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Original Article

Effect of Supplementation of Different Levels of *Urtica dioica* Seeds to the Diet on the Immune Response and Microbial Composition of the Gastrointestinal Tract of Broiler Chickens

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Abstract

This experiment was conducted in the poultry field of the Department of Animal Production, College of Agriculture, Al-Qasim Green University in order to assess the effect of adding different levels of *Urtica dioica* seeds to the diet on the immune response and microbial composition of the gastrointestinal tract of broiler chickens. This study was performed on 180 one-day-old unsexed broiler chickens (Ross 380) which were randomly divided into four treatments, with 45 birds per treatment and 3 replicates in each treatment (15 birds per replicate). The treatments were conducted as follows: First treatment (control) without the addition of *Urtica dioica* seeds to the diet, second treatment: the addition of 5g/kg *Urtica dioica* seeds, third treatment: the addition of 10g/kg *Urtica dioica* seeds, and treatment Fourth: the addition of 15g/kg *Urtica dioica* seeds. The experiment included the following traits: antibody titer against Newcastle disease, investigating sensitivity against Newcastle disease, the relative weight of bursa of Fabricius, bursa of Fabricius index, as well as estimating the total number of bacteria, *Coliform* bacteria, and *Lactobacillus* bacteria. The results indicated that the addition of *Urtica dioica* seeds led to significant improvement in cellular immunity (DHT) and antibody titer against Newcastle disease (ELISA), as well as significant improvement in the relative weight of bursa of Fabricius and bursa of Fabricius index, a significant decrease in the logarithmic number of total aerobic bacteria and *Coliform* bacteria, as well as a significant increase in the logarithmic number of *Lactobacillus* bacteria in the Duodenum contents for the small intestine and the Ceca, compared to the control treatment. Based on the obtained results, it can be concluded that the addition of *Urtica dioica* seeds to the diet can improve the immune traits and microbial compositions of the digestive tract of broiler chickens.

Keywords: Broiler chickens, Immune response, Microbial content, *Urtica dioica* seeds

1. Introduction

The poultry industry has received assiduous attention in the agricultural sector due to the production of white meat, eggs, and other by-products, raising living standards. Poultry has a better food conversion efficiency, as well as a greater impact on the amount of feed consumed and the average weight gain for the body, compared to other farm animals. We have recently witnessed the introduction of many medicinal plants in the feeding of broiler chickens and laying hens, including Parsley and lemon grass leaves (1, 2), moringa, and oregano leaves (3), as well a white tea and *Laurus nobilis* leaves (4, 5). One of these plants is *Urtica dioica*, the English name for the Nettle. Its percentage is about 1%-3%; moreover, it possesses many active substances, such as flavonoids, polyphenols, and phenolic acids (6). In addition, it has antioxidant properties since it contains phenolic compounds and antibacterial traits (7). Mansoub (8) indicated that the

addition of *Urtica dioica* in the diets of laying hens intended for egg production led to lower concentrations of malondialdehyde in serum, compared to the control treatment. Mansoub (9) pointed to improve feed conversion ratio of chicks fed diets containing *Urtica dioica*, compared to the control treatment. In light of the aforementioned issues and the absence of research on the use of this plant in poultry diets, the present study aimed to determine the effect of *Urtica dioica* seeds added to the diet on the immune response and microbial composition of the gastrointestinal tract of broiler chickens and identify the best percentages that can be used in the diet.

2. Materials and Methods

This study was conducted in the field of Poultry birds belonging to the Department of Animal Production at the College of Agriculture, Al-Qasim Green University from 21/4/2020 to 25/5/2020 (35 days). This study was performed on 180 one-day-old unsexed broiler chickens (Ross 380) which were randomly divided into four treatments, with 45 birds per treatment and 3 replicates in each treatment (15 birds per replicate). The birds were freely fed twice, the initiator diet from 1-21 days and the final diet from 22 to 35 days as displayed in table 1. The *Urtica dioica* seeds were added to the diet (manual mixing) from the age of one day as follows: First treatment (control) without the addition of *Urtica dioica* seeds to the diet, second treatment: supplementation of 5g/kg *Urtica dioica* seeds, third treatment: supplementation of 10g /kg *Urtica dioica* seeds, and fourth treatment: the addition of 15g/kg *Urtica dioica* seeds. The following traits were estimated at 35 days: antibody titer against Newcastle disease (ELISA) according to Voller, Bartlett (10), investigating sensitivity against Newcastle disease, the relative weight of bursa of Fabricius and bursa of Fabricius index according to Lucio and Hitchner (11) method, as well as estimating the total number of bacteria, *Coliform* bacteria, and *Lactobacillus* bacteria. The completely randomized design was used to assess the effect of different treatments on the studied traits. Significant differences between the means were compared using the Duncan's Multiple Range Test (12), and the Statistical package for social sciences (SAS, 2016) was used to analyze the data.

