

Research Article

Dose dependence studies of Aniseed (*Pimpinella anisum*) and Ginger (*Zingiber officinale*) extract mixture on growth promoting and immunomodulatory effects in broiler chicks

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Citation

Fazal Raziq, Muhammad Mushtaq, Erum Bughio, Muhammad Tahir Khan, Arif Hameed, Hafiz Allah Bachaya, Mushtaq Ahmad Gondal and Mubasher Rauf. Dose dependence studies of Aniseed (*Pimpinella anisum*) and Ginger (*Zingiber officinale*) extract mixture on growth promoting and immunomodulatory effects in broiler chicks. Pure and Applied Biology. Vol. 12, Issue 2, pp197-205. <http://dx.doi.org/10.19045/bspab.2023.120021>

Received: 28/01/2022

Revised: 24/06/2022

Accepted: 14/07/2022

Online First: 04/08/2022

Abstract

Dose dependency studies (2gms + 4gms), (2.5gms + 5gms) and (3gms + 6gms) allowed us to observe dose dependent responses of aniseed and ginger extract mixture in drinking water on the immune response and over all performance of broiler chicks. Two hundred and forty (240), day old broiler chicks were randomly distributed into four groups, designated as A, B, C and D and each group was further divided into two subgroups. Each subgroup was replicated three times each with 10 checks per replicate for different treatments. One of the subgroup was vaccinated with new castle disease (ND), infectious bronchitis (IB) and infectious bursal disease (IBD) vaccine according to the schedule. Group A was kept as a control, while group B, C and D were treated with an extract mixture of aniseed and ginger at the rate of (2gms + 4gms), (2.5gms + 5gms) and (3gms + 6gms)/litter of drinking water respectively. Body weight and feed conversion

ratio (FCR) were significantly ($P < 0.05$) better in group D as compared to other groups. Non-significant ($P > 0.05$) difference was recorded in mean feed intake and water intake. Mean antibody titer against ND, IB and IBD was significantly ($P < 0.05$) better in group D and vaccinated groups as compare to other groups. It is concluded that extract mixture of aniseed and ginger @ (3gms+6gms) added in poultry birds drinking water is better for increasing body weight gain, best FCR and improving immune response. Similarly, it is comparatively safe, free of side effects, eco-friendly and locally available.

Keywords: Aniseed; Antibody titer; Body weight; Dose; Immune response

Introduction

Food of animal origin is an important part of human diet and is essential for proper maintenance of health. The minimum daily animal protein requirement of a normal man is 53 grams [1]. The scarcity of animals and two meatless days are the major causes of shortage of animal protein in Pakistan. Commercial poultry production plays a vital role in providing quality protein in shortest possible time in the form of meat and eggs. Poultry industry has a great potential to fill the gape between supply and demand of protein of animal origin due to rapid increase in human population of Pakistan.

In Pakistan, poultry industry is confronting with many problems, affecting poultry production unlike developed countries in the world. The alarming problems are the onset of various diseases and high cost of production. To control the spread of diseases and reduce cost of per unit production, certain synthetic feed additives and antibiotics are used; as a result, the harmful microorganisms developed resistance against diseases. The residual effect of antibiotics in meat and egg is a big threat both for human and poultry health. To overcome all these problems, phytobiotics are the potential alternative to improve the immunity and production performance of poultry birds [2].

Due to favorable climatic condition, considerable numbers of many herbaceous medicinal plants are found in Pakistan. These plants have been found to possess antimicrobial, antifungal, anti-tumor, immune stimulator and antioxidants activity.

These plants have been used as anthelmintic, analgesic, growth promoter and stimulate digestion to enhance liver function by producing specific enzymes.

Aniseed (*Pimpinella anisum*) is an annual herb, cultivated in Egypt and Greece. It is also cultivated in tropical regions of Asia. Aniseed contains various amounts of nutrients such as moisture, protein, fat, total dietary fibre, carbohydrate, energy and volatile oil [3]. Aniseed has been studied as an anti-bacterial, anti-pyretic, analgesic, anthelmintic, anti-oxidant, antifungal and natural growth promoter [4-9]. Moreover, the plant and especially its fruit oil, has been used for treatment of some of the diseases like rhinitis, cough and other symptoms of the common cold [10]. Aniseed is a growth promoter, immune stimulator with better FCR [11].

