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# Promising Immunomodulation Potential of *Ganoderma lucidium* and Oregano as Mixture on Broiler Chicken

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**Abstract** | Immuno-stimulant component recent research is very important to reduce using un-necessary vaccination and antibiotic abused. The effects of combination of *Ganoderma lucidium* and *Origanum vulgare* on broilers chicken are not completely understood. In this study, four broiler chicken groups around 3 week age each group contain 10 chicken was divided randomly. First group was feed with (2g/ Kg feed) from *Ganoderma lucidium* (G group), second group was treated with ( 2g/Kg feed) *Origanum vulgare* (O Group) in a feed and the third group was treated with mixture of both *Ganoderma lucidium* and *Origanum vulgare* with 1gm/Kg from each (GO group) and the fourth group was the control group (C group). The hematological parameter (haematocrit, Hb, RBC, MCV, MHC and MHCH) were measured. The immuno-stimulation in each group were measured using total, differential WBC count, heterophil to lymphocyte ratio, bursa index, spleen index, bursa to spleen ratio and phagocytosis index using carbon clearance test. This study showed that haematological parameters (haematocrit, Hb, RBC, MCV, MHC and MHCH) were all within the normal range values in all the groups. The mixture of plant and mashroom insignificantly increased the total WBCs compared with G group. Mixture and Ganoderma groups was significantly decreased the heterphiles/lymphocytes ratio in comparing with C ($p$=.004, .03 respectively). The eosinophils were significantly increased in the GO group in comparing to G and C groups ($p$=.04, .05 respectively). G group result in significantly increase ($p$<.05) of lymphocytes count compared with other groups including the C group. While the O group showed significantly decreases in lymphocytes ($p$<0.05) compared with other groups. In Monocyte count the O group showed significantly ($p$<.05) increase the count comparing with other groups. Heterophil to lymphocyte ratio (H/L) was showed significantly decreases (in both G group ($p$=.03) and GO group ($p$=.004). G and O groups induced significant increase in bursa index when compared with C group ($p$= .0003 and .0004 respectively) and the GO combined additives ($p$=.004 and .0004 respectively). The spleen index was significant decrease in GO groups when compared with C group ($p$=.002, .014 and 0.016 respectively). Brusa to spleen ratio (B/S) significantly increases ($p$<.05) in G and O groups compare with a C group while the GO group decrease the ratio in comparing to G and O groups ($p$= .006 and .004 respectively). In regarding phagocytosis index, only GO group showed an increased significance in comparing to C group ($p$=.003). The G and O groups showed insignificant increase in phagocytosis index. In conclusion, the combination *Ganoderma lucidium and Origanum vulgare* are promising to be used in broiler ration and could be used as potential immune-regulator.

**Keywords** | Immunomodulation, broiler, herbal, mushroom, Ganoderma, Oregano

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## INTRODUCTION

The increment in poultry protein demand to cover the human requirements in the past few decades lead to using intensive rearing with different growth and production enhancement tools. This leads to defensive adverse effects on immunological parameters of poultry and increasing in the number of highly resistant microorganism (RU et al., 2015). In addition, antibiotic residues in poultry meat are dangerous to human health, therefore the researcher are continuously looking for safety and low cost natural alternatives. The first step in this issue was performed by European Commission which support the scientific community to focus on finding antibiotic alternative from the aromatic plants and extracts which day after day many scientific confirmation has been emphasis their beneficial productivity and health effects on animal (Franciosini et al., 2016).

Among many different natural resources that may benefit in this issue are the botanical and Mushroom, Although the different methods of plant and mushroom extraction effects the complex compounds in extracts and consequently the mode of action, but generally, the complex mixtures targets different physiological pathways. The using of single compound purified from plant or mushroom represent by modern pharmacological point of view, magic bullet for targeting certain cellular pathway. Several studies prove that the synergistic interaction of compounds in herbal plants and mushroom may present enhancing by that the action of one or both of them. This synergistic mode may attributes in some cases to the enhancement of absorption and bioavailability or to reduction of some mediators that effect the target pathway of another extracts (Martel et al., 2017). Despite of that, there are growing attention, devoted to environmental issues, led to new techniques capable of extracting plant bioactive compounds without using non-renewable resources (i.e. solvents) with a consequent reduction in the environmental impact of wastes because the plant material, remaining after solvent extraction, is considered hazardous waste (Setti and Zanichelli, 2009).

In poultry production, it is vital to enhance immunity to prevent infectious diseases. Minimizing immunosuppression and its impact is additionally crucial strategy for success in the broiler industry. However, strategies to control immunosuppression are largely based on vaccination programs for poultry and management to minimize stress during rearing (Hashemi and Davoodi, 2012). Utilization of natural immunostimulants is one solution to improve the immunity of animals and to decrease their susceptibility to infectious diseases (Liu, 1999).

Increasing number of researches reported the immunostimulation effects of Oregano (Hernandez-Coronado et al., 2019; Avila-Ramos et al., 2012; Mathlouthi et al., 2012; Giannenas et al., 2003; Botsoglou et al., 2002) or Ganoderma (Ahad et al., 2016; Ogbe et al., 2009; Liu et al., 2015; Pi et al., 2014; Ojiezeh and Eghafona, 2015). , so the aim of this study was:to study the effects of Oregano and Ganoderma mixture on some immunological parameters in broiler chicken and to assist potential synergistic and/or immunomodulation effects of combination of this natural immunostimulants.

## MATERIALS AND METHODS

### Feed additives and preparation of ration

The effects of two feed additives, *Ganoderma lucidium* and *Origanum Vulgare,* were investigated in the present study. The *Ganoderma lucidium* were obtained from Dxn company branch at Dhamar city, while the Oregano was obtained from local herbal shop. *Ganoderma lucidium* and *Origanum Vulgare* grinded finally and were added into feeds in a ratio of 0.2% (2g/Kg) (Ogbe et al., 2008; Sofyan et al., 2012; Ri et al., 2017; Rahman et al., 2018). a combination of the additives were added into feeds in a ratio of 0.1% (1g/Kg) for each additive. The chicks were received on sugar solution of 5% for 6 hours. The feeding program were, *ad libdo* feeding, starter feed from one day to 14 days, grower feed from 15 day to 28 day and finisher from 29 to 42 day. The broilers' feeders were from commercial source (Alfalah Corporation, Dhamar, Yemen). The lighting program were 23 hours per day at one day old and reduced 10 minutes per day. Initial brooding temperature was 33 °C in the first week of age and reduced gradually 2 °C per wk and reached 22 °C at the end of experimental period.

### Chickens and experimental design

Forty broiler chicks of the Ross breed were obtained from local provider (AL-Flah Hatchery, Ibn Al-Haj poultry incorporation) at age of one-day old. Birds were kept on deep beds and allowed to feed and drink ad libitum. At arrival, chicks were randomly allocated into four experimental groups, 10 chicks in each group. Three experimental groups were used to investigate the effects of *Ganoderma lucidium, Origanum Vulgare* and combination of them on immune status of birds (G, O and GO groups respectively). The fourth group was used as a control (C group).

