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Evaluation of Nettle *(Urtica dioica)* and Ginger *(Zingiber officinale)* Powder on Serum Antioxidants and Immune responses of Broiler Chicks

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ABSTRACT

This study was performed to investigate the effect of dietary supplementation with nettle (Urtica dioica) and ginger (Zingiber officinale) as an antibiotic growth promoter on serum antioxidant capacity and immunity of broiler chicks. A total of 450 day-old broilers (Ross 308) were randomly assigned to six dietary treatments with five replicates. The dietary treatments consisted of the basal diet as control; antibiotic growth promoter, 100 mg/kg vitamin E, 2 g/kg nettle powder; 4 g/kg ginger powder and 2 g/kg ginger+2 g/kg nettle powder. At day 8 Newcastle and Influenza viruses were injected and at day 25 two birds per pen selected and sheep red blood cell (SRBC) were injected to venous, and then at day 30 antibody titer against Newcastle, Influenza and SRBC were measured. At day 42, two broilers were selected and blood samples were collected for determination serum antioxidant capacity. Antibody titers against Newcastle and Influenza viruses and SRBC were not significantly influenced by dietary treatments (P>0.05). Serum antioxidant capacity significantly elevated by nettle or ginger (P< 0.05). In conclusion, nettle or ginger cannot be used as an immunomodulator but can improve serum antioxidant capacity of broiler chicks.

Key words: Broiler chick, Nettle, Ginger, Antioxidant, Immunity.

INTRODUCTION

At the end of 1940 decade American farmer started using antibiotics as feed additive [1]. Antibiotics cause to bacterial resistance, chemical compound residual into meat and their side effect on human. Gradually antibiotic growth promoter usage had limited till beginning of January 2006 in Europe and after that banned [2]. In future antibiotic usage as a growth promoter will be forbidden in the world, therefore antibiotic substitution is unavoidable. Medicinal plants and or their components are one of the antibiotic growth promoter substitutes. Plant contain essential oils and their mode of action as follow: a) antimicrobial property; b) antioxidant; c) stimulating the production of digestive enzyme d) nitrogen absorption e) reduction of ammonia producing in intestine. Medicinal plants reduce harmful bacteria in gastrointestinal tract, reduce amino acid and protein degradation, with this mechanism most of the amino acids and proteins will be absorbed and save; following this mechanism carcass percentage will be better, protein conversion to fat and fat deposition will be reduced [3]. Some advantages of medical plants are: a) their usage is simple, b) most of them have no side effect on animal performance c) have no residual in animal products. Some trials showed that plant secondary metabolites such as: Isoprenes derivatives, Flavonoids, Glucosinolats act in animal body like antibiotics or antioxidants [4].

Farzami et al [5] showed that nettle extract increases insulin secretion in hyglicemic mousses. Tomson [6] in his recent experiment showed adding dried nettle in broilers diet at 0.2% level had positive effect on final weight. Nettle protect small intestine epithelium, this protection is for blood turning improvement and or improvement in nervous system performance by reduction in sorbitol production. Some ingredients in nettle and other medical plants like Carvacrol and Thymol reduce blood cholesterol and triglyceride [7]. In accordance with Nasiri et al [8] findings, 0.5% nettle powder in starter and grower phases of broiler chicks improved carcass parameters. While, Khosravi et al [9] reported nettle extract cannot suitable replacement instead of antibiotic. In Rukhani et al [10] experiment, ginger uses in poultry diet caused to an increase serum enzymes activity such as superoxide dismutase, catalase and glutathione peroxidase, these enzymes are important antioxidants enzymes. Also, Koachare et al [11] declared, ginger increased number of lactic acid bacteria in jejunum of poultry, final weight and improved feed conversion ratio. Sadeghi et al [12] declared, ginger powder has an antibacterial property such as gingerioul and other ingredients have positive effect on broilers growth performance and immune systems. Tabatabaei et al [13] stated 0.5% and maximum 0.7% ginger especially in starter phase can improve broilers performance and fortifying their immunity system. Arkan et al [14] in their experiment used ginger and expressed, 0.2% level significantly decreased feed conversion ratio in grower phase. Fewest feed intake was achieved by increase ginger level from 0.1% to 0.2%. Ginger in two levels (0.1 and 0.2%) significantly reduced blood glucose, cholesterol and triglyceride, but treatments had no effect on amount of serum protein. Zhang et al [15] showed, ginger in broiler feed improved serum antioxidant activity. In another experiment ginger increased feed palatability and feed intake [16]. In accordance with Hashimoto et al [17] ginger effectively can stimulate digestion and intestine peristalsis movement.

Thus, this study was conducted to evaluate the effect of nettle and ginger powder as an antibiotic growth promoter on antioxidants and immunity of broiler chicks.

