Effect of adding crushed *Pimpinella anisum*, *Nigella sativa* seeds and *Thymus vulgaris* mixture to antibiotics-free rations of vaccinated and non-vaccinated male broilers on growth performance, antibody titer and haematological profile

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Abstract

This research explores an experimental study conducted to investigate the effect of crushed *Pimpinella anisum* (PA), *Nigella sativa* (NS) seeds and *Thymus vulgaris* (TV) mixture as a feed additive on growth performance and mortality rate (MR), selected antibodies titer (Ab’s) and blood haematological profile of vaccinated and non-vaccinated Lohman male broiler chicks fed free-antibiotics ration. A total of 400 one-day-old chicks were distributed into 16 groups (4 treatment × 4 replicates × 25 chicks). The experiment lasted from 1 to 42 days of age. The statistical findings of this experiment prove that the use of medicinal plants mixture improves live body weight, body weight gain, feed conversion ratio and MR of vaccinated male broilers at 21 and 42 days of age. Antibodies titer against infectious bronchitis and infectious bursal disease of non-vaccinated and vaccinated Lohman male broilers were significantly improved at d 21 and 42, as a result of the addition of medicinal plant mixture to the basal ration. Concerning Newcastle disease, the use of PA, NS and TV mixture did not result in any additional improvement of Ab’s compared with the use of vaccines. The addition of medicinal plants mixture increased WBCs, RBCs, thrombocytes count and Hb concentration of vaccinated and non-vaccinated male broilers at 21 days of age. Meanwhile, heterophils, lymphocytes and monocytes of vaccinated male broilers (VMB) were significantly improved by adding medicinal plant mixture to their basal diet. Moreover, at 42 days of age the use of PA, NS seeds and TV mixture indicated significant increase in total WBCs, lymphocytes and monocytes count of VMB and non-vaccinated male broiler (NVMB). No significant differences were noticed in RBCs and Hct as a result of feeding crushed medicinal plants mixture.

Introduction

Poultry industry is one of the most dynamic branches of world agribusiness trade. Antibiotics are frequently used for the treatment and control of diseases and even as growth promoters to improve poultry performance. Addition of certain antibiotics fed at low levels for an extended period of time is a common practice in the poultry industry and provides economic benefits by increasing weight gain and improving feed efficiency from 1 to 5% (Thromke and Elwinger, 1998). Jones and Ricket (2003) cited that a total of 32 antimicrobial compounds are approved for usage in broiler feeds in USA, without a veterinary prescription. Recently, published data indicated that indiscriminate use of in-feed antibiotics increases several strains resistance to antibiotics used for human health and enhances transferring the resistance to other bacteria (Botsoglou and Fleitouris, 2001; Moser et al., 2003). In fact, it has been reported to phase out these antibiotics from the European Union market since January 2006. Nowadays, the possibility of using new natural alternative additives instead of antibiotics in poultry rations is being researched. One of the alternatives is represented by medicinal plants such as *Thymus vulgaris* leaves, *Nigella sativa* seeds, *Pimpinella anisum* L seeds and dianthus or their extracts (Gill, 1999; Dickens et al., 2000). All mentioned disadvantages of live attenuated and traditionally inactivated vaccines triggered some researchers to use natural oils as an adjuvant. However, plant extracts contain many active components, particularly essential oils, which have a wide range of pharmacological activities (Lewis et al., 2003). The essential oils of these medicinal plants have shown antibacterial (Mitsh et al., 2004), anticcicodial (Jamroz et al., 2002), antifungal (Jantan et al., 2003) and antioxidant (Botsoglou et al., 2004) activities. Moreover, Cifci et al. (2005) reported that herbs have antiviral properties and stimulate the noderene and immune system. Therefore, the present study was designed to answer the following question: can a mixture of some selected medicinal plants, particularly *Nigella sativa*, *Pimpinella anisum* seeds and *Thymus vulgaris*, replace vaccines and antibiotics in broiler rations?

