), cholesterol (Chol.) and glucose were determined using commercial kits. The globulin (G) value was obtained by subtracting the values of albumin from the corresponding values of total protein. Also, albumin/ globulin (A / G ratio) values were obtained by dividing the values of albumin on the values of globulins according to Coles, (1974). Glutathione peroxidase activity (GPx) was measured colorimetrically, in erythrocytes as stated by procedures of Rotruck *et al.* (1973) and serum MDA concentration was measured in accordance with Yagi (1984) using spectrophotometer at 520 nm and expressed as n mal/ ml TBARS (thionbarbituric reaction substances) index. Serum high-density lipoprotein (HDL) and low-density lipoprotein (LDL) were determined according to methods described by Stein and Myers (1995).

**Economic Efficiency:**

The economic efficiency of the experimental treatments of the present study was calculated from the input – output analysis (Heady and Jensen, 1954), assuming that the other head costs were constant.

**Statistical analysis:**

Statistical analysis was done by one way analysis of variance using SPSS (2011) Software 20 for windows applying the following model:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where:

$Y_{ij}$  = individual observation,

$\mu$  = overall mean,

$T_i$  = the fixed effect of  $i^{\text{th}}$  treatments,

$e_{ij}$  = the experimental random error.

Differences among treatments means were separated by Duncan's multiple range test (Duncan, 1955).

**RESULTS AND DISCUSSION****Growth performance:**

Results of the effect of clove, peppermint, cinnamon oils (0.3%) or their mixture (0.1% clove+ 0.1% peppermint+ 0.1% cinnamon oils) supplementation on broiler growth performance at 1, 21, 35 and whole periods (1-35) day are shown in Tables 2 and 3. Body weight, body weight gain and performance index were significantly increased ( $P \leq 0.05$ ) by essential oils (EOs) supplementation in broiler diets for all experimental diets. Chicks fed 0.1% clove + 0.1% peppermint + 0.1% cinnamon oils; mixture EOs had the highest body weight gain ( $T_5$ ; 2151.21) in comparison with control ( $T_1$ ; 1898.77g) and other treatments  $T_2$ ; 2051.96,  $T_3$ ; 2063.12 and  $T_4$ ; 2030.15g, respectively.

Data describing the effect of dietary essential oils each alone or their mixture on feed intake (FI, g / bird/ day) of broiler chicks during the period from 1-35days of age are presented in Table3. During the entire experimental period from 1 –35days of age, chicks fed the basal diet with mixture essential oil,  $T_5$  had significantly decreased feed intake (107.00g) in comparison with the control group and other experimental groups. While, feed conversion ratio was significantly improved ( $P \leq 0.05$ ) by essential oils mixture supplementation from (1 – 35) days of age being, 2.07, 1.87, 1.89, 1.89 and 1.81 for  $T_1$ ,  $T_2$ ,  $T_3$ ,  $T_4$  and  $T_5$ , respectively. The highest performance index (PI, 121.36%) was recorded in chicks fed the mixture of essential oils ( $T_5$ ) compared to 93.92% in the control group at 35 days of age (Table 3). The enhancing effect of essential oils on the feed conversion and growth performance was due to improving the immune system, regulating the gut micro flora, increasing endogenous digestive enzymes secretion and eliciting antioxidant, antibacterial and anti-viral properties (Kishawy *et al.*, 2019 and Abo Ghanima *et al.*, 2020). The beneficial positive effect of peppermint could be attributed to its content of menthol in a concentration dependent manner. It seems that the presence of active compounds such as menthol in peppermint leaves stimulate appetite and improve feed conversion in broiler chicks. The active components (cineole, citral, geraniol, linalool, and menthol) are shown to possess antimicrobial and antioxidant activities as well as to improve digestion and absorption of dietary nutrients (Bupesh *et al.*, 2007) that might have improved the growth performance of broiler chicks in this study.