MATERIALS AND METHODS

Four hundred and fifty day-old broiler chicks (Ross 308) were weighed on arrival time and randomly assigned to six treatments with five replicates of 15 chicks based on completely randomized design. Feed and water were provided *ad libitum* throughout the experiment. The lighting program consisted of 23 hours light and 1 hour darkness. The experimental diets consisted of 1) basal diet as a control group; 2) basal diet plus 0.5 g/kg flavophospholipol as antibiotic growth promoter; 3) basal diet plus 100 mg/kg vitamin E; 4) basal diet plus 2 g/kg nettle powder; 5) basal diet plus 4 g/kg ginger powder; 6) basal diet plus 2 g/kg nettle + 2 g/kg ginger. Table 1 lists the basal diets formulated for broilers according to manual of Ross 308. Average daily feed intake, daily weight gain and feed conversion ratio were measured at the end of each phase. On day 28 and 42 two birds per replicate with average body weight close to group weight was selected, weighed and slaughtered for determination of lymphoid organs (spleen and bursa of Fabercius) weight.

For immunological stimulus against Newcastle disease virus (NDV) and avian influenza virus (AIV, H9N2 subtype) subcutaneously with 0.2 mL per chick at 8 days of age. On days 30, two birds were randomly selected and blood samples were taken by brachial puncture vein for analysis of antibody titers against Newcastle disease viruses and Influenza viruses by the hemagglutination inhibition test and expressed in log2.

On day 25 two birds per pen randomly selected and sheep red blood cell (SRBC) diluted at 1% in sterile phosphate buffered saline solution, was injected at 1 mL dose per bird. Serum were collected 5 days post-SRBC injection, and specific antibodies against SRBC were measured by hemagglutination technique and expressed in log2.

For determination of serum antioxidant capacity of broilers, at 42 d two birds per pen were selected and blood sample were taken via vein puncture. Blood samples were centrifuged at 2000 rpm for 10 minutes to obtain serum and serum antioxidant capacity was measured according to method of karakay et al [18].

Data were subjected to the analysis of variance appropriate for a completely randomized design using General Linear Model (GLM) procedure of SAS institute [19]. If a significant effect was detected, differences between treatments were separated using LSD test. Statements of statistical significance are based on a probability of $P< 0.05$.

RESULTS AND DISCUSSION

Serum antioxidant and immunity

The effect of experimental diets on serum antioxidant and immunity is presented in Table 2. There were no significant difference in antibody titer against Newcastle and Influenza viruses (P>0.05). Although, numerically greatest antibody titer against Newcastle disease viruses and Influenza viruses observed in vitamin E treatment and antibody titer against sheep red blood cell gained in nettle treatment. Nasiri and coworkers [20] applied different levels of Nettle (*Urtica diocia*) on various parameters including immunity parameters of broilers and reported experimental diets did not have any significant effects on immunity parameters of broilers. Serum antioxidant capacity were significantly influenced by experimental treatments (P<0.05). Govahi et al [21] stated total activities of serum antioxidant of broilers significantly increased in 6 g *Ferulago angulate sub. carduchrom* significantly increased compared to antibiotic treatments (P<0.05). Maximum serum antioxidant capacity was observed in vitamin E treatment (1.286 µm/L) and minimum were observed in control group (0.935 µml/L). According to present results, serum antioxidant capacity in three treatments comparison with control group significantly increased, while there was no significant difference with vitamin E treatment. Medicinal plants lead to antioxidant activity improvement. This results according to Rukhani et al [10] that stated ginger in poultry diet cause to increase activity of serum enzymes like superoxide dismutase, catalase and glutathione peroxidase, these are important antioxidant enzymes. Also, Booth and Bradford [22] reported nettle can have strong antioxidant property, because this plant has noticeable amount of vitamin E and phenolic compound. Karakay et al [18] expressed phenolic compound in nettle has antioxidant property and when they use in small amount comparison to large amount, their activity will be better. Contemporary to results of present experiment, Fekri-Yazdi et al [23] used *Tribulus terrestris* L. as an antibiotic growth promoter substitute, broilers that received 1 or 5 g/kg had higher antibody titer against Avian Influenza Virus compared to control group.

The results related to lymphoid organs are summarized in Table 3. In 28 day, significant difference was not observed for spleen and bursa, while bursa weight had significant difference in 42 day (P<0.05). In agree with present results, Toghyani et al [24] in their evaluation about using cinnamon and garlic as an antibiotic growth promoter substitution in broilers' diet, the weights of lymphoid organs in broilers fed diets contain different level of cinnamon and garlic were not significant. Also, they did not observe significant difference in immune responses. As cinnamon and garlic have potential to act as an antimicrobial substance, immune responses were expected to elevated but positive or negative changes were not observed, maybe high amount of herbal plants needed to stimulate humoral immune responses. Contrary to Toghyani et al [24], Rahimi et al [25] reported that relative weight of bursa in broiler fed garlic significantly increased but relative weight spleen was affected. Antibody response to SRBC was higher in coneflower group (P< 0.05). Antibody response to Newcastle disease vaccine was unaffected by the treatments but antibody levels were improved in the coneflower group (P< 0.05).