Immunological performance of feed additives was evaluated using total and differential count of WBC, heterophil/lymphocyte ratio (Gross and Siegel, 1983), indices of immune organs, carbon clearance and phagocytosis index-(Abbas et al., 2017, Dorhoi et al., 2006, Zheng et al., 2013). At age of 45 days, blood samples were taken.

### Sample collection

At age of 42 days, blood samples form 24 chickens (six chicken per group) were collected from wing vein into EDTA containing vacutainer. Blood parameters measurements were performed according to the procedures recommended by Samour (2015). Accordingly, heterophil to lymphocyte (H/L) ratio was calculated (Gross and Siegel 1983). Heterophil to lymphocyte ratio was used as indication of stress (Lentfer et al., 2015, Jahanian and Rasouli, 2015, Gross and Siegel, 1983, Arczewska-Włosek et al., 2018).

### Immunological parameters

**Phagocytosis index:** The phagocytic function of the mononuclear phagocytes system in chickens was evaluated with carbon clearance test. Six broilers from each treatment group were randomly selected at day 42 and used to measure carbon clearance. Indian ink was diluted in normal saline at rate of 1:3 and injected in right wing vein of broiler chickens at a rate of 2 ml/Kg. Three and ten minutes post injection, 100 µl of blood was collected from left wing vein into tubes containing 10 ml of 0.1% sodium carbonate. Optical density was measured at wavelength of 640 nm using PD-303UV (APEL, JAPAN) (Shahzad et al., 2015, Zheng et al., 2013). The carbon clearance index (K) were calculated as follows:

$k = \log OD_1 - \log OD_2)/(t_2 - t_1)$

Where $OD_1$ is the optical density at 3 min ($t_1$) and $OD_2$ is the optical density at 10 min ($t_2$)

The phagocytosis indices were calculated from the carbon clearance (*K*), total weights of carcass (W) and weight of both spleen and liver ($W_{ls}$) as following:-

$\alpha = W/W_{ls} \times K^{1/3}$, (Zheng et al., 2013)

### Immunological organs indices

At age of 45 day, chickens were weighed and humanly sacrificed. Internal organs including bursa of Fabricius, spleen and liver, were removed surgically and weighed. Organ weight was presented as percentage of the live body weight.

### Statistical analysis

Data were analyzed using SPSS (V22.0; IBM SPSS Statistics Inc., Chigaco, IL, US). All study groups were subjected to Analysis Of Variance (ANOVA). Least Significant Different (LSD) was used to separate the means were significant difference exist. Differences were considered significant at $p < .05$

## RESULTS

Haematological parameters (haematocrit, Hb, RBC, MCV, MHC and MHCH) were all within the normal range values in all the groups. Although were no significant differences among study groups in RBCs, MCV, MCH and MCHC, the addition of *O.licidium* induced significant decreased both hematocrite (Ht) (*P*=.025) and hemoglobin (*P*=.034) in comparison to control, (Table 1).

There was insignificant increase in eosinophil count due to addition of *O. vulgare*, while there was insignificant decrease in eosinophil count due to addition of *G. lucidium* as Table 2 The GO combined additives induced further increase in eosinophil count which was significant when compared with control (*P*<.05) and *G.lucidium* (*P*=.04) groups. In contrast to eosinophil count, there was significant increase in lymphocyte count due to addition of *G. lucidium* (*P*=.002) and significant decrease due to addition of *O. vulgare* (*P*=.01). The GO combined additives showed insignificant decrease in lymphocyte count (*P*=.518) when compared to control but significantly increased (*P*=.043) when compared to *O. vulgare* group.

There was significant decrease in heterophil count due to addition of *G. lucidium* (*p*=.039). The GO combined additives induced further decrease in heterophil count which was significant when compared with control group (*p*=.0004) while the addition of *O. vulgare* induced insignificant reduction in heterophil when compared with control (*p*=.053). Regarding heterophil/lymphocyte ratio, there was significant decrease due to addition of *G. lucidium* (*p*=.029) and the GO combined additives (*p*=.004). Decrease was insignificant due to addition of *O. vulgare* (*p*=.140).

There was significant increase in monocyte count due to addition of *O. vulgare* (*p*<.05) compared to other groups. Addition of *G. lucidium* alone or in combination with *O. vulgare* showed no effects on monocyte count. Overall, *G. lucidium* and *O. vulgare* showed insignificant decrease in TWB, while the GO combined additives showed insignificant increase in TWBC (*p*=.08) (Table 2).

*G. lucidium* and *O. vulgare* induced significant increase in bursa index when compared with control group (*p*= .0003 and .0004 respectively) and the GO combined additives decrease the bursa index in comparing to *G. lucidium* and *O. vulgare* (*p*=.004 and .0004 respectively) as shown table3. On the other hand, the spleen index was significant decrease in *G. lucidium* , *O. vulgare* and GO groups when compared with control (*p*=.002, .014 and .016 respectively). Significant increase in bursa to spleen (B/S) ratio were induced by *G. lucidium* and *O. vulgare* when compared with control group (*p*= .004 for both) and the GO combined additives significantly decrease the ratio in comparing to *G. lucidium* and *O. vulgare* (*p*= .006 and .004 respectively). The GO combined additives was significantly superior in increasing phagocytosis index when compared

**Table 1:** The Effects of *G. lucidum* and/or *O. vulgare on* hematological parameters of broiler chicken.

| Treatment | RBC [1012 l-1] | Ht [l1-1] | Hb [g l-1] | MCV (Fl) | MCH (Pg) | MCHC (g/dL) |
|---|---|---|---|---|---|---|
| G | 2.5 | 34.11 a | 11.46a | 135.4 | 45.5 | 33.48 |
| O | 2.6 | 35.01 | 11.83 | 135.5 | 45.8 | 33.73 |
| GO | 2.6 | 34.75 | 11.69 | 134.1 | 45.5 | 33.81 |
| C | 2.7 | 36.28 b | 12.16 b | 135 | 45.5 | 33.49 |
| SEM | 0.0366 | 0.33 | 0.11 | 1.3306 | 0.4351 | 0.1 |

a, b, c, d means in columns with different superscripts differ significantly at $p \leq 0.05$ for main treatment; G—*Ganoderma lucidium*; O—Origanum vulgare; GO—Ganoderma & Oregano mixture; C—Control; RBC—red blood cells; Ht—haematocrit; Hb—haemoglobin;MCV—mean corpuscular volume; MCH—mean corpuscular hemoglobin; MCHC—mean corpuscular haemoglobin concentration; SEM—standard errors mean.