The obtained results confirmed the previous findings of several researches; Al-Kassie (2010) showed that broilers fed on diet supplemented with cinnamon oil had a body weight gain significantly higher than the control (without cinnamon oil). In addition, Devi *et al.* (2018) showed that supplementation with a combination of cinnamon oil and ajwain essential oil in broiler diets significantly

increased body weight at 42 days of age. In an earlier study, Khattak *et al.* (2014) found that feeding broilers a blend of EO (basil, caraway, laurel, lemon, oregano, sage, tea and thyme) had a beneficial effect

on broiler performance. Others reported that dietary supplementation with EO improves the growth performance of broilers (Saleh *et al.*, 2018 and Witkowska *et al.*, 2019).

**Table 2. Effect of dietary clove, peppermint, cinnamon oils and their blends supplementation on body weight and body weight gain**

| Dietary treatments <sup>1</sup> | Body weight (g)     |                              |                              | Body weight gain (g)        |                            |                             |
|---------------------------------|---------------------|------------------------------|------------------------------|-----------------------------|----------------------------|-----------------------------|
|                                 | Initial body weight | 21day                        | 35day                        | 1-21days                    | 22-35days                  | 1-35days                    |
| T <sub>1</sub> (Control)        | 45.39±0.35          | 1019.46 <sup>c</sup> ±20.24  | 1944.16 <sup>c</sup> ±12.71  | 975.06 <sup>c</sup> ±10.47  | 924.70 <sup>c</sup> ±9.98  | 1898.77 <sup>d</sup> ±10.95 |
| T <sub>2</sub>                  | 45.37±0.32          | 1134.33 <sup>ab</sup> ±19.17 | 2097.33 <sup>b</sup> ±12.35  | 1087.96 <sup>b</sup> ±10.18 | 963.00 <sup>ab</sup> ±9.04 | 2051.96 <sup>b</sup> ±10.19 |
| T <sub>3</sub>                  | 45.32±0.36          | 1129.36 <sup>b</sup> ±16.18  | 2108.44 <sup>ab</sup> ±10.18 | 1084.14 <sup>b</sup> ±8.53  | 979.08 <sup>a</sup> ±8.43  | 2063.12 <sup>b</sup> ±11.34 |
| T <sub>4</sub>                  | 45.26±0.32          | 1122.00 <sup>b</sup> ±15.55  | 2075.41 <sup>ab</sup> ±10.86 | 1076.90 <sup>b</sup> ±8.53  | 953.41 <sup>ab</sup> ±8.56 | 2030.15 <sup>c</sup> ±9.95  |
| T <sub>5</sub>                  | 45.32±0.31          | 1210.53 <sup>a</sup> ±13.49  | 2196.53 <sup>a</sup> ±11.30  | 1165.28 <sup>a</sup> ±8.19  | 986.05 <sup>a</sup> ±8.49  | 2151.21 <sup>a</sup> ±10.95 |

<sup>1</sup>T<sub>1</sub>; control; basal diet without any supplementation, T<sub>2</sub>; basal diet + 0.3% clove, T<sub>3</sub>; basal diet + 1% peppermint, T<sub>4</sub>; basal diet + cinnamon oils and T<sub>5</sub>; basal diet + 0.1% blends of essential oils, <sup>2</sup> means ± S.E. of 3 replicates / treatment, <sup>3</sup>a, b, c and .... etc. means within the same column with different superscripts are significantly different (P ≤ 0.05).

Likewise, it is reported that broilers fed EO (myrtle leaf oil, oregano oil, sage leaf oil, laurel leaf oil, citrus peel oil and fennel seed oil) showed significant improvement in FCR (Cabuk *et al.*, 2005). Recent studies (Wade *et al.*, 2018) showed that supplementation of EO (thyme extract) in broiler diet increased secretion of digestive enzymes i.e. amylase and chymotrypsin which results into increased rate of absorption in intestine and consequently improves feed utilization. Also, Moustafa *et al.* (2020) reported that supplementation of 100 mg/ kg cinnamon and clove oils had significantly improved feed conversion (FCR) of broiler chickens.

