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BENEFICIAL EFFECTS OF CHRYSIN AND GINGER ON BLOOD BIOCHEMICAL PARAMETERS, HEMATOLOGY AND IMMUNE RESPONSE OF BROILERS

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Abstract

A study using 80 one-day-old Ross 308 broiler chickens was undertaken to assess the impact of orally administered chrysin, ginger and their combination on blood biochemistry (total protein, triglyceride and AST: aspartate aminotransferase activity), hematology (RBC: red blood cells, WBC: white blood cells, HbC: hemoglobin concentration and PCV: packed cell volume) and immune response against Newcastle disease (ND), infectious bronchitis virus (IBV) and infectious bursal disease (IBD). Experimental birds (n= 20 chicks in each treatment) received basal diet (control) or basal diet plus capsulated forms of either of chrysin (40 mg), ginger (80 mg) or their mixture (40 mg of chrysin plus 80 mg of ginger) for 42 days. At the end of experiment, blood samples (n=6 birds per treatment) were collected and tissue samples (n=4 birds per treatment) including spleen and bursa of Fabricius were carefully removed and weighed. According to the results, blood total protein was not significantly (P=0.32) affected by treated groups, however, chrysin and its mixture with ginger had significantly ($P<0.01$) higher AST activity compared to other groups. Triglyceride, by contrast, had significantly ($P<0.01$) lower value in treated groups than control group. In compared with control group, while red blood cells were significantly ($P=0.01$) lower in additive groups, however, WBC were significantly ($P<0.01$) increased in the same groups. Neither did HbC (P=0.13) nor PCV (P=0.12) significantly alter in the treated groups. With regard to immune response, chrysin, ginger or their mixture significantly ($P<0.01$) enhanced antibody response against ND, IBV and IBD. Birds received ginger or its mixture with chrysin had significantly higher spleen and bursa relative weights compared to their respective control groups ($P<0.05$). Taken together, chrysin, ginger or their mixture could be a proper feed additive in broilers' diet.

Keywords: Broilers, Chrysin, Ginger, Blood Parameters, Immune Response

Introduction

There is an increasing public concern over potentially harmful drug residue in human diet as a result of using chemical additives and antibiotics in poultry industry. Worldwide restriction has also been imposed against antibiotic use in poultry farms due to the emergence of antibiotic resistance by pathogen bacteria (Oleforuh-Okoleh *et al.,* 2015). Therefore, finding safe and effective alternatives that benefit both farmers and consumers is recommended. Having multiple positive pharmacological and physiological effects, plant-derived substances have received considerable interest among researchers and therefore studies on finding safe and reliable sources are on the rise.

Chrysin, a polyphenolic phytochemical, with multiple biological properties naturally occurs in honey, bee propolis and various plants (Mantawy *et al.,* 2014). Dietary supplementation of propolis as a main source of chrysin had positive effects on hematological parameters of broilers, but attributing this effect to chrysin may not be true and study on pure source of chrysin would be worthwhile. Anticancer effect of chrysin in human (Khoo *et al.,* 2010) as well as its stimulating effects on the reproductive performance of male rat (Ciftci *et al.,* 2012) and rooster (Amin Altawash *et al.,* 2017) have been well documented before, but there is not enough data concerning its impacts on hematology and immune system of broiler chicks.

Ginger (*Zingiber officinale*) rhizome is a common food additive contains several biologically active compounds lending it antioxidant, antimicrobial, immunomodulatory and antilipidemic properties (Ali *et al.,* 2008). These advantages persuade researchers to further assess dietary inclusion of ginger in broilers diet as a safe and reliable alternative for currently used harmful chemicals. Although there are some reports on the effect of ginger on the productive performance of broilers (Zhang *et al.,* 2009; Qorbanpour *et al.,* 2018), literature is lacking in assessing its effectiveness along with less-known phytochemical such as chrysin. Consequently, this study was aimed to find the impact of chrysin, ginger or their combination on some selected physiological parameters of blood in broiler chicks.

Materials and Methods

Animal ethics and treatments

All procedures in the current study were confirmed by the Animal Ethics Committee in the Department of Animal Production, Faculty of Agriculture, University of AL-Qadisiyah, Al-Diwaniyah, Iraq.

