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Influence of some medicinal plants on the humoral immunity of broiler chickens

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Abstract

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Infectious diseases cause devastating economic losses in the poultry industry. The aim of the current study was to evaluate blood serum concentrations of lysozyme, alfa interferon, gamma interferon, immunoglobulin G, and immunoglobulin M, the activity of the alternative pathway of complement activation (APCA), and betalysin activity in chicken broilers, whose rations were supplemented with either 2% dry herbs or 0.02% herbal essential oils. A total of 390, one-day-old of age male broilers poults were randomly allocated to 13 groups, any group 30 chickens (3 replicates – 10 birds per treatment) until 39 days old, namely control (C) and 12 experimentals. The experimental groups received a basal diet plus 2% dry herbs or 0.02% essential oils (EO) supplements obtained from the herbs (*Matricaria chamomilla*; *Rosmarinus officinalis*; *Lavandula angustifolia*; *Origanum vulgare*; *Thymus vulgaris*; *Hypericum perforatum*). In the groups treated with dry herbs highest lysozyme concentrations were determined in chamomile, APCA and betalysin in lavender, alpha interferon in thyme, interferon gamma in control group, immunoglobulin IgG in oregano, and immunoglobulin IgM in chamomile. In groups treated with essential oils (EO), lysozyme concentration was the highest in the group treated with rosemary, APCA in lavender, betalysin in thyme, alpha interferon in thyme, immunoglobulin IgG in St. John's wort, and immunoglobulin IgM in the control group. It could be concluded that studied herbs possess an important immunomodulating potential in chickens, which could improve their health.

Keywords: chickens; medicinal plants; humoral immunity

Introduction

For the last few decades, the ban on using antibiotics in livestock husbandry and poultry farming has increased the interest in biotechnological and natural products with the purpose to improve birds' productivity, health, quality, and safety of produce (Liu et al., 2011). Medicinal plants constitute a new class of growth promoters that recently has gained importance in the food industry for the production of functional foods. Research has been focused mainly on the effect of medicinal and aromatic plants on mortality, stress hormones, blood, and muscle metabolism, and even the immune system function of domestic animals. Rasouli et al. (2020) treated chicken broilers with different doses of water extract of salvia (*Salvia officinalis* L.) and found dose-dependent enhancement of the immune response of broilers. They also reported for the bactericidal effect of sage extract against *Escherichia coli*. Similar results for the positive effect of salvia on the immune system in chickens were reported by Farhadi et al. (2020). On a global scale, numerous studies on the effects of herbs and herbal products on various production traits in broiler chickens are published (Ocak et al., 2008; Moorthy et al., 2009; Ali, 2014; Mohamed, 2015). Wallace et al. (2010) demonstrated that plant extracts and different probiotics from leaves, roots, tubers, or fruits of herbs, spices, and other plants were excellent growth promoters in poultry farming. Some researchers reported chamomile effects on specific immunity of broiler chickens (Roby, 2013; Munir et al., 2014; Stanojevic et al., 2016). Matouskova et al. (2016) reported that encapsulated extracts of various herbs, including rosemary, had a marked antibacterial effect, which is increased in the presence of lysozyme. Mohamed et al. (2020) deliberate the different practical applications of a few medical herbs to improve the health state of poultry particularly as thermoregulatory and immunomodulatory agents. Hashemipour et al. (2013) investigated the effect of phytocompounds carvacrol, cinnamaldehyde, and Capsicum oleoresin, on translational regulation of genes associated with immunology, physiology, and metabolism in an in vivo model of coccidiosis in chickens. The results provided clear evidence that isolated phytoderivatives had immunostimulating properties in chickens. Jiang et al. (2012) investigated the effect of methanolic extract from Saint John's wort on specific humoral immune responses in chickens vaccinated against various avian influenza strains.

The aim of the current study was to evaluate blood serum concentrations of lysozyme, alfa interferon, gamma interferon, immunoglobulin G, and immunoglobulin M, the activity of the alternative pathway of complement activation (APCA), and betalysin activity in chicken broilers, whose rations were supplemented with either 2% dry herbs or 0.02% herbal essential oils.