**Table 2:** The effects of f *G. lucidum* and/or *O. vulgare* feed additive on leukogram of broiler chicken

| Treatment | TWBC [109 l-1] | H [%] | L [%] | MONO [%] | EOS [%] | BASO [%] | H/L |
|---|---|---|---|---|---|---|---|
| G | 5.50 | 12.55 [a] | 65.88 [a] | 11.22 [a] | 10.33 [a] | 0 | 0.19 [a] |
| O | 6.01 | 12.70 | 53.37 [b] | 14.50 [b] | 19.38 [a] | 0 | 0.25 |
| GO | 6.33 | 8.60 [a] | 57.7 [c] | 11.10 [a] | 22.60 [b] | 0 | 0.15 [a] |
| C | 6.02 | 17.66 [b] | 59 [cd] | 11.44 [a] | 11.89 [a] | 0 | 0.32 [b] |
| SEM | 0.17 | 0.97 | 1.0 | 0.5 | 0.97 | 0 | 0.21 |

a, b, c, d means in columns with different superscripts differ significantly at $p \leq 0.05$ for main treatment; G—*Ganoderma lucidium*; O—Origanum vulgare; GO—Mixture of Ganoderma and Oregano; C—Control;; WBC—white blood cells; ; H—heterophils; L—lymphocytes; MONO—monocytes; EOS—eosinophils; BASO—basophils; H/L—heterophils/lymphocytes; SEM—standard errors mean.

to control group ($p$=.003). Both *G. lucidium* and *O. vulgare* induced increase in phagocytosis index, though it was not significant when compared with control group ($p$=.06 and .051 respectively) (Table 3).

**Table 3:** The immunological indices of broiler chicken fed on additive of *G. lucidum* and/or *O. vulgare*

| Treatment | SI | BI | B/S | PI |
|---|---|---|---|---|
| G | 0.077 [a] | 0.258 [a] | 3.530 | 38.60 |
| O | 0.090 [a] | 0.321 [a] | 3.745 | 39.55 |
| GO | 0.087 [a] | 0.114 [bc] | 1.349 | 52.81 [a] |
| C | 0.147 [b] | 0.043 [bc] | 0.309 | 17.94 [b] |
| SEM | 0.010 | 0.031 | 0.419 | 4.30 |

a, b, c, d means in columns with different superscripts differ significantly at $p \leq 0.05$ for main treatment; G—*Ganoderma lucidium*; O—*Origanum vulgare*; GO—*Ganoderma lucidium* & *Oregano vulgare* mixture; C—Control; SI—spleen index; BI—bursa of Fabricus index; B/S—bursa to spleen ratio; PI—Phagocytosis Index,: SEM—standard errors mean.

## DISCUSSION

Immunomodulation. Immune-based therapies are gaining more importance than monovalent approaches which having limited benefits. The herbal products have privileged and high structural diversity with numerous biological activities (Ezzat Abd El-Hack et al., 2016, Christaki et al., 2020). In this study the immunomodulation effects of G.lucidium and O.vulgare with their mixture were evaluated.

Results showed that the hematological values of broilers were inconsistent and seemed to bear no direct relationship. It should be taken in consideration that the fluctuation and wide range in normal hematological values in poultry as normal phenomonia which have been reported in many research (Reece et al., 2015, Orawan and Aengwanich, 2007, Onibi et al., 2011, Ogbe et al., 2010, Ogbe et al., 2003, Islam et al., 2004).

This study revealed that the mixture of Ganoderma and Oregano insignificantly increase the total WBCs which may be indicative of hematopoietic stem cell stimulation (Ganeshpurkar and Saluja, 2017). Overall, *G. lucidium* and *O. vulgare* showed insignificant decrease in TWBC. The GO combined additives showed insignificant increase in TWBC. Similar findings were also reported. Extract of wild Ganoderma species was used to treat Eimeria tenella-infected broilers, result showed no significant effects on total WBC count (Ogbe et al., 2010). Similarly, wild Ganoderma lucidum together with other herbs were administered into broilers and showed no significant effects on TWBC count (Ogbe and Affiku, 2012). Likewise, addition of oyster mushroom to broiler feed at rates of 1, 10, 20 gm/kg showed no significant effects on total WBC (Toghyani et al., 2012). Feeding broilers on Oregano oil at rates of 60, 100 and 200, 300, 500 ppm induce no change

in TWBC count (Mohiti-Asli and Ghanaatparast-Rashti, 2017; Hashemipour et al., 2013). Similarly, broilers supplied with Mexican Oregano oil at a rate of 0.4gm/kg of diet showed no change in total WBC count (Alagawany et al., 2018). In the present study, there was highly significant decrease in heterophil count due to addition of the combined additives, while the lymphocyte count was significant increased and decreased due to addition of *G. lucidum* and *O. vulgare* , respectively. This suggest that the *G. lucidium* is more effective as immunostimulant than *O. vulgare*. Although the GO combined reveal significant increase in lymphocytes when compare with *O. vulgare* . This reflected on the H/L ratio that were significantly decreased due to addition of *G. lucidium* and to lesser extent on the GO combined additives group.

Unfortunately, effects of *G. lucidum* on H/L ratio was not fully covered in previous studies. Willis et al. (2013) showed that addition of *G. lucidum* to chicken feeds at rate of at 5% and 10% induce increase of heterophil and decrease of lymphocyte and increase in H/L ratio Studies on oyster mushroom showed that its addition to broiler feed at a rate of 2% result in significant decrease of heterophil to lymphocyte ratio (Fard et al., 2014). While similar experiment on broiler showed that use of 10 and 20 g/kg of the oyster mushroom showed no effect on H/L ratio (Toghyani et al., 2012). Regarding effect of Oregano on H/L ratio, our results are in contrast to previous investigations where addition of ≤300 ppm to chicken feed induced significant decrease in H/L ratio while addition of higher rates induce no change in H/L ratio. Similarly, provision of broilers feeds with 60, 100 and 200 ppm of the Oregano essential oil was reported to significantly (*P*=.005) decrease H/L ratio (Hashemipour et al., 2013). Addition of 300 of the Oregano essential oil to broiler feeds result in significant decrease the heterophil and H/L ratio at 300 ppm but not at 500 ppm (Mohiti-Asli and Ghanaatparast-Rashti, 2017). While addition of Mexican Oregano oil to feeds of broilers at rate of 0.4gm/kg (=400ppm) induced slightly lower lymphocyte and monocyte count (Alagawany et al., 2018). Increase in H/L ratio may result from increase in circulating heterophil and decrease in circulating lymphocyte counts in stress condition (Maxwell and Robertson; 1995, Post et al., 2003).