Ginger (*Zingiber officinale*) is an annual herb, cultivated in southeastern Asia in tropical and subtropical regions. It is cultivated in the U.S., India, china, West Indies and tropical regions. Ginger contains various amounts of nutrients such as protein, lipids and minerals. Ginger has been used as anti-rheumatic, anti-oxidant, anti-inflammatory and anti-microbial [12-14]. Ginger has been also used as growth promoter, immune stimulator, and decrease mortality with better FCR [15].

Keeping in view the medicinal value of aniseed and ginger a research study was conducted to investigate aniseed and ginger growth performance and immunostimulant on broiler chicks.

Materials and Methods

Location and meteorological data

A research study was conducted to investigate the effect of different levels of aniseed (*Pimpinella anisum*) and ginger (*Zingiber officinale*) extract in drinking water on the overall performance, immunity and lipid profile of broiler chicks. This study was undertaken at Poultry Unit of Poultry Science Department, Faculty of Animal Husbandry and Veterinary Sciences, The University of Agriculture Peshawar-Pakistan.

Ethics

The care and use of birds and all experimental protocols were in accordance with the laws and regulations of Pakistan which were approved by Ethical Review Committee for Biomedical Research, The University of Agriculture Peshawar-Pakistan via (Letter No. 6785-A/UAP, Dated: 06/01/2021).

Bird, s husbandry

Birds were maintained in an independent open-sided laying housed with the east to west dimension measuring 6.10×6.10 m (37.21 m²), equipped with two rows of 3-tiered laying cages measuring 5.18×1.52 m (47.42 m²) with sloping wire floor to facilitate egg collection. The ventilation, humidity, and house temperature were controlled using ceiling fans, curtains, and other helpful manual techniques. Variations in daily temperature (°F) and humidity (%) were noted using a wet and dry bulb hygrometer (Mason's type, Zeal, England) and later an average of the temperature and humidity were derived on weekly basis (Fig. 1). The removable dropping trays were fitted under the mesh floor for the removal of faecal material. Feeding of the birds was done through removable individual trough feeders installed outside the cage and watering through the automatic nipple drinker system fitted therein. Birds were offered a commercial laying ration (Table 1)

at 06:00 AM with an allowance of 100 g / bird /day recommended by NRC, 1994 and availability of fresh water was ensured with nipple drinking system throughout the experimental period [16].

Bird, treatments and feeding

The experiment was conducted in a Completely Randomized Block Design with two factors i.e. 4 levels of aniseed and ginger and in vaccinated and non vaccinated chicks. Two hundred and forty commercial day old broiler chicks of approximately the same weight were purchased from a local market. These day old chicks were divided into four groups as A, B, C and D and these each group were further divided into two sub-groups for different treatments. Each subgroup was replicated three times with ten chicks / replicate. The birds of one of subgroup were vaccinated. The chicks were reared in pens provided with feeders, drinkers and lights. Group A was kept as a control while group B, C and D were treated with an extract mixture of aniseed and ginger at the rate of (2gms+4gms), (2.5gms+5gms and (3gms+6gms)/litter of drinking water respectively.

The extract mixture was prepared according to the method described by [17]. All the broiler chicks were allowed *ad libitum* access to feed and water through out the experiment. Feed intake, water intake and mortality were recorded daily and body weight gain on weekly bases. Feed conversion ratio (FCR) was calculated by dividing amount of feed intake with corresponded the body weight gain. All the birds of sub group 1 were vaccinated with ND, IB and IBD vaccine according to the schedule while all the birds of sub group 2 were kept unvaccinated. After complete feeding trial three birds from each subgroup was selected, weighed and slaughtered. After calculating the dressing percentage, the bird was then cut into pieces, lymphoid organs (spleen, thymus and bursa) were also

recorded separately.

At the end of experiment blood samples were collected for each replicate in test tubes. These tubes were kept in centrifuge machine at the rate of 4000 rpm for 10 minutes to separate serum. Serum was taken in small tubes, labeled for identity and stored in refrigerator for antibody titer against ND, IB and IBD using ELIZA kit.