Additionally, it had been shown that dietary supplementation of 100 and 200 mg/ kg of clove oil for broiler chickens had the greater performance values compared to broilers fed un-supplemented diet (Agostini *et al.*, 2012). With the same concept, clove oil has been found to be effective in improving broiler chicken growth performance in previous experiments (Azadegan *et al.*, 2014). The positive effect of clove oil on growth performance could be due to the active materials in clove (eugenol) that are considered as digestion stimulatory factors, in addition to their antimicrobial activity against bacteria found in intestine (Cabuk *et al.*, 2005). Also, it has been reported that essential oils blocked effect of pathogens in the digestive system, improved feed intake, and feed conversion ratio (Gular *et al.*, 2005). Additionally, it has been reported that clove oil may improve the absorption of trace minerals necessary for protein and carbohydrate metabolism, and the synthesis of fatty acid and cholesterol, besides, contain omega3 fatty acid in lesser amounts so, could improve broiler performance (Mukhtar, 2011).

However, other authors did not find positive effect on body weight gain or feed conversion with application of EOs or its main components (Cross *et al.*, 2002 and Demir *et al.*, 2008).

#### **Carcass characteristics and immune organs:**

The effect of different sources of the tested essential oils either alone or their mixture supplementation on carcass characteristics of Ross –

308broiler chicks at 35 days of age are presented in Table (4). Statistical analysis of data revealed that pre-slaughtering weight was significantly higher for the different sources of essential oil supplementation than the control group. The highest value of pre-slaughter weight was for mixture essential oil (T<sub>5</sub>, 2176.66g) than the control group (T<sub>1</sub>, 1935.54g). At 5 weeks of age, there were significant differences among dietary treatments on dressing percentage compared to the control group (T<sub>1</sub>). The highest percent of dressing (78.03%) was observed in group five that have 0.1% clove + 0.1% peppermint + 0.1% cinnamon oils.

The lower values of abdominal fat were recorded for the essential oils supplemented treatments (T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub> and T<sub>5</sub>) compared to the control group (T<sub>1</sub>). Different sources of essential oils supplementation significantly affected the carcass traits as giblets percentage (liver and gizzard), but heart percentage did not significantly differ among all dietary treatments. Improvement in production of poultry meat depends mainly on the increase in muscle proportion, the enhancements in growth and reduction in abdominal fat (Musa *et al.*, 2006). Khattak *et al.* (2014) found that there was an increase in carcass weight, breast weight and breast meat with the supplementation of a natural blend of essential oil, being basil, lemon, caraway, oregano, laurel, sage, thyme and tea. These results indicated that there are beneficial effects on carcass traits of broilers with dietary supplementation of essential oils. However, dietary supplementation of essential oils at 300 mg/kg showed non-significant effect on characteristics of carcass (Alp *et al.*, 2012).

Data of Table 4, represents the effect of dietary essential oils (clove, peppermint, cinnamon) either singly or their mixture on immune organs (spleen, bursa and thymus). Immune organs were significantly improved with sources of dietary essential oil compared to the control group (T<sub>1</sub>). The increase in lymphoid percent may be attributed to the production of specific or non-specific antibodies against different antigens, since lymphoid sinophil and

heterophil are responsible for achieving the defense mechanism and immune response introduced into body (El-Feki, 1987). These results agree with those of Witkowska *et al.* (2019), Alto *et al.* (2018) and

Omar *et al.* (2020) who reported that supplementing essential oils to broiler diets had significantly higher thymus and spleen% compared with those fed the control diet.

**Table 3. Effect of dietary clove, peppermint, cinnamon oils and their blends supplementation on feedintake, feed Conversion and performance index of broiler chicks during experimental periods (Mean±SE)**