There was significant increase (*p*=.00001) in eosinophil count due to addition of GO combined additives. Addition of *G. lucidum* to chicken feeds at rate 5% and 10% induce no change in eosinophil count (Willis et al., 2013). On the other, administration of Mexico Oregano oil to broiler feeds at rate of 0.4gm/kg induce insignificant increase in eosinophil count (Méndez Zamora et al., 2017). This may result from the higher dose of Oregano administered in the present study. Eosinophils and basophils have been described in avian systems and in mammals play an important role in defense against helminthes and in allergy responses. However, compared to other leukocytes such as heterophils and macrophages the functionality of eosinophils and basophils in chickens and other avian species has not been well determined. Using a range of intravenous injected immunostimulatory materials (e.g. horse serum, material from spontaneously occurring eosinophilia chicken, etc.), other researchers was unable to increase the levels of eosinophils in chickens. However intra-dermal injection of chickens challenged with dinitrochlorobenzene (DNCB) or citraconic anhydride (CA) increased eosinophil and heterophil levels in the skin indicating a potential role of eosinophils in early inflammatory responses (Maxwell, 1987).

There was significant increase in monocyte count due to addition of *O. vulgare* (p=0.030) while there was no change due to addition of *G. lucidum*. This is in agreement with result shown by Willis and collegues where addition of *G. lucidum* to chicken feeds at rate 5% and 5% result in no change in monocyte count (Willis et al., 2013) Similarly the addition of 600 and 900 ppm of Oregano cause significant increase in monocyte count (Eler et al., 2019). On the other hand, addition of 300 and 500ppm of the Oregano essential oil to broiler feeds result in no effect on monocyte count (Mohiti-Asli and Ghanaatparast-Rashti, 2017). addition of 0.4gm/kg of feed induced no significant change in relative monocyte (Méndez Zamora et al., 2017). The Oregano essential oil (OEO) 400 mg/ kg as natural antioxidant improved AFs induced oxidative stress, TWBCs, heterophil%, lymphocyte%, monocyte% and eosinophil % toward control values.

It's known that oxidative stress influence hypothalamicpituitary-adrenal axis as well as the increased NO level that catalysed cortisol synthesis (Minka et al., 2012). Moreover, oxidative stress could affect differentiation and phagocytic ability of leucocytes especially monocytes against infection (Ponnappan and Ponnappan, 2011). Daily administration of corticosterone to chicken result in decrease of lymphocyte and eosinophil and increase in heterophil count (Glick B, 1958).

It has been reported that stress in chickens induce increase in plasma corticosterone concentration, decrease in total leukocyte count and increase in H/L ratio (Post et al., 2003, Mehaisen et al., 2017, Shini et al., 2009, Elnesr et al., 2019, Babacanoğlu et al., 2013). Decrease in lymphocyte and increase in heterophil and H/L ratio in response to stress and cortcosterone in chicken were previously documented (Lentfer et al., 2015, Jahanian and Rasouli, 2015, Gross and Siegel, 1983, Glick, 1961, Glick, 1958, Arczewska-Włosek et al., 2018). This give an evidence that the

increasing in monocytes at peripheral blood indicate immune stimulation at non-infection-normal condition. GO combined additive antagoise the effect of stress as shown in H/L ratio under non-infection status.

In contrast to many researches, our results showed significant increase in bursa index and bursa to spleen ratio as result of *G. lucidum* and O. *vulgare*, while GO significantly decrease bursa index and bursa to spleen ratio. The spleen index was significant decrease in three treatments. Addition of 5% and 10% *G. lucidum* to feed of broiler did not induce significant change in bursa weight (Willis et al., 2013), Aqueous extracts of wild *G. lucidum* and other herbs were mixed at equal volume and administered in broiler feed at rates of 5% w/v at age of 2 weeks, 10% w/v at age of 4 weeks and 20% w/v at age of 6 weeks. Result showed no significant difference in weights of visceral organs, including spleen (Ogbe and Affiku, 2012). Similarly, addition of 1 or 2% of oyster mushroom to broiler feeds induce no change in weight of bursa, spleen or bursa to spleen ratio (Toghyani et al., 2012, Kavyani et al., 2012, Fard et al., 2014).

Similarly, several studies were performed on the effects of Oregano active ingredients, Oregano oil or Oregano powder on weight of lymphoid organ in broiler chicken or bursa: spleen ratio. No change in weight of bursa or spleen were induced by addition of Oregano active ingredient thymol and carnvacrol at rate of 60, 100, 200 ppm (Hashemipour et al., 2013); 150 mg of the Oregano powder/kg diet . (Ri et al., 2017); 250 and 500 mg of the Oregano oil/kg diet (Basmacioğlu Malayoğlu et al., 2010); 300 mg of Oregano essential oil /kg (OEO) diet (Kırkpınar et al., 2011). Combination of OEO with garlic oil at rates of 150mg of both/kg diet induce no change in weight of bursa and spleen (Kırkpınar et al., 2011). On the other hand, combination of OEO with garlic and thyme oils at rates of 10mg or 20mg of each/kg diet for 15 days induce no change in weight of bursa but significant increase in weight of spleen. Extending feeding period for 35 days abolish the effect on spleen weight (Abou-Elkhair et al., 2014).

Thymus gland, bursa of Fabricius and spleen are important immune organs of broilers which are involved in humoral and cell-mediated immunities and as generative organ for T and B cells. Generally, cell growth, development and division caused animal immune organ weight increase, and the immune organ weights reflected the immune functions. The greater bursa weight commonly reflects better health status and also the better anatomical response to immune system changes due to stress (Willis et al., 2013). Beside that There is a strong correlation between the relative size of bursa and the average levels of IgG antibody expression (Yonash et al., 2002; Glick et al., 1956). The Bursa of Fabricius is an essential lymphoid organ in chickens that plays an important role in adaptive immunity. Bursa size can reflect the immunological health status of birds (Glick, 1979). Healthy broilers produce larger bursas than broilers whose immune systems have been compromised (Glick, 1979). Glick et al. (1956), also reported that the Bursa of Fabricius plays an important role in antibody production. Therefore, it may be perceived that unchallenged broilers in treatment groups with higher bursa have superior immune systems compared to broilers whose bursa weights were smaller. Although, there is no apparent evidence that bursa size had any significant influence on the lymphocyte values or antibody titers of unchallenged broilers in this study, there has been a noticeable immunological response to *Eimeria* infections in both challenged and unchallenged broilers. In addition, there have been reports that have indicated positive effects on the integrity of the immune system of chickens given medicinal mushrooms in their diets (Guo et al., 2004; Willis et al., 2012; Willis et al., 2013).

Therefore, it may be suggested that broiler diets supplemented with certain mushrooms can exert positive health attributes via bursa performance. Immune stimulation by using herbal extracts may reduce the animal's susceptibility to infectious diseases (Alagawany et al., 2015). Not all trials have been positive, for example Bozkurt et al. (2009) studied the effect of 1.0 g/kg diet of Oregano on the performance of broilers and found that bursa was not affected by supplementation.