Statistical analysis

The data recorded for all parameters was statistically analyzed by the standard procedure of analysis of variance using Completely Randomized Block Design with two factors, as described by [18]. The statistical package SAS was used to perform the analysis on computer [19].

Results

Aniseed (*Pimpinella anisum*) and Ginger (*Zingiber officinale*) extract mixture on growth promoting effects

There was no significant effect of aniseed (*P. Anisum*) and ginger (*Z. officinale*) extract on water intake and feed intake, however significant ($P < 0.05$) difference in the mean body weight gain and FCR value was recorded among the treatments and non-significant between the vaccinated and non vaccinated subgroups (Table 2).

Aniseed (*Pimpinella anisum*) and Ginger (*Zingiber officinale*) extract mixture on immunomodulatory effects

Aniseed (*P. anisum*) and ginger (*Z. officinale*) extract mixture had no effect on the mean weight of lymphoid organs (spleen, bursa and thymus) (Table 3).

Mean ELISA and HI antibody titer against ND, IB and IBD was significantly ($P < 0.05$) higher for group D among the treatments and significant between the vaccinated and non-vaccinated (Table 3).

Table 1. Composition of the ration offered to the experimental birds

| Ingredient | Inclusion rate (g/100g) |
|----------------------|-------------------------|
| Corn | 62.30 |
| Guar Meal | 3.00 |
| Raw rice Bran | 4.00 |
| Soybean Meal 44% | 1.31 |
| Rape Seed Meal | 2.00 |
| DL-Methionine | 0.23 |
| L-threonine | 0.08 |
| Calcium Carbonate | 8.29 |
| Salt | 0.11 |
| Corn Gluten | 1.00 |
| Canola Meal | 8.00 |
| Cotton Seed Meal | 4.00 |
| Lysine Sulphate | 0.36 |
| Premix | 0.30 |
| L-Tryptophan | 0.01 |
| Fish Meal 47% | 1.00 |
| Feather Meal 54% | 4.00 |
| Quantum 600FTU | 0.01 |
| Total | 100.00 |
| Crude Protein% | 16.5% |
| Metabolisable Energy | 2902 Kcal/Kg |

Table 2. Mean Feed intake, water intake, body weight gains and FCR in broiler chicks fed different levels of aniseed and ginger

| Groups | Feed intake | Water intake | Body weight gain | FCR |
|-------------|-------------|--------------|-----------------------|--------------------|
| | Mean | Mean | Mean | Mean |
| A | 2582.13 | 6706.90 | 1043.80 ^a | 2.48 ^a |
| B | 2541.30 | 6907.12 | 1098.87 ^{ab} | 2.32 ^{ab} |
| C | 2541.30 | 6647.58 | 1135.25 ^{bc} | 2.27 ^b |
| D | 2539.53 | 6933.40 | 1182.02 ^c | 2.15 ^b |
| Vaccination | | | | |
| Vac | 2588.43 | 6778.51 | 1109.11 | 2.34 |
| Non-Vac | 2526.76 | 6818.99 | 1120.86 | 2.26 |

Mean in the columns with superscripts are significantly different at a = 0.05

Table 3. Mean Antibody titer against IB, IBD and ND and relative weight of lymphoid organs (bursa, spleen and thymus) in broiler chicks fed different levels of aniseed and ginger

| Groups | IB | IBD | ND | Bursa | Spleen | Thymus |
|-------------|-------------------|----------------------|-------------------|-------|--------|--------|
| | Mean | Mean | Mean | Mean | Mean | Mean |
| A | 3.83 ^a | 1113.00 ^a | 0.33 ^a | 1.90 | 1.18 | 2.18 |
| B | 5.16 ^a | 1370.00 ^b | 0.50 ^b | 1.93 | 1.55 | 2.21 |
| C | 5.66 ^a | 1532.00 ^b | 1.83 ^c | 2.08 | 1.51 | 1.62 |
| D | 5.83 ^b | 2462.50 ^b | 3.00 ^c | 1.80 | 1.37 | 1.94 |
| Vaccination | | | | | | |
| Vac | 5.91 ^a | 1714.75 | 2.25 ^a | 1.96 | 1.46 | 2.29 |
| Non-Vac | 4.33 ^b | 1524.00 | 0.58 ^b | 1.89 | 1.34 | 1.68 |