Table 1. Ingredients and composition of the basal diets.

Diet composition %	Starter (1-14 d)	Grower (14-28 d)	Finisher (28-42 d)
Corn	55.18	60.1	66.8
Soybean meal	39	34.50	28.25
Soybean oil	1.50	1.50	1.30
Mono calcium phosphate[1]	1.50	1.40	1.30
Calcium carbonate	1.74	1.50	1.40
Salt	0.30	0.30	0.30
DL-Methionine	0.32	0.24	0.20
L-Lysin.HCl	0.20	0.18	0.18
L-Threonine	0.06	0.05	0.04
Vitamin premix[a]	0.10	0.10	0.10
Mineral premix[b]	0.10	0.10	0.10
Calculated composition			
Metabolizable energy (Kcal/kg)	2900	2950	3000
Crude protein %	21.85	20.45	18.15
Calcium %	0.97	0.86	0.81
Available phosphorous %	0.46	0.43	0.40
Methione+cysteine	1.01	0.90	0.80
Lysine %	1.35	1.16	1.03
Threonine %	0.83	0.81	0.63

[1] *Monocalcium phosphate contained: 23% phosphorous and 15% calcium.*

[a] 2.5 kilogram of vitamin premix contained: vitamin A, 14000000 IU; vitamin D3, 6000000 IU; vitamin E, 40000 mg; vitamin K3 2640 mg; thiamine, 3000 mg; riboflavin, 6800 mg; panthothenic acid, 20 g, niacin, 50 g; pyridoxine, 6800 mg; cyanocobalamin, 15 mg; biotin, 200 mg; folic acid, 1900 mg; choline chloride, 400 g.
[b] 2.5 kilogram of mineral premix contained: Mn, 100 g; Zn, 100 g; Fe, 51 g; Cu, 15 g; I, 1000 mg; Se, 350 mg.

Table 2. Effect of experimental diets on serum antioxidant capacity and immune responses of broiler chicks

Dietary treatments	Antibody titers(log_2)			Total antioxidant capacity (mmol/dl)
	Newcastle	Influenza	SRBC[1]	
Control	4.62	5.37	7.12	0.935[b]
Antibiotic	4.70	5.40	7.30	0.907[b]
Vitamin E	4.80	5.70	7.00	1.286[a]
Nettle	4.50	5.25	7.58	1.142[a]
Ginger	4.50	5.00	7.20	1.197[a]
Nettle + Ginger	4.37	5.00	7.00	1.199[a]
SEM [2]	0.116	0.138	0.0892	0.0262

[1] Sheep Red Blood Cell.
[2] SEM: standard error of mean.
[a-b] Values in the same column not sharing a common superscript differ significantly (P<0.05).

Table 3. Effect of dietary treatments on average weight of lymphoid organs of broiler chicks at different ages (Percentage of live weight).

Dietary treatments	28 d		42 d	
	Spleen	Bursa of Fabricius	Spleen	Bursa of Fabricius
Control	0.113	0.052	0.107 [ab]	0.067 [b]
Antibiotic	0.146	0.051	0.124 [a]	0.087 [ab]
Vitamin E	0.134	0.029	0.096 [b]	0.074 [ab]
Nettle	0.126	0.054	0.117 [ab]	0.094 [ab]
Ginger	0.128	0.023	0.095 [b]	0.077 [ab]
Nettle + Ginger	0.119	0.063	0.101 [ab]	0.137 [a]
SEM[1]	0.0063	0.001	0.0096	0.0092

[1] SEM: standard error of mean.
[a-b] Values in the same column not sharing a common superscript differ significantly (P< 0.05).

Similarity, Azadegan Mehr [26] which used clove essential oils and probiotic on lymphoid organs and immune response in broiler chicks reported treatment had no pronounced effect on relative weight of bursa of Fabricius, thymus, spleen at 42 days of age. Mostly, beneficial effects of herbal plants on immune system of animals is because of their secondary metabolites [27]. Fekri Yazdi et al [28] used anise seed as an alternative to antibiotic growth promoter in broiler's diet and reported spleen's weight was not affected by dietary treatments although bursa weight was higher for broilers fed with diet contained 1 g anise/kg. Has been reported medicinal plants like anise have antifungal, antimicrobial, and antioxidant activities [29, 30]. Najafi and Taherpour [31] performed an experiment on medicinal herbs and stated that ginger, cinnamon, symbiotic and antibiotic had no significant effect on relative weight of lymphoid organs in broilers at 42 days. Higher spleen weight was observed in antibiotic and nettle treatments. According to these results, Koachere et al [11] stated, ginger can improve immune system.

CONCLUSION

The results of this study demonstrated dietary nettle or ginger cannot be used as an immunomodulator in broiler chicks but can improve serum antioxidant capacity.

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