Materials and Methods

Experimental Design

Chickens (hybrid Ross 308) were reared in the poultry farm of the Agricultural Institute, Stara Zagora. A total of 390, day-old of age male poults were randomly allocated to 13 groups, any group containing 30 chickens (3 replicates, 10 birds per treatment with initial body weight 49.78 ± 0.2 g) until 39 days old, namely control (C) and 12 experimentals. The treatment of poults starts from the 1st day and lasts until 39 days of age. The control group received a basal diet without herbs (nutritional program NRC was used). The composition of the basal diet is presented in Table 1.

All diets were in mashed form. The experimental groups received a basal diet plus 2% supplemented with dry herbs or 0.02% essential oils (Eos) obtained from the same herbs. They are *Matricaria chamomilla (M. chamomilla)*; *Rosmarinus officinalis (R. officinalis)*; *Lavandula angustifolia (L. angustifolia)*; *Origanum vulgare (O. vulgare)*; *Thymus vulgaris (T. vulgaris)*; *Hypericum perforatum (H. perforatum)*. Commercial Eos (Nature energies LTD, Bulgaria; ALTEYA ORGANICS LTD, Bulgaria) was used. Birds had *ad libitum* access to feed and water and lighting were provided continuously. The experiments were conducted within standard ethical norms and no birds were subjected to undue stress. The minimum requirements for the protection and welfare of experimental animals and the requirements for facilities for their use, keeping, and/or supply are set out in Ordinance № 20 of 1.11.2012 on the minimum requirements for protection and welfare of experimental animals and the requirements for sites for use (8.1.2018), breeding and/or delivery, which transposes Directive 2010/63/EU.

Table 1. Nutritional value of compound feed

Nutritional value	Starter 1-14 day	Grower 15-28 day	Finisher 29-39 day
Crude protein, %	22.47	21.01	19.02
Crude fat, %	7.03	8.10	9.08
Metabol. energy, kcal/kg	2912.80	3042.19	3111.17
Crude fiber, %	3.79	3.76	3.47
Ca, %	1.03	0.90	0.85
Utilizable phosphorus, %	0.50	0.45	0.42
Methionine, %	0.51	0.45	0.40
Lysine, %	1.44	1.25	1.05

Assay methods

At the end of the fattening at 39 days of age, blood samples for analyses were collected from v. subcutaneous ulnaris from 6 chickens from each group to assay some parameters of humoral innate immunity. Serum lysozyme concentrations were determined by the method of Lie et al. (1985). (Table 1), the alternative pathway of complement activation (APCA) was evaluated by the method of Sotirov (1986) and betalysin activity was assessed by the method of Buharin et al. (1977). Our previous publication described these methods in detail (Bozakova et al., 2020). Concentrations of alfa interferon (IFN-α), gamma interferon (IFN-γ), immunoglobulin G (IgG), and immunoglobulin M (IgM) in chicken's sera were determined by ELISA tests mentioned below:

1. Chicken interferon alfa (IFN-α) ELISA kit; cat. № CSB-E10071ch, China.
2. Chicken interferon gamma (IFN-γ) ELISA kit; cat. № CSB-F08550ch, China.
3. Chicken IgG ELISA kit; Lot: L210401953, Antibodies, USA.
4. Chicken IgM ELISA kit cat. № CSB-E16200c, China.

Statistical analysis

Data were processed by one-way analysis of variance (ANOVA) with the fixed effect model using the Data analysis tool pack, Microsoft Excel 2016, Microsoft Corporation Ltd. at a level of significance P < 0.05.

Results

The results presented in Tables 2 and 3 show that in the case of dried herbs, the highest concentration of lysozyme is observed in chamomile followed by the control, oregano, and other herbs. In lavender, the levels are the lowest, but the differences between the groups are insignificant. Chickens treated with extracts of the same herbs have almost the same concentration of lysozyme in the blood serum, except for oregano, which has a significantly higher difference than other herbs. When comparing the values of the indicator between dried herbs and their extracts, only one significant difference is found in lavender, which is in favor of dried herbs.