Mean in the columns with superscripts are significantly different at a = 0.05

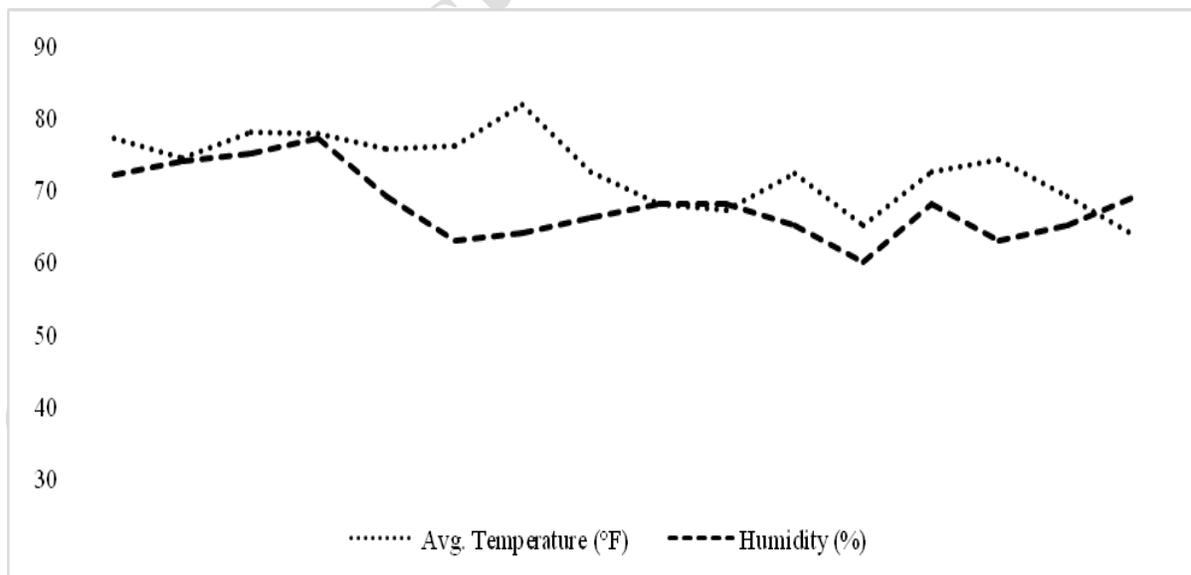


Figure 1. Variations in temperature (°F) and humidity (%) by a wet and dry bulb hygrometer (Mason's type, Zeal, England)

Discussion

Our current trial's findings are supported by, who recorded a non-significant effect on broiler feed intake by aniseed infusion [20]. Growth performance was significantly affected by ginger and there was a tendency of broilers consuming a ginger-supplemented diet to grow faster during the grower phase as compared with broilers fed the control diet. It shows that anise improved palatability of broiler diets [21]. Probably, the enhancing effect of anise on growth and FCR of broilers was due to its carminative, spasmolytic and expectorant effects [22]. Similarly, herbal mixture Livol improved weight gains, FCR and reduced mortality rate of broiler chicks [23]. Similarly, broiler, s chick higher body weight gains and better FCR while receiving aniseed [24]. This variation might be due to the presence of some active compounds like asanethole and so on, which stimulates digestive system [25].