Eighty one-day-old Ross 308 broiler chicks acquired from a local hatchery and were at random assigned to four treatments (n= 20 birds per each treatment) including basal diet (control) and basal diet plus capsulated form of ginger (80 mg), chrysin (40 mg) or their combination (80 mg of ginger + 40 mg of chrysin). Basal diet was prepared according to the NRC recommendation (Table 1). Experimental period started following two weeks of adaptation (1 to 2 weeks) and lasted till six weeks of age. Birds received feed and water ad libitum throughout the experiment.

Ginger rhizome was purchased from local market and dried in a dryer at 60ºC for 5 days. Using mill (Panasonic M176, China), it was ground into a micron particle for 5 minutes. Other materials including chrysin were bought from Sigma company (St. Louis, Mo, USA) and Merck (Darmstadt, Germany) unless otherwise indicated.

Table 1 :. Basal diet composition fed to experimental birds

Ingredients (%)	Starter (1-21 days)	Finisher (22-42 days)
Corn	47	56
Soybean meal	37	35
Wheat	8	0
vegetable oil	3	4.5
Limestone	2	1.5
Salt	0.2	0.2
Premix*	2.8	2.8
Total	100	100
Calculated nutrients		
Energy (kcal/kg of diet)	2910	3050
Crude protein (%)	22.6	21.2
Calcium (%)	0.85	0.7
Phosphorus (%)	0.47	0.50
Lysine (%)	1.02	0.95
Methionine + Cysteine (%)	0.83	0.75

*Premix contains vitamins (vit. A: 2400000 IU, vit E 16000 IU, vit. K3 800 mg, vit D3 1000000 IU, riboflavin 1600 mg, niacin 8000 mg, thiamine 600 mg, vit. B12 6mg, folic acid 400 mg, vitamin B6 1000 mg) and minerals (Mn 18000 mg, Zn 14000 mg, Fe 1200 mg, Cu 2000 mg, Se 60 mg) per kg of diet.

Sampling and analysis of traits

On the last day of experiment, six birds were randomly chosen and blood samples were obtained from the brachial vein in heparinized tube and centrifuged (3000 rpm, 15 min) to separate plasma samples which then were stored in -20 °C until analysis. Samples were thawed and triglyceride level was determined using enzymatic method (Spinreact kit, Santa Coloma, Spain) in which triglyceride was completely hydrolyzed to release its glycerol and measured spectrophotometrically (Qorbanpour *et al.,* 2018). Total protein of plasma was measured using Biuret method (Lubran, 1978). Biochemical test to determine AST activity was determined by procedure described by (Reitman and Frankel, 1957).

Samples (n= 5 birds per treatment) subjected to hematological parameters including PCV, RBC, WBC and HbC were determined in tubes containing EDTA as a coagulant and by Wintrobe hematocrit, Neubauer haemocytometer and cyanomethaemoglobin methods, respectively (Olorede *et al.,* 1996).

In order to assess immune response, briefly six birds from each treatment were randomly injected sheep red blood cells (0.5 mL of 5% suspension diluted in phosphate buffered saline) at 35 days and blood samples were collected from brachial veins 7 days later at 42 days. Using hemagglutination assay, total antibody response against ND, IBV and IBD was determined (van Heugten and Spears, 1997).

On the last day of experiment, four birds from each treatment were randomly selected and individually weighed (CAMRY ACS-40-JE11, Hong Kong) immediately before being slaughtered. The bursa of Fabricius and spleen from each bird were carefully removed and weighed (Kern ACS-40-JE11, Germany). The relative weights were estimated as the organ weight/body weight ratio (Madej *et al.,* 2015).

Statistical analysis

Shapiro–Wilk test was used to exam the normality of datum and transformation conducted where appropriate. Data were analyzed by the GLM procedures of SAS. All of means were compared by Tukey's test with a statistical significance value of $P < 0.05$.