Materials and methods

Experimental birds and rearing conditions

Four hundred one-day-old Lohman male broiler chicks (MB) were purchased from a commercial hatchery. The average weight of chick was 30.0±1.5 g. Chicks were randomly allocated into 16 pens (1.15×2.10 m) in an open sided house (4 treatments × 4 replicates × 25 chicks). Chicks had free access to feed and water and kept under Lohman recommended procedure. No antibiotics were used through all the experimental period. Half of the group was vaccinated against Newcastle (ND) clone 30 and Lasota and infectious bronchitis (IB) strain H120 at 9 and 23 days of age, and against infectious bursal disease (Gumboro) at 13 and 28 days of age. The remaining half was not vaccinated against any disease.
Experimental rations

Lohman male broilers received corn-soybean based rations. The dietary treatments consisted of the same starter and finished antibiotics-free diets (from d 1 to 21 and from d 22 to 24, respectively), supplemented or not with 2.0% crushed *Pimpinella anisum* (PA), *Nigella sativa* seeds (NS) and *Thymus vulgaris* leaves (TV) mixture (1:1:1) as feed additive. These rations were offered to either vaccinated or non-vaccinated male broilers. All rations were formulated to cover the requirements according to strain guide. Basal diets are presented in Table 1. Randomized samples (3 replicates) from each starter and finisher rations were collected for proximate analysis by the procedure described by AOAC (1990).

Measurements

*In vivo* performance

Chicks were weighed at arrival time and the average weight was recorded as one-day-old weight (30.0±0.75 g). Live body weight (BW) and cumulative feed intake (CFI) was measured at d 21 and 42 of age. Feed conversion ratio (FCR) was calculated also at d 21 and 42 of age. Mortality as well was calculated.

Selected antibodies titer (Ab’s)

At d 21 and 42 of age Ab’s titer against Newcastle (ND), infectious bronchitis (IB) and infectious bursal (IBD) diseases were quantified from 5 randomly selected chickens from each replicate within each treatment. Antibody titer was assayed by link immunosorbent assay (ELISA) for IBD and IBD haemaglutination inhibition (HI) test for ND as described by Thayer and Beard (1998).

Blood haematology

At d 21 and 42, blood samples were collected from wing vein of 5 chickens from each replicate within each treatment. Half of the blood received into EDTA anticoagulant tubes for the determination of hematological parameter, white blood cells (WBCs) and red blood cells (RBCs) count, haemoglobin (Hb) content and haematocrit (Hct) using the method described by Wintrobe (1976). The second half of blood was collected in non-heparinized tubes and centrifuged at 4000 rpm for 15 min. Clear serum was used for immunity measurements.

Statistical analysis

Collected data were statistically analyzed by analysis of variance (ANOVA) by the statistical package SPSS for Windows (2001), significant means were subjected to a multiple comparison test (Duncan, 1955) at 0.01 and 0.05 levels (Snedecor and Cochran 1980).

### Results

#### Growth performance

The present results (Tables 2 and 3) prove that the addition of 2.0% crushed PA, NS and TV mixture to starter diet supplemented with selected crushed medicinal plants mixture from 1 to 21 days of age (means ± SE). The addition of 2% crushed medicinal plants mixture to finisher diet supplemented with selected crushed medicinal plants mixture from 1 to 21 days of age (means ± SE).

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**Table 1. Ingredients and chemical composition of the basal diets.**

|               | Starter | Finisher |
|---------------|---------|----------|
| Ingredients   |         |          |
| Corn, %       | 61.50   | 69.50    |
| Soybean meal, %| 34.00   | 23.70    |
| Broiler concentrate*, % | 2.00 | 4.00 |
| CaCO₃, %      | 1.25    | 1.23     |
| Dicalcium phosphate, % | 0.72 | 1.00 |
| NaCl, %       | 0.25    | 0.25     |
| Lysine, %     | 0.03    | -        |
| Methionine, % | -       | 0.07     |
| Vitamin mineral premix**, % | 0.25 | 0.25 |

Calculated feeding value

| Measured parameters | T1 Basal ration + | T2 Basal ration + | T3 Basal ration + | T4 Basal ration + |
|---------------------|-------------------|-------------------|-------------------|-------------------|
| DM, %               | 91.50             | 91.20             |                   |                   |
| Crude protein, %    | 22.20             | 18.80             |                   |                   |

*Broilers concentrate: crude protein 54%, crude fat 24%, ME 3900Kcal/kg diet, calcium 1.2%, phosphorous 0.9%, cystine 1.45, methionine 0.6% and lysine 1.5%.