| Items                                       | Periods   | Dietary treatments <sup>1</sup> |                           |                            |                           |                           |
|---|-----------|---------------------------------|---------------------------|----------------------------|---------------------------|---------------------------|
|   |           | T <sub>1</sub>                  | T <sub>2</sub>            | T <sub>3</sub>             | T <sub>4</sub>            | T <sub>5</sub>            |
| Feed intake, FI (g/bird/day)                | (1–21) d  | 94.11 <sup>a</sup> ±0.58        | 92.14 <sup>c</sup> ±0.59  | 94.25 <sup>b</sup> ±0.76   | 92.35 <sup>c</sup> ±0.39  | 90.22 <sup>d</sup> ±0.28  |
|   | (22–35) d | 130.08 <sup>a</sup> ±0.49       | 127.29 <sup>b</sup> ±0.46 | 128.08 <sup>ab</sup> ±0.22 | 127.38 <sup>b</sup> ±0.42 | 124.74 <sup>d</sup> ±0.37 |
|   | (1-35) d  | 112.09 <sup>a</sup> ±0.57       | 109.72 <sup>b</sup> ±0.47 | 111.60 <sup>ab</sup> ±0.35 | 109.83 <sup>b</sup> ±0.55 | 107.00 <sup>d</sup> ±0.56 |
| Feed conversion ratio, FCR (g feed/ g gain) | (1–21) d  | 2.02 <sup>a</sup> ±0.01         | 1.78 <sup>c</sup> ±0.02   | 1.83 <sup>b</sup> ±0.02    | 1.80 <sup>b</sup> ±0.02   | 1.63 <sup>d</sup> ±0.02   |
|   | (22–35) d | 1.83 <sup>a</sup> ±0.03         | 1.71 <sup>c</sup> ±0.08   | 1.70 <sup>b</sup> ±0.08    | 1.74 <sup>b</sup> ±0.08   | 1.65 <sup>d</sup> ±0.08   |
|   | (1-35) d  | 2.07 <sup>a</sup> ±0.18         | 1.87 <sup>c</sup> ±0.09   | 1.89 <sup>b</sup> ±0.02    | 1.89 <sup>b</sup> ±0.02   | 1.81 <sup>d</sup> ±0.01   |
| Performance index, PI, (%)                  | 21d       | 50.47 <sup>d</sup> ±1.43        | 63.73 <sup>b</sup> ±1.73  | 61.71 <sup>c</sup> ±1.69   | 62.33 <sup>c</sup> ±1.72  | 74.27 <sup>a</sup> ±1.41  |
|   | 35d       | 93.92 <sup>d</sup> ±3.21        | 112.16 <sup>b</sup> ±2.26 | 111.56 <sup>c</sup> ±2.74  | 109.81 <sup>c</sup> ±2.74 | 121.36 <sup>a</sup> ±2.33 |

<sup>1</sup>T<sub>1</sub>; control; basal diet without any supplementation, T<sub>2</sub>; basal diet + 0.3% clove, T<sub>3</sub>; basal diet + 1% peppermint, T<sub>4</sub>; basal diet + cinnamon oils and T<sub>5</sub>; basal diet+ 0.1% blends of essential oils, <sup>2</sup> means ± S.E. of 3 replicates / treatment, <sup>3</sup>a, b, c and ....etc. means within the same row with different superscripts are significantly different (P ≤ 0.05).

**Blood Serum parameters and antioxidant activity:**

Results presented in Table 5 showed that there was a significant improvement in serum total protein, albumin, globulin and A/ G ratio by addition of essential oils blend (0.1% clove + 0.1% peppermint +0.1% cinnamon oils, T<sub>5</sub>) at 35 days of age; as well as serum level of high density lipoprotein (HDL) in comparison with the control group. Also, serum antioxidant status of glutathione peroxidase activities (GPx) showed significant increase by addition of different essential oil sources Table (5). The highest value of GPx concentration was increased by addition

of 0.1% clove + 0.1% peppermint + 0.1% cinnamon oils mixture (T<sub>5</sub>) in comparison with the control group. The obtained results on serum antioxidant status shed light upon the selenium function as a major component of the antioxidant system which participates in controlling the body glutathione (Witkowska *et al.*, 2019). Moreover, lower serum cholesterol, total lipids and low density lipoprotein (LDL), as well as lipid peroxidation (MDA) concentration were obtained for broiler chicks fed the diets containing 0.1% blends of essential oils (T<sub>5</sub>), compared to the control group.