In the groups treated with dry herbs, the alternative pathway of complement activation (APCA) had the highest activity in the group treated with lavender (P < 0.001), followed by rosemary. The values for chamomile and thyme are relatively lower. In the groups treated with herbal extracts, the activity of APCA was highest in oregano (P < 0.01), followed by chamomile. When comparing the values of APCA among dry herbs and extracts of them the highest activity is characterized by chamomile (P < 0.01). These results show that the studied herbs significantly affect the activity of this important factor of natural immunity in chickens.

The data for betalysin (dry herbs) show that the highest activity of the indicator is in the groups treated with chamomile, rosemary, and St. John's wort (P < 0.001), and the lowest value of the indicator is in the control group. In the groups treated with herbal extracts, the activity was highest in oregano, followed by lavender, and the lowest in rosemary. However, no significant difference between the studied groups has been established for this indicator.

The results for alpha interferon show that in dried herbs the highest level is in thyme (P < 0.05), followed by the control group and oregano, and the lowest in chamomile. In the groups treated with herbal extracts, a higher value of the indicator is observed in thyme. However, the differences are unreliable.

Interferon-gamma in dried herbs has the highest value in the control group (P < 0.001), closely followed by chamomile and St. John's wort, and oregano has the lowest value. In the case of herbal extracts, again the control group had the highest value, followed by rosemary (P < 0.01). Dried chamomile and dried lavender compared to their extracts have significantly higher values of interferon gamma (P < 0.001).

Immunoglobulin IgG in dried herbs has the highest value in oregano (P < 0.05), followed by St. John's wort, and chamomile has the lowest value. In herbal extracts, St. John's wort has the highest value (P < 0.01), followed by rosemary. St. John's wort extract compared to its dry version has significantly higher values of immunoglobulin IgG (P < 0.001).

Immunoglobulin IgM in dried herbs has the highest value in chamomile (P < 0.01), followed by rosemary. For herbal extracts, the control group had the highest value (P < 0.001), followed by chamomile. Compared to immunoglobulin IgG, the opposite effect of herbs is observed in immunoglobulin IgM – chickens treated with dried herbs have higher values of immunoglobulin IgM than those treated with extracts from them.

Discussion

Matouskova et al. (2016) reported that encapsulated extract of various herbs, including chamomile had a marked antibacterial effect that is increased in the presence of lysozyme. Our results confirmed that dry chamomile and chamomile essential oil possessed immunomodulating properties on blood serum lysozyme in chicken broilers.

Primo et al. (2018) let us know that lysozymes are enzymes that break down the bacterial cell wall and disrupt the bacterial life cycle by cleaving the linkage between the N-acetylglucosamine and N-acetylmuramylpentapeptide carbohydrates. So, adding medical herbs to the diet of chicken broilers will increase the serum lysozyme concentrations and will improve their resistance to infectious diseases. This fact explains the similarities between our results and the one obtained by Matouskova et al. (2016). Other researchers reported chamomile effects on specific immunity of broiler chickens (Roby, 2013; Munir et al., 2014; Stanojevic et al., 2016). In the available literature, no research studies were found on the effects of dried chamomile and chamomile essential oils on APCA activity. It is acknowledged that APCA is the primary humoral means for control of viruses, virus-infected cells, Gram-negative bacteria, cancer cells etc. (Sotirov et al., 1998; Andonova et al., 2001; Goundasheva et al., 2002; Yotova et al., 2004; Bozakova et al., 2018). Matouskova et al. (2016) reported that encapsulated extracts of various herbs, including rosemary, had a marked antibacterial effect, which is increased in the presence of lysozyme. Our results confirmed that dried rosemary and especially rosemary essential oil exerted an immunomodulating effect on serum lysozyme concentrations in chicken broilers (Tables 2 and 3). This correspondence in the results can be explained

Table 2. Effect of some dry medical herbs on humoral immunity in chicken broilers (m ± SEM)