**Vitamin-Mineral Premix: vitamin A 2,000,000 U; Vitamin D3 400,000 U; Vitamin E 400 mg; Vitamin B1 200 mg; Vitamin B2 800 mg; Nicotinic acid 4000 mg; Pantothenic acid 2000 mg; Vitamin K 300 mg; Folic acid 200 mg; Vitamin B6 300 mg; Co 50 mg; Cu 1600 mg; Fe 6421 mg; I 156 mg; Mn 12,800 mg; Se 32 mg; Zn 9000 mg; Choline chloride 100 mg.

**Table 2. Growth performance of vaccinated and non-vaccinated male broilers fed basal diet supplemented with selected crushed medicinal plants mixture from 1 to 21 days of age (means ± SE).**

| Measured parameters | T1 Basal ration | T2 Basal ration + | T3 Basal ration + | T4 Basal ration + |
|---------------------|-----------------|-------------------|-------------------|-------------------|
| Live body weight, kg | 0.575±0.010⁸    | 0.613±0.010⁸     | 0.610±0.010⁸     | 0.648±0.010⁹     |
| Body weight gain, kg | 0.545±0.010⁸    | 0.583±0.010⁸     | 0.580±0.010⁸     | 0.618±0.010⁹     |
| Cumulative feed intake, kg | 0.884±0.010⁸ | 0.876±0.020⁹ | 0.812±0.020⁹ | 0.840±0.010⁹ |
| Feed conversion ratio | 1.622±0.030⁸ | 1.503±0.030⁹ | 1.400±0.030⁰ | 1.360±0.030² |

**Table 3. Growth performance of vaccinated and non vaccinated male broilers fed basal diet supplemented with selected crushed medicinal plants mixture from 1 to 42 days of age (means ± SE).**

| Measured parameters | T1 Basal diet | T2 Basal diet + | T3 Basal diet + | T4 Basal diet + |
|---------------------|--------------|----------------|----------------|----------------|
| Live body weight, kg | 2.0±0.01⁸   | 2.08±0.02⁹   | 2.1±0.20³⁰   | 2.16±0.03³⁰  |
| Body weight gain, kg | 1.97±0.01¹   | 2.05±0.02³   | 2.07±0.20³   | 2.13±0.30³³  |
| Cumulative feed intake, kg | 3.75±0.07⁷   | 3.71±0.10³   | 3.81±0.02³   | 3.65±0.04³   |
| Feed conversion ratio | 1.96±0.01¹   | 1.81±0.02³   | 1.84±0.03³   | 1.78±0.02³   |
| Mortality, %         | 2.00±0.02²   | 0.67±0.01⁴   | 0.52±0.01⁴   | 0.000         |

**Table 2. Growth performance of vaccinated and non-vaccinated male broilers fed basal diet supplemented with selected crushed medicinal plants mixture from 1 to 21 days of age (means ± SE).**

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TV mixture to the basal diet of VMB (T2 and T3) at 21 and 42 days of age improved (P<0.05) their growth performance in terms of LBW, BWG. Furthermore, CFI and FCR was the highest (P<0.05) for NVMB fed unsupplemented basal diet. However, MR was the highest for NVMB fed the basal ration.

**Selected antibodies titer (Ab’s)**

The data presented in Tables 4 and 5 indicated that at d 21 and 42 the NVMB fed the unsupplemented basal diet had the lowest P value (<0.05) Ab’s titer against all studied diseases. Meanwhile, at d 21 the addition of crushed PA, NS seeds and TV mixture to the basal diet of either NVMB or VMB improved (P<0.05) Ab’s titer against IB and IBD compared to those of NVMB and VMB groups fed the unsupplemented basal diet (Table 4).