The primary task of 'phagocytosis' is amputation of foreign bodies and microorganisms along with the eradication of injured and dead cells, malignant cells, and inorganic particles (White and Gallin, 1986). Carbon clearance test is one of the necessary methods to assess phagocytosis immunomodulatory effects of drugs and phytoconstituents in different animal model including chicken (Nudo and Catap, 2011; Kundu et al., 2015). increasing in phagocytosis is index is indicative of stronger immune response (Sun et al., 2008).

Separated additives of *G. lucidum* and *O. vulgare* or combination of them induce insignificant increase in carbon clearance and phagocytosis index in comparing to Control Group. Unfortunately, phagocytosis and carbon clearance were not tested in previous studies on *G. lucidum* and *O. vulgare*. Investigating the effects of oral administration of aqueous methanol extracts of roots of *Glycyrrhiza glabra*, leaves of *Artemisia brevifolia* and *Ageratum conyzoides* at doses of 100, 200 and 300 mg/kg body weight of broilers. carbon clearance was low at low doses and increase with higher doses to a level similar to those result from treatment with vitamin E (Hussain et al., 2017). Acamovic and Brooker (2005) reported an immune stimulato-

ry effect with thymol and Oregano oil fed in broiler diets in terms of mononuclear phagocyte proliferation, as well as in cell mediated and humoral immunity. In a study by (Perez-Roses et al., 2015), it was reported that immunity partially participates in resistance against different infections. Phagocyte proliferation, cellular and humoral immune responses in broiler chickens could be improved by using herb oils, which elevated the ability of defence system to interact with infectious agents.

Although, there are many evidence on the phagocytosis activation ability of *G. lucidium.* Both the classical and alternative complement pathways were activated by an alkali extract obtained from *G. lucidum* cultivated mycelium. In the carbon clearance test, this fraction also activated the reticuloendothelial system of mice and enhanced haemolytic plaque producing cells in the spleen. In RAW 264.7 macrophages, Ganoderan, a D-glucan isolated from *G. lucidum*, increased NO generation. Raw 264.7 cell lines treated with GAN had their cell growth reduced. These findings suggest that the higher fungus' -glucan-related polysaccharides activate macrophages and produce NO, a key chemical messenger for the generation of a variety of biological responses. GLB, a protein-polysaccharide fraction derived from the developing tips of *G. lucidum*, is a potent macrophage stimulant. GLB (100 g/ml) boosted the phagocytic activity of BALB/c mouse peritoneal macrophages and chicken macrophage BM2CL cells against FITC-labeled *C. albicans* by 55.2 percent and 21.2 percent, respectively, when examined using a flow cytometer. GLB boosted the spreading and expression of MHC class II molecules in BM2CL cells and mouse peritoneal macrophages as well (Sanodiya et al., 2009).

Reishi mushroom extracts boosted leukocyte respiratory burst activity, phagocytic activity, and lysozyme synthesis, all of which are antimicrobial enzymes. The phenotypic functions of macrophages such as phagocytic absorption, generation of NO and other reactive oxygen species, cytokine gene expression, and morphological modifications were all up-regulated by glucan. Many immune cells which have TLR 4 on their surface, can mediate the immunological response. Ganoderma polysaccharide appears to be a ligand for these receptors as well as an activator. Many of the effects identified in dendritic cells may be blocked by blocking the TLR4 receptor; this, together with the activation of TLR4 and the presence of certain polysaccharides, suggests that receptor activation is a fundamental mechanism of action. On macrophages, TLR4 activation has been seen, with downstream effects that boost macrophage phagocytic capacities. Polysaccharides and proteoglycans may be involved in these actions. It's been proposed that a polysaccharide fraction F3 containing fucose binds to TLR4 on macrophages, activating proteins like ERK, which regulates meiosis, mitosis, and postmitotic functions, as well as JNK and p38, which regulate cell proliferation/differentiation, inflammation, and cytokine production (Bhardwaj et al., 2014).

## CONCLUSION

In: conclusion, it is known that an unbalanced immunitory system provides the premise for a great number of diseases in both humans and animals the combination of Ganoderma and Oregano in broiler chicken may be promising immunomodulation and/or immunobalancer feed additive, although further studies are required to assess different biological effects and the best optimal synergistic concentration.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## NOVELTY STATEMENT

Up to our knowledge, this is the first study to evaluate the effects of combined feed additives, which include G. lucidium and O. vulgare, on the haematological and immunological parameters of broiler chicks. Obtained findings showed these phytobiotic feed additives are promising

## AUTHORS CONTRIBUTION

Fateh Badi: conceptualization and experimental design; performed the experiment; data curation. Fateh Badi, Anwar Al-Kubati, Naif Al-Gabri: validation, data analysis, writing, and editing the manuscript. All authors have read and agreed to publish this version of the manuscript.

## REFERENCES

Abbas A., Iqbal Z., Abbas R. Z., Khan M. K. Khan J. A. (2017). Immunomodulatory activity of Pinus radiata extract against coccidiosis in broiler chicken. Pak. Vet. J., 37: 145-149.

Abou-Elkhair R., Gaafar K. M., Elbahy N., Helal M. A., Mahboub H. D., Sameh G. (2014). Bioactive effect of dietary supplementation with essential oils blend of Oregano, thyme and garlic oils on performance of broilers infected with Eimeria species. Global Vet., 13: 977-985.

Acamovic T., Brooker J. (2005). Biochemistry of plant secondary metabolites and their effects in animals. Proceed. Nutrit. Societ., 64: 403-412. https://doi.org/10.1079/PNS2005449

Ahad S., Tanveer S., Malik T. A. (2016). Anticoccidial activity of aqueous extract of a wild mushroom (Ganoderma applanatum) during experimentally induced coccidial infection in broiler chicken. J. Parasit. Dis., 40: 408-414. https://doi.org/10.1007/s12639-014-0518-3

Alagawany M., El-Hack M., Farag M. R., Tiwari R., Dhama K. (2015). Biological effects and modes of action of carvacrol in animal and poultry pro-duction and health-a review. Adv Anim. Vet. Sci., 3: 73-84. https://doi.org/10.14737/journal.aavs/2015/3.2s.73.84

Alagawany M., EL-HACK M.A., Farag M., Shaheen H., Abdel-Latif M., Noreldin A., Patra A.(2018). The usefulness of Oregano and its derivatives in poultry nutrition. World's Poult. Sci. J., 74: 463-474. https://doi.org/10.1017/S0043933918000454

Arczewska-Włosek A., Świątkiewicz S., Ognik K., Józefiak D. (2018). Effect of dietary crude protein level and supplemental herbal extract blend on selected blood variables in broiler chickens vaccinated against coccidiosis. Animals., 8: 208. https://doi.org/10.3390/ani8110208