Dry herbs (2%)	Lysozyme, mg/L	APCA (CH50)	Betalysin, %	Alfa interferon, ng/ml	Gamma interferon, ng/ml	IgG, ng/ml	IgM, ng/ml
Control	0.49±0.07	518.14±17.22	28.22±12.59	41.88±9.06	278.91±14.88^{ac**}	1959.47±482.11	853.47±61.02
M. chamomilla	0.52±0.13	484.23±10.62	42.01±8.8	22.46±6.81	274.44±22.78	1934.13±610.36	899.47±46.17^{a**}
R. officinalis	0.46±0.08	616.74±14.92^{c***}	34.4±15.72	23.34±4.8	212.26±16.96	3411.79±805	887.13±36.15
L. angustifolia	0.29±0.06^{c*}	624.47±15.99^{a***}	28.65±11.23	31.29±5.82	242.06±30.53	2618.46±485.13	736.8±54.14
O. vulgare	0.49±0.14	509.39±18.06	29.18±9.66	39.53±6.04	166.5±33.51	3367.01±770.64^{a*}	801.87±81.24
T. vulgaris	0.31±0.07	491.14±15.85^{a**}	30.2±10.15	45.12±8.73^{a*}	212.26±24.88	2036.35±327.37	730.07±83.14
H. perforatum	0.35±0.1	582.68±7.19	32.41±6.43	40.41±3	254.04±27.28	3098.35±648.58	711±70.48

*** – P < 0.001 – the superscripts within a column indicate statistically significant differences among the groups.
** – P < 0.01
* – P < 0.05
a – between dry herbs
c – between dry herbs and Eos
Note: All data are given as mean ± standard error mean (for example 0.49±0.07). P – values are given at the bottom of the tables.

Table. 3. Effect of essential oils (EOs) obtained from medical herbs on humoral immunity in chicken broilers (m ± SEM)

Eos 0.2%	Lysozyme, mg/L	APCA (CH50)	Betalysin, %	Alfa interferon, ng/ml	Gamma interferon, ng/ml	IgG, ng/ml	IgM, ng/ml
Control	0.49±0.07	518.14±17.22	28.22±12.59	41.88±9.06	278.91±14.88	1959.47±482.11	853.47±61.02
M. chamomilla	0.24±0.05	561.66±13.03^{c***}	17.77±4.67	46.3±15.6	165±30.74	4140.9±868.19	486.64±55.48
R. officinalis	0.38±0.05	546.56±17.28	15.15±5.14	40.12±8.27	221±35.45^{a**}	4799.89±1386.9	439.52±86.84
L. angustifolia	0.18±0.05	449.49±13.73	30.24±11.77	55.72±11.3	151.91±35.27	3827.45±1229.9	395.77±62.97
O. vulgare	0.45±0.1^{b**}	592.09±19.67^{b**}	41.02±17.73	56.6±14.55	158.09±22.99	3770.01±810.95	350.9±63.89
T. vulgaris	0.4±0.1	504.64±11.44	17.2±6.04	64.25±18.1	179.93±25.79	2381.91±664.46	322.85±37.14
H. perforatum	0.31±0.04	573.49±22.1	23.84±7.68	58.66±8.01	127.7±21.15	5650.66±1062.6^{bc**}	265.64±100.3

*** – P < 0.001 – the superscripts within a column indicate statistically significant differences among the groups.
** – P < 0.01
* – P < 0.05
a – between dry herbs
b – between EOs
c – between dry herbs and Eos
Note: All data are given as mean ± standard error mean (for example 0.49±0.07). P – values are given at the bottom of the tables.

again by the information published by Primo et al. (2018). Ayoub et al. (2019) have investigated the effects of dried and ground leaves from moringa (*Moringa oleifera*), rosemary (*Rosmarinus officinalis*) and Curcuma (*Curcuma longa*) on immune parameters of Nile tilapia before and after infection with *Aeromonas hydrophila*. The results showed that serum concentrations of albumin, globulins, and total protein were significantly higher compared to those in the control group. Also, serum lysozyme and respiratory activity were statistically significantly higher in treated groups than in control fish. Shokrollahi et al. (2015) investigated the effect of rosemary extract (*Rosmarinus officinalis*) on weight, hematological parameters, and cell-mediated immune response in newborn goat kids. The results confirmed that rosemary essential oil added to the milk of kids exerted a positive effect on their immunity. Franciosini et al. (2016) established the positive effect of aqueous extracts of oregano (*Origanum vulgare* L.) and rosemary (*Rosmarinus officinalis* L.) on immune functions and the intestinal microbial population of broiler chickens. Yousefi et al. (2020) reported that the application of lavender extract (LE) increased total white blood cell counts, plasma globulins, APCA activity, and serum lysozyme concentrations in carp before and after stress. Hashemipour et al. (2013) evaluated the effect of a phytogenic product containing a mixture of equal parts of thymol and carvacrol applied at 4 dose rates (0, 60, 100, and 200 mg/kg) on broiler chickens. The authors concluded that a combination of thymol and carvacrol improved the immune response of broilers. Haghighi et al. (2018) and Ali et al. (2018) investigated the effect of oregano extract on total serum protein, albumin and globiluns, respiratory activity, phagocytic activity, and serum lysozyme in rainbow and reported improved growth performance, increased serum lysozyme concentrations, increased total antibodies, and better survival rate. Chun et al.