**Table 4. Effect of dietary clove, peppermint, cinnamon oils and their blends supplementation on carcass characteristics and immune organs of broiler chickens at 35 days of age ( Mean±SE)**

| Items                    | Dietary treatments <sup>1</sup> |                             |                             |                             |                                |
|--------------------------|---------------------------------|-----------------------------|-----------------------------|-----------------------------|--------------------------------|
|                          | T <sub>1</sub>                  | T <sub>2</sub>              | T <sub>3</sub>              | T <sub>4</sub>              | T <sub>5</sub>                 |
| Pre-slaughter weight, g. | 1935.54 <sup>c</sup> ±17.59     | 1998.65 <sup>b</sup> ±19.05 | 2113.40 <sup>b</sup> ±17.05 | 2081.10 <sup>c</sup> ±16.65 | 2176.66 <sup>a2,3</sup> ±18.08 |
| Dressing percentage, %.  | 72.15 <sup>d</sup> ±1.74        | 73.24 <sup>c</sup> ±1.13    | 76.93 <sup>b</sup> ±1.00    | 75.00 <sup>ab</sup> ±1.12   | 78.03 <sup>a</sup> ±0.88       |
| Abdominal fat, %.        | 1.54 <sup>a</sup> ±0.04         | 1.44 <sup>b</sup> ±0.05     | 1.38 <sup>b</sup> ±0.05     | 1.32 <sup>bc</sup> ±0.04    | 1.25 <sup>c</sup> ±0.04        |
| Liver, %.                | 3.00 <sup>d</sup> ±0.16         | 3.45 <sup>c</sup> ±0.16     | 3.50 <sup>b</sup> ±0.14     | 3.52 <sup>b</sup> ±0.18     | 3.77 <sup>a</sup> ±0.14        |
| Gizzard%.                | 3.34 <sup>d</sup> ±0.02         | 3.78 <sup>c</sup> ±0.02     | 3.70 <sup>c</sup> ±0.01     | 4.52 <sup>a</sup> ±0.02     | 4.37 <sup>b</sup> ±0.01        |
| Heart, %.                | 0.51 ±0.01                      | 0.50 ±0.01                  | 0.52 ±0.02                  | 0.54 ±0.01                  | 0.55 ±0.01                     |
| Giblets %.               | 6.85 ±0.02                      | 7.73 <sup>b</sup> ±0.01     | 7.72 <sup>b</sup> ±0.01     | 8.58 <sup>a</sup> ±0.03     | 8.69 <sup>a</sup> ±0.03        |
| <b>Immune organs</b>     |                                 |                             |                             |                             |                                |
| Thymus, %.               | 0.16 <sup>d</sup> ±0.005        | 0.20 <sup>b</sup> ±0.02     | 0.19 <sup>c</sup> ±0.02     | 0.21 <sup>b</sup> ±0.01     | 0.27 <sup>a</sup> ±0.01        |
| Spleen, %.               | 0.09 ±0.01                      | 0.10 ±0.04                  | 0.10 ±0.004                 | 0.09 ±0.01                  | 0.11 ±0.01                     |
| Bursa, %.                | 0.16 <sup>c</sup> ±0.01         | 0.189 <sup>b</sup> ±0.01    | 0.17 <sup>b</sup> ±0.01     | 0.19 <sup>a</sup> ±0.01     | 0.18 <sup>a</sup> ±0.01        |

<sup>1</sup> T<sub>1</sub>; control; basal diet without any supplementation, T<sub>2</sub>; basal diet + 0.3% clove, T<sub>3</sub>; basal diet + 1% peppermint, T<sub>4</sub>; basal diet + cinnamon oils and T<sub>5</sub>; basal diet+ 0.1% mixture of essential oils, <sup>2</sup> means ± S. E. of 3 replicates / treatment., <sup>3</sup>a, b, c and .... etc. means within the same row with different superscripts are significantly different (P ≤ 0.05).

Results of the present experiment agree with those of Al-Kassie (2010) and Ciftci *et al.* (2010) who reported that serum levels of glutathione peroxidase activity were increased significantly in broiler diets that contained cinnamon oils. While serum malondialdehyde (MDA) level, cholesterol levels were decreased significantly in cinnamon oil

groups compared to the control group. Similar findings were reported by Yang *et al.* (2019) who revealed that broilers supplemented with 50, 100, 200, 400, 800 mg of cinnamon oil/kg diet had significant impacts on liver MDA contents at 21 days of age. Additionally, Sarica *et al.*(2014) the orized that quail diets combined with the supplementation of

essential oils resulted in a reduction of plasma total cholesterol level compared with the basal diet.