Results and Discussion

The results of blood biochemicals and hematology of broilers fed chrysin, ginger and their mixture are presented in Table 2. As the obtained results show, total protein content was not significantly altered; however, triglyceride concentration significantly ($P<0.01$) decreased in the feed additive groups. In consistent with our findings, blood total protein did not alter significantly in rats treated with chrysin compared with untreated ones (Malarvili and Veerappan 2014). Chrysin showed significant antihyperglycemic and antidiabetic effects in nude diabetic mice (Ramírez-Espinosa *et al.,* 2018). In fact, chrysin which was as effective as metformin in reducing blood triglyceride level via the activation of PPAR-γ and this in turn enhances insulin sensitization and glucose metabolism. The effect of ginger on total protein content of plasma in broilers is controversial. On one hand the positive effect of ginger at 5 g/kg of diet on blood total protein was reported by (Zhang *et al.,* 2009), but on the other hand dietary supplementation of ginger at 60 g/kg decreased broilers' total protein content (Al-Homidan, 2005). A non-significant effect of dietary ginger on blood total protein in broilers was also previously reported (Mohamed *et al.,* 2012). This discrepancy may due to the various levels of administration. In the study of Saeid *et al.* (2010), broilers received ginger at the rate of 0.4% or 0.6% of drinking water had significantly lower cholesterol, triglyceride and glucose concentration. Triglyceride-lowering mechanism of ginger could mediate through its insulin sensitizer activity and thus stimulating effect of insulin on lipoprotein lipase activity (Saeid *et al.,* 2010). In addition, ginger inhibit the conversion of extra carbohydrates into TG by down-regulating the expression of hepatic carbohydrate response element-binding protein (ChREBP), one of the main contributing factors in hypertriglyceridemia and fatty liver disease (Gao *et al.,* 2012).

In the present study, birds received chrysin, ginger or their mixture had significantly higher aspartate aminotransferase activity compared to control ones (Table 2). Chrysin pretreatment in mice subjected to intraperitoneal injection of carbon tetrachloride, as an acute liver damage inducer, showed potent hepatoprotective activity through reducing serum AST and ALT activities (Hermenean *et al.,* 2017). In addition, supplementation of layers diet with propolis at 100 or 150 mg/kg of diet reduced alanine aminotransferase and aspartate aminotransferase activity (Galal *et al.,* 2008; Abdel–Mohsein *et al.,* 2014). Ginger could effectively protect mice liver against piroxicam-induced histopathological changes and also decreased elevated serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) (Badawi, 2018).

In the present study, when compared to control group, RBC significantly decreased, but WBC significantly increased in feed additive groups (Table 2). On the contrary, hemoglobin concentration and PCV percent did not change significantly. Propolis, as a main source of chrysin, had no significant effect on the biochemical and hematological parameters in rat (Sforcin *et al.,* 1995), but it significantly reduced red blood cell count and hemoglobin concentration in men after a period of 30 days of supplementation (Jasprica *et al.,* 2007). Rats received a single various doses of chrysin had a significantly lower RBC compared to sham group (Halina Borawska *et al.,* 2014). This was in contrast with Olorede *et al.* (1996) report in which aqueous extract of ginger, garlic or their combination increased PCV, Hb and RBC in broilers. Dietary supplementation of propolis in broilers (Shihab 2012) (2-3 g/kg of diet), layers (Cetin *et al.* 2010) (3g/kg of diet) and ducks (2 g/kg of diet) (Abdel-Rahman 2013) enhanced red blood cell counts. In one study carbon tetrachloride treatment in rats resulted in higher blood cellular constituents (RBC, WBC and Hb), enzymes activities (gamma glutamyltransferase, alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase) and metabolites' concentration (triglycerides and cholesterol) as compared to control group. However, methanol extract of ginger (250 and 500 mg/kg) significantly restored those alterations (Atta *et al.,* 2010).

The effects of two feed additives and their combination on bird immune response are shown in Table 3. The antibody titers against ND, IBV and IBD at 42 days of age were significantly enhanced in chrysin, ginger and their combination groups. Orally administration of chrysin could promote immune response in leukemic mouse. In fact, chrysin promoted the immune response via both humoral immune response (increasing B-cell population and promotion of macrophage activities) and cellular immune response (increasing the activity of NK cells) (Lin *et al.,* 2012). In addition, IgM and IgG concentrations in broilers (Shihab 2012) and layers (Cetin *et al.,* 2010) significantly increased following dietary inclusion of 3 g/kg of propolis. Saeed *et al.* (2017) proposed that these effects might due to flavonoids and benzene compounds of propolis. Immunoregulatory effect of chrysin mediated via various mechanisms. For instance, it acts as an antagonist of NF-kB which involved in the expression of encoding of genes the cytokines of pro inflammatory (IL-1, IL-2, IL-6, TNF-α) and enzymes (COX-2 and iNOS). Therefore, it could be a good therapeutic candidate for people with immune system problems (Zeinali *et al.* 2017) In agreement with our study, dietary inclusion of ginger at 0.02 could significantly increase antibody titer against Newcastle vaccine in broilers (Valiollahi *et al.* 2014). On the other hand, antibody response against virus of Newcastle disease and avian influenza at 28 and 42 days of age were not significantly affected in birds received ginger at 0.15%, 0.20% and 0.25% of diet (Qorbanpour *et al.* 2018). Immunomodulating effects of ginger was reported in Kausar *et al.* (1999) study in which carminative mixture containing ginger increased primary and secondary antibody responses against Newcastle disease in broilers.