(2001) carried out a unique experiment that demonstrated anticomplementary activity (inhibition of complement system activation) of a polysaccharide isolated from thyme leaves (*Thymus vulgaris* L.). The isolated polysaccharide has inactivated both pathways of complement activation – the classical and the alternative. Jiang et al. (2012) investigated the effect of methanolic extract from Saint John's wort on specific humoral immune responses in chickens vaccinated against various avian influenza strains. They found that the application of the extract as a dietary supplement during the immunization period enhanced the effect of vaccination against avian influenza. Landy et al. (2012) carried out an interesting experiment to investigate of effects of using dried ground aerial parts from Saint John's wort as an alternative to nutritional antibiotics in poultry farming. It was observed that this herb improved feed conversion, increased antibody titer against avian influenza, and decreased blood cholesterol concentration compared to chickens treated with a nutritional antibiotic (flavophospholipol). Other researchers (Shang et al., 2012) attempted to treat chickens infected with Gumboro disease virus (IBDV BC-6/85) by applying Saint John's wort extract. According to the results, Saint John's wort extract applied at 1330 and 667.9 mg/kg resulted in a significant therapeutic response and improvement of immune functions of infected chickens.

Some authors try to explain the mechanisms of immunostimulation of medicinal plants. According to Kumar et al. (2012), the main substances of medical plants are alkaloids, polysaccharides, cannabinoids, triterpenoid saponins, and glycosides. Mentioned substances that act on the immune system in different ways for example increasing serum immunoglobulin levels, neutrophil adhesion, phagocytosis, the total number of T-helper and T-suppressor cells, activation of macrophages, inhibition of C3 convertase of the classical complement pathway, induction of cytokine (TNF-a, IFN-g), lymphoid cells stimulation, cellular immune function enhancement and nonspecific cellular immune system effect, increase immunoglobulin production, nonspecific immunity mediators and natural killer cell numbers. Sharma et al. (2017) in their review also showed that alkaloids, flavonoids, terpenoids, polysaccharides, lactones, and glycoside products possessed immunomodulatory properties. For example, the root extract of *Astragalus membranaceus* was found to lower IL-6 *in vitro* human model (IL-6 is an inflammatory and impending deterioration marker). Garlic (*Allium sativum*) is found to lower IL-1 and IL-6, TNF, and IL-8 acting as an anti-inflammatory inhibitor and boosting effect on IL-10 which is an antagonist to pro-inflammatory cytokines. Megna et al. (2012) tested the effect of Echinacea, Rhodiola, and Ginseng on the immune system of athletes especially in endurance sports in relation to exercise, and reported that *Echinacea purpurea* is stimulating on all cytokines as to the root, while the leaf has an immunosuppressive action. *Rhodiola rosea* stimulates all the cell lines of the immune system like Echinacea. Trinh et al. (2020) investigated the effect of herbal formulation KM1608 (alcohol extract from *Saussurea lappa, Terminalia chebula*, and *Zingiber officinale*) on RAW264.7 murine macrophages and showed that KM1608 stimulates the expression of immune cytokines (interferon (IFN)-α, β, IL-1β, IL-6, IL-10 in macrophages. Bozakova et al. (2018) reported that preparation Immunobeta (CHEMIFARMA S.p.A., Animal nutrition products, Italy) which consists of ß-glucans and mannan oligosaccharides enhanced serum lysozyme, IgM, and IgG concentrations, the activity of APCA and betalysine, in layer hens. The immunomodulatory effect of ß-glucans and mannan oligosaccharides is based on the activation of neutrophils, B cells, T cells, and natural killer cells. They also enhance cytotoxic activity and inflammatory cytokines of primary macrophages and RAW264.7 cell lines (Kim et al., 2011).