Also, different responses among various types and levels of medicinal plants used as growth promoting substance in broiler diets were shown by [26]. Similarly, there is a synergetic effect among nigella sativa seed and chamomile flowers or harmala seeds in growth performance of broiler chicks and this was correlated with improving digestibility of nutrients [27]. Finding of the present research study might be due to its strong antimicrobial properties [28]. Similarly, there is an agreement with the findings, better weight gain in broiler chicks, fed different levels of aniseed and antibiotics [9]. This variation might be due to some chemicals which are responsible for ileal digestibility and may also increase the pancreatic lipase effect and amylase [29, 30]. Similarly, aniseed, fed to broiler chicks, resulted in higher body weight gain than control group [24]. Similarly, there is a potential alternative growth promoter [31]. Similarly, an improvement of 8.1% and

daily weight gain and 7.7% in FCR in seventeen day old pullets fed a diet supplemented with a herbal plants extract feed weight gain [32]. This variation might be due to chemicals inside aniseed that investigated that are responsible for harmful bacteria destruction in the chicken's GIT so increases digestion and absorption and hence results in better body weight gain [33]. Similarly, broiler, s chick higher body weight gains and better FCR while receiving aniseed [24]. This variation might be due to the presence of some active compounds like asanethole and so on, which stimulates digestive system [25].

Similarly, medicinal herb extract, given in drinking water had no significant effect on the mean weight of spleen, bursa and thymus of broiler chicks [15]. In poultry industry, medicinal plants play a key role in poultry immunity boosting. This boost up immunity is used in infectious diseases reduction or its prevention. Broiler birds are prone to various environmental stressors that negatively affect bird's immunity and minimize their resistance to different diseases probably due to oxidative damage of lymphoid tissues that result in impaired antibodies production [34]. This variation occurs might be due to medicinal plant antioxidant nature and hence improves immunity against different diseases [35].

It has been noted that the mixing of Anise and ginger to poultry birds diet which is responsible for the improvement of immune system due to boosting in immune globulin level (IgG, IgA and IgM). Anise may stimulate immunity of broiler to stress conditions [36]. There is an improved broiler antibody titer against Newcastle disease by receiving aniseed [36, 37]. Similarly, aniseed addition to basal diet play an important role in broiler, s immunomodulatory activity both for humoral as well as cellular immune response [38]. This variation might be due to this

improvement in immunity may be due to aniseed which is rich in some chemicals that acts as antioxidants and may enhance immune function [39]. Ginger and aniseed extract respectively and observed higher antibody titer against ND, IB and IBD than control group in broiler chicks [15]. Similarly, ginger powder plays an important role in boosting of broiler antibody titer against NDV [40]. Similarly, there is a higher bursal weight broiler, chicks fed on ginger powder [20]. This variation might be higher antibody titers against IBD. Similarly, herbal plant extract improves broiler, s IBD titer [41].

Conclusion

It is concluded from the present study that supplementation of Aniseed (*P. anisum*) and ginger (*Z. officinale*) extract mixture in broiler ration significantly improved the all performance, immunity of broiler chicks and hence it is recommended that extract mixture of aniseed and ginger added @ (3gms+6gms) of drinking water could effectively be used to improve body weight gain, immunity and better FCR.

Authors' contributions

Conceived and designed the experiment: M Mushtaq & F Raziq, Performed the experiment: M Mushtaq, Analyzed the data: M Mushtaq, Contributed materials/ analysis/ tools: MT Khan, HA Bachaya, M Rauf, MA Gondal & E Bughio, Wrote the paper: F Raziq, M Mushtaq & A Hameed.

Acknowledgement

The authors gratefully acknowledge the Department of Poultry Science, University of Agriculture, Peshawar, KPK-Pakistan for supporting the trial.