The data of d 42 (Table 5) indicated that the addition of crushed PA, NS seeds and TV to the basal diet of VMB increased HI titer (P<0.05) compared with NVMB fed the supplemented diet and with NVMB fed the basal diet alone. No significant differences were noticed in HI titer of VMB and NVMB fed the unsupplemented or the supplemented diet. Addition of crushed PA, NS seeds and TV mixture to the basal diet improved (P<0.05) Ab’s of NVMB and VMB against IB compared with NVMB and VMB the unsupplemented basal diet. However, Ab’s of VMB against IBD was improved (P<0.05) as a result of the medicinal plants mixture as feed additives.

**Hematological profile**

Results of d 21 (Table 6) showed that the addition of crushed PA, NS seeds and TV mixture to the basal diet of vaccinated and NVMB increased (P<0.05) their WBC’s, RBC’s, thrombocytes and haemoglobin (Hb) compared with those fed the basal diet, either vaccinated or non-vaccinated. VMB fed basal diet supplemented with PA, NS seeds and TV mixture had the highest (P<0.05) heterophils, lymphocytes and monocytes counts among all treatments; however, the highest (P<0.05) Hct count was recorded for NVMB fed the supplemented basal diet. At d 42, it was noticed that the VMB fed the basal diet without supplementation or NVMB fed the basal diet with medicinal plants mixture had the highest (P<0.05) total WBC’s, lymphocytes and monocytes count. On the other hand, the use of crushed PA, NS seeds and TV as feed additive in the basal diet of NVMB improved (P<0.05) their heterophils, thrombocytes values among all treatments. Moreover, no significant differences were noticed in RBC’s and Hct at d 42 (Table 7). Hb concentration was the highest (P<0.05) for non-vaccinated and vaccinated male broilers that were fed the supplemented basal diet.

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**Discussion**

**Growth performance**

Crushed AP, NS seeds and TV mixture had additional positive effect on growth performance when used in basal diet of VMB. The favorable effects of adding PA, NS seeds and TV mixture to the basal diet of VMB could be attributed to the active and valuable components of such plants particularly essential oils. The previous studies in this regards reported that the major components of *Thymus vulgaris* essential oils are carvacrol (5-isopropyl-2-methylphenol) and thymol (5-methyl-2-isopropyl), which can improve animal performance (Masada, 1976; Hertrampf, 2001; Alcicek

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**Table 4. Antibodies titer against infectious bronchitis, infectious bursal and Newcastle diseases of male broilers from 1 to 21 days of age (means ± SE).**

|          | T1 Basal ration | T2 Basal ration + medicinal plants mixture | T3 Basal ration | T4 Basal ration + vaccines + medicinal plants mixture |
|----------|-----------------|-------------------------------------------|-----------------|-----------------------------------------------------|
| Ab’s     |                 |                                           |                 |                                                     |
| Infectious bronchitis disease ELISA (EUs) | 61.7±6.63<sup>c</sup> | 101.9±4.92<sup>b</sup> | 115.9±5.70<sup>a</sup> | 125.9±7.65<sup>a</sup> |
| Infectious bursal disease ELISA (EUs)   | 79.9±3.63<sup>c</sup> | 167.3±6.35<sup>b</sup> | 190.4±8.92<sup>a</sup> | 195.1±7.71<sup>a</sup> |
| Newcastle disease HI     | 2.50±0.21<sup>b</sup> | 4.71±0.41<sup>a</sup> | 4.29±0.26<sup>a</sup> | 5.00±0.29<sup>a</sup> |