Moreover, Abudabos *et al.* (2018) noted that plasma total protein, and globulin were significantly increased in broiler chicks growing diets supplemented with a mixture of cinnamaldehyde, anise, carvacol, thyme, yucca extract and oregano essential oils. The reduction of cholesterol in the groups fed essential oil components may be due to its suppressing impact on 3-hydroxy-3-methylglutaryl coenzyme A reductase (Elson *et al.*, 1989) which is a key enzyme in the synthesis of cholesterol (Goldstein and Brown, 1990). To summarize, the possible mechanism of essential oils in limiting and preventing cell membrane destruction by oxidative and production of free radicals, consequently reduces MDA formation. Laying hens fed diet supplemented with 100 mg EOs +500 mg mannan oligosaccharide (MOS/kg) diet had significant higher values of HDL cholesterol and GSH-Px activity in plasma, while,

those fed control diet had higher values of total lipids, triglycerides and LDL cholesterol in plasma (Emam *et al.*, 2016).

In this connection, Omar *et al.* (2020) reported that chicks fed diets supplemented with 0.2g EOs/kg diet had lower values of total lipids, triglycerides and total cholesterol compared to the control group. Essential oil was observed to exert a hypocholesterolemic effect. The decrease in cholesterol levels may be due to an inhibition of the hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity, which is a key regulatory enzyme in cholesterol synthesis (Hong *et al.*, 2020). It is known that the absence or presence of cholesterol effects of essential oils in an animal depend on breed, gender, age and the composition of the feed (Lee *et al.*, 2003).

However, Mohammadi *et al.* (2014) showed that dietary clove oil supplementation did not affect serum levels of cholesterol, HDL and LDL.

**Table 5. Effect of dietary clove, peppermint, cinnamon oils and their blends supplementation on blood biochemical and antioxidants statuses of broiler chickens at 35 days of age ( Mean±SE)**

| Items                             | Dietary treatments        |                           |                           |                           |                            |
|-----------------------------------|---------------------------|---------------------------|---------------------------|---------------------------|----------------------------|
|                                   | T <sub>1</sub>            | T <sub>2</sub>            | T <sub>3</sub>            | T <sub>4</sub>            | T <sub>5</sub>             |
| <b>Total protein, g / dL.</b>     | 4.01 <sup>c</sup> ±0.02   | 4.33 <sup>bc</sup> ±0.04  | 4.36 <sup>b</sup> ±0.02   | 4.49 <sup>ab</sup> ±0.02  | 4.61 <sup>a</sup> ±0.04    |
| <b>Albumen (A), g / dL.</b>       | 2.21 <sup>e</sup> ±0.01   | 2.45 <sup>d</sup> ±0.02   | 2.43 <sup>c</sup> ±0.03   | 2.52 <sup>b</sup> ±0.02   | 2.64 <sup>a</sup> ±0.01    |
| <b>Globulin (G), g / dL.</b>      | 1.80 <sup>b</sup> ±0.01   | 1.88 <sup>ab</sup> ±0.03  | 1.93 <sup>a</sup> ±0.01   | 1.97 <sup>a</sup> ±0.02   | 1.97 <sup>a</sup> ±0.01    |
| <b>A / G ratio</b>                | 1.23 <sup>c</sup> ±0.01   | 1.30 <sup>a</sup> ±0.01   | 1.26 <sup>b</sup> ±0.01   | 1.28 <sup>ab</sup> ±0.01  | 1.34 <sup>a</sup> ±0.01    |
| <b>Total lipids, mg / dL.</b>     | 428.55 <sup>a</sup> ±0.21 | 345.61 <sup>b</sup> ±0.16 | 329.17 <sup>c</sup> ±0.21 | 319.01 <sup>d</sup> ±0.11 | 310.20 <sup>d</sup> ±0.12  |
| <b>Total cholesterol, mg/ dL.</b> | 167.01 <sup>a</sup> ±0.23 | 151.02 <sup>b</sup> ±0.21 | 137.04 <sup>c</sup> ±0.11 | 129.03 <sup>d</sup> ±0.32 | 1122.10 <sup>e</sup> ±0.17 |
| <b>HDL (mg/dL)</b>                | 43.66 <sup>c</sup> ±0.54  | 51.33 <sup>b</sup> ±0.55  | 54.20 <sup>ab</sup> ±0.62 | 55.39 <sup>ab</sup> ±0.62 | 59.69 <sup>a</sup> ±0.629  |
| <b>LDL (mg/dL)</b>                | 56.24 <sup>a</sup> ±0.33  | 42.29 <sup>b</sup> ±0.37  | 37.03 <sup>c</sup> ±0.39  | 40.18 <sup>b</sup> ±0.34  | 31.59 <sup>d</sup> ±0.43   |
| <b>GPx activity (mg/dL)</b>       | 1.69 <sup>c</sup> ±0.01   | 2.76 <sup>a</sup> ±0.02   | 2.66 <sup>b</sup> ±0.01   | 2.68 <sup>b</sup> ±0.01   | 2.79 <sup>a</sup> ±0.01    |
| <b>MDA mg/dl.</b>                 | 0.97 <sup>a</sup> ±0.02   | 0.87 <sup>b</sup> ±0.04   | 0.85 <sup>b</sup> ±0.02   | 0.74 <sup>c</sup> ±0.02   | 0.58 <sup>d</sup> ±0.04    |