References

1. Khan MA, & Al-Kanhalm MA (1998). Dietary energy and protein requirements for Saudi Arabia: a methodological approach. *EMH-Eastern Medit Health J* 4(1): 68-75.
2. Gheisar MM & Kim IH (2017). Phytobiotics in poultry and swine nutrition– a review. *Italian J Anim Sci* 17(2): 92-99.
3. Farhat GN, Affara NI & Gali-Muhtasib HU (2001). Seasonal changes in the composition of the essential oil extract of east Mediterranean sage (*Salvia libanotica*) and its toxicity in mice. *Toxicol* 39(10): 1601-1605.
4. Tabanca N, Bedir E, Kirimer N, Baser KH & Khan SI (2003). Antimicrobial compounds from *Pimpinella* species growing in Turkey. *Planta Medica* 69: 933-938.
5. Afifi NA, Ramandan A, El-Kashour EA & El-Banna HA (1994). Some pharmacological activities of essential oil from certain unbelliferous fruits. *Vet Med J Giza, Cairo Uni* 42(1): 85-92.
6. Bhatti MA, Khan MTJ, Ahmad B, Jamshaid M & Ahmad W (1996). Antibacterial activity of aniseed (*Pimpinella anisum*). *Fitoterapia* 67: 372-374.
7. Gülcin I, Oktay M, Kireççi E & Küfrevioğlu I (2003). Screening of antioxidant and antimicrobial activities of aniseed (*Pimpinella anisum* L.) seed extract. *Food Chem* 83(1): 371-382.
8. Soliman KM & Badea RI (2002). Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi. *Food Chem Toxicol* 40(1): 1669-1675.
9. Mehmet C, Talat G, Dalkiliç B & Ertas ON (2005). The Effect of Anise Oil (*Pimpinella anisum* L.) On Broiler Performance. *Int J Poult Sci* 4(1): 851-855.
10. Schilcher HHP (2000). Efficient phytotherapy. Herbal medicines in the upper respiratory tract for catarrh. *Herba Polonica* 46(1): 52-57.
11. Durrani FR, Sultan A, Sajjad A, Chand N & Khattak A (2007). Efficacy of Aniseed extract as immune stimulant and growth promoter in broiler chicks.

- Pak J Biol Sci* 10(20): 3718-3721.
12. Srivastava KC & Mustafa T (1989). Ginger (*Zingiber officinale*) and rheumatic disorders. *Med Hypoth* 29(1): 25-28.
 13. Masuda T, Jitoe A & Mabry TJ (1995). Isolation and structure determination of cassumunarins A, B, and C: new anti-inflammatory antioxidants from a tropical ginger, *Zingiber cassumunar*. *J Ame Oil Chemist's Soc* 72(9): 1053-1057.
 14. Onyeagba RA, Ugboogu OC, Okeke CU & Iroakasi O (2004). Studies on the antimicrobial effects of garlic (*Allium sativum* Linn), ginger (*Zingiber officinale* Roscoe) and lime (*Citrus aurantifolia* Linn). *Afri J Biotech* 3(10): 552-554.
 15. Arshad M, Kakar AH, Durrani FR, Akhtar A & Shakirullah M (2012). Economical and immunological impact of Ginger (*Z. Officinale*) extract on broiler chicks. *Pakistan J Sci* 64(2): 46-48.
 16. NRC (1994). Nutrients requirements of poultry. 9th revised ed., National Academy Press. Washington, D.C., USA.
 17. Leila SFM (1977). A manual on some Philippine medicinal plants (preparation of drug materials), *Bot Soc UP* 20(1): 78-81.
 18. Steel RGD & Torrie JH (1981). Principles and procedures of statistics: A biometrical approach. 2nd. Ed. McGraw-Hill, Singapore.
 19. SAS. Institute (1998). SAS User's Guide: Statistics. SAS Institute, Inc., Cary, NC.
 20. Ghasemi HA & Taherpour K (2015). Comparison of broiler performance, blood biochemistry, hematology and immune response when feed diets were supplemented with ginger essential oils or mannan-oligosaccharide. *Iranian J Vet Med* 9(2): 195-205.
 21. Al-Kassie GAM (2008). The effect of Anise and rosemary on the microbial balance in gastro intestinal tract for broiler chicks. *Int J Poult Sci* 7: 610-612.
 22. Zepernick B, Langhommer L & Lu'dcke JBP (1984). Lexikon der offiziellen arzneipflanzen. De Gruyter, Berlin, New York.
 23. Sapra KL & Mehta RK (1990). A comparative study on additive of livol (Herbal Growth Promoter) and some chemical growth promoters in the diets of broiler chickens. *Indian J Anim Prod and Mangement* 6(2): 115-118.
 24. El-Deek AA, Attia YA & Hannfy MM (2003). Effect of aniseed (*Pimpinella anisum*), ginger (*Zingiber officinale roscoe*) and fennel (*Foeniculum vulgare*) and their mixture on performance of broilers. *Archiv Fur Geflügelkunde* 67: 92-96.
 25. Giannenas I, Florou-Paneri P, Papazahariadou M, Christaki E & Botsoglou NA (2003). Effect of dietary supplementation with oregano essential oil on performance of broilers after experimental infection with *Eimeria tenella*. *Arch Anim Nutr* 57(1): 99-106.
 26. Abaza MIK, Asar MA, El-shaarrawi GE & Hassan MF (2003). Effect of using Nigella seed, chamomile flowers, thyme flowers and harmala seed as feed additives on performance of broiler. *Egyptian J Agr Res* 81(4): 735-750.
 27. Abaza MIK, Ali NM & Hassan SM (2004). Nutritional and physiological studies on improving the utilization of wheat bran in laying hen diets. *Egyptian Poult Sci J* 24(4): 1015-1031.
 28. Gangrade SK, Shrivastava RD, Sharma OP, Jain NK & Trivedi KC (1990). In vitro antifungal effect of the essential oils. *Indian Perfum* 34: 46-48.