**Table 5. Antibodies titer against infectious bronchitis, infectious bursal and Newcastle diseases of male broilers fed different dietary treatments from 1 to 42 days of age (means ± SE).**

|          | T1 Basal ration | T2 Basal ration + medicinal plants mixture | T3 Basal ration | T4 Basal ration + vaccines + medicinal plants mixture |
|----------|-----------------|-------------------------------------------|-----------------|-----------------------------------------------------|
| Ab’s     |                 |                                           |                 |                                                     |
| Infectious bronchitis disease ELISA (EUs) | 39.29±5.23<sup>c</sup> | 82.71±8.21<sup>b</sup> | 115.9±6.99<sup>a</sup> | 130.9±9.63<sup>a</sup> |
| Infectious bursal disease ELISA (EUs)   | 48.83±3.75<sup>c</sup> | 135.2±8.10<sup>d</sup> | 166.3±8.82<sup>c</sup> | 214.2±8.72<sup>a</sup> |
| Newcastle disease HI     | 0.14±0.08<sup>c</sup> | 3.62±0.42<sup>b</sup> | 3.48±0.19<sup>a</sup> | 4.10±0.23<sup>a</sup> |

**Table 6. Blood haematological profile of male broilers fed selected medicinal plants mixture from 1 to 21 days of age (means ± SE).**

| Blood haematology parameters | T1 Basal ration | T2 Basal ration + medicinal plants mixture | T3 Basal ration | T4 Basal ration + medicinal plants mixture |
|-----------------------------|-----------------|-------------------------------------------|-----------------|-----------------------------------------------------|
| WBCs, ×10<sup>3</sup>/µL    | 7.92±0.77<sup>c</sup> | 8.72±0.23<sup>c</sup> | 14.12±0.20<sup>a</sup> | 15.26±1.15<sup>a</sup> |
| Heterophils, ×10<sup>3</sup>/µL | 5.37±0.44<sup>c</sup> | 5.75±0.21<sup>d</sup> | 8.88±0.20<sup>b</sup> | 10.61±0.08<sup>a</sup> |
| Lymphocytes, ×10<sup>3</sup>/µL | 2.26±0.07<sup>c</sup> | 2.62±0.10<sup>d</sup> | 3.89±0.04<sup>a</sup> | 4.16±0.05<sup>a</sup> |
| Monocytes, ×10<sup>3</sup>/µL | 0.29±0.01<sup>c</sup> | 0.35±0.01<sup>d</sup> | 0.34±0.02<sup>a</sup> | 0.49±0.03<sup>a</sup> |
| Thrombocytes, ×10<sup>3</sup>/µL | 26.50±0.43<sup>c</sup> | 22.06±0.12<sup>b</sup> | 36.58±0.09<sup>a</sup> | 34.00±0.32<sup>a</sup> |
| RBCs, ×10<sup>6</sup>/µL    | 2.88±0.12<sup>c</sup> | 2.76±0.03<sup>b</sup> | 3.16±0.17<sup>a</sup> | 3.12±0.03<sup>a</sup> |
| Hb, g/dL                    | 10.0±0.10<sup>c</sup> | 10.29±0.28<sup>b</sup> | 12.45±0.47<sup>a</sup> | 12.97±0.39<sup>a</sup> |
| Hct, ×10<sup>3</sup>/µL     | 34.00±0.25<sup>c</sup> | 30.69±3.41<sup>d</sup> | 41.37±0.89<sup>a</sup> | 36.83±0.58<sup>a</sup> |

<sup>a-e</sup>Means with different superscripts in the same row are significantly different at P<0.05.
A further factor that could explain the favorable effects of such medicinal plants is the presence of a mixture of linolenic, linoleic and oleic acids in crushed Nigella sativa seeds, well known as essential fatty acids (Babayan et al., 1978). Several researchers discovered that crushed of Pimpinella anisium seeds, Nigella sativa seeds and Thymus vulgaris mixture stimulate animal digestive system, particularly protein, fats and cellulosule digestion (Jamroz and Kamel, 2002; Cabuk et al., 2003; Ramakrishna et al., 2003). In addition, Nigella sativa seeds have choleric effects producing a definite increase in bile flow (Mahfouz et al., 1960). Bile is recognized as an emulsifying agent activating the pancreatic lipase that helps in the digestion and absorption of fats and fats soluble vitamins (Crossland, 1980). On the other hand, it has been reported that Pimpinella anisium oil significantly improves daily live BWG and FCR of male broiler (Giannenas et al., 2003; Ciftci et al., 2005).