Table 1. Percentage of Feed materials included in the formation of the Initial Diet and Final Diet Used in the Experiment with the Calculated Chemical Composition for Both Diets

Feed material	Initial diets (1-21 days)%	Final diets (22-35 days)%
Yellow corn	48.2	58.7
Local wheat	8	7.5
Soybean meal (44% protein)	28.5	20.5
The concentrated Protein*	10	10
Vegetable oil (sunflower)	4	2.5
limestone	1	0.5
Food salt	0.3	0.3
Total	100 %	100 %
Calculated Chemical Analysis **		
Metabolic Energy (kg/kg)	3079.85	3102.6
crude protein (%)	21.56	18.87
Lysine (%)	1.04	0.85
Methionine+Cysteine (%)	0.455	0.42
Raw fiber%	3.54	3.2
Calcium (%)	1.28	1.07
phosphorus availability (%)	0.42	0.41

* the concentrated protein, each kilogram contains: 2200 kcal/kg metabolic energy, 40% crude protein, 8% fat, 3.5% fiber, 25% ash, 8% calcium, 3.1 phosphorus availability, 1.2% lysine, 1.2% Methionine, 1.8% Methionine + 70 mg, 30 mg vitamin B1, 300 mg vitamin E, 2500 IU D3, A cysteine, 2% chlorine, 10,000 IU 12 mg folic acid, 250 B12, B 120 mg pantothenic acid, 400 mg niacin, 50 mg vitamin B2 and 6, 5000 mg Choline chloride, 450 mg iron, 70 mg copper, 600 mg, C 600 mcg biotin, 1000 mg special vitamin, 750 manganese, 5 mg iodine, 1 g cobalt and antioxidants

** The chemical composition was calculated according to the analysis of feed materials mentioned in (NRC, 1994).

3. Results and Discussion

Table 2 displays the effect of adding *Urtica dioica* seeds to the diet on the immune response in broiler chickens. Based on this table, a significant improvement ($P \leq 0.05$) was observed in the traits of cellular immunity (DHT), antibody titer against Newcastle disease (ELISA), and the relative weight of bursa of Fabricius in the fourth treatment, compared to the other treatments. A significant improvement ($P \leq 0.05$) was observed for the two treatments (second and third), compared to the first treatment. As for the trait of the bursa of the Fabricius index, the improvement for the fourth treatment was significant ($P \leq 0.05$), compared to the rest of the treatments. The treatments (second and third) showed a significant ($P \leq 0.05$) improvement in the same trait, compared to the first treatment (control). All *Urtica dioica* seeds treatments added to the diet gave the highest immune performance, compared to the control treatment, since medical plants have a great role to play in raising the immune response, stimulating this system in birds, and increasing its effectiveness by raising the level of antibodies against pathogens. The significant increase in the relative weight of the bursa of Fabricius and the bursa of Fabricius index in *Urtica dioica* seeds treatments, compared to that in the control treatment, indicates a significant improvement in the activity of the bursa since it is responsible for humoral immunity in birds and bursa vesicles are involved in the maturation of B cells that are responsible for antibody production (13). The increase in their size points to an increase in their production of B cells, thereby increasing the production of antibodies, which raise the immune response.