Avila-Ramos F., Pro-Martinez A., Sosa-Montes E., Cuca-Garcia J. M., Becerril-Perez C. M., Figueroa-Velasco J. L., Narciso-Gaytan C. (2012). Effects of dietary Oregano essential oil and vitamin E on the lipid oxidation stability of cooked chicken breast meat. Poult. Sci., 91: 505-11. https://doi.org/10.3382/ps.2011-01731

Babacanoğlu E., Yalçin S., Uysal S. (2013). Evaluation of a stress model induced by dietary corticosterone supplementation in broiler breeders: effects on egg yolk corticosterone concentration and biochemical blood parameters. Brit. Poultr. Sci., 54: 677-685. https://doi.org/10.1080/00071668.2013.847901

Basmacioğlu Malayoğlu H., Baysal Ş., Misirlioğlu Z., Polat M., Yilmaz H., Turan N. (2010). Effects of Oregano essential oil with or without feed enzymes on growth performance, digestive enzyme, nutrient digestibility, lipid metabolism and immune response of broilers fed on wheat–soybean meal diets. Br. Poult. Sci., 51: 67-80. https://doi.org/10.1080/00071660903573702

Bhardwaj N., Katyal P., K Sharma, A. (2014). Suppression of inflammatory and allergic responses by pharmacologically potent fungus Ganoderma lucidum. Recent patents inflammat. Allerg. Drug Discover., 8: 104-117. https://doi.org/10.2174/1872213X08666140619110657

Botsoglou N. A., Florou-Paneri P., Christaki E., Fletouris D. J., Spais A. B. (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: 223-30. https://doi.org/10.1080/00071660120121436

Bozkurt M., Alçiçek A., Cabuk M., Küçükyilmaz K., Catli A. (2009). Effect of an herbal essential oil mixture on growth, laying traits, and egg hatching characteristics of broiler breeders. Poult. Sci., 88, 2368-2374. https://doi.org/10.3382/ps.2009-00048

Christaki E., Giannenas I., Bonos E., Florou-Paneri P. (2020). Innovative uses of aromatic plants as natural supplements in nutrition. Feed Addit.. Elsevier. https://doi.org/10.1016/B978-0-12-814700-9.00002-9

Dorhoi A., Dobrean V., Zăhan M., Virag P. (2006). Modulatory effects of several herbal extracts on avian peripheral blood cell immune responses. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, 20: 352-358. https://doi.org/10.1002/ptr.1859

Eler G., Gomes A., Trindade B., Almeida L., Dilelis F., Cardoso, V., Lima C. (2019). Oregano essential oil in the diet of broilers: performance, carcass characteristics, and blood parameters. South African Journal of Animal Science, 49, 753-762. https://doi.org/10.4314/sajas.v49i4.17

Elnesr, S. S., Elwan, H. A. M., Xu, Q. Q., Xie, C., Dong, X. Y. & Zou, X. T. 2019. Effects of in ovo injection of sulfur-containing amino acids on heat shock protein 70, corticosterone hormone, antioxidant indices, and lipid profile of newly hatched broiler chicks exposed to heat stress during incubation. Poult Sci. https://doi.org/10.3382/ps/pey609

Ezzat Abd El-Hack, M., Alagawany, M., Ragab Farag, M., Tiwari, R., Karthik, K., Dhama, K., Zorriehzahra, J. & Adel, M. 2016. Beneficial impacts of thymol essential oil on health and production of animals, fish and poultry: a review. Journal of Essential Oil Research, 28, 365-382. https://doi.org/10.1080/10412905.2016.1153002

Fard S. H., Toghyani M., Tabeidian S. A. (2014). Effect of oyster mushroom wastes on performance, immune responses and intestinal morphology of broiler chickens. Int. J. Recycling Organic Waste Agricult., 3: 141-146. https://doi.org/10.1007/s40093-014-0076-9

Franciosini M. P., Casagrande-Proietti, P., Forte C., Beghelli D., Acuti G., Zanichelli D., dal Bosco A., Castellini C., Trabalza-Marinucci M. (2016). Effects of Oregano (Origanum vulgare L.) and rosemary (Rosmarinus officinalis L.) aqueous extracts on broiler performance, immune function and intestinal microbial population. J. Appl. Anim. Res., 44: 474-479. https://doi.org/10.1080/09712119.2015.1091322

Ganeshpurkar A., Saluja A. K. (2017). Experimental animal models used for evaluation of potential immunomodulators: A mini review. Bulletin of Faculty of Pharmacy, Cairo University, 55, 211-216. https://doi.org/10.1016/j.bfopcu.2017.08.002

Giannenas I., Florou-Paneri P., Papazahariadou M., Christaki E., Botsoglou N. A., Spais A. B. (2003). Effect of dietary supplementation with Oregano essential oil on performance of broilers after experimental infection with Eimeria tenella. Arch Tierernahr, 57: 99-106. https://doi.org/10.1080/0003942031000107299

Glick B. (1958). The effect of cortisone acetate on the leukocytes of young chickens. Poult. Sci., 37: 1446-1452. https://doi.org/10.3382/ps.0371446

Glick B. (1961). The effect of bovine growth hormone, desoxycorticosterone acetate, and cortisone acetate on the white blood cell counts of 2-week-old chickens. Poult. Sci,. 40: 1537-1539. https://doi.org/10.3382/ps.0401537

Glick B. (1979). The avian immune system. Avian di., 23: 282-289. https://doi.org/10.2307/1589557

Glick B., Chang T. S., Jaap R. G. (1956). The bursa of Fabricius and antibody production. Poult. Sci., 35: 224-225. https://doi.org/10.3382/ps.0350224

Gross W., Siegel H. (1983). Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. Avian Dis., 972-979. https://doi.org/10.2307/1590198

Guo F., Williams B., Kwakkel R., Li H., Li X., Luo J., Li W., Verstegen M. (2004). Effects of mushroom and herb polysaccharides, as alternatives for an antibiotic, on the cecal microbial ecosystem in broiler chickens. Poult. Sci., 83: 175-