29. Reyer H, Zentek J, Männer K, Youssef IMI & Julian TA (2017). Possible molecular mechanisms by which an essential oil blend from star anise, rosemary, thyme and oregano and saponins increase the performance and ileal protein. *J Agr Food Chem* 65(1): 6821-6830.
30. Ramakrishna RR, Platel K & Srinivasan K (2003). In vitro influence of spices and spice-active principles on digestive enzymes of rat pancreas and small intestine. *Nahrung* 47(1): 408-412.
31. Kubo I (2004). Anethole, a synergist of polygodial and warburganal against *Candida albicans*. WOCMAP 1-Medicinal and aromatic plants conference: part 2. 332(4): 191-198.
32. Jamroz D & Kamel C (2002). Plant extracts enhance broiler performance. *J Anim Sci* 80(1): 41-46.
33. Narimani-Rad M, Nobakht A, Shahryar HA, Kamani J & Lotfi A (2011). Influence of dietary supplemented medicinal plants mixture (*Ziziphora*, *Oregano* and *Peppermint*) on performance and carcass characterization of broiler chickens. *J Med Plants Re* 5(1): 5626-5629.
34. Quinteiro-Filho WM, Ribeiro A, Ferraz-de-Paula V, Pinheiro ML & Sakai M (2010). Heat stress impairs performance parameters, induces intestinal injury and decreases macrophage activity in broiler chickens. *Poult Sci* 89(4): 1905-1914.
35. Botsoglou NA, Florou-Paneri P, Christaki E, Fletouris DJ & Spais AB (2002). Effect of dietary oregano essential oil on performance of chickens and on Iron-induced lipid oxidation of breast, thigh and abdominal fat tissues. *Br Poult Sci* 43(2): 223-230.
36. Al-Shammari KIA & Batkowska J (2017). Effect of Various Concentrations of an Anise Seed Powder (*Pimpinella Anisum* L.) Supplement on Selected Hematological and Biochemical Parameters of Broiler Chickens. *Brazilian J Poult Sci* 19(6): 41-46.
37. Al-Beitawi NA, El-Ghousein SS & Abdullah HN (2009). Antibiotic growth promoters and Anise seeds in broiler diets. *Jordan J Agr Sci* 5(1): 472-481.
38. Al-hajj MS, Alhobaishi M, Ger EI, Nabi AR & Al-Mufarrej SI (2015). Immune responsiveness and performance of broiler chickens fed a diet supplemented with high levels of Chinese Star Anise fruit (*Ifficiimverum* Hook. f). *J Anim Vet Adv* 14: 36-42.
39. Acamovic T & Brooker JD (2005). Biochemistry of plant secondary metabolites and their effects in animals. *Proc Nutr Soc* 64: 403-412.
40. Azhir D, Zakeri A & Kargare-Rezapour A (2012). Effect of ginger powder rhizome on humoral immunity of broiler chickens. *Europe J Exper Biol* 2(1): 2090-2092.
41. Chand N, Muhammad D, Durrani FR, Qureshi MS & Ullah SS (2011). Protective Effects of Milk Thistle (*Silybum marianum*) against Aflatoxin B₁ in Broiler Chicks. Asian-Australasian. *J Anim Sci* 24(7): 1011-1018.