Table 3 illustrates the effect of adding *Urtica dioica* seeds to the diet on the logarithmic total number of aerobic bacteria, *Coliform*, and *Lactobacillus* (cfu/gr) of the duodenal and ceca contents for broiler chickens. This table shows a significant decrease in the total number of aerobic bacteria and *Coliform* bacteria in the duodenum and cecum in favor of the fourth treatment, compared to the other treatments. Moreover, a significant ($P \leq 0.05$) decrease was observed in the total number of aerobic bacteria and *Coliform* bacteria in the two treatments (second and third), compared to that in the control treatment (first). In addition, a significant improvement ($P \leq 0.05$) was detected in favor of the fourth treatment in the number of *Lactobacillus* bacteria for the two regions of the duodenum and cecum, compared to other treatments. The significant improvement ($P \leq 0.05$) continued in the logarithmic number of *Lactobacillus* in favor of the treatments (third and second), compared to that in the first treatment (control). Nonetheless, there were no significant differences between the two treatments (second and third) in the two regions of the duodenum and cecum in the numbers of the aerobic bacteria, *Coliform* bacteria, and *Lactobacillus* bacteria. The treatments of *Urtica dioica* seeds added to the poultry diets yielded the best results in the decrease of the total number of aerobic bacteria and *Coliform* bacteria, as well as an increase in the numbers of *Lactobacillus*. This can be ascribed to the fact that *Urtica dioica* seeds contain phenolic compounds that have antibacterial activity against the positive and negative pathogenic bacteria (14). Most of the active compounds affect the structural and functional traits of the cell membrane in microorganisms and the permeability of the membranes, thereby affecting the performance and vitality of those organisms. Accordingly, *Lactobacillus* outnumbered pathogenic bacteria and, therefore, supported microbiological balance within the intestines (15).

Table 2. Effect of adding *Urtica dioica* seeds to the diet on the immune response in broiler chickens (Mean±Standard error)

Treatments	Cellular Immunity (DTH)	Newcastle immunity (ELISA)	The relative weight of the bursa of Fabricius	Bursa of Fabricius index
First treatment (control)	0.015±0.166^{c}	230.4±2619.5^{c}	0.001±0.065^{c}	0±1.000^{d}
Second treatment (5 g *Urtica dioica* seeds)	0.013±0.218^{b}	215.5±2946.6^{b}	0.001±0.098^{b}	0.013±1.508^{c}
Third treatment (10 g *Urtica dioica* seeds)	0.011±0.223^{b}	203.8±2955.8^{b}	0.002±0.101^{b}	0.011±1.554^{b}
Fourth treatment (15 g *Urtica dioica* seeds)	0.011±0.254^{a}	200.9±3017.4^{a}	0.001±0.124^{a}	0.011±1.908^{a}
Significant level	*	*	*	*

• There were significant differences at the level of ($P \leq 0.05$)

Table 3. Effect of adding *Urtica dioica* seeds to the diet on the logarithmic number of total aerobic bacteria, *coliform* bacteria, and *Lactobacillus* bacteria for the contents of the duodenum and the ceca in broiler chickens (mean±Standard error)

Treatments	Duodenum			Ceca		
	Total aerobic bacteria	*Coliform* bacteria	*Lactobacillus* bacteria	Total aerobic bacteria	*Coliform* bacteria	*Lactobacillus* bacteria
First treatment (control)	0.06±6.27^{a}	0.13±13.31^{a}	0.05±3.04^{c}	0.04±3.97^{a}	0.08±8.53^{a}	0.03±2.82^{c}
Second treatment (5 g *Urtica Dioica* seeds)	0.05±3.61^{b}	0.11±11.82^{b}	0.04±4.35^{b}	0.03±3.41^{b}	0.07±7.16^{b}	0.02±3.56^{b}
Third treatment (10 g *Urtica Dioica* seeds)	0.04±3.57^{b}	0.09±11.73^{b}	0.03±4.55^{b}	0.03±3.35^{b}	0.07±6.88^{b}	0.03±3.78^{b}
Fourth treatment (15 g *Urtica Dioica* seeds)	0.05±2.35^{c}	0.09±9.58^{c}	0.04±4.73^{a}	0.04±3.16^{c}	0.05±6.61^{c}	0.02±3.89^{a}
Significant level	*	*	*	*	*	*

• There were significant differences at the level of ($P \leq 0.05$)

Authors' Contribution

Study concept and design: R. J. A.
Acquisition of data: R. J. A.
Analysis and interpretation of data: N. A. A.
Drafting of the manuscript: N. A. A.
Critical revision of the manuscript for important intellectual content: N. A. A.
Statistical analysis: N. A. A.
Administrative, technical, and material support: R. J. A.

Ethics

The study design was approved by the ethics committee of the Wasit University, Wasit. Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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