The relative weight gain of lymphoid organs is a marker of improvement in the function of immune system. In our study, birds fed sole ginger had significantly higher relative spleen weight compared to control group (Figure 1). In addition, birds received ginger or its combination with chrysin had significantly heavier bursa of Fabricius than control group. This was in consistent with other study in which mean spleen weight and antibody titer against ND showed a significant increment in broilers fed ginger at 1 gr/kg of diet (Taghdisi and Hejazi 2019).

Table 2 : Blood biochemical and hematological parameters in broilers fed chrysin, ginger or their mixture

Traits	Treatments* (Means± SE)				*P* value
	Control	Chrysin	Ginger	Chrysin+Ginger	
Total protein (g/dL)	5.98±0.21	5.51±0.61	5.48±0.12	6.40±0.43	0.32
Triglyceride (mg/dL)	135.40^{a}±6.95	107.80^{b}±7.04	105.20^{b}±7.17	89.60^{b}±7.25	<0.01
AST activity (U/L)	201.40^{b}±10.50	211.60^{b}±6.64	255.60^{a}±7.07	246.00^{a}±12.10	<0.01
Red blood cells $\times 10^{6}$	2.77^{a}±0.09	2.21^{b}±0.12	2.29^{b}±0.08	2.34^{b}±0.14	0.01
White blood cells$\times 10^{3}$	68.54^{b}±1.10	79.74^{a}±3.44	76.68^{a}±1.48	83.12^{a}±1.27	<0.01
Hemoglobin concentration (g/dL)	11.46±0.44	10.20±0.48	10.86±0.43	10.12±0.35	0.13
Packed cell volume(%)	32.62±1.54	29.60±1.31	30.10±1.80	27.36±1.00	0.12

*Means with different superscripts within same row are significantly (P<0.05) different.

Table 3 : Antibody response against some common diseases in broilers fed chrysin, ginger or their mixture

Traits	Treatments* (Means± SE)				*P* value
	Control	Chrysin	Ginger	Chrysin+Ginger	
Newcastle disease	569.33^{b} ±52.76	5110.66^{a}±399.33	4778.83^{a}±367.06	5092.16^{a}±549.65	<0.01
Infectious bronchitis virus	461.50^{b}±113.01	3561.00^{a}±981.90	4690.00^{a}±734.10	3243.50^{a}±794.00	<0.01
Infectious bursal disease	2139.33^{b}±260.35	8912.83^{a}±909.53	8000.00^{a}±693.95	8291.83^{a}±1243.54	<0.01

*Means with different superscripts within same row are significantly (P<0.05) different.

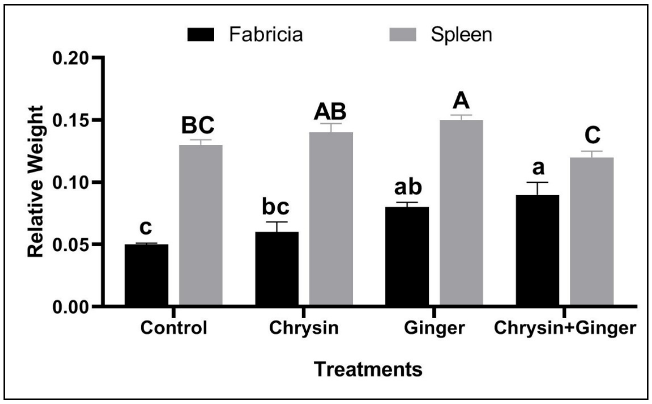

Fig. 1 : Relative weights of spleen and bursa of Fabricius in broiler chicks fed chrysin, ginger or their combination. Bars lacking a common letter are significantly (*P*<0.05) different.

Conclusion

The results of this study suggest that chrysin and ginger are effective feed additives to improve health status of broilers via modulating their blood parameters, immune response and also lymphatic organs' development. Given that these compounds can be appropriate alternatives to commonly used chemicals and antibiotics in poultry industry. However, further studies are recommended to optimize their dietary inclusion rate and assess their impacts on the growth excution and microbiological profiles of broiler digestive tract.

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