<sup>1</sup> T<sub>1</sub>; control; basal diet without any supplementation, T<sub>2</sub>; basal diet + 0.3% clove, T<sub>3</sub>; basal diet + 1% peppermint, T<sub>4</sub>; basal diet + cinnamon oils and T<sub>5</sub>; basal diet+ 0.1% mixture of essential oils, <sup>2</sup> means ± S. E. of 3 replicates / treatment., <sup>3</sup>a, b, c and .... etc. means within the same row with different superscripts are significantly different (P ≤ 0.05).

#### **Economic efficiency:**

Economic efficiency and relative economic efficiency are shown in Table 6. The highest economic efficiency and relative economic efficiency was found in the 5th treatment, as chicks fed diet containing combined supplementation of essential oils as compared with those fed the control diet. Chicks fed with essential oils (0.1% clove + 0.1% peppermint + 0.1% cinnamon oils) mixture had the highest economic and relative efficiency values being

(1.04 and 133%). This may have been due to the fact that the treatments to which single or blends essential oils were added were heavier in body weight with better feed conversion ratio compared to the control diet. The broiler production economics have been found variable on incorporation of EOs in diet. Recently, Wade *et al.* (2018) and Omar *et al.* (2020) showed that Chicks fed with essential oils (EOs) had the highest economic and relative efficiency compared to the control group.

**Table 6. Economic efficiency and relative economic efficiency of broiler chicks as affected by dietary clove, peppermint, cinnamon oils and their blends supplementation of broiler chickens during experimental period**

| Items                                | Dietary treatments <sup>1</sup> |                |                |                |                |
|--------------------------------------|---------------------------------|----------------|----------------|----------------|----------------|
|                                      | T <sub>1</sub>                  | T <sub>2</sub> | T <sub>3</sub> | T <sub>4</sub> | T <sub>5</sub> |
| Initial body weight, g.              | 45.39                           | 45.37          | 45.32          | 45.26          | 45.32          |
| Final body weight, Kg.               | 1.94                            | 2.08           | 2.11           | 2.08           | 2.19           |
| Body weight gain, Kg.                | 1.89                            | 2.03           | 2.06           | 2.03           | 2.15           |
| Total revenue <sup>2</sup> , L.E.    | 50.44                           | 54.08          | 54.86          | 54.08          | 55.90          |
| Feed intake, kg.                     | 3.92                            | 3.84           | 3.91           | 3.84           | 3.75           |
| Price of one feed, L.E.              | 7.25                            | 7.27           | 7.31           | 7.28           | 7.30           |
| Feed cost, L.E.                      | 28.42                           | 27.92          | 28.58          | 27.96          | 27.38          |
| Net revenue <sup>3</sup> , L.E.      | 22.02                           | 26.16          | 26.28          | 26.12          | 28.52          |
| Economical efficiency <sup>4</sup> . | 0.78                            | 0.94           | 0.92           | 0.94           | 1.04           |
| Relative economic efficiency.        | 100                             | 121            | 118            | 121            | 133            |