The studies of Jiang et al. (2011) show that pre-treatment with chicken IFN-α before AIV infection (Avian influenza virus) significantly reduces virus replication in both chicken and turkey lung cells and to a lesser extent in cells of a duck. Virus growth was reduced by approximately 200-fold in chicken and turkey cells and 30-fold in duck cells after 48 hours of incubation. Taken together, these studies show that chicken IFN-α reduces virus replication, reduces the host's innate immune response after infection, and is biologically active in other bird species. Mo et al. (2001) investigated the in vitro and in vivo effects of chicken interferon alfa on IBDV (infectious bursal disease virus) infection. Recombinant interferon inhibits IBDV plaque formation in a dose-dependent manner and improves IBDV infection and Newcastle disease virus. Galal et al. (2016) believe that the addition of herbal oils to poultry feed is an alternative and complementary approach to conventional viral control strategies in poultry farming. In their study, they used oregano oil in various doses. Positive regulation of both NDV and AIV-HI specific antibody titers existed when birds were given oregano oil along with NDV and AI vaccination, especially in the case of a higher dose. A study of the effect of OEO on chicken interferon-alpha components of the signaling pathway after NDV vaccination using real-time quantitative PCR (qRT-PCR) does not reveal an obvious positive immunomodulatory effect on the level of transcripts of resistance to myxovirus 1 (Mx1), except at 29 days of age. On the other hand, dose-dependent increased regulation of both IFN regulatory factor 7 (IRF-7) and interferon-alpha RNA levels has been observed. Both doses of OEO can protect birds from nervousness because of a challenge with vvNDV. In conclusion, oral addition with OEO has beneficial effects on avian growth as well as humoral and innate immune responses.

Song et al. (1997) reported that IFN-γ inhibited vesicular stomatitis virus (VSV) mediated by the cytotoxicity of chicken embryonic fibroblast cells (CEF) and increased the expression of many antigens on the surface of cell surface macrophages, including protein-based complexes Class I and Class II histocompatibility (MHC). These results indicate that chicken IFN-γ has antiviral activity and regulates macrophage activity. However, technologies need to be developed to stimulate the production of interferon-gamma in birds. Yeh et al. (2004) used recombinant chicken interferon-gamma (ChIFN-gamma) to determine its in vitro effects on chicken immune cells. They found ChIFN-gamma-induced nitric oxide (NO) production, increased regulation of cell surface Ia expression, and inhibition of Newcastle disease virus replication in NCSU and HD11 cells (chicken macrophage cell lines). In addition, ChIFN-gamma has an anti-proliferative effect on RP9 cells and the chicken B cell line. Finally, ChIFN-gamma inhibits the mitogenic proliferation of normal chicken spleen cells and induces cells to generate NO. Inhibition of viral replication and mitogenic proliferation of normal cells are associated with NO production. They concluded that recombinant chicken ChIFN-gamma modulates chicken immune cells.

Coccidiosis is known to be a constant enemy of poultry farming. Shah et al. (2010) investigated the immunogenicity of the cSZ-2 vaccine against *Eimeria acervulina* in combination with chicken interferon-gamma. The results showed that cSZ-2 in combination with interferon-gamma could protect chickens from coccidiosis by significantly reducing weight loss and the release of oocysts, reflecting partial protection against *E. tenella* infection.

Conclusion

We reported in the section Results that the highest concentration of lysozyme is in chamomile, APCA had the highest activity in the group treated with lavender, betalysin shows the highest activity in the groups treated with chamomile, alpha interferon has the highest level is in thyme, interferon-gamma has the highest value in the control group, immunoglobulin IgG has the highest value in oregano, immunoglobulin IgM has the highest value in chamomile. Based on obtained results, it could be concluded that studied herbs possess an important immunomodulating potential in chicken broilers, which could improve their health and consequently, their productive performance.

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