182. https://doi.org/10.1093/ps/83.2.175
Hashemi S., Davoodi H. (2012). Herbal plants as new immuno-stimulator in poultry industry: A review. Asian J. Anim. Vet. Adv., 7: 105-116. https://doi.org/10.3923/ajava.2012.105.116
Hashemipour H., Kermanshahi H., Golian A., Veldkamp T. (2013). Effect of thymol and carvacrol feed supplementation on performance, antioxidant enzyme activities, fatty acid composition, digestive enzyme activities, and immune response in broiler chickens. Poult. Sci., 92: 2059-2069. https://doi.org/10.3382/ps.2012-02685
Hernandez-Coronado A. C., Silva-Vazquez R., Rangel-Nava Z. E., Hernandez-Martinez C. A., Kawas-Garza J. R., Hume M. E., Mendez-Zamora G. (2019). Mexican Oregano essential oils given in drinking water on performance, carcass traits, and meat quality of broilers. Poult. Sci. https://doi.org/10.3382/ps/pez094
Hussain K., Iqbal Z., Zahid Abbas R., Kasib Khan M., Kashif Saleemi M. (2017). Immunomodulatory activity of Glycyrrhiza glabra extract against mixed Eimeria infection in chickens. Int. .J. Agricult. Biol., 19. https://doi.org/10.17957/IJAB/15.0397
Islam M., Lucky N., Islam M., Ahad A., Das B., Rahman M., Siddiui M. (2004). Haematological parameters of Fayoumi, Assil and local chickens reared in Sylhet region in Bangladesh. Int. J. Poult. Sci, 3: 144-147. https://doi.org/10.3923/ijps.2004.144.147
Jahanian R., Rasouli E. (2015). Dietary chromium methionine supplementation could alleviate immunosuppressive effects of heat stress in broiler chicks. J. Anim. Sci., 93: 3355-3363. https://doi.org/10.2527/jas.2014-8807
Kavyani A., Shahne A. Z., PorReza J., Haji-abadi S. J., Landy N. (2012). Evaluation of dried powder of mushroom (Agaricus bisporus) as an antibiotic growth promoter substitution on performance, carcass traits and humoral immune responses in broiler chickens. J. Med. Plants Res., 6: 94-100. https://doi.org/10.5897/JMPR11.1168
Kırkpınar F., Ünlü H. B. , Özdemir G. (2011). Effects of Oregano and garlic essential oils on performance, carcase, organ and blood characteristics and intestinal microflora of broilers. Livest. Sci., 137: 219-225. https://doi.org/10.1016/j.livsci.2010.11.010
Kundu A., Singh D., Mohapatra S. (2015). In vivo phagocytic activity in various indigenous and exotic breeds of chicken for genetic selection. Indian J. Anim. Sci., 85: 1.
Lentfer, T., Pendl H., Gebhardt-Henrich S., Fröhlich E., Von Borell E. (2015). H/L ratio as a measurement of stress in laying hens–methodology and reliability. Br. Poult. Sci., 56: 157-163. https://doi.org/10.1080/00071668.2015.1008993
Liu X. (1999). Stress and immunity. Poult. Immunol., 230-252.
Liu Y. H., Lin Y. S., Lin K. L., Lu Y. L., Chen C. H., Chien M. Y., Shang H. F., Lin S. Y., Hou W. C. (2015). Effects of hot-water extracts from Ganoderma lucidum residues and solid-state fermentation residues on prebiotic and immune-stimulatory activities in vitro and the powdered residues used as broiler feed additives in vivo. Bot Stud., 56: 17. https://doi.org/10.1186/s40529-015-0097-3
Martel J., Ko Y.-F., Ojcius D. M., Lu C.-C., Chang C.-J., Lin C.-S., Lai H.-C. Young J. D. (2017). Immunomodulatory properties of plants and mushrooms. Trends Pharmacolog. Sci.,38: 967-981. https://doi.org/10.1016/j.tips.2017.07.006
Mathlouthi N., Bouzaienne T., Oueslati I., Recoquillay F., Hamdi M., Urdaci M., Bergaoui R. (2012). Use of rosemary, Oregano, and a commercial blend of essential oils in broiler chickens: in vitro antimicrobial activities and effects on growth performance. J. Anim. Sci., 90: 813-23. https://doi.org/10.2527/jas.2010-3646
Maxwell M. (1987). The avian eosinophil—a review. World's Poult. Sci. J., 43: 190-207. https://doi.org/10.1079/WPS19870013
Maxwell M., Robertson G. (1995). The avian basophilic leukocyte: a review. World's Poult. Sci. J., 51: 307-325. https://doi.org/10.1079/WPS19950021
Mehaisen G. M., Eshak M. G., Elkaiaty A. M., Atta A.-R. M., Mashaly M. M., Abass A. O. (2017). Comprehensive growth performance, immune function, plasma biochemistry, gene expressions and cell death morphology responses to a daily corticosterone injection course in broiler chickens. PloS one., 12, e0172684. https://doi.org/10.1371/journal.pone.0172684
Méndez Zamora G., Durán Meléndez L. A., Hume M. E. Silva Vázquez R. (2017). Performance, blood parameters, and carcass yield of broiler chickens supplemented with Mexican Oregano oil. Revista Brasil. de Zoot., 46: 515-520. https://doi.org/10.1590/s1806-92902017000600006
Minka N. S., Adeiza A. A., Hassan F. B., Ayo J. O. (2012). Effects of melatonin and transportation on rectal temperature, heterophil/lymphocyte ratio and behaviour of Japanese male quails (Coturnix japonica). NY Sci J, 5, 52-59.
Mohiti-Asli M., Ghanaatparast-Rashti M. (2017). Comparison of the effect of two phytogenic compounds on growth performance and immune response of broilers. J. Appl. Anim. Res., 45: 603-608. https://doi.org/10.1080/09712119.2016.1243119
Nudo L. P., Catap E. S. (2011). Immunostimulatory effects of Uncaria perrottetii (A. Rich.) Merr.(Rubiaceae) vinebark aqueous extract in Balb/C mice. J. Ethnopharmacol., 133: 613-620. https://doi.org/10.1016/j.jep.2010.10.044
Ogbe A., Adeyefa C., Joshua R. (2003). Growth rate and haematological parameters of broiler chickens vaccinated with IBD (Gumboro) vaccines exposed to different handling temperature. J. Sci. Technol. Res.. 2: 36-38.
Ogbe A., John P.A. (2012). Effect of Polyherbal Aqueous Extracts (Moringa Oleifera, Gum Arabic and Wild *Ganoderma lucidum*) in Comparison with Antibiotic on Growth Performance and Haematological Parameters of Broiler Chickens. Res. J. Rec. Sci.. 1. 10-18.
Ogbe A., Atawodi S., Abdu P., Oguntayo B., Dus N. (2010). Oral treatment of Eimeria tenella-infected broilers using aqueous extract of wild mushroom (Ganoderma sp): Effect on haematological parameters and histopathology lesions. African J. Biotechnol., 9: 8923-8927.
Ogbe A. O., Atawodi S. E., Abdu P. A., Sannusi A., Itodo A. E. (2009). Changes in weight gain, faecal oocyst count and packed cell volume of Eimeria tenella-infected broilers treated with a wild mushroom (Ganoderma lucidum) aqueous extract. J. S. Afr. Vet. Assoc, 80: 97-102. https://doi.org/10.4102/jsava.v80i2.179
Ogbe A., Mgbojikwe L., Owoade A., Atawodi S., Abdu, P. 2008. The Effect of a Wild Mushroom (Ganoderma Lucidum) Supplementation of Feed on the Immune Response of Pullet Chickens to Infectious Bursal Disease Vaccine. Electronic J. Environ Agricult. Food Chem., 7: 2844-2855.
Ojiezeh, T. I. & Eghafona, N. 2015. Humoral responses of broiler chickens challenged with NDV following supplemental treatment with extracts of Aloe vera, Alma millsoni,