<sup>1</sup>T<sub>1</sub>; control; basal diet without any supplementation, T<sub>2</sub>; basal diet + 0.3% clove, T<sub>3</sub>; basal diet + 1% peppermint, T<sub>4</sub>; basal diet + cinnamon oils and T<sub>5</sub>; basal diet+ 0.1% mixture of essential oils, <sup>2</sup>Total revenue= live body weight × marketing price (26L.E. according to prices in September, 2021), <sup>3</sup>Net revenue= total revenue-feed cost, <sup>4</sup>Economical efficiency= net revenue/ feed cost.

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## تأثير إضافة زيوت القرنفل، النعناع والقرفة وخليطهم على أداء النمو وخصائص الذبيحة والصفات البيوكيميائية للدم ومضادات الأكسدة لكتاكيت التسمين

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اجريت هذه الدراسة بهدف تقييم تأثير إضافة الزيوت العطرية لكل من زيت القرنفل والنعناع والقرفة وخليطها على أداء النمو وصفات الذبيحة وبعض القياسات البيوكيميائية للدم ومضادات الأكسدة والكفاءة الاقتصادية. استخدم ٣٠٠ ككتوت غير مجنسة (روس - ٣٠٨) ككتوت تسمين بعمر يوم واحد تم تغذيتها من ١-٣٥ يوم حيث تم تقسيمها عشوائياً إلى خمس معاملات غذائية كل منها في ٣ مكررات وبكل مكرر ٢٠ ككتوت (٦٠ ككتوت لكل مجموعة) بتصميم عشوائي كامل. تغذت المجموعة الأولى (الكنترول) على العليقة الأساسية بدون أى إضافات، بينما تغذت المعاملات الثانية والثالثة والرابعة على العليقة الأساسية مضافاً إليها زيوت القرنفل والنعناع والقرفة كل بمستوى ٠,٣ جم / كجم من كل منها، على التوالي، بينما تغذت المعاملة الخامسة على العليقة الأساسية مضافاً إليها مخلوط زيوت القرنفل، النعناع والقرفة بمستوى ٠,١ جم / كجم غذاء لكل منها. أشارت النتائج إلى أن إضافة مخلوط الزيوت العطرية أدى إلى تحسن كبير في وزن الجسم والوزن المكتسب ونسبة التحويل الغذائي ودليل الأداء خلال فترات التجربة. تحسنت معنوياً صفات الذبيحة وصفات الأجزاء المأكولة وبعض نسب أعضاء المناعة في الكتاكتيت المغذاه على علائق بها مخلوط الزيوت العطرية مقارنة بالكنترول، بينما انخفضت نسبة دهون البطن في المعاملات المضاف لها الزيوت العطرية مفردة أو خليطها مقارنة بالمعاملة لکنترول. سجلت الطيور المغذاه على العلائق المضاف لها الزيوت العطرية (المعاملة الثانية إلى الخامسة) أعلى تركيزاً للبروتين والجلوبيولين، نشاط انزيم الجلوتاثيون (GPx) وكذلك مستوى البروتين الدهني عالي الكثافة (HDL) في الدم وخاصة المعاملة الخامسة مقارنة بالمجموعة بالکنترول. لوحظ انخفاض تركيز الكوليسترول والدهون الثلاثية والبروتين الدهني منخفض الكثافة (LDL) وبيروكسيد الدهون (MDA) في الدم للطيور المغذاه على خليط الزيوت العطرية مقارنة بباقي المعاملات. بشكل عام، تحسن أداء النمو وبعض صفات الذبيحة والصفات البيوكيميائية ونشاط انزيمات مضادات الأكسدة بإضافة خليط الزيوت العطرية (٠,١ جم / كجم عليقة من كل من زيوت القرنفل والنعناع والقرفة) لعلائق كتاكتيت التسمين مع عدم وجود تأثير ضار على الصحة لكتاكيت التسمين تحت ظروف التجربة.