However, it also seems that the improvement effect of Pimpinella anisium seeds might be due to the improvement of apparent whole tract and ileal digestibility of the nutrients (Hernandez et al., 2004) and increases the effect of pancreatic lipase and amylase (Ramakrishna et al., 2003).

The present findings are in agreement with Abu-Egla et al. (2001); El-Ghammary et al. (2002) and Hassan et al. (2004), who reported that BW and BWG significantly increased at 7 weeks of age as a result of adding crushed Pimpinella anisium, Nigella sativa seeds or Thymus vulgaris individually or as a mixture, to the basal rations of broiler chickens.

**Selected antibodies titer**

Antibodies (Ab’s) are glycoproteins specifically to the antigen that are induced their information. They are produced when immunogenic molecules are introduced into the host’s lymphoid system and present in the serum body fluids, and attached to the surface of certain cell types (Weir and Stewart, 1993).

The significant improvement in Ab’s titer against IB, IBD and ND diseases might be attributed to the presence of different active components and high feeding value of these plants. Bayram et al. (2007) reported that anisaldehyd, estragol, eugenol and methicheelcol are main components of Pimpinella anisium, while Masada (1976) found that carvacrol, thymol and anathol are the main components of Thymus vulgaris. These active components are known to possess antimicrobial, antiviral, antifungal and parasitic activities (Cabuk et al., 2003; Tabanca et al., 2003; Valero and Salmeron, 2003). On the other hand, Al-Beitawi et al. (2009) reported that replacing bacitracin methylene disalicylate (BMD) by crushed NSS improved significantly Ab’s titer of IBD and ND, but not IB. This improvement is ascribed to NSS oil components such as thymoquinone, nigellimine, thymol, carvacrol and nigelicline. This finding is consistent with Al-Jabre et al. (2003), who found that volatile oils of NSS have 67 constituents able to induce pharmacological effects against Escherichia coli and Staphylococcus aureus. However, collected data in different studies with poultry have shown that when a diet is protein or amino acids deficient, the concentration of circulating Ab’s to specific challenge organisms is low. Recently, Kidd et al. (2001) reported that a dietary arginine concentration near the 1.25% level passed on to the chicks that can protect them against infections until 3 to 4 weeks; and then given an inactivated oil adjuvant vaccine 6 to 8 week later, this results in high antibody level passed on to the chicks that can protect them against infections until 3 to 4 weeks; and is explaining the high level of Ab’s against IB and IBD at 21 days of age.

The present results are in agreement with previous findings of Dorman and Deans (2000) and Burt and Reinders (2003), who discovered that thymol, carvacrol, eugenol and anathol affect pathogenic organism in the GI tract. Ather (2000) reported that broilers performance was improved by using a poly herbal premix of which were anise seeds and rosemary. Moreover, it has been reviewed that medicinal plants such as Nigella sativa seeds and fenugreek have highly significant effect on the immune response of broilers (EL-Kaity et al., 2002).
The present results agree with those obtained by Osman and El-Barody (1999), who reported that total WBC's, lymphocytes, heterophils and basophils were significantly improved by increasing *Nigella sativa* seeds up to 0.8% in broiler rations. In a more recent work, conducted to evaluate the effect of using combination of some medicinal plants (of which *Nigella sativa* seeds was on productive and physiological traits of White Bovans poullets), El-Khaiaty et al. (2002) reported that WBC's count were significantly increased compared with control group. We can assume that addition of these medicinal plants, but not their oils, significantly increased Hb concentration of vaccinated and non-vaccinated male broilers. The composition of *Pimpinella anisum*, *Nigella sativa* seeds and *Thymus vulgaris* rather than essential oils might be the reason. Haemoglobin is the main components of erythrocytes. Furthermore, Rindler (1990) and Abu-Jadayil et al. (1999) reported that dry crushed *Thymus vulgaris* was particularly rich in iron (121.4 mg and 117.2 mg/100 g dry matter). Moreover, the improvement in selected haematological parameters (Hb and Hct) might be due to the high content of tocopherols, vitamins and minerals particularly B1, B2, B6, niacin, folic acid, calcium, iron, sodium and potassium (Nergiz and Otles, 1993). Our results were inconsistent with previous findings of Hassan et al. (2004), who reported that supplemented basal diet with 0.2% crushed *Nigella sativa* seeds, crushed *Thymus vulgaris* and crushed *Cinnamon barks* or their mixture 1:1:1 caused a significant increase in Hb content of chickens blood. Similar results were obtained also by Ibrahim et al. (2000) in growing rabbits. The present results disagree with those obtained by Al-Homidan et al. (2002), who did not find significant effect on PCV, Hb or RBCs from adding crushed *Nigella sativa* seeds to the basal diet of broilers.