Ganoderma lucidum and Archachatina marginata. Cent Eur J Immunol, 40, 300-6. https://doi.org/10.5114/ceji.2015.54590

Onibi G., Bobadoye A., Folorunso O. (2011). Haematological indices, serum cholesterol and meat quality of broiler chickens fed diets with palm oil sludge substituting maize. Agric. Biol. JN America, 2: 552-558. https://doi.org/10.5251/abjna.2011.2.3.552.558

Orawan C., Aengwanich W. (2007). Blood cell characteristics, hematological values and average daily gained weight of Thai indigenous, Thai indigenous crossbred and broiler chickens. Pakistan J. Biolog. Sci.: PJBS, 10: 302-309. https://doi.org/10.3923/pjbs.2007.302.309

Perez-Roses R., Risco E., Vila R., Penalver P., Canigueral S. (2015). Effect of some essential oils on phagocytosis and complement system activity. J. Agricult. Food Chem., 63: 1496-1504. https://doi.org/10.1021/jf504761m

Pi C. C., Wang H. Y., Lu C. Y., Lu F. L., Chen C. J. (2014). Ganoderma formosanum polysaccharides attenuate Th2 inflammation and airway hyperresponsiveness in a murine model of allergic asthma. Springerplus., 3: 297. https://doi.org/10.1186/2193-1801-3-297

Ponnappan S., Ponnappan U. (2011). Aging and immune function: molecular mechanisms to interventions. https://doi.org/10.1089/ars.2010.3228

Post J., Rebel J., ter Huurne A. (2003). Automated blood cell count: a sensitive and reliable method to study corticosterone-related stress in broilers. Poult. Sci., 82: 591-595. https://doi.org/10.1093/ps/82.4.591

Rahman A., Cetingul I. S., Bayram I., Uyarlar C., Akkaya A. B., Gultepe E. E., Keles H., Ulucan A., Hayat Z. (2018). Effect of Supplementation of Oregano (Origanum Onites) Dried Leaves on the Intestinal Properties in Japanese Quails. Pakistan J. Zool., 50. https://doi.org/10.17582/journal.pjz/2018.50.4.sc12

Reece W. O., Erickson H. H., Goff J. P., Uemura E. E. (2015). Dukes' physiology of domestic animals, John Wiley & Sons.

Ri C.-S., Jiang X.-R., Kim M.-H., Wang J., Zhang H.-J., Wu S.-G., Bontempo V., Qi G.-H. (2017). Effects of dietary Oregano powder supplementation on the growth performance, antioxidant status and meat quality of broiler chicks. Italian J. Anim. Sci., 16: 246-252. https://doi.org/10.1080/1828051X.2016.1274243

RU A. M., Farag M., Alam G., Laudadio V., Tufarelli V. (2015). Multiple beneficial applications and modes of action of herbs in poultry health and productiona review. Int. J. Pharmacol., 11: 152176Docherty. https://doi.org/10.3923/ijp.2015.152.176

Samour J. (2015). Avian medicine, Elsevier Health Sciences.

Sanodiya B. S., Thakur G. S., Baghel R. K., Prasad G., Bisen P. (2009). Ganoderma lucidum: a potent pharmacological macrofungus. Curr. Pharmaceuti. Biotechnol., 10: 717-742. https://doi.org/10.2174/138920109789978757

Setti L., Zanichelli D. (2009). Bioliquefaction as a bio-refinery's approach for the production of natural bioactive compounds for functional cosmetics. Waste Recove.: Strateg. Techniq. Applicat. Europe. 122-128.

Shahzad A., Khan A., Khan M. Z., Mahmood F., Gul S., Saleemi M.K. (2015). Immuno-pathologic effects of oral administration of chlorpyrifos in broiler chicks. J. Immunotoxicol., 12: 16-23. https://doi.org/10.3109/1547691X.2013.866706

Shini S., Shini A., Huff G. (2009). Effects of chronic and repeated corticosterone administration in rearing chickens on physiology, the onset of lay and egg production of hens. Physiol. Behav., 98: 73-77. https://doi.org/10.1016/j.physbeh.2009.04.012

Sofyan A., Angwar M., Herdian H., Istiqomah L., Febrisiantosa A., Julendra H., Wibowo M., Untari T. (2012). Performance Enhancement and Immunity Profile of Broiler Treated Feed Additive Containing Lactic Acid Bacteria and Ganoderma Lucidum. Media Peternakan, 35: 201-201. https://doi.org/10.5398/medpet.2012.35.3.201

Sun S., Pan Q., Hui X., Zhang B., Wu H., Li,H., Xu W., Zhang Q., Li J., Deng X. (2008). Stronger in vitro phagocytosis by monocytes-macrophages is indicative of greater pathogen clearance and antibody levels in vivo. Poult. Sci., 87: 1725-1733. https://doi.org/10.3382/ps.2007-00202

Toghyani M., Tohidi M., Gheisari A., Tabeidian A., Toghyani M. (2012). Evaluation of oyster mushroom (Pleurotus ostreatus) as a biological growth promoter on performance, humoral immunity, and blood characteristics of broiler chicks. J. Poult. Sci., 49: 183-190. https://doi.org/10.2141/jpsa.011068

White C. J., Gallin J. I. (1986). Phagocyte defects. Clin. Immunol. Immunopathol., 40: 50-61. https://doi.org/10.1016/0090-1229(86)90068-1

Willis W., Wall D., Isikhuemhen O., Jackson J., Ibrahim S., Hurley S., Anike F. (2013). Effect of level and type of mushroom on performance, blood parameters and natural coccidiosis infection in floor-reared broilers. Open Mycol. J., 7. https://doi.org/10.2174/1874437001307010001

Willis W. L., Wall D., Isikhuemhen O. S., Ibrahim S., Minor R. C., Jackson J., Hurley S. L., Anike F. (2012). Effect of different mushrooms fed to Eimeria-challenged broilers on rearing performance. Int. J. Poult. Sci., 11: 433. https://doi.org/10.3923/ijps.2012.433.437

Yonash N., Bacon L., Smith E. (2002). Concentration of immnoglobulin G in plasma varies among 6C. 7 recombinant congenic strains of chickens. Poult. Sci., 81: 1104-1108. https://doi.org/10.1093/ps/81.8.1104

Zheng L., Ma Y., Gu L., Yuan D., Shi M., Guo X., Zhan X. (2013). Growth performance, antioxidant status, and nonspecific immunity in broilers under different lighting regimens. J. Appl. Poult. Rese., 22: 798-807. https://doi.org/10.3382/japr.2012-00713