**Conclusions**

The result of the present study demonstrated that the addition of PA, NS and TV mixture improves growth performance of vaccinated male broilers at 21 and 42 days of age. Based on the present findings, we can conclude that Ab's titer against infectious bronchitis and infectious bursal disease of non-vaccinated male broilers and vaccinated male broilers were improved at 21 and 42 days of age, as a result of the medicinal plant mixture addition to the basal ration. Moreover, at 42 days of age the addition of PA, NS and TV mixture increase total WBCs, lymphocyte and monocyte count of non-vaccinated and vaccinated male broilers. However, to answer the original question, more studies are needed to prove the present findings and explain the working mechanism of the active component of *Pimpinella anisum*, *Nigella sativa* and *Thymus vulgaris*. Further studies are also required to evaluate the effect of such plants on a particular disease.

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Haematological profile

Haematological profile is an important index of the physiological state of the animal. The ability to interpret the state of blood profile in normal and disease condition is among its primary task. The increase in total WBCs and heterophils counts of vaccinated or non-vaccinated male broilers fed basal diet supplemented by 2.0% crushed *Pimpinella anisum*, *Nigella sativa* seeds and *Thymus vulgaris* mixture noticed at 21 days of age may be due to the active components of these medicinal plants. Esonu et al. (2001) had reported that haematological constituents reflect the physiological responsiveness of the animal to its internal and external environments include feed and feeding. Previous researchers discovered the effect of various feeds on the haematological profile of livestock's and concluded that unconventional sources affect animal physiology (Emenalom and Udehibie, 1998). Furthermore, Mahfouz and El-Dakhakhny (1960) discovered that the essential oils in *Nigella sativa* seeds contain crystalline compounds named nigellone or thymoquinone, which has protective effect against diseases. However, the increase in total count of WBCs could be also related to an increase in the membrane protection from autoxidation.

Concerning heterophils, lymphocytes and monocytes counts at 21 days of age, it can be noticed that VMB fed the supplemented basal had significantly the highest values among all treatments. It is well documented that lymphocytes and monocytes perform a specific function against viral infections and several diseases (Fischbach and Dunning, 2004). RBCs, Hb, Hct and thrombocytes counts at 21 days of age showed that vaccinated and non-vaccinated male broilers fed the basal diet after the addition of 2.0% crushed medicinal plants mixture had significantly the highest counts, assuming that the composition of such medicinal plants other than oils might be effective for increase of these blood components. However, it has been reported that amino acids are required to form a single protein called globin, which is considered to be a part of haemoglobin (Fischbach and Dunning, 2004).

The WBCs known to be useful guide to the severity of disease fight infection and defend the body (Fischbach and Dunning, 2004). The favourable effect obtained in total WBCs or in differential counts (heterophils, lymphocytes, monocytes and thrombocytes) might be attributed to the main components of such medicinal plants, particularly essential oils. Furthermore, Zaher et al. (2008) postulated that crushed *Nigella sativa* seeds has an immunostimulant effect and used